STUDIES ON PLANT GROWTH PROMOTING BACTERIA AND RECYCLING OF CROP RESIDUES FOR SUSTAINABLE AGRICULTURE

Thesis submitted by

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To Osmania University
for the award of degree of
Doctor of Philosophy
in Microbiology
2005

To My Family

DECLARATION

I, **Hameeda Bee**, Department of Microbiology, Osmania University, Hyderabad, do hereby declare that the research work embodied in this thesis entitled **"Studies on plant growth promoting bacteria and recycling of crop residues for sustainable agriculture"** submitted to Osmania University for award of the degree of Doctor of Philosophy in Microbiology is a bonafide record of work done by me during the period of research carried out under the supervision of Dr. Gopal Reddy, Chairman Board of Studies, Department of Microbiology, Osmania University, Hyderabad. This thesis has not formed in whole or part, the basis for the award of any degree or diploma to any other University prior to this date.

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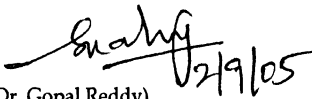M.Sc., M.Phil., Ph.D.,
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CERTIFICATE

This is to certify that the Ph.D. thesis entitled **"Studies on Plant Growth Promoting Bacteria and Recycling of Crop Residues for Sustainable Agriculture"** submitted for the award of degree of Doctor of Philosophy in Microbiology, Osmania University by **Ms. Hameeda Bee** is the original research work carried out under our supervision. This work has not been formed the basis for any degree or diploma of any university or institute prior to this date.

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CHAPTER - I

INTRODUCTION

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1.1 INTRODUCTION

Current trends in agriculture are focused on the diminution of the use of chemical pesticides and inorganic fertilizers, compelling the search for alternative ways to improve soil fertility and crop production. In recent years much attention has been paid to natural agricultural practices in expectation of moving towards environmentally sustainable development (Wani and Lee 1996; Sturz *et al.* 2000). The application of crop residues (Pare *et al.* 2000) and the appropriate management of microbial inoculants as biofertilizers and/or antagonists of phytopathogens (Bashan 1998a; Vessey 2003; Correa *et al.* 2004) are being considered among the diverse technological practices of low-input agricultural systems for decomposition and mineralization of organic matter and release of nutrients (Altieri 1994) for enhancing plant growth.

Man's use of land has aggravated the loss of soil organic carbon from cultivated soils. The practices and conditions that favor higher evolution of carbon dioxide oppose maintenance of organic carbon (also known as carbon sequestration) in soils and vice versa (Heenan *et al.* 1995; Probert *et al.* 2001). Depletion in soil organic carbon is further accentuated by the activities of people, livestock, deforestation, overgrazing, burning of crop residues and vegetation. Disuse of organic manure including removal of crop residues, monoculture without cover crops, fallow vegetation leads to low productivity (Bationo *et al.* 2000; Beri and Gupta 2003a; Biederbeck *et al.* 2005). Several studies have concluded that low soil fertility is often the major constraint for production of both food grains and natural vegetation (Bekunda *et al.* 1997; Singer *et al.* 2000). Although, yields increase due to application

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of chemical fertilizers, they alone cannot sustain crop yields in long run due to heavy cost and decrease in soil quality (Pankhurst *et al.* 1997; Giller *et al.* 1997; Doane *et al.* 2003). Improving soil organic matter is therefore, vital in sustenance of soil quality and agricultural productivity (Reeves 1997; Follett 2001; West and Post 2002). The management strategies for improving soil organic matter and crop productivity include the use of N-fixing cover crops, application of crop residues as surface mulch, compost, manures etc. (Bezdicek and Granatstein 1989; Reeves 1994; Conklin *et al.* 2002; Jordan 2004).

Crop residues and animal excreta are the organic wastes generated by agricultural activities. Proper disposal of these wastes by recycling can supply nutrients to plants and improve soil conditions and environmental quality (Bharadwaj 1995; Bansal and Kapoor 2000). Composting, which is preferentially aerobic or predominantly aerobic, is one method of utilization of organic wastes to produce manure, rich in plant nutrients. The nutrient content of composts varies considerably depending on type of raw materials used, method of composting and maturity. Decomposition is by diverse microbial populations that are highly interactive and syntrophic, with community structures that change rapidly as conditions change (Miller 1993). Vermicompost or composting with earthworms is an alternative to traditional composting. It is the process of turning organic debris into worm castings (Edwards 1998; Arancon *et al.* 2004a). Compared to the parent material, composts have less soluble salts, greater cation exchange capacity, increased humic acid contents and high microbial activity (Albanell *et al.* 1988; Atiyeh *et al.* 2002; Contreras *et al.* 2005).

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Plant growth promoting and biocontrol agents such as *Bacillus* spp. *Enterobacter* spp., *Flavobacterium* spp., *Pseudomonas* spp., *Streptomyces* spp., *Penicillium* spp., *Trichoderma* spp. and *Gliocladium virens* have been identified in compost amended substrates (Nelson *et al.* 1983; Nakasaki *et al.* 1985; Chung and Hoitink 1990; Hardy and Sivasithamparam 1991). Beneficial effects induced by composts and vermicomposts are due to activities of diverse microbial population present in the rhizosphere. Some of these microorganisms produce plant growth hormones and stimulate plant growth directly (Tomati *et al.* 1990). Others produce natural chelators called siderophores that, along with water-soluble humic substances in composts, keep iron at high concentration in available form to plants in soil (Chen *et al.* 1998). Beneficial plant-microbe interactions in the rhizosphere are the determinants of plant health and soil fertility (Jeffries *et al.* 2003; Egamberdiyeva and Hoflich 2004).

Soil-plant-microbe interactions are complex and there are many ways in which the outcome can influence the plant health and productivity (Kennedy 1998). These interactions may be detrimental, beneficial and neutral to the plants. However, the focus is to exploit the beneficial bacteria that enhance plant growth. These are known as plant growth promoting bacteria (PGPB; Bashan and Holguin 1998b) and represent a wide variety of soil bacteria which, when grown in association with a host plant, result in stimulation of plant growth directly and/or indirectly. Direct growth promotion is by nitrogen fixation (Kennedy *et al.* 1997), solubilization of mineral phosphates and mineralization of other nutrients (De Freitas *et al.* 1997; Rodriguez and Fraga 1999), ability to produce 1-amino-cyclopropane-1-carboxylate (ACC) deaminase (Glick *et al.* 1995), hormones like indole acetic acid (IAA) (Pattern

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and Glick 1996), gibberellic acid (Gutierrez Manero *et al.* 2001) and cytokinins (Tien *et al.* 1979; Timmusk *et al.* 1999) that increase root length and plant growth. Indirect mechanisms include antagonism against plant pathogenic fungi by producing siderophores, chitinase, ß-1,3-glucanase, antibiotics, fluorescent pigments and cyanide (Scher and Baker 1982; Leong 1986; Voisard *et al.* 1989; Pal *et al.* 2001).

After nitrogen, phosphorous (P) is the major plant growth-limiting nutrient despite being abundance in soils in both inorganic and organic forms. Many soils throughout the world are P-deficient because the free phosphorous concentration (the form available to plants) even in fertile soils is around 10µM at pH 6.5 where it is most soluble (Arnou 1953). Phosphorous is present in micromolar or lesser quantities in soil solutions, due to high reactivity of soluble P with calcium (Ca), iron (Fe) and aluminium (Al) (Ozanne 1980). Organic P may also make up a large fraction of soluble P, as much as 50% in soils, with high organic matter content (Barber 1984). Phytate, a hexaphosphate salt of inositol, is the major form of organic P that accumulates in soil as insoluble complex molecules with Ca, Fe and Al (Alexander 1977). To circumvent the problem of P deficiency, agricultural producers rely on application of large quantities of chemical P to maximize yields, which may lead to a potential phosphate crisis (Abelson 1999). Fertilizer P is produced globally by the mining and chemical processing of rock phosphate (RP) ore (Robinson 1980). Their production process requires energy worth US $4 billion per annum in order to meet the global need (Goldstein *et al.* 1993) and the situation is further compounded by the fact that 75-90% of added P fertilizer is precipitated in soils (Vig and Dev 1984). The biological means of enhancing the agronomic effectiveness of RP applied as

phosphatic fertilizers are inoculation of seeds with phosphate solubilizing microorganisms (PSMs) and composting organic wastes with RP and PSMs (phosphocompost) (Nyirongo *et al.* 1999). Considering the quantitative importance of phosphorous in plant nutrition and given that phosphorous availability and PGPB are known to trigger changes in both plant nutrition and growth, phosphatic biofertilizers are gaining importance.

P-solubilization by microorganisms depends on the carbon sources present in the rhizosphere (Katznelson and Bose 1959). The availability of adequate and appropriate carbon source in soil is known to be one of the most limiting factors for survival, growth and functioning of microorganisms added to soil (Kucey *et al.* 1989). PSMs can solubilize the unavailable P by secreting organic acids and acid or alkaline phosphatases in the rhizosphere (Gyaneshwar *et al.* 1999). The major mechanism of mineral phosphate solubilization (MPS) by PSMs is by reducing the pH of their surroundings, either by the release of organic acids or protons (Bagyaraj *et al.* 2000). Among the organic acids, gluconic acid forms the metabolic basis of MPS phenotype in some Gram-negative bacteria (Goldstein 2003). Gluconic acid is produced by the oxidative metabolism of glucose by glucose dehydrogenase (Gcd), which requires 2.7,9-tricarboxy-1H-pyrrolo [2,3]-quinoline-4,5-dione (PQQ) as redox co-factor (Liu *et al.* 1992). Gluconic acid is further oxidized to 2-ketogluconic acid, one of the strongest naturally occurring organic acids (pKa ~ 2.6). Because this pathway operates in the periplasmic space, the extracellular environment can become highly acidic. MPS bacteria using the direct oxidation pathway can release

significant amounts of Pi from calcium phosphates via acidification of their extracellular environment (van Schie *et al.* 1985; Goldstein 1999).

In most soils, where crop residues are applied, cellulose is the largest renewable carbon source available (approximately 150 billion tones of organic material is photosynthesized annually), and is found in close association with other compounds, such as hemicellulose, lignin and other polysaccharides, which makes its bioconversion more difficult (Person *et al.* 1990). Most microorganisms use plant biomass (including crop residues) as their food or source of energy. As bacteria, fungi and yeasts can grow on solid substrate in absence of free water, they can be used in bioprocessing of crop residues (Rimbault 1998). Direct conversion and/or bioprocess of RP in the presence of cellulose would revolutionize the fertilizer industry, both in terms of product type and environmental pollution.

The plant response to PGPB is obviously a very complex phenomenon resulting from the combination of mechanisms, which effect several aspects of mineral nutrition, root development and colonization potential of the introduced bacteria (Chebotar *et al.* 2001; Sundara *et al.* 2002; Hontzeas *et al.* 2004). The interactions between plant growth promoting and phosphate solubilizing bacteria with rhizobia may be exploited for enhancing the biological nitrogen fixation and crop yield (Alagawadi and Gaur, 1988; Chanway *et al.* 1989). Arbascular mycorrhizal (AM) fungi are soil microorganisms that form a symbiotic association with a wide range of host plants including angiosperms, gymnosperms, pteridophytes and bryophytes

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(Smith and Read 1997). AM are an important component of the soil microflora promoting nutrient uptake in plants in exchange for carbon compounds.

Extensive use of fungicides to manage the soil borne plant pathogens has disturbed the ecological balance of soil, leading to groundwater contamination and health risk to human (Loper and Ishimaru 1991). Biocontrol agents, especially of the genera *Pseudomonas* and *Bacillus*, may be an ecologically sound alternative to chemical seed treatment (Martin and Loper 1999; Emmert and Handelsman 1999; Schmidt *et al.* 2004). Seed treatment with biocontrol agents is an economically viable option of disease control on most of the crops. Seed treatment by bio-priming results in enhanced germination, increase microbial population that inhibit the pathogen propagules on seeds thereby protecting the plant (van Dijk and Nelson 1998).

Sclerotium rolfsii Sacc. is a polyphagous soil borne plant pathogenic fungus that causes collar rot, root rot, blights, wilts and damping off in various crops (Punja 1985). *Macrophomina phaseolina* (Tassi) Goid is one of the most destructive seed and soil-borne plant pathogens causing charcoal rot, dry-root rot, wilt, leaf blight, stem blight and damping off diseases in a wide range of host plants (Singh *et al.* 1990). Recently, there has been growing interest in combining biocontrol agents with other chemical components to enhance their activity against certain phytopathogens (Siddiqui *et al.* 2001). The addition of mineral and carbon sources influences production of antibiotics and enhances antagonistic activity of bacteria (Latour *et al.* 1996; Duffy and Defago 1999).

Sustainable agriculture involves the successful management of agricultural resources to satisfy human needs, while maintaining or enhancing the quality of environment and conserving natural resources. Application of low-cost inputs can be made efficient by value-addition using the scientific knowledge to improve the crop productivity. Different perceptions exist to know what constitutes a sustainable management system. If properly characterized, soil quality can serve as an indicator of on-going conservation and degradation process. It depends highly on the nutrient content, biological and microbiological component of the soil eco-system and influences crop yield and quality (Parr *et al.* 1992; Halvorson *et al.* 1996). Soil biological parameters may hold potential as early and sensitive indicators of soil ecological stress or restoration (Dick and Tabatabai 1992). Soil microorganisms are potentially one of the most sensitive biological markers available and should, therefore, be useful for classification of disturbed or contaminated systems (Powlson *et al.* 1987).

In the present work bacteria were isolated from three different composts farm waste compost, rice straw compost, *Gliricidia* vermicompost and macrofauna present in farm waste compost. All the bacterial isolates were screened for the different plant growth promoting traits such as P solubilization, phytase activity, siderophore, IAA, ACC deaminase, chitinase and HCN production. Two selective plant growth-promoting (phosphate solubilizing) bacteria EB 67 and CDB 35 were characterized for their glucose dehyrogenase (Gcd) activity and solubilization of P in presence of various carbon sources and soil conditions. In addition their cellulase activity and P released was estimated in submerged and solid-state conditions in presence of

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different crop residues. Twelve bacterial isolates with plant growth promoting and antagonistic ability in plate culture conditions were studied for growth of pearl millet cultivar ICMV 155 and sorghum cultivar CSV15 in glasshouse conditions. Compatibility of plant growth promoting bacteria with the existing microbial inoculants like *Rhizobium* and Arbascular mycorrhizae was evaluated in glasshouse conditions. Efficacy of two antagonistic isolates EB 13 and CDB 35 against the two soil borne plant pathogenic fungi *S. rolfsii* and *M. phaseolina* was studied. Performance of the two phosphate solubilizing bacteria EB 67 and CDB 35 to colonize maize rhizosphere, growth and yield of maize was studied in glasshouse and field conditions. Further, an effort was made to identify and select suitable indicators, which can act as early signals of changes in soil quality of four different crop-husbandry systems (T1, T2, T3 and T4) of a long-term field experiment in BW3 field at ICRISAT. The methods used in the long-term field experiment reported here include no tillage, value-added compost, plant growth promoting bacteria (EB 35, EB 67 and CDB 35 of this study in addition to the other required microbial inoculants) and biomass as surface mulch and integrated pest management in two (T1 and T2) of the four field treatments studied. The third treatment included conventional system (tillage, integrated nutrient management) (T3) and fourth treatment (T4) where both integrated nutrient management (similar to T3) and farm waste as biomass (similar to T2) was practiced. Emphasis was given to biological, microbiological, nutrient and productive potentials as indicators of soil quality and the sustainability index of the four different crop husbandary systems T1, T2, T3 and T4 was determined.

1.2 OBJECTIVES

The present study is focused on characterization of plant growth promoting bacteria from a microbial rich source i.e., composts. Emphasis was to select plant growth promoting bacteria and antagonistic bacteria. Selected bacterial strains *Bacillus licheniformis* EB 13 and *Pseudomonas* sp. CDB 35 were evaluated for antagonistic activity and *Serratia marcescens* EB 67 and *Pseudomonas* sp. CDB 35 for their phosphate solubilizing and plant growth activity. This thesis work is done with following objectives:

➤ To prepare composts using different crop residues, isolate and screen bacteria from composts for plant growth promoting and antagonistic traits and identification of selected plant growth promoting bacteria (PGPB).

➤ Characterization of selected phosphate-solubilizing bacteria (PSB) in rock phosphate (RP) medium and soil conditions.

➤ Evaluation of selected bacterial isolates for plant growth promoting activity, interaction studies with *Rhizobium* and Mycorrhizae. Efficacy of two antagonistic bacteria *Bacillus licheniformis* EB 13 and *Pseudomonas* sp. CDB 35 to suppress the soil borne plant pathogenic fungi (*S. rolfsii* and *M. phaseolina*).

➤ To study the effect of composts on plant growth and biocontrol activity.

➤ Evaluation of efficient phosphate solubilizing (*Serratia marcescens* EB 67 and *Pseudomonas* sp. CDB 35) bacteria in glasshouse and field conditions.

➤ Characterization of soil indicators of different crop husbandry systems of an on-going long-term field experiment to evaluate the sustainability index.

1.3 PRESENTATION OF THE THESIS

The thesis is presented in six chapters followed by references. Chapter I – Introduction; Chapter II – Review of Literature; Chapter III – Materials and Methods; Chapter IV – Results; Chapter V – Discussion; and Chapter VI – Summary and Conclusions.

At the end, the literature studied for the thesis is arranged in alphabetical order of the names of authors, year of publication, article title, journal name (abbreviated), volume number and both initial and final page numbers according to the guidelines of the American Society for Microbiologists. Reference by the same author (s) are arranged chronologically and if more than one publication by the same author (s) published in the same year, then it is distinguished in the text and reference by the letters a, b, c, etc. after the year of publication.

A list of papers presented at various symposia/conferences, research publications/communications is given after references. The list of tables, figures and photographs are given at the end of the thesis.

CHAPTER – II

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Intensification of agriculture is widely recognized as one of the most significant alterations to the global environment by humans. Adverse impacts of agriculture include loss of biodiversity, eutrophication of surface water, contamination of ground water and loss of organic matter (OECD 1999). Concerns have developed, however, over the long-term sustainability and environmental consequences of intensifying agricultural production. Sustainable agriculture is defined as an integrated system of plant production that will make the most use of nonrenewable sources, integrate natural biological cycles and over the long term, satisfy human food and fiber needs; enhance environmental quality, sustain the economic viability of farm operations and enhance the quality of life for farmers and ranchers and society as a whole (Mahaffee and Kloepper 1994; Haggag 2002).

The management of soil organic matter requires inputs of organic manures, crop residues, green manures and other organic wastes (Beri *et al.* 2003b; Manici *et al.* 2004). Composting is one method of utilization of these organic wastes to produce manure, rich in plant nutrients. The role of earthworms in maintaining soil fertility is well known and emphasis is laid on vermicomposts by earthworms (Orozco *et al.* 1996). Beneficial properties of compost-amended plant growth medium are suppression of soil-borne plant pathogens and enhanced microbial activity (Craft and Nelson 1996). In natural systems most of the microorganisms depend upon addition of carbon compounds in plant materials (roots, stems and leaves) and faeces of soil and litter dwelling animals (earthworms, termites, millipedes, centipedes etc.). The presence of microorganisms in earthworm casts (Parle 1963a,

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1963b), termitaria soil (Sriveni *et al.* 2004), and faeces of millipedes (Anderson and Bignell 1980) are known. These macrofauna incorporate the plant materials in to the soil system and contribute to the cycling of carbon, nitrogen and sulphur, facilitating the release of nutrients in forms taken up by the plant and microorganisms (Ineson *et al.* 1982; Ingham *et al.* 1985).

The utilization of bacteria to stimulate plant growth in agriculture has been exploited since ancient times. Nevertheless, it was through the mixing of different soils and adding as inoculants to seeds (Tisdale and Nelson 1975; Smith 1992). Later on, the first patent ("Nitragin") was registered for plant inoculation with *Rhizobium* spp. (Bashan 1998a). However, inoculation with *Bacillus megaterium* for phosphate solubilization and *Azotobacter* (Macdonald 1989) had inconclusive results and was later abandoned. Major outbreaks in plant inoculation technology happened in late 1970s when *Azospirillum* for plant growth (Bashan and Holguin 1997) and *Pseudomonas* spp. for biocontrol ability were intensively investigated (Kloepper and Schroth 1981; Defago *et al.* 1992). In recent years, several bacterial genera, such as *Bacillus, Paenibacillus, Flavobacterium, Acetobacter, Serratia and Delftia* mostly isolated from rhizosphere and non-rhizosphere soil, rhizoplane etc. were evaluated as plant growth promoting bacteria (Tang 1994; Bai *et al.* 2002; Han *et al.* 2005). These microorganisms have the potential to reduce the application of agro-chemicals and maintain biotic diversity in the plant-associated bio-community.

2.1   CROP RESIDUES

Organic matter is an important soil constituent influencing a number of components linked with crop productivity. It is widely accepted that high soil organic matter (SOM) means high potential productivity and health of the soil. Amount of SOM in most arid, semi arid and sub-tropical soils is low and rarely exceeds 0.6%, yet its influence on soil properties is of great significance (Virmani *et al.* 1982). Intensive cropping and tillage systems have led to substantial decrease in SOM levels in the world. This decrease in SOM seems associated with decline in soil productivity. This strongly suggests a degradation of soil base in addition to other determinants for production. The management of nutrients to maintain productivity and quality in organic farming systems is a challenge that must be met through a combination of organic amendments and management of SOM, which consists of C (carbon) (55%), N (nitrogen) (5-6%) and P (phosphorous) and S (sulphur) (1%). Because SOM contains C, it is an energy source for microorganisms, similar to fresh plant residue input (Campbell *et al.* 1991; Horwath 2005).

An estimated 72% of the crop residues are returned to the land each year in the United States (Power and Papendick 1985). Through these approximately 72% of the nitrogen accumulated in the residue and 67% of both phosphorous (P) and potassium (K) are returned to the land. Such information from developing countries is not available. But from the common agricultural practices it seems that much of the nutrients in the crop residues are not returned to the land, thus resulting in poor soil quality over the years, despite use of chemical fertilizers. On all India basis, response of food grain crops to the applied fertilizers ($N+P_2O_5+K_2O$) has declined

immensely from 47.1 in 1959-60 to 7.7 kg grain kg^{-1} applied fertilizer in 1997-98 (Beri *et al.* 2003a).

Processing of the resulting crop biomass for food, fiber and other products of direct economic value leaves behind a large amount of residual material. The term crop "residue", with the connotation of something left over that nobody wants, gives a false impression of its value, as crop residues perform various functions in the mixed agricultural systems. Residues from field and plantation crops constitute a potent source of carbon and plant nutrients, the two inputs essentially required for sustainable productivity of soils. The available crop residues have been used in many ways like animal fodder, bedding, cardboard industry, household and industrial fuel. In addition to the above uses, there are residues of some crops which need disposal (Mani and Marimuthu 1992; Powell and Unger 1997).

Farmers resort to *in situ* burning of crop residue that remains scattered in the field. Significant quantities are burnt in some areas in several developing countries (Sidhu *et al.* 1998). In Punjab, about 81% of rice and about 48% of wheat residues are being burnt annually. The burning of residues results in loss of nutrients like C, N and S and partial losses of P, K and molybdenum (Mo) to the atmosphere. It is estimated that about 0.351 million mega tons (Mg) of nutrients in the straw and husks are completely or partially burnt down in Punjab (Beri *et al.* 1999), which comprises more than 50 per cent of total nutrients consumed in Punjab and the cost of which comes to about Rs. 233 crores at the prevailing prices, value of nitrogen being about Rs. 64.4 crores. This leads to loss of nutrients and environmental pollution; including

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production of substantial quantities of carbondioxide (CO_2), a greenhouse gas that adversely affects the environment. Instead of burning, these crop residues can be composted or directly managed in the field. Residues returned to the land may maintain or increase SOM levels, increase the waterholding capacity of the soil and provide a conducive habitat for growth and activity of microorganism and soil fauna (Parmelee *et al.* 1990; Smith *et al.* 1993).

2.2 MICROBIAL DECOMPOSITION & RECYCLING OF ORGANIC WASTES

2.2.1 Composts and Vermicomposts

Compost is prepared by biological degradation of organic material under controlled, aerobic conditions (Baca *et al.* 1992; Eghball *et al.* 1997). Composting is often divided into three phases (Finstein *et al.* 1983; Goodfellow and Williams 1983; Hoitink and Fahy 1986). The initial phase occurs during the first 24-48 hours as temperature gradually rises to 45-50 °C and mostly sugars and other easily biodegradable substances are utilized. During the second phase, high temperatures of 55-70 °C prevail and less biodegradable cellulosic substances are destroyed. Thermophilic microorganisms predominate during this stage of composting and most of the plant pathogens are killed during this phase (Bollen 1993; Farrell 1993). Compost piles must be turned frequently to expose all parts to high temperature to produce a homogenous product free of pathogens and weed seeds. Unfortunately, most beneficial microorganisms also are killed during high temperature phase of composting. Curing, the third phase, begins as the concentration of readily biodegradable components in wastes decline due to which decomposition rate, heat

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output and temperature decrease. Plant growth promoting bacteria and biocontrol agents naturally colonize during curing stage when 45-55% of moisture is maintained (Hoitink *et al.* 1996).

However, in recent years, researchers have become progressively interested in using another biological process, termed vermicompost which does not include a thermophilic stage, but involves the use of earthworms for breaking down and stabilizing the organic wastes (Chan and Griffiths 1988; Atiyeh *et al.* 2001). Vermicompost is peat like with high porosity, aeration and waterholding capacity. It has soluble nutrients, plant growth regulators and high microbial activity (Krishnamoorthy and Vajrabhiah 1986; Tomati and Galli 1995).

However, both composts and vermicomposts are distinct processes, particularly concerning the optimum temperatures for each process and the types of microbial communities that predominate during active processing (i.e. thermophilic bacteria in composting, mesophilic bacteria and fungi in vermicomposting). The wastes processed by the two systems are also quite different and should have a great potential in the horticultural and agricultural industries as media for plant growth (Subler *et al.* 1998).

2.2.2 Uses of Composts

From literature study, the reasons to support the use of compost in agriculture as a fertilizer are as follows:

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- Compost is needed as substitute for limited natural resources (e.g. peat, mineral fertilizer). Nevertheless, recycling of plant material (produced) contributes to sustainable agriculture.

- In agricultural soils application of organic matter by compost amendments is not needed but useful, especially in the livestock-less farming systems.

- Benefits of compost application in agriculture mainly result from its content of organic matter and plant nutrients.

- Compost is known to be product rich in microbes; often containing 10^7 to 10^8 bacteria per gram dry matter (Postma *et al.* 2003). Since agricultural soils also have similar population of bacteria, their addition through compost may not be impressive. However, the composition of the microflora in compost might be different, and the soil will be enriched with a variety of nutrients that will become available for the indigenous microflora and hence plants.

- Composts suppress soil-borne plant diseases (Hoitink 1980).

However, there are few disadvantages of improper compost preparations.

- Risks in compost application are due to input of pollutants (heavy metals), overload of nutrients and accumulation of organic nitrogen fractions (Carlson *et al.* 1975).
- Few types of compost might have noxious effects on crops because of the high content of polyphenolic compounds and humic acids (Inbar *et al.* 1990;
- Valdrighi *et al.* 1995).

There is a need to balance benefits and risks of compost amendments to agricultural soils through good practice of fertilization and limitations of pollutant inputs.

Various types of rural organic wastes such as excreta (dung), biogas slurry, poultry, piggery wastes and bone meal can be decomposed along with the farm wastes/crop

residues. Most of these composts are prepared with integration of mineral fertilizers within the framework of integrated plant nutrition system (IPNS) to improve and sustain soil fertility and crop productivity (Mishra 1992; Ajwa and Tabatabai 1994). The potential availability of rural wastes per year is estimated to be 600 million tones, out of which only 310-350 million tones of compost is prepared by traditional and improved methods (Gaur *et al.* 1995). Urban organic wastes include municipal solid wastes, sewage sludge and huge quantity of industrial effluents, which can be decomposed and used for plant growth (Mamo *et al.* 1999; Masciandaro *et al.* 2000). Rasal *et al.* (1988) produced good quality enriched sugarcane thrash compost by combining the technology of rapid composting and enrichment with N-fixing *Azotobacter*, cellulolytic and phosphate-solubilizing cultures with addition of rock phosphate (Manna *et al.* 1997). The use of phosphate rocks by composting it with different kinds of organic compounds improved its agronomic effectiveness. Phosphate rock composted products are usually referred to as phospho-composts (Singh and Amberger 1991; Sekhar and Aery 2001). Moreover, agronomic effectiveness of composts could be increased through the application of organic manure in conjunction with rock phosphate (Sharma *et al.* 1995).

Composts have been successfully used as an economically feasible substrate for mushroom cultivation and as packing material in odour-removing biofilters for treatments of wastes from food and other industries. The capacity of compost microflora to degrade a wide variety of organic contaminants has been clearly demonstrated (Sukesan and Watwood 1998). In addition to this, composts have the potential for improving plant growth when added to soil. Composts promoted the

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growth of marigold and tomato seedlings in glasshouse conditions (Atiyeh *et al.* 2000b). Municipal solid waste enhanced the enzyme activities, C and N content when added to the soil (Crecchio *et al.* 2001). Vermicompost stimulated mycorrhizal colonization of roots of sorghum (Cavender *et al.* 2003). Composts can be effective in controlling diseases caused by soil borne plant pathogens like *Pythium, Phytophthora, Fusarium, Rhizoctonia, Sclerotium* spp. and also root-knot nematodes (Chen *et al.* 1988; Hardy and Sivasithamparam 1995). Composts enrich microorganisms that are capable to promote plant growth and inhibit root pathogens (Kwok *et al.* 1987; Alvarez *et al.* 1995; Boehm *et al.* 1993).

2.3   PLANT GROWTH PROMOTING & ANTAGONISTIC TRAITS OF BACTERIA

2.3.1 Mobilization of soil phosphorous

Phosphorous is second only to nitrogen in mineral nutrients most commonly limiting the growth of plants. Soils have large reserves of total P, but the amount available to plants is a small proportion (Stevenson and Cole 1999). Many soil microorganisms are able to solubilize unavailable forms of bound P. Visual detection and semi-quantitative estimation of phosphate solubilizing ability of micro-organisms is possible by plate screening methods, that show clearing zone around the microbial colonies in media containing insoluble mineral phosphates (tricalcium phosphate or hydroxyapatite) as sole P source (Louw and Webley 1959; Nautiyal 1999).

Mineralization of organic P is achieved by phytases (i.e. phosphatases which show high specificity toward phytate or myo inositol hexa phosphate). Various plant

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species do not possess significant amount of extracellular phytase (Hayes *et al.* 1999; Richardson 2001a) and hence cannot use phytate as P source. Hence, the growth and P nutrition in plants can be improved by microbial phytases. Phytases have been isolated and characterized from *Bacillus subtilis* (Kerovuo *et al.* 1998), *Klebsiella terrigena* (Greiner *et al.* 1997), *Pseudomonas* spp. (Richardson and Hadobas 1997) and *Enterobacter* sp. 4 (Yoon *et al.* 1996).

2.3.2 Indole acetic acid production

Organic substances capable of regulating plant growth produced either endogeneously or applied exogeneously are called plant growth regulators. They regulate growth by affecting physiological and morphological processes at very low concentrations (Arshad and Frankenberger 1998). Several microorganisms are capable of producing auxins, cytokinins, gibberilins, ethylene or abscisic acid (Lebuhn *et al.* 1997). Bacteria synthesize indole acetic acid (IAA) predominantly by an alternate tryptophan-dependant pathway, through indole pyruvic acid. However, the role of bacterial IAA in plant growth remains undetermined. Promotion of root growth is one of the major markers by which the beneficial effect of plant growth-promoting bacteria is measured (Glick *et al.* 1995). The establishment of roots, whether by elongation of primary roots or by proliferation of lateral and adventitious roots, is advantageous for young seedlings as it increases their ability to anchor themselves to the soil and to obtain water and nutrients from the environment, thus enhancing their survival (Pattern and Glick 2002). Most PGPB synthesize IAA and their effect on plants mimics that of exogeneous IAA.

2.3.3 ACC deaminase

A number of PGPB contain the enzyme 1-amino-cyclopropane-1-carboxylate (ACC) deaminase and this enzyme could cleave ACC, the immediate precursor to ethylene in the biosynthetic pathway for ethylene in plants (Jacobson *et al.* 1994; Glick *et al.* 1998). ACC deaminase activity would decrease ethylene production in the roots of host plants and results in root lengthening. For most of the plants, ethylene is required to break seed dormancy (Esashi 1991) but after germination, high-level ethylene would inhibit root elongation (Jackson 1991). PGPB that contain the enzyme ACC deaminase, when bound to the seed coat of a developing seedling, act as a mechanism for ensuring that the ethylene level does not become elevated to the point where initial root growth is impaired. By facilitation the formation of longer roots, these bacteria may enhance the survival of some seedling, especially during the first few days after the seeds are planted (Penrose and Glick 2003). The plants treated with ACC deaminase producing bacteria are dramatically more resistant to the deleterious effects of stress ethylene that is synthesized as a consequence of stressful conditions such as flooding, heavy metals, the presence of phytopathogens, drought and high-salt (Grichko *et al.* 2000; Wang *et al.* 2000; Grichko and Glick 2001). These bacteria are beneficial to plant growth since in the natural environment plants are often subjected to stress due to ethylene production.

2.3.4 Siderophores and Fe nutrition

Iron is an essential nutrient of plants, but it is relatively insoluble in soil solutions. Plant roots prefer to absorb iron as the more reduced ferrous (Fe^{2+}) ion, but the ferric ion (Fe^{3+}) is more common in well-aerated soil although it is easily precipitated in

iron-oxide forms (Lindsay 1991). Plants commonly excrete soluble organic compounds (chelators and phytosiderophores), which bind Fe^{3+} and help to maintain it in solution. Chelators deliver the Fe^{3+} to the root surface where it is reduced to Fe^{2+} and immediately absorbed. Phytosiderophores are absorbed with the Fe^{3+} across the plasmalemma (von Wiren et al. 2000). Under Fe deficiency conditions, many microorganisms produce siderophores for Fe acquisition (Neilands 1981). Microbial siderophores are substances of low molecular weight (generally less than 1500 daltons), which strongly bind Fe^{3+} and supply iron to the microbial cell. However, there is a controversy of bacterial Fe^{3+}-siderophore uptake to the iron nutrition of plants. Some believe that contribution of these siderophores to the overall iron requirement of plants is small, while others suggest an important role, especially in calcareous soils (Bar-Ness et al. 1991; Wang et al. 1993; Masalha et al. 2000).

Majority of research on microbial siderophores is associated with their biocontrol activities due to their competitive effects with plant pathogens (Kloepper et al. 1980). Bacteria can prevent the proliferation of phytopathogens, through the production of siderophores that bind Fe^{3+} that is available in the rhizosphere and as a result effectively prevent any fungal pathogens in vicinity from proliferating because of lack of iron (O'Sullivan and O'Gara 1992). The ability of siderophores to act as effective "disease-suppressive" agents is affected by the particular crop plant, specific phytopathogen being suppressed, the soil composition, the bacteria that synthesize siderophore and the affinity of specific siderophore for iron. Composition

of composts has been well characterized and includes many microorganisms that synthesize siderophores (Loper and Buyer 1991).

2.3.5 Enzymes

Plants respond to pathogen attack by synthesizing pathogenesis related (PR) proteins that can hydrolyze cell wall of some fungal pathogens (Mauch *et al*. 1988). Similarly, some biocontrol PGPB have been found to produce enzymes including ß-1, 3 glucanase, chitinase, protease, lipase that can lyse the fungal cells (Chet and Inbar 1994). Lim *et al*. (1991) isolated a strain of *Pseudomonas stutzeri* that produced extracellular chitinase and laminarinase and found that these enzymes could digest and lyse *Fusarium solani* mycelia thereby preventing the fungus from causing crop loss due to root rot. Similarly, Fridlender *et al*. (1993) were able to reduce the incidence of plant disease caused by the phytopathogenic fungi *Rhizoctonia solani*, *Sclerotium rolfsii* and *Pythium ultimum* by using ß-1, 3 glucanase producing strain *Pseudomonas cepacia*, which was able to damage fungal mycelia.

2.3.6 Anti-fungal metabolites

The most widely studied group of bacteria with respect to the production of antibiotics is the fluorescent pseudomonads. The first antibiotic described as being implicated in biocontrol were phenazine derivatives produced by fluorescent *Pseudomonas* (Weller and Cook 1983). The antibiotics synthesized by the biocontrol pseudomonad include 2,4-diacetyphloroglucinol, herbicolin, oomycin, phenazines, pyoluteorin and pyrrolnitrin (Handelsman and Stabb 1996). Volatile compounds such as hydrocyanic acid (HCN) is produced by many bacterial strains and has been considered as important metabolites in biocontrol (Walsh *et al*. 2001; Bano and

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Musarrat 2003; Fernando *et al.* 2005). *Pseudomonas* spp. that produced HCN *in vitro* inhibited the pathogenic fungi, *Thielabiopsis basicola* and suppressed the black root-rot of tobacco (Dowling and O'Gara 1994). Mineral and carbon sources added as soil amendments influenced the antagonistic ability of *Pseudomonas* spp. (Shaukhat and Siddiqui 2003).

2.4 PHOSPHORUS (P) AVAILABILITY

Phosphorus (P) is one of the major essential macronutrients for biological growth and development. Its cycle in biosphere is described as 'open' or 'sedimentary', because there is no interchange with the atmosphere (Richardson 1994). Microorganisms are involved in a range of processes that affect transformation of soil P and are thus an integral component of P cycle (Fig. 2.1). Rhizosphere microorganisms' are effective in releasing P from inorganic and organic pools of total soil P through solubilization, mineralization and play a vital role for transfer of P from poorly available soil pools to plant available form. Plants absorb P in two soluble forms: the monobasic ($H_2PO_4^-$) and the dibasic (HPO_4^{2-}) ions (Daniel *et al.* 1998). Phosphorous comprises of 0.2% of plant's dry weight and is component of essential molecules such as nucleic acids, phospholipids, ATP (Theodorou and Plaxton 1993). Crop plants achieve maximum growth in solution culture at 0.03mM of P or less. The cytoplasmic concentration of Pi in plants is maintained at 10 mM independent of external Pi concentration. In plants, Pi absorbed by the roots is transported through xylem to younger leaves. Concentration of Pi in xylem is less when plants are grown in P starved conditions and vice versa. Pi uptake by plant

cells is an energy dependent process. It is hypothesized that Pi is co-transported with

one or more protons into the plant cell (Ullrich and Novacky 1984).
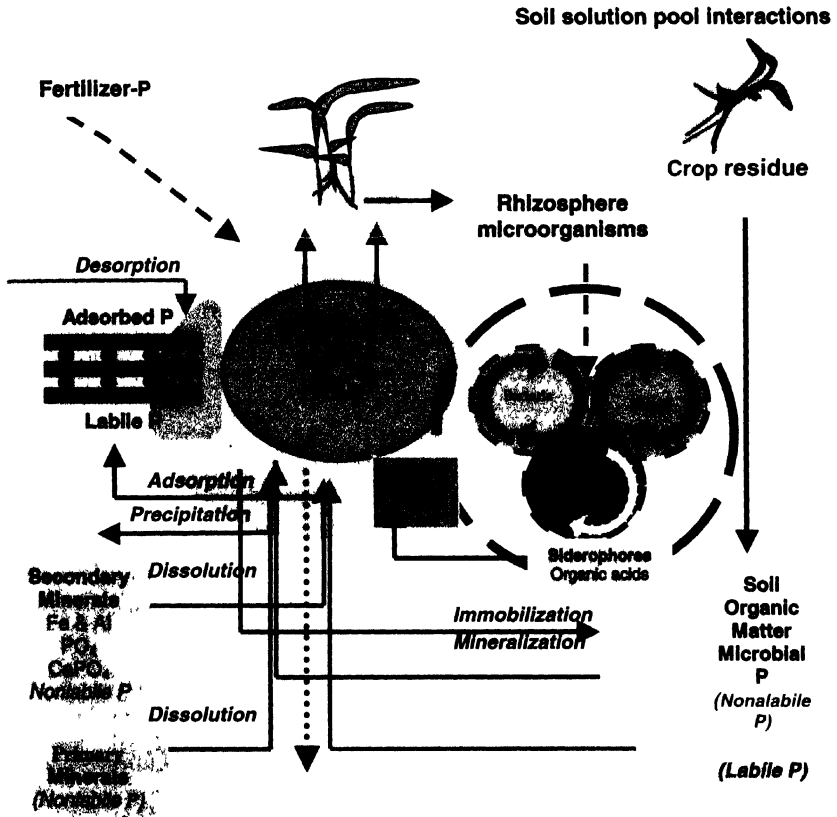
Figure 2.1. Phosphorus cycle

Plants have two Pi uptake systems, one with high affinity phosphate transporters,

which can be increased or de-repressed by Pi starvation and other consists of Pi

transporters, which have low affinity for Pi but are expressed constitutively

(Furihata *et al.* 1992). In conditions of severe Pi depletion, plants grow more roots,

increase rate of Pi uptake, deplete vacuolar stores of Pi and high affinity P transporters are expressed (Lee *et al.* 1990).

2.5   PHOSPHATE SOLUBILIZING MICROORGANISMS (PSMs)

PSMs are ubiquitous and their numbers vary from soil to soil and enhance P availability when present in rhizosphere. Phosphate solubilizing bacteria (PSB) generally, outnumber the P solubilizing fungi by 2-150 fold (Kucey 1983). The concept of using pure cultures of soil microorganisms to increase P nutrition of plants through increased solubility of Ca-phosphates is not new (Gerretsen 1948). Predominant among these organisms are *Bacillus*, *Pseudomonas*, *Penicillium* and *Aspergillus* spp. Majority of soil P is found in insoluble form and soils like Indian alkaline Vertisols are rich in Ca-P complexes and have high buffering capacity. Given the global importance of limited phosphate supplies in many agricultural soils, it is important to isolate and characterize PSMs, which can secrete high concentrations of organic acids in buffered medium.

2.6   MECHANISMS OF P SOLUBILIZATION

Microorganisms show preference to particular carbon source and the highest ability to solubilize P was observed in presence of glucose. Solubilization of inorganic P is due to production of organic acid and phosphatases (Kim *et al.* 1998a). Microorganisms are effective in releasing P from mineral phosphates by producing organic acids such as citric, oxalic, tartaric, gluconic, ketogluconic, lactic and succinic etc. (Sperber 1958; Duff *et al.* 1969; Chung *et al.* 2005). The production of phosphatase

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is controlled by complex regulatory mechanisms and the enzyme is detected only under specific conditions. The principal mechanism for the regulation of phosphatase production is the regulation of inorganic phosphate (Pi) concentration. *Pseudomonas* spp. and *Enterobacter* spp. are known to produce alkaline phosphatase, which is repressed by phosphate (Gugi *et al.* 1991; Thaller *et al.* 1995).

Glucose may be provided by the plant either as a secretion product or as a result of biodegradation of sloughed off carbohydrate polymer. Gluconic acid secretion in PSMs is mediated by oxidation of glucose to gluconic acid by a pyrolloquinone quinoline (PQQ)-dependent D-glucose dehydrogenase (EC 1.1.99.17) (Gcd). It is found on the outer face of cytoplasmic membrane of various Gram-negative bacteria. Gcd does direct oxidation of glucose and other aldose sugars (Goldstein 1994) such that oxidized product is released in the periplasm. Glucose oxidized to gluconic acid can further be oxidized to 2-ketogluconic acid or 2,5-diketogluconic acid (Anderson *et al.* 1985) (Fig. 2.2). While oxidizing glucose, Gcd transfers two electrons to electron transport chain via cofactor pyrroloquinoline-quinone (PQQ). Further two protons are also generated in the process, which contribute to generate proton motive force across the membrane. It transfers electrons from aldose sugar to the electron transport chain mediated by co-factor PQQ. Protons generated by the periplasmic oxidation mediated by glucose dehydrogenase (GDH) contribute directly to the transmembrane proton motive force (Duine 1991). Gcd from many bacterial sources has been shown to possess broad substrate specificity and catalyze the oxidation of several aldosugars to their respective lactones (Goodwin and Anthony 1998).
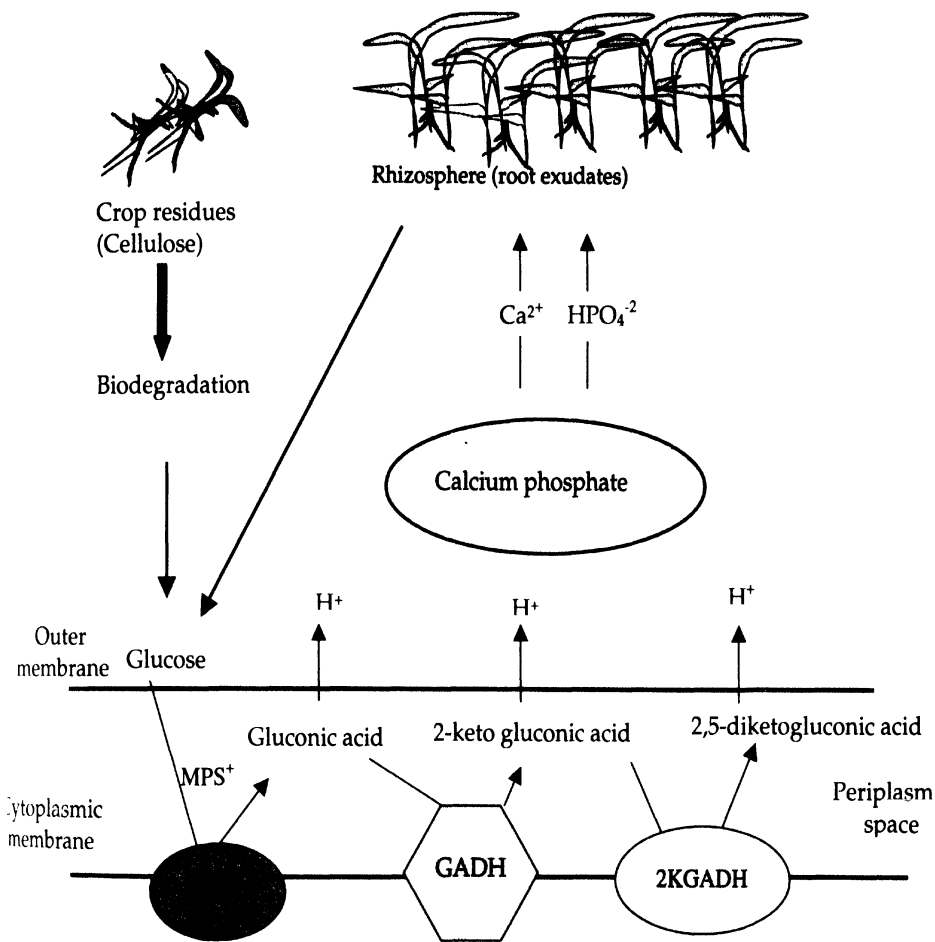
Figure 2.2. Direct oxidation pathway in release of phosphate (Pi) from calcium phosphate by mineral phosphate solubilizing bacteria (MPS+) at or near rhizosphere. GDH = glucose dehyrogenase, GADH = gluconate dehydrogenase, 2KGADH = 2-keto gluconate dehydrogenase.

Two types of Gcd enzymes have been identified till date; Gcd A and Gcd B that differ in their subcellular localization and substrate specificity (Cleton-Jansen *et al.* 1989). Gcd A, the predominant isozyme, has been found in organisms such as

Acinetobacter calcoaceticus, Escherichia coli (Ameyama *et al.* 1986), *Gluconobacter oxydans* (Ameyama *et al.* 1981) and *Pseudomonas fluorescence* (Matsushita *et al.* 1980) whereas Gcd type B enzyme has been demonstrated only in *A.calcoaceticus.*

Agricultural residues are rich source of cellulose, hemicellulose and lignin in an average ratio of 4:3:3, although the exact percentage of these components may vary (Sitton *et al.* 1979). Cellulose is an unbranched glucose polymer, composed of anhydro-D-glucose units linked by 1,4- ß-D glucoside bonds, which can be hydrolyzed by cellulolytic enzymes produced by bacteria and fungi (Robson and Chimbliss 1989). Due to its polymeric nature bioprocessing of cellulose is limited. Cellulolytic bacteria include aerobic species such as *Pseudomonas* and actinomycetes, facultative anaerobes such as *Bacillus & Cellulomonas* and strict anaerobes such as *Clostridium.* PSMs, found in the earthworm (*Eudrilus eugeniae*), casts showed cellulase activity and released P using carboxymethyl-cellulose as carbon source in the medium (Mba 1997).

Increased mineralization of organic P occurs due to incorporation of crop residues, crop rotations and increases the rate of P cycling through the microbial biomass (McLaughlin *et al.* 1988). Incorporation of organic residues through legume rotation resulted in higher biological activity and increased microbial P uptake and release. However, the contribution of P released through these processes needs to be evaluated in relation to plant uptake. Elucidation as to whether or not the availability of this P can be synchronized with plant requirements or be targeted to rhizosphere remains a significant challenge (Oberson *et al.* 2001).

2.7   PLANT-GROWTH PROMOTING BACTERIA (PGPB)

Beneficial free-living soil bacteria in the rhizosphere are generally referred to as plant growth-promoting bacteria (PGPB) and are found in association with the roots of various plants (Kloepper *et al.* 1991; Sajjad *et al.* 2001). In the era of sustainable agricultural production, the interactions in the rhizosphere by soil microorganisms play a pivotal role in transformation, mobilization and solubilization from a limited nutrient pool in the soil and ensuing uptake of essential nutrients by the crop plants (Bolton *et al.* 1993; Mantelin and Touraine 2004). The beneficial effects of biocontrol PGPB towards various phytopathogens have been extensively studied. Soil-borne plant pathogenic fungi infect several crops like cereals, legumes, oilseeds, vegetables and fruits. James (1981) reported that soilborne diseases of economic crops alone cause 13-20% annual loss in production representing US$ 50 million. As exact estimates are not found in India, it can be assumed that more than 50 per cent of crop loss is due to soil inhabiting pathogenic microorganisms (Sen 2000). Fungal genera like *Sclerotium rolfsii*, *Macrophomina phaseolina*, *Fusarium solani* and *Fusarium oxysporum* etc. have been recognized to play a major role in the root disease complex causing seed decay, damping off, root rot, seedling blight, collar rot, crown rot, foot rots and wilt (Cook and Baker 1983).

Thus, the need of the hour is to enhance the efficiency of scanty amount of external inputs by employing the best combinations of beneficial microbes for improved crop production. The use of PGPB-inoculants as biofertilizers and/or antagonists of plant pathogens provide a promising alternative to chemical fertilizers and pesticides. Seed treatment with plant growth promoting rhizobacteria (PGPR) increased the

growth of maize (Jacoud *et al.* 1999) wheat (Khalid *et al.* 2004) and several other crops (Belimov *et al.* 2004).

2.8 MUTUAL SYMBIOTIC INTERACTIONS OF PGPB WITH *RHIZOBIUM* AND MYCORRHIZAE

Rhizobium or *Bradyrhizobium* are widely used in agriculture for crop improvement because of their ability to fix atmospheric nitrogen. Mutual symbiotic interaction of both ecological and agronomical importance occurs between leguminous plants and rhizobia, involving the development of root nodule (Brevin 1991). Co-inoculation of plant growth promoting bacteria with *Rhizobium* spp. stimulates chickpea growth, nodulation and nitrogen fixation (Parmar and Dadarwal 1999). Common mechanisms used by PGPB to alter nodule formation or biological nitrogen fixation include the release of auxins, gibberillins, cytokinins and ethylene or the alteration of endogen levels in the plant (Hirsch *et al.* 1997). The effects of some phytohormones are indirect, as they stimulate root growth, providing further sites for infection and nodulation. Systemic inductions of secondary metabolites such as flavonoids are implicated due to inoculation of PGPB (Andrade *et al.* 1998). However, other compounds with less known functions such as tabtoxinine-beta lactam (Knight and Langston 1988) and B group vitamins (Marek-Kozaczuk and Skorupska 2001), produced by *Pseudomonas* spp. could also be involved in these bacterial effects. Co-inoculation of *Rhizobium, Azospirillum,* and *Azotobacter* with PSMs also showed synergistic effect on plant growth and crop yields (Barea *et al.* 1975; Kundu and Gaur 1981).

Symbiotic association between plant roots and fungal mycelia is termed as mycorrhiza (fungus root, plural-mycorrhizae). These fungi are found associated with majority of agricultural crops. Arbascular mycorrhizae (AM) occur over a broad ecological range from aquatic to desert environments (Mosse 1981). They are ubiquitous in geographic distribution occurring with plants growing in arctic, temperate and tropical regions alike. These fungi belong to the genera *Endogone, Glomus, Entrophosphora, Gigaspora, Acaulospora, Scutellispora*, are obligate symbionts and are grown in association with living tissues (Al-Raddad 1995). The most widely used is pot culture, where the fungi are usually maintained in conjunction with suitable host plant roots (Ferguson and Woodhead 1982).

Arbascular mycorrhizal fungi possess special structures, globose or oval terminal swellings known as vesicles and arbuscules that infect and spread inside the root. The arbuscules help in transfer of nutrients from the fungus to the root system and the vesicles, which are 'sac-like' structures and store P as phospholipids. There is little host specificity for AM but the competitive ability of a given species with native strains may influence the dominance of a certain endomycrorrhizal fungus in a root system. AM have been associated with increased plant growth and with enhanced accumulation of plant nutrients, particularly P, zinc (Zn), and S mainly through soil exploration by mycorrhizal hyphae (Abbot *et al.* 1984; Wani *et al.* 1991). Related approaches, viz. soil less culture, hydroponic culture, aeroponic culture and axenic root organ culture are well reviewed (Jarstfer and Sylvia 1993). The making of AM inoculants is relatively expensive and involves extended culture periods of several months. Hence, the development of rapid and more efficient culture system remains

an important challenge for commercialization. Synergistic effects of AM fungi and plant growth promoting rhizobacteria and yeast on root colonization and sporulation have been documented (Tilak *et al.* 1990; Larsen and Jakobsen 1996).

The beneficial effects of mycorrhizae on plant growth have often been related to the increase in uptake of immobile nutrients; especially P and the mechanisms for increase in uptake of P for mycorrhizal and non-mycorrhizal plants are examined. The mechanisms that have been suggested for the increase in the uptake of P by mycorrhizal plants include exploration of larger soil volume, faster movement of P into mycorrhizal hyphae and solubilization of phosphorous. Exploration of larger soil volume by mycorrhizal plants is achieved by decreasing the distance that P ions must diffuse to plant roots and by increasing the surface area for absorption. Faster movement of P into mycorrhizal hyphae is achieved by increasing the affinity for P ions and by decreasing the threshold concentration required for absorption of P. Solubilization of soil P is achieved by the release of organic acids and phoshphatase enzymes (Bolan 1991).

2.9   BIOCONTROL OF SOIL-BORNE PLANT PATHOGENS

Biological control of plant pathogens is an attractive alternative to the strong dependence of modern agriculture on chemical fungicides, which cause environmental pollution and development of resistant strains. Species of *Pseudomonas* and *Bacillus* have been known for long for their capacity to reduce plant diseases caused by fungal pathogens and some have been tested for biocontrol potential in many glasshouse and field trials (Wilson and Wisniewski 1989; Sadfi *et*

al. 2001; Dey *et al.* 2004). These biocontrol bacteria have been reported to destroy cells of fungal pathogen after or slightly before invasion. They show necrotrophy and utilize nutrients from the dying or dead host. The invasion is often initiated by attack and lysis of hyphae (Defago and Haas 1990).

Bacteria show their antagonistic effect against fungal pathogens by antibiosis, competition or exploitation, which is subdivided into predation and direct parasitism (Pleban *et al.* 1997; Muninbazi and Bullerman 1998; Walker *et al.* 1998). Several *Bacillus* spp. produce enzymes that degrade chitin, an insoluble linear polymer of ß-1, 4-N-acetylglucosamine (GlcNAc), which is the major component of fungal cell wall. Among these species, *Bacillus circulans* (Watanabe *et al.* 1990), *Bacillus licheniformis, Bacillus cereus* (Trachuk *et al.* 1996) cited as potential biocontrol agents, have been reported to secrete chitnase. Evidence that these chitinolytic enzymes play a major role in the biocontrol of fungal pathogens has been demonstrated in many systems involving bacterial and fungal antagonists (Lorito *et al.* 1993). *Pseudomonas* spp. have received attention as biocontrol agents due to their catabolic versatility, excellent root colonizing ability and capacity to produce a wide range of antifungal metabolites. In addition to this, few *Pseudomonas* spp. have shown to elicit a disease-resistance response in crop species, a phenomenon known as induced systemic resistance (ISR) (van Loon *et al.* 1998).

Seed priming is now a widely used commercial process developed to help accelerate germination and improve seedling uniformity in many crop and ornamental plants, especially where they are grown under unfavorable environmental conditions or in

soils infested with pathogens (Taylor and Harman 1990). There are three technologies to achieve priming (Halmer 2003). Historically, the most widely used is osmoconditioning or osmopriming where seeds are placed in aerated solutions containing mannitol, inorganic salts or polyethylene glycol of low water potential or exposed to the solutions on the surface of paper or other fibers. A second system, solid-matrix priming, is carried out by mixing seeds with insoluble matrix particles (fine dried clay-sieved to 40 mesh) and water in a predetermined ratio allowing seeds to imbibe to an equilibrium hydration level. A third procedure, sometimes termed hydropriming, covers the approaches of gradual addition of a limited amount of water or imbibition in water for a short period (also known as steeping), which may include incubation in humid air. In biopriming, the inoculum applied as antagonist is in close proximity to the sites of pathogen entry, in the seed and the emerging seedling. In such application, much less antagonist inoculum is needed than for soil treatment, which will reduce crop production costs (Wright *et al.* 2003).

Sclerotium rolfsii Sacc. is a devastating soil-borne plant pathogenic fungus with a wide host range (Sarma *et al.* 2002); atleast 500 species in 100 families are susceptible. The most common hosts are legumes, crucifers and cucurbits. *S.rolfsii* occurs in tropics, subtropics and other warm temperatures. It primarily attacks host stems, although it may infect any part of a plant under favorable environmental conditions including roots, fruits, petioles, leaves and flowers. Collar rot caused by *S. rolfsii* is an important disease worldwide affecting chickpea production drastically. Its control using host plant resistance has not been very effective. Thus, considerable emphasis is placed to use fungal and bacterial antagonistic organisms, either alone

or in combination with fungicides, for the control of soil/seed borne diseases (Singh et al. 2003). The antagonistic ability of biocontrol agents depends upon their survival in the environment and on several other factors particularly its interaction with other soil microorganisms.

Macrophomina phaseolina (Tassi) Goid is a pathogen with an exceptionally broad host range that includes over 500 plant species. Agronomically important hosts include soybean (*Glycine max* [L.] Merril), corn (*Zea mays* [L.], sorghum (*Sorghum bicolor* [L.] Moench) and cotton (*Gossypium Hirsutum* [L.]. It causes charcoal rot disease and considerable yield loss. In severe cases, whole plant becomes defoliated and results in complete loss of leaves (Singh and Srivastava 1984). Combined use of different biocontrol agensts or integration of biocontrol agents with other disease management options, has improved disease protection and the activity spectrum of biocontrol agents (Jetiyanon and Kloepper 2002).

2.10 PHOSPHATE SOLUBILIZING BACTERIA (PSB)-PLANT INTERACTIONS

Most agricultural soils contain large reserves of phosphorous, a considerable part of which is accumulated and fixed as a consequence of regular application of chemical (P) fertilizers. However, a large portion of soluble inorganic phosphate applied to soil as chemical fertilizer is rapidly immobilized soon after application and becomes unavailable to plants (Dey 1988). The phenomenon of fixation and precipitation of P in soil is generally dependant on pH and soil type. Two major types of soils namely alkaline Vertisols and acidic Alfisols, both show P deficiency but they have qualitative difference in the form of P fixed in them (Ae et al. 1991). Inorganic P car

occur in calcium (Ca), iron (Fe) or aluminum minerals *i.e.* Ca-P, Fe-P or Al-P, which

are insoluble hence unavailable to plants (Fig. 2.3).

(a) 23% 60% 17%

■ Ca-P ▣ Al-P ■ Fe-P

(b) 36% 44% 20%

■ Ca-P ▢ Al-P ■ Fe-P
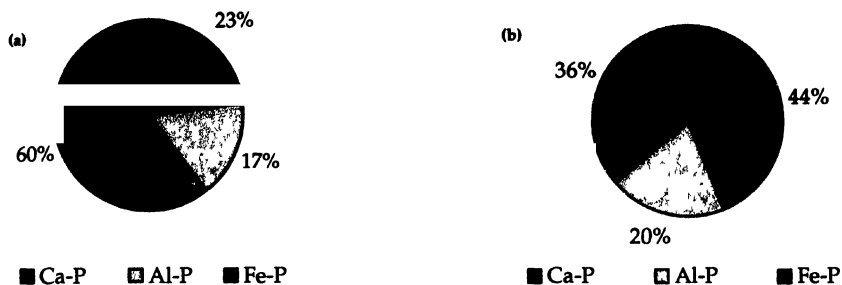
Figure 2.3 Proportion of various bound form of P in (a) Alfisols and (b) Vertisols

The biggest reserves of P are rocks and other deposits, such as primary apatite and

other minerals formed during the geological age. Phosphatic rock deposits in India

provide a cheap source of phosphatic fertilizer for crop production (Halder *et al.*

1990). The current demand of these is about four million tones, out of which 95% is

consumed in agricultural sector as a source of phosphatic fertilizers. Domestic

production in the country of about 1.4 million ton/ per year could meet hardly 35%

of the total demand, while the remaining 65% bulk demand was met through import

which involved Rs. 477.35 crores and Rs. 661.20 crores respectively during 1996-97

and 1997-98. The domestic demand of phosphates has been estimated to be 57.88

lakh tones and 81.18 lakh tones for years 2006-07 and 2011-12, while the production

of phosphate rock are projected to be 20 lakh tones and 25 lakh tones respectively

(TIFAC 2002). Fertilizer P is produced globally by the mining and chemical

processing of rock phosphate ore (RPO), a nonrenewable natural resource. Reserves of rock phosphate, which is the raw material to make chemical P, could be depleted in as little as 60-80 years. Hence attempts to augment the fertilizer value of rock phosphate by the use of microbial inoculants was found effective, since organic acid liberated by the activity of heterotrophic microflora was responsible for dissolution of insoluble phosphates (Datta *et al.* 1982), (Kumar and Singh 2001).

Examples of recently studied PSMs-plant inoculation associations include *Azotobacter chroococcum* and wheat (Kumar and Narula 1999), *Bacillus circulans* and wheat (Singh and Kapoor 1999), *Enterobacter agglomerans* and tomato (Kim *et al.* 1998b), *Rhizobium leguminosarum* bv. *phaseoli* and maize (Chabot *et al.* 1996). *Penicillium bilaii* and wheat (Asea *et al.* 1988). Studies with PSMs showed enhanced growth and increased P content, but the performances varied (Kucey *et al.* 1989). Many reasons were proposed to account for the variations in the effectiveness of PSMs inoculation on plant growth are: (i) survival and colonization of inoculated PSMs in rhizosphere; (ii) competition with native microorganisms; (iii) buffering capacity of soils; and (iv) low organic acid secreting PSMs.

2.11 SURVIVAL AND PERFORMANCE OF INOCULATED BACTERIA IN SOIL

The competitiveness of a PSM in natural environment will depend upon its ability to survive and multiply in soil. However, understanding performance of PSMs in soil is the most limiting factor and is difficult to predict the behavior and efficacy of the inoculated bacteria in a particular location. In general, the population of the introduced microbe declines rapidly in soils (Ho and Ko 1985). The biotic factors that

affect the survival of the inoculated microbes include competition, predation and root growth that provide substrate to the microbes. The abiotic factors include soil texture, pH, temperature, moisture content and substrate availability in the soils (van Elsas *et al.* 1991). The biotic factors play a very important role in the survival of inoculated strains as the decline observed in non-sterile soils can often be abolished in sterile soils (Heijnen and van Veen 1991). The success of introduced bacterium in soil can be monitored using intrinsic antibiotic resistance markers (Rupela *et al.* 1981). This technique could be used to see the heterogeneity of indigenous populations, change in the population over cropping system and to trace back the particular inoculant strain. The persistence of *Rhizobium japonicum* in a field was monitored for 56 weeks, following inoculation (Ellis *et al.* 1984). The inoculated species were detected within the first seven weeks compared to the indigenous *Rhizobium* population. Similar pattern was observed for survival of *Pseudomonas* species in field conditions (Dupler and Baker 1984).

2.12 SUSTAINABLE INDICATORS OF SOIL QUALITY

Crop residues are applied to soil as source of nutrients, a practice that fits well with the current world-wide trends towards conservation in agriculture and particularly, with growing interest of local farmers in alternative agriculture practices, such as organic farming and zero- or reduced tillage (Roldan *et al.* 2005). These practices optimize the rate of turnover and recycle organic matter, nutrients and offer several other physical, chemical and biological soil improvements. Crop residue management has a profound influence on nutrient-supplying power of soils not only

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to next growing season but it may last for several years (Borie *et al.* 2002; Johansson *et al.* 2004).

Increased monoculture production of cash grain crops and greater reliance on chemical fertilizers and pesticides to maintain grain yields lead to decline in soil productivity and increased environmental contamination. Motivation for shifting from chemically intensive management to alternative practices include: (i) concern for protecting soil, human and animal health from the potential hazard of pesticides; (ii) concern for protecting environmental and soil resources; and (iii) a need to lower production costs. Organic farming systems rely upon legume-cropping containing crop rotations, crop and animal residues and mechanical cultivation to maintain soil tilth, nutrient supply, control insects, weeds and other pests (USDA 1980).

The use of synthetic chemical fertilizers and pesticides disturb chemical and biological balance in soil and is considered to be harmful to microorganisms and other life forms and is avoided. Herbicides use is known to selectively suppress activity of microorganisms responsible for N_2 fixation and nitrification for 4 to 12 weeks in soil (Bollen 1961). Soil reclamation by the addition of topsoil and revegetation was found to increase microbial population similar to levels found in undisturbed soils. Decline in microbial populations soon after soils are reclaimed can be attributed to utilization of soil organic matter (Fresquez *et al.* 1986). Reclamation of degraded lands often begins with non-mycorrhizal plants with the mycorrhizal inoculums and fungal population increasing with each stage (Cundell 1977; Rao and Venkateswarlu 1981;).

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Soil quality is one of the significant agroecosystem components for which management efforts must intensify in order to achieve sustainability. It depends on a large number of physical, chemical and biochemical properties and its characterization requires the selection of properties more sensitive to changes in management practices (Elliott *et al.* 1996). Biological and biochemical soil properties fulfill this requirement and are also sensitive to environmental stress (Dick and Gupta 1994); for these reasons they are appropriate for estimating soil quality (Yakovchenko *et al.* 1996). The various proposed physical indicators of soil quality are texture, structure, bulk density, infiltration rate and water holding capacity, aggregate stability and depth of soil. Most of these physical attributes assess the functioning of soil with respect to retention, leaching and transport characteristics of water and chemicals through the soil. Degraded soil with respect to physical conditions have low or excessive permeability, poor water retention and transmission characteristics, poor structure including crusting, sealing, compaction and hardening, obstruction to tillage operations, shallow depth, etc. Use of organic amendments, such as farmyard manure, compost, plant residues etc. is known to improve soil physical properties of puddle soils (Chaudhary and Ghildyal 1969; Sahoo *et al.* 1970; Fagi and De Datta 1983; Bhagat and Verma 1991). This improvement in physical conditions of soil with the addition of organic sources was attributed to the decomposition product of organic matter known as polysaccharides which bind several soil particles together in discrete structural units, that allow more water to infiltrate and decrease the runoff loses (high permeability). A positive

correlation of organic carbon with aggregate stability and negative correlation with bulk density has been indicated by Christensen (1986).

The chemical indicators of soil are soil organic carbon, pH, electrical conductivity, cation exchange capacity (CEC), extractable nitrogen, phosphorous and potassium. The chemical degradation of soils occurs mainly as a result of loss of soil buffering capacity, soil organic matter, CEC and exchangeable Ca^{2+} and Mg^{2+} as well as soil nutrient depletion and accumulation of Al^{3+} and Fe^{3+} to toxic levels. An improvement in the buffering capacity of soil and increase in organic carbon, electrical conductivity and available nitrogen, phosphorous and potassium was observed with the addition of organic amendments to the soil in the form of farmyard manure, compost and plant residues etc. (Nambiar and Abrol 1989; De Datta 1990; Clark *et al.* 1998).

Various biological indicators are related to soil quality, for example microbial biomass, soil respiration, soil enzyme activities etc (Andrews *et al.* 2003). Soil microbial biomass is an important component of soil organic matter that regulates the transformation and storage of nutrients. Microbially mediated processes affect nutrient cycling, soil fertility and global C change and soil organic matter turnover. The size and activity of soil microbial biomass must be assessed to fully understand nutrient fluxes in managed and natural ecosystems. Microbial biomass (MB) measurements have been used to estimate the biological status of soil. Soil microbial biomass is closely related to organic matter content and is an integrative measure of soil environment thereby minimizes the number of indicator measurements needed

to predict changes in soil quality. Because C and N in the microbial biomass (MBC and MBN respectively) reflect changes in management practices long before changes in total soil C or N are detectable. Fauci and Dick (1994) carried out the microbial biomass studies to see the effects of long-term management and recent soil amendments on the soil quality. Microbial biomass has been shown to be a sensitive indicator of differences in sustainable cropping systems (Anderson and Domsch 1990).

Soil enzyme activities are very sensitive to both natural and anthropogenic disturbances and show a quick response to the induced change (Dick 1994). Therefore, enzyme activities can be considered effective indicators of soil quality changes resulting from environmental stress or management practices. Enzyme activities are responsive to the different agricultural soil conservation practices such as no-tillage, organic amendments (Dick 1992; Perucci 1992), crop rotation (Miller and Dick 1995) and organic cultivation (Quilchano and Maranon 2002). The enzyme activity of soils results from the activity of accumulated enzymes and those in proliferating microorganisms. Dehydrogenase plays an essential role in the initial stages of oxidation of soil organic matter by transferring electrons or hydrogen substrates to acceptors (Camina *et al.* 1998). The activity of dehydrogenases serves as an indicator of the microbial redox systems in soils and can be considered a good measure of microbial oxidase activity (Tabatabai 1982). Dehydrogenase activity positively correlates with respiratory activity in soils (von Mersi and Schinner 1991). Acid phosphatase and alkaline phosphatases show optimum activities in acids and alkaline ranges respectively and play a role in mineralization of organic P.

Soil quality has emerged as the central concept for examining and integrating relationships and functions of various parameters in soil, which are important in the context of sustainable land use and management (Karlen *et al.* 1997). However soil quality cannot be measured directly, but must be inferred from measuring changes in its attributes referred as indicators (Wick *et al.* 1998). Individual soil biological and biochemical properties are not useful measures of soil quality as they vary both seasonally and spatially (Skujins 1978; Nannipieri *et al.* 1990; Doran and Parkin, 1994, Gil-Sotres 2005). Stefanic *et al.* (1984) proposed the biological activity index of fertility (BIF), which is based on combining soil dehydrogenase and catalase activity as:

$$BIF = \frac{DA + KCA}{2}$$

where,

DA and CA represent dehydrogenase and catalase activity, respectively and K is a proportional coefficient. In another approach, Beck (1984) proposed Enzyme Activity Number (EAN) as biological index based on 5 different enzymes given by the expression.

$$EAN = 0.2 \left(TAF\ (mg) + \frac{Catalase\ (\%)}{10} + \frac{\mu g\ phenol}{40} + \frac{\mu g\ amino\text{-}N}{2} + \frac{amylase\ (\%)}{20} \right.$$

The use of different units of measurement for deriving a single index does not support the universal applicability of such indices. Moreover, the methods used by Stefanic *et al.* (1984) and Beck (1984) was not as sensitive as currently used to determine enzyme activity in soil (Nannipieri 1994). In case of EAN index, the choice of amylase rather than cellulase activity was also criticized because cellulose is more important than starch in plant residues (Stevenson and Cole 1999). These indices do

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not take into account the content of main nutrients in soil and thus its nutrient supplying capacity that is important for plant growth.

Nannipieri *et al.* (2002) discussed two approaches to assess the soil microbial activity, one is the use of single index and the other is based on measurements of number of soil enzyme activities to estimate microbial functional diversity. He emphasized integrating enzyme activities limiting the rate of metabolic processes. Microorganisms promote physiochemical changes in the soil, such as the stabilization of soil organic matter, N_2 fixation and other alterations in soil properties necessary for plant growth. Soil organisms are one of the most sensitive biological markers available and the most useful for classifying disturbed or contaminated systems since diversity can be affected by minute changes in the ecosystem. Visser and Parkinson (1992) have indicated that studies of soil microorganisms as related to soil quality can be made at one of the three organizational levels: population, community or ecosystem. Microbial populations apparently can provide advance evidence of subtle changes in soil long before it can be accurately measured by changes in organic matter.

Microbial diversity has been studied with respect to species richness, keystone species and functional groups. Keystone species are defined based on the concept that 'a limited number of organisms and group of organisms seem to control the critical processes necessary for ecosystem functioning'. Fatty acid methyl ester (FAME) analysis and terminal restriction fragment length polymorphism (T-RFLP)

analysis have been used as keystone to characterize microbial community structure in soils maintained under continuous long-term fertilizer conditions (Suzuki *et al.* 2005). Sustainability index of different fertilizer management practices was assessed based on soil nutrient, biological and crop index. It was based on the area of the triangle in which nutrient index (calculated from various soil chemical parameters), microbial index (calculated from soil microbial and biochemical activities) and crop index (calculated from crop yield) of soil represented the three vertices of a triangle (Kang *et al.* 2005).

In view of the above literature, studies were carried out to re-cycle crop residues under controlled aerobic conditions to prepare composts. Three composts, farm waste compost, rice straw compost and *Gliricidia* vermicompost were prepared and 207 bacteria were isolated. They were screened for plant growth promoting and antagonistic traits. Phosphate solubilizing bacteria (PSB) were screened in RP buffered medium and efficient P solubilizers were selected. The two PSB, *Serratia marcescens* EB 67 and *Pseudomonas* sp. CDB 35 were able to utilize cellulose, crop residues and solubilize RP in laboratory conditions. Soil microbial activity was enhanced in presence of different organic carbon sources when these two isolates were inoculated. Characterization of the bacterial isolates for plant growth traits in laboratory conditions can be used to select the bacteria for glasshouse studies. Ten bacterial isolates showed improvement in plant growth of pearl millet and sorghum in pots using unsterilized soil. Bacterial isolates were compatible with existing microbial inoculants (*Rhizobium* and Mycorrhizae) and can be tested on varied hosts

to improve plant growth when applied. Antagonistic bacteria *Bacillus licheniformis* EB 13 and *Pseudomonas* sp. CDB 35 suppressed two plant pathogenic fungi (*Sclerotium rolfsii* and *Macrophomina phaseolina*). Phosphate solubilizing bacteria *Serratia marcescens* EB 67 and *Pseudomonas* sp. CDB 35 colonized the rhizosphere of maize and improved plant growth in field conditions when inoculated as seed treatment. These bacteria survived in peat for longer durations and may be supplied as peat-based inoculants.

Studies involving different cropping systems, where composts, microbial inoculants and crop residues, were applied from the past four years observed an improvement in soil properties characterized when compared to cropping system that received chemical fertilizers. In the present study, we have used a polygon approach to evaluate the sustainability of four different crop husbandry systems emphasizing the soil nutrient, biological, microbiological and crop production as indicators. This approach is based on measurement of soil biological index (calculated from soil enzyme activities (acid phosphatase, alkaline phosphatase, dehydrogenase, soil respiration, biomass C, biomass N), soil microbial index (calculated from population of bacteria, fungi, actinomycetes, *Pseudomonas*, PSB, siderophore, phytase producers and antagonistic bacteria), soil nutrient index (calculated from organic C, total N and P, available P and K) and crop index (dry matter and crop yield of legume and cereal crop raised during that period).

CHAPTER III

MATERIALS AND METHODS

3 MATERIALS AND METHODS

3.1 CHEMICALS AND RAW MATERIALS

The chemicals and raw materials used in this study were obtained from different sources. General chemicals like ammonium chloride, ammonium sulphate, boric acid, calcium chloride, citric acid, copper sulphate, ferric chloride, ferrous sulphate, ferrous sodium EDTA, gluconic acid, magnesium sulphate, potassium chloride, potassium iodide, potassium nitrate, potassium phosphate, sodium chloride, sodium phosphate, tarataric acid, tris salt, zinc sulphate etc. and H_2SO_4, HCl, NaOH, KOH, chloroform, methanol were from Qualigens India. 1-amino-cyclopropane-1-carboxylic acid, chrome azurol S dye, 8- Hydroxy quinoline, hexadecyltrimethyl ammonium bromide, phytic acid, PIPES [Piperazine 1,4 Bis (2-ethane sulphonic acid)] buffer, tryptophan are from Sigma Chemicals, USA. All media components like casaminoacid, cellulose, glucose, Luria broth, nutrient broth, peptone, yeast extract, agar etc. were from Himedia, Mumbai, India. All the crop residues used in the study were obtained from farms at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, AP, India.

3.2 PREPARATION OF COMPOSTS

Composts were prepared using three different crop residues in controlled conditions.

3.2.1 Farm waste compost and the associated macrofauna

Farm waste compost (FWC) was prepared in an above-soil surface brick chamber (150 cm x 90 cm x 100 cm) that received farm and kitchen waste (Photograph 3.1) at ICRISAT. The compost was prepared by keeping the contents moist by regular watering. A small population of earthworms (about 35 mm long and 2 mm diameter), centipedes (about 15 mm long and 3 mm diameter), slugs (about 40 mm long and 15 mm wide) and snails (about 12 mm long and 1 mm diameter at the narrow end and 4 mm diameter at the wider end) that were naturally present in the compost product were used in the study. Centipedes, slug and snails were similar to garden fauna and their preliminary identification was done.

3.2.2 Rice straw compost

A scale up study to prepare rice straw compost (RSC) in heaps was designed at ICRISAT, unlike the old method of using digesters for compost preparation (Rupela *et al.* 2003a). Multiple heaps (5 m long x 1.5 m wide x 1.5 m high) of 500 kg capacity was prepared for composting (Photograph 3.2). Five to 10 kg bundles of air-dried rice-straw were tied up in plastic nets or twine and dipped in water (every 1 kg dry straw soaked in 1.5 L water) for 2 to 3 minutes and then allowed to drain for five minutes. The dripping were captured on a tarpaulin sheet and recycled. Moistened straw was allowed to stay in a heap and covered with polythene sheet to reduce evaporation losses and to prevent it from getting extra moisture from rain during the composting period. Each heap was covered on all sides with at least 10 cm thick layer of moistened rice-straw (non-experimental). Care was taken to maintain

wetness of contents and water was applied using a specially designed lance. Temperature and relative humidity (RH%) was recorded using Hobo data logger (from Onset Computer Corporation, USA), which helped to decide the time for watering. After maturity period of compost, contents of the heap were passed through a pulverizer and used.

3.2.3 *Gliricidia* vermicompost

Gliricidia vermicompost (GVC) was prepared in cement cylinder (90 cm diameter x 50 cm height) with leaves and twigs of *Gliricidia sepium* (Jacq.), by soaking in 1% cow dung slurry. After two weeks, about 500 earthworms (*Eisenia fetida*) were released into the same tank. Seven layers of foliage of 10 cm thickness of each were added after decomposition of lower layer.

Table 3.1.   Composts used in the study

Main materials	Additives	Primary fermentation	
		Operational period	Maximum temperature
Farm waste (Leaves, twigs)	Kitchen waste	40 days	52 °C
Rice straw	-	45-50 days	55-60 °C
Gliricidia (Leaves, twigs)	Earthworms Cow dung	40 days	48-52 °C

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Photograph 3.1. Preparation of farm-waste compost (FWC) in brick chamber.
(View showing earthworms naturally present in compost).

Photograph 3.2. Preparation of rice straw compost (RSC) in heaps.

Photograph 3.3. Preparation of *Gliricidia* vermicompost (GVC) in cement cylinders.

3.3   ENUMERATION OF MICROORGANISMS IN COMPOSTS

Each compost preparation took about 45-50 days at 70% moisture. Four – eight weeks after maturity, 10 g of the compost was taken and appropriate dilutions were plated on NA for bacteria and quarter strength potato dextrose agar (PDA) with streptomycin (500 µg mL^{-1}) for fungi. A small population of macrofauna (10-15 kg compost) such as earthworms, centipedes, slugs and snails were naturally present in FWC. Each macrofauna were placed separately in sterilized high density polyethylene (HDPE) bags (size: 20 x 11cm) after washing with sterile distilled water for collecting excreta. It took about 4 h (in case of earthworms and centipedes) to 24 h (in case of slugs and snails) and one gram of excreta was mixed in nine mL saline and appropriate dilutions were plated on NA and PDA. Ten macrofauna were placed in Petriplate, desiccated to death and appropriate dilutions in saline were plated on NA and PDA for isolating bacteria and fungi from body surface. Plates of NA and PDA were incubated at 30 ± 2 °C and observed once a day for four days. Microbial population was calculated as $\log_{10}$ per gram dry weight compost. Population on body surface of macrofauna was expressed as $\log_{10}$ macrofauna^{-1} and in excreta as macrofauna^{-1} h^{-1}.

3.3.1   Isolation, purification and preservation of the isolated microorganisms

Representative bacteria from each source were further isolated onto NA media. All the bacterial isolates were purified and preserved by freeze-drying. The isolates from the FWC and the macrofauna associated with it were numbered with a prefix EB (data on bacterial isolates from macrofauna was pooled as one group). CDB as prefix to the isolates from RSC and BWB to those from GVC.

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3.3.2 Microscopic studies

A smear of each organism was prepared using the inoculum from 24 hour old culture and standard procedure for Gram staining was used and slides were observed under light microscope (Zeiss MC 80 DX).

3.4 MEDIA EMPLOYED TO SCREEN PLANT GROWTH PROMOTING AND ANTAGONISTIC BACTERIA (*IN VITRO*)

3.4.1 Preparation of inoculum

Bacterial isolates from glycerol stocks were activated with few subcultures and were inoculated to three mL of Luria Bertani (LB) broth (Sambrook *et al.* 1989) and incubated at $30\pm1°$ C for 24 h on a rotary shaker. About 250 µL of inoculum was added to the well of sterilized multiple pin inoculator that transferred ~10^3 cells to media plates (as given below) and allowed the simultaneous inoculation of 25 isolates (as shown in Photograph 3.4) (Josey *et al.* 1979).
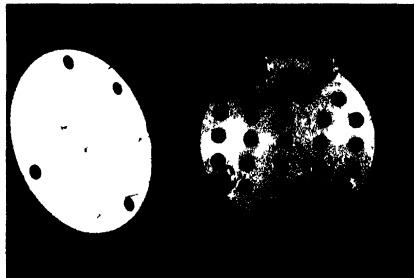
Photograph 3.4. Multiple pin-inoculator used in screening assays.

3.4.2 Preparation of media to screen plant growth promoting bacteria (PGPB)

Different media were used to screen the PGPB. The media were prepared as mentioned and 2% agar was added for preparing plates. All media were sterilized by autoclaving at 121° C for 15 minutes. After inoculation all the plates were incubated at 30±2° C for 24 to 144 h as required based on the trait under study. Bacteria positive for plant growth promoting trait were rated as 0, 1, 2 and 3. Studies were conducted in triplicate and repeated twice.

Modified M9 medium (g/L) (Booth 1971) for cellulose utilization

Cellulose[a]	:	10
$MgSO_47H_2O$	:	0.5
$Na_2HPO_4.7H_2O$	:	12.8
KH_2PO_4	:	3
NaCl	:	0.5
NH_4Cl	:	1
$CaCl_2$	:	0.150
Ferric chloride	:	trace
pH to 7.2-7.4		

[a] finely powdered, washed, oven-dried rice straw powder (1 mm size) was also used as carbon source to screen the cellulose degrading bacteria.

Rock phosphate (RP) buffered medium (mL/ L). (Gyaneshwar *et al.* 1998) for PSB

Glucose (2M)	:	50
NH_4Cl or KNO_3 (1M)	:	3
Tris. Cl (1M)	:	100
$MgSO_4$(1M)	:	0.025
$FeSO_4. 7H_2O$	:	3.5 mg
$ZnSO_4. 7H_2O$	:	0.16 mg
$CuSO_4. 5H_2O$	:	0.08 mg
H_3BO_3	:	0.5 mg
$CaCl_2. 2H_2O$	:	0.03 mg
$MnSO_4. 4H_2O$	:	0.4 mg
Mussorie RP	:	1 g
Methyl red	:	0.01 g
pH	:	8.0

Phytic acid medium   (g/L) (Richardson and Hadobas 1997) for phytase activity

Arabinose	:	1
Phytic Acid*	:	10
$(NH_4)_2SO_4$	:	1
$MgSO_4.7H_2O$	:	0.1
KCl	:	7
$CaCl_2.2H_2O$	:	0.1
FeNa EDTA(0.1M)	:	1 mL

*sterilization by membrane filtration, pH-5
pH 7.0 adjusted with sterilized 1.8 M NaOH, just before preparing the plates.

Iron free medium (g/L) (Schwynn and Neilands 1987) for siderophore production

Dextrose	:	10
K_2HPO_4	:	4
KH_2PO_4	:	1
$(NH_4)_2SO_4$	:	1
$Mg SO_4.7H_2O$	:	0.5 mL of 20% Solution
Salt Solution*	:	0.1mL
Chromeazurol S (CAS)[a] dye	:	100mL
Casamino acid	:	3
pH 6.8		

*$(ZnSO_4.7H_2O$- 4.4 mg; $Cu SO_4.5H_2O$-40 mg; $MnSO_4.4H_2O$- 41 mg; KI-42 mg)

[a] CAS solution is prepared using 60.5 mg dissolved in 50 ml of water. 10 mL of $FeCl_3$ (16.2 mg of $FeCl_3$ in 36µL of conc. HCl and make upto 100 mL) was added to the above solution. Under stirring add this solution to 40 mL of hexadecyl trimethyl ammonium bromide (72.9 mg in 40 mL of water). Resultant dark blue liquid was autoclaved separately and added before preparing plates. To remove iron from media add 5 mg of 8-Hydroxyquinoline to 1 mL of $CHCl_3$. Add 30.24 g of PIPES buffer and adjust pH to 6.8 with 50% NaOH.

Pseudomonas **isolation agar (g/L) for fluorescent pseudomonads**

Casein enzymatic hydrolysate	:	10
Proteose peptone	:	10
K_2HPO_4	:	1.5
$MgSO_4$	:	1.5
Glycerol	:	10mL
pH 7.0		

Indole producers

LB agar medium amended with 5mM 1-tryptophan. After inoculation onto the medium, the inoculated points were overlaid with 10-mm-diameter nitrocellulose membrane (NCM) disk (Millipore, USA) (Bric et al. 1991). After incubation, the NCM were overlaid on a Whatman No. 2 filter paper saturated with Salkowski reagent (Gordon and Weber 1951).

Dworkin foster minimal medium (g/L) (Jacobson et al. 1994) for ACC deaminase

Glucose	:	0.2
Gluconic acid	:	0.2
Citric acid	:	0.2
KH_2PO_4	:	4
Na2HPO$_4$	:	6
$MgSO_4$	:	0.2
$FeSO_4$	:	0.001
H_3BO_3	:	10μg
$MnSO_4$	:	10 μg
$ZnSO_4$	:	70 μg
$CuSO_4$	:	50 μg
MoO_3	:	10 μg
ACC*	:	3 mM

*sterilization by membrane filtration

Kings B medium (g/L) (King et al. 1954) for Cyanide production

Proteose peptone	:	10
Casamino acids	:	5
K_2HPO_4	:	2
Glycine	:	4.4

Thirty-five mm Petri plate was used and 100 μl of 24 h grown culture was inoculated. Whatman No. 2 filter paper impregnated with 0.5% picric acid and 2% sodium carbonate was placed inside the lid of each Petri dish (Bakker and Schippers 1987) and plates were incubated.

Chitin medium (g/L) (Hirano and Nagao 1988) for chitinase activity

Chitin	:	15
Yeast extract	:	3
$MgSO_4. 7H_2O$	:	3
KH_2PO_4	:	13.6
$(NH_4) SO_4$	:	1
pH	:	7

Potato dextrose agar (PDA medium) (g/L)

Potato	:	200
Dextrose	:	20
$MgSO_4. 7H_2O$:		0.2

Glucose casamino acid yeast extract (GCY) (g/L)

Glucose	:	15
Casamino acids	:	1.5
Yeast extract	:	1.0
KH_2PO_4	:	1.0
$MgSO_4. 7H_2O$	:	1.0

Chloroneb mercuric chloride – Rose Bengal agar (CMRA medium g/L) (Dubey and Maheshwari, 2004)

Potato	:	200
Dextrose	:	20
$MgSO_4$	:	0.2
Chloroneb	:	312 mg
$HgCl_2$	:	8.5 mg
Rose Bengal	:	112 mg
Streptomycin sulphate	:	40 mg
pH - 6		

3.4.3 Screening for antagonistic bacteria

All the 207 isolates were screened for in *vitro* antagonism against *S. rolfsii*, *M. phaseolina*, *F. solani* and *F. oxysporum* on PDA medium using dual culture technique. In plate assay, an agar block (five mm diameter) was cut from an actively growing (96 h old) fungal culture and placed on the surface of fresh agar medium at the center of Petri plate. A loopful of 24 h old culture of each bacterium was streaked in a straight line on one edge of a 90-mm diameter Petri plate. Plates inoculated with the same fungus without bacteria were used as control. For each treatment three

replications were maintained. Plates were incubated at 30±1 °C and the inhibition zone between the two cultures was measured 5 days after inoculation. Percent inhibition of fungi was calculated by using the formula as given below:

$$I = \frac{100\ (C-T)}{C}$$

where,

I = Inhibition % of mycelial growth (growth reduction over control)
C= radial growth of fungus in the control plate (mm)
T = radial growth of fungus on the plate inoculated with bacteria (mm)

Based on *in vitro* plant growth promoting and antagonistic character twenty-three bacterial isolates were selected for further studies.

3.5 SELECTION AND IDENTIFICATION OF PLANT GROWTH PROMOTING BACTERIA (PGPB)

Twenty-three isolates with more than one plant growth-promoting trait were selected for further plant growth studies by paper towel method.

3.5.1 Preparation of peat based formulation

All bacterial isolates used for plant growth studies were prepared as peat based inoculants. Neutralized peat (Biocare Technology Pvt. Ltd., Australia) (30 g) was packed in HDPE bags and sterilized by autoclaving at 121 °C for 20 minutes. 30 mL of bacterial cells (10^8-10^9) harvested from mid-log phase culture grown in LB broth were injected aseptically into an individual pack and covered with a label at the injecting point. Inoculated packets were thoroughly kneaded (mixed) to ensure uniform adsorption of the bacterial cells into the carrier material and incubated at

$30\pm1°$ C for a period of ten days. Later on, the peat packets were preserved at room

temperature (26° C) or cold room (4° C), based on further use.

3.5.2 Seed bacterization

Seeds were surface sterilized with 3% sodium hypochlorite (Na OCl_2) for five

minutes and washed five times with sterilized distilled water. The seeds were

treated with peat-based inoculum of bacteria (10^8-10^9 gm^{-1} peat) using 1% carboxy

methylcellulose (CMC) as adhesive and dried in laminar airflow before testing for

plant growth assay. The viable cell count as determined by dilution plating was 10^6-

10^7 colony forming unit (CFU) per seed.

3.5.3 Seed vigor index

Germination test was carried out in paper (roll) towel according to International

Seed Testing Association (ISTA, 1993). Vigor index was calculated as:

Vigor index = (Mean root length + Mean shoot length) x Germination (%)

3.5.4 Growth of pearl millet (*Pennisetum glaucum* L.) and sorghum (*Sorghum bicolor* L. Moench) inoculated with bacterial isolates using paper towel method

Twenty-three isolates were evaluated for growth of pearl millet cultivar ICMV 155

under sterilized conditions. After bacterization 50 seeds were placed in each

germination paper and were incubated in glasshouse. Temperature was recorded

with hobo data logger and ranged between 22-32°C (average 26°C). *Azotobacter*

chroococcum HT-54 known to promote plant growth was used as reference strain

(Alka *et al.* 2001). Seeds treated only with peat served as control. Four replications for

each treatment were maintained. After 10 days seed vigor index and plant weight (wet biomass) was recorded. Based on growth of pearl millet, *in vitro*, twelve isolates were selected and evaluated for growth of sorghum cultivar CSV 15. After bacterization fifty seeds were placed in each germination paper and incubated in glasshouse. After 10 days seed vigor index and plant weight (wet biomass) was recorded.

3.5.5 Identification of selected bacteria

Based on plant growth studies of pearl millet, twenty-two bacterial isolates were selected for identification studies. Identification was done by staining, morphology, cultural, growth and biochemical characters based on Bergeys manual of determinative bacteriology (Krieg and Holt 1984) and Handbook of microbiology (Gordon 1977).

3.6   ESTIMATIONS

3.6.1 Phosphate estimation

Phosphate was estimated by colorimetric method (Ames 1964) using (250 µg/mL) potassium dihydrogen phosphate as standard. Culture broth from RP media was collected (after drop in pH) and centrifuged at 10,000 rpm. 100 µl of sample (supernatant) was taken in test tube (6 x 1 cm) and made up to 450 µl with distilled water. To this 750 µl of ammonium molybdate-ascorbic acid mixture was added. All the tubes were incubated at room temperature for one hour till blue color develops and absorption was recorded at 820 nm against blank (100 µl of distilled water).

3.6.2 HPLC analysis for organic acids

The culture supernatant was filtered using 0.22 μm pore size nylon- 66 membrane filters and subjected to high-performance liquid chromatography with following specifications: HPLC (LaChrom) (Merck), reverse phase, ion pairing column (RP-18). The flow rate was one ml/min. The mobile phase consisted of 0.1% phosphoric acid. Detection of organic acid was done using UV-VIS detector at 210 nm. The HPLC profile of the culture supernatants was analyzed by comparing with the elution profile of standard organic acids.

3.6.3 Protein estimation

Total protein was estimated using modified Lowry's method (Peterson 1977) using one mg/mL of bovine serum albumin as standard. 25 μl of sample (cells) was taken in tubes kept in ice and made up to two mL with distilled water. To this 0.5 mL of 1N NaOH was added and incubated at 100°C (boiling water bath-BWB) for five minutes. To this 2.5 mL of complex reagent [5% Na_2CO_3 (72.1 mL) and 0.5% $CuSO_4.5H_2O$ in 1% sodium potassium tartarate (2.9 mL)] was added. After 10 minutes, 0.5-mL freshly prepared Folins reagent (1:1 diluted) was added. The tubes were incubated at room temperature for 30 minutes till color developed and A750 was recorded against blank (distilled water instead of cells).

3.6.4 Glucose estimation

Glucose was estimated by dinitrosalicylic (DNS) acid method (Miller 1959) using 1000 μg/mL. One mL of sample (supernatant) was added to one mL of DNS reagent and incubated for 10 minutes at 100° C. The tubes were removed and cooled to room

temperature. A color change from orange to brown indicates reducing sugars. The absorption was recorded at 560 nm against blank (one mL distilled water).

3.6.5 Chemical estimation of composts and soil

Composts and soils used in the study were analyzed for pH (using pH meter), organic carbon (OC) % (TOC analyzer, Primacs), Total N (Modified Kjeldahl method), Total and available P and K (Okalebo *et al.* 1993).

3.6.6 P estimation of soil

Phosphate was estimated by Olsens P method (Olsen and Sommers 1982) using K_2HPO_4 as standard. Five grams of soil was taken in 150 mL plastic nalgene bottles and 50 mL of sodium bicarbonate (0.5M, pH 8.5) was added. Bottles were shaken for 30 minutes on a shaker and the sample was filtered through Whatman No. 2 filter paper. From the filtrate five mL sample was taken in 25-mL volumetric flask and 5N H_2SO_4 was added till pH of extract was five. Volume of extract was made up to 21 mL with distilled water and four mL of reagent (ammonium molybdate-ascorbic acid mixture) was added. Absorption was taken at 882 nm.

3.7 ENZYME ASSAYS

3.7.1 Glucose dehydrogenase

Glucose dehydrogenase (Gcd) activity (Matsushita and Ameyama 1982) was measured in reaction mixture that contained one mL of Tris-HCl buffer (0.05M), 500 µl distilled water, 100 µl of phenazine methosulphate and appropriate concentration of enzyme (25 µl). Then it was adjusted to zero absorbance and 40 µl of

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diclorophenol indophenol (DCIP) was added. After the absorbance reached to a stable value, 100 μl of substrate (carbon source) was added and change in color due to reduction of diclorophenol indophenol (DCIP) at A600 was recorded.

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3.7.2 Cellulase assay

One mL of supernatant (crude enzyme) was taken in test tube (15 x 1.5 cm) with ten mg cellulose in two mL of Tris-HCl buffer (pH 7.2), for determination of cellulase activity. The tubes were incubated for 30 minutes at 37°C and one mL of 1N NaOH was added to inhibit the reaction. Later on, to the supernatant one mL of DNS was added and OD was taken at 600 nm. Cellulase activity is expressed as one unit of enzyme equivalent to 1μ mole of reducing sugar (glucose equivalents) released per minute under assay conditions.

3.7.3 Soil enzymes

Soil samples were airdried, hand poundered and passed through two mm sieve before enzyme assays.

3.7.3.1 Soil dehydrogenase assay

Dehydrogenase assay was determined by the method described by Casida *et al.* (1964). It involved determination of Triphenyl formazan (TPF) produced by the reduction of 2, 3, 5-Triphenyltetrazolium chloride (TTC). Six grams of soil was mixed with 0.2 g of $CaCO_3$ in centrifuge tube to which, one mL of 3% aqueous solution of TTC and 3.5 mL of distilled water was added. The tubes were incubated at 37°C for 24 h. Oxidation of TTC resulted in red colored Triphenyl formazan (TPF), which was extracted with methanol. Each soil sample was extracted thrice with 40, 40 and 20

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mL of methanol each time. Intensity of red color was measured by spectrophotometer at 485-nm absorption. Dehydrogenase activity was expressed as µg of TPF produced during the incubation period of 24 h per gram of dry soil and was calculated as:

$$\text{Dehydrogenase activity } (\mu g \text{ TPF } g^{-1} 24 \text{ } h^{-1}) = \frac{40 \times C1 + 40 \times C2 + 20 \times C3}{6}$$

where,

C1 = Concentration of TFP (ppm) as read from the standard curve + 40 mL of methanol + volume of TTC and distilled water used in the assay.

C2 = Concentration of TFP (ppm) as read from the standard curve + 40 mL of methanol + volume of TTC and distilled water used in the assay.

C3 = Concentration of TFP (ppm) as read from the standard curve + 20 mL of methanol + volume of TTC and distilled water used in the assay.

3.7.3.2 Soil phosphatase assay

The enzymatic activity of *phosphomonoesterases* was determined by the method described by Eivazi and Tabatabai (1977). In this method, colorimetric measurement of *p*-nitrophenol formed by the enzymatic hydrolysis of *p*-nitrophenyl phosphate gives an estimation of *phosphomonoesterases* in soil. One gram of soil (two mm) was taken in test tube (20 x 2.5 cm) and 0.2 mL toluene, four mL of modified universal buffer (MUB) and one mL of *p*-nitrophenyl phosphate (substrate) prepared in MUB was added and contents were mixed by swirling for few seconds. Test tube was stoppered and incubated for one hour at 37°C. MUB was prepared with 12.1 g of tris (hydroxymethyl) aminomehtane (THAM), 11.6 g of maleic acid, 14 g of citric acid and 6.3 g of boric acid in 488 mL of 1N NaOH and diluted to one litre. For acid phosphatase activity pH of buffer and substrate was adjusted to 6.5 and for alkaline phosphatase pH-11. After one hour, the stopper was removed and one mL of $CaCl_2$

(0.5 M), four mL of NaOH (0.5M) and 25 mL of distilled water was added and soil suspension was filtered through a Whatman No. 2 filter paper. Intensity of yellow color of *p*-nitrophenol formed was measured by spectrophotometer at 420-nm absorption. The activity of phosphatase was expressed as µg of *p*-nitrophenol formed during incubation period of one h per gram of dry soil.

Phosphatase activity (µg *p*-nitrophenol g^{-1} h^{-1}) = C x absorbance value

(C=Concentration of *p*-nitrophenol as read from the standard curve)

3.8 GROWTH & PERFORMANCE OF PHOSPHATE SOLUBILIZING BACTERIA

3.8.1 Selection of efficient phosphate solubilizing bacteria (PSB)

Of the 207 isolates, five bacterial isolates (EB 27, EB 67, EB 75, CDB 35 and BWB 21) showed P solubilization in RP buffered agar medium. Quantitative study was carried out inoculating five isolates in RP broth. Growth was monitored using spectrophotometer, (Shimazu) A600 nm, pH was recorded using pH meter. Organic acid was detected using HPLC and P released was estimated by Ames method. The experiment had two replications and repeated thrice.

3.8.2 Performance of efficient PSB *Serratia marcescens* EB 67 and *Pseudomonas* sp. CDB 35 in presence of different carbon sources

Two bacterial isolates EB 67 and CDB 35 based on P solubilizing ability were tested for growth, drop in pH and P using different carbon sources. RP buffered medium with D-glucose, xylose, galactose, mannose, maltose, cellobiose, L-arabinose at 100 mM concentration and mixture of each carbon source was autoclaved at 108 °C for 20 minutes. The broth was inoculated with an overnight grown culture of EB 67 and

CDB 35, which was washed twice with saline and subsequently diluted 1:100 times. 250 μl of this dilution was used to inoculate 50 mL broth in 250 mL flask. The organisms were allowed to grow aerobically at 30±1 °C on rotary shaker at 200 rpm. Absorption at A600 nm was taken for 0 h, 24 h, 48 h, 72 h and 96 h to monitor bacterial growth. Broth was centrifuged and the supernatant was used to record decrease in pH, P estimation by Ames method and organic acid using HPLC (Table 3.2). EB 67 and CDB 35 were grown on RP buffered medium for 30 hours with different carbon sources (100 mM). After growth, cells were harvested (5000 x g for 10 minutes), washed with sterilized saline and resuspended in Tris-HCl (0.05M) pH 8.75 and the whole cell suspensions were used as source of enzyme in Gcd assay. the cells were grown on glucose and different sugars were used to measure the Gcd activity. The experiment had two replications and repeated thrice.

Table 3.2.   Retention time (minutes) of organic acids in presence of different sugars in RP medium inoculated with the two PSB

Isolates	Glucose	Galactose	Xylose	Mannose	Cellobiose	Mixture
EB 67	2.35	2.27,2.63	2.28,2.62	2.29,2.60	2.37, 2.75	2.37, 2.75
CDB 35	2.36	2.28,2.61	2.37,2.79	2.29,2.63	2.24, 2.27	2.24, 2.27

There were two peaks (due to acid production) with the retention times mentioned above in all the carbon sources tested except glucose.

3.8.3. Soil used in the studies

Vertisol was obtained from two different field locations (BR1D and BP2C) at ICRISAT, Patancheru. Soil from these two fields was used for different *in vitro* and glasshouse experiments carried out in this study. The pH of soil solution from BR 1D was 7.8, OC%-0.7, total N-759 ppm, total P-323 ppm and available P-1.8 ppm (low P

soil). BP2C soil had pH 7.8, OC%-1.1, total N-1200 ppm, total P-484 ppm and available P-5 ppm (medium P soil). Both the soil samples were used separately for the studies.

3.8.4 Effect of nutrient supplementation on growth and P release by native microorganisms and inoculated PSB EB 67 and CDB 35 in Vertisol

Vertisol was suspended at 0.5 g/mL in 100 mL medium containing 100 mM glucose, 10 mM NH_4Cl and RP (1 mg/mL) in 250 mL flask. Samples were collected at 0 h, 12 h, 24 h, 36 h and 48 h for estimating native population of PSB on RP buffered medium. Soil was centrifuged at 5000 rpm for 10 min and supernatant was used to record change in pH and estimate P. The experiment had two replications and repeated twice.

To see the effect of inoculated PSB in soil conditions, the native microorganisms were eliminated by autoclaving at 121°C for 15 minutes. For inoculation, an overnight grown culture of the bacteria was centrifuged at 5000 rpm for 10 minutes and the cell pellet was washed twice with saline and subsequently diluted to 100 folds. 500 µl of diluted saline was used to inoculate 100 mL medium in 250 mL flask. Both the bacterial isolates were allowed to grow aerobically in the soil suspension at 30° C on rotary shaker at 200 rpm. Soil samples were collected at 0 h, 12 h, 24 h, 36 h and 48 h to monitor counts of inoculated bacteria, determine pH and RP solubilized.

3.8.5 Solubilization of RP in presence of crop residues by EB 67 and CDB 35

3.8.5.1 Preparation of crop residues

Five crop residue materials rice straw, rice root, mixture of rice straw and root, pigeonpea root and grass (*Cynodoctilan* sp.) were soaked in water for two days and

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washed thoroughly to remove the soluble nutrients. It was oven dried at 55° C and grinded with cross beater 0.5 mesh size (Photograph 3.5).
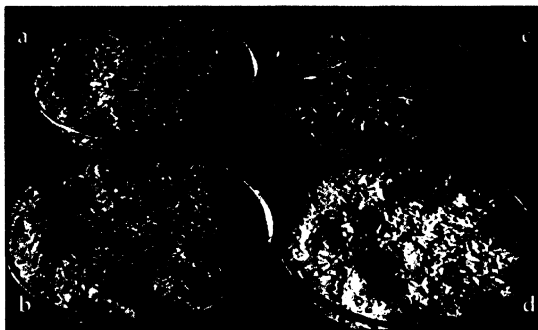
Photograph 3.5. Crop residues used in the study: a) rice straw b) rice root
c) grass d) pigeonpea root.

3.8.5.2. Preparation of water extracted rice straw

About 250 g ground rice straw was put separately in two litre conical flask, containing 1000 mL of distilled water and autoclaved at 121 °C for 30 minutes. Supernatant was decanted and the residue was thoroughly washed with distilled water until coloring compounds were removed. The materials were dried at 60 °C to constant weight.

3.8.5.3. Preparation of Alkali treated rice straw

About 250 grams ground rice straw was dispensed into two litre conical flask containing 1000 mL of 1% NaOH and autoclaved at 121 °C for 15 minutes. The supernatant was decanted and the residue was neutralized with 1% H_2SO_4. The fractions were thoroughly washed with distilled water until the coloring compounds were removed. The materials were dried at 60° C to constant weight.

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3.8.6 Cellulase activity, reducing sugars released and solubilization of RP in submerged and solid-state conditions in presence of different crop residues

In RP buffered medium glucose was replaced with different crop residues like rice straw, rice root, mixture of rice straw and root, pigeonpea root and grass separately (1%). For submerged medium conditions, 100 mL broth with each crop residue was taken in 250 mL flask and media were sterilized by autoclaving at 121° C for 15 minutes. For inoculation, an overnight grown culture of the bacteria was centrifunged at 5000 rpm for 10 minutes and the cell pellet was washed twice with saline and subsequently diluted to 100 folds. 500 µl of this dilution was used to inoculate 100 mL broth in 250 mL flask. Incubation was carried out aerobically at $30\pm1°$ C for a period of 12 days. The sample was collected at day 4, 6, 8 and 12, centrifuged at 15,000 rpm for 30 minutes and the supernatant was used to record cellulase enzyme activity; reducing sugars and P released. Each treatment had two replications and was repeated twice.

For solid-state conditions (SSC), 10 g of each crop residue (untreated rice straw, water extracted rice straw and alkali treated rice root, mixture of rice straw and root, pigeonpea root and grass), 1 g - NH_4Cl, 1g - RP and water was used as moistening liquid (80%) in 250 mL flask. The media was sterilized by autoclaving at 121° C for 15 minutes. For inoculation, an overnight grown culture of bacteria was centrifuged at 5000 rpm for 10 minutes and the cell pellet was washed twice with saline and subsequently diluted to 100 folds. 500 µl of saline was used as inoculum. Incubation was carried out aerobically at $30\pm1°$ C for eight days. After incubation 40 mL of water was added to each flask and filtered using masuline cloth. The sample was

used to record cellulase activity; reducing sugars and soluble P. Each treatment had two replications and was repeated twice.

3.8.7 Microcosm experiment: To see the effect of different organic carbon sources on microbial activities inoculated with PSB *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35

Microcosm experiment was set up in amber colored bottles of 150 mL capacity (Photograph 3.6). Hundred grams of soil (unsterilized soil of BR1D field) was taken in the bottle and RP was added (0.1%) and evenly mixed.

Photograph 3.6. Microcosm experiment set up for estimating microbial activity in soil inoculated with PSB in presence of different organic carbon sources.

Different organic carbon sources were added to each bottle and various treatments were as follows:

i.   Soil + RP

ii.   Soil + carbon source (glucose - 1%), N source (NH$_4$Cl - 0.1%) + RP

iii.   Soil + organic acids (mixture of gluconic, citric and tartaric acid 1%) + RP

iv.   Soil + rice straw (RS 1%) + RP

v.   Soil + rice root (RR 1%) + RP

vi.   Soil + Grass (GS 1%) + RP

vii.   Soil + pigeonpea root (PR 1%) + RP

Peat based inoculum of bacteria 10^7-10^8 CFU was added and covered with parafilm (punctured). Each treatment had three replications. Moisture was maintained to field capacity level by taking weight of the bottles every week. The bottles were incubated in glasshouse at temperature 22°-32° C (average 26° C). Bottles were harvested on day 6, 12, 24 and 48. Soil was used to monitor viability of bacteria on RP buffered antibiotic medium (benomyl was added to inhibit the fungal growth), drop in pH, acid and alkaline phosphatase activity, P estimation and microbial biomass C was determined.

3.8.8 Intrinsic antibiotic markers of EB 67 and CDB 35

Antibiotic resistant markers were developed for both the bacterial isolates EB 67 and CDB 35 by testing their tolerance to intrinsic levels of different antibiotics (Erythromycin, vancomycin, trimethoprim, nalidixic acid, gentamycin, streptomycin and rifamycin) on LB agar and RP buffered (50 mM) medium. A marker was developed with combination of resistant levels of different antibiotics to trace back the bacteria from soil. The isolates were subcultured 10 times onto the respective antibiotic LB agar and RP buffered (50mM) media plates to confirm their stability of antibiotic resistance.

3.9 SELECTION OF ANTAGONISTIC BACTERIA

3.9.1 Antagonistic bacteria

Eighteen of the 207 isolates were selected based on *in vitro* antagonism to soil-borne plant pathogenic fungi (*S. rolfsii, M. phaseolina, F. solani and F. oxysporum*) on PDA and were further tested on two media (GCY and KBM) against all four fungi.

3.9.2 Antifungal activity in liquid culture

Four antagonistic isolates (EB 13, CDB 15, CDB 35 and CDB 47) based on *in vitro* studies were tested for antifungal activity in dual liquid culture method against *S. rolfsii, M. phaseolina, F. solani and F. oxysporum* in GCY medium. Actively growing fungal culture in GCY medium was homogenized with a Tissumizer Mark II (from Tekmar, Cincinnati, Ohio, USA) blender. One mL of fungal suspension was inoculated into 100 mL of GCY broth in 250 mL conical flask. The culture was incubated on a rotary shaker (120 rpm, Model G25, New Brunswick, Sci. Inc. NJ, USA) at 30 ± 1 °C. After 24 h of fungal growth, one mL of freshly grown bacterial culture was inoculated. The flasks were incubated on shaker at 30 ± 1 °C. For control, the fungus alone was inoculated. The experiment was repeated twice with three replications each time. After 96 hours, dual cultures were passed through the preweighed Whatman No. 1 filter paper. It was dried for 24 h at 65°C and weights were measured. Biomass reduction (inhibition percentage) of fungi over control was calculated. A drop of culture from each flask was taken on a glass slide and stained with lactophenol cotton blue and observed under light microscope for morphological changes for *S. rolfsii* and *M. phaseolina*.

3.9.3 Detection of antifungal volatile metabolites

Production of volatile compounds, by selected 18 bacterial isolates against *S. rolfsii* and *M. phaseolina* was studied by sealed method as described by Fiddmann and Rossall (1993). From a 24 hour culture of bacteria, 100 µL inoculum was spread on GCY medium in a Petri plate. After incubation at 30 ± 1 °C for 24 hours, a second Petri plate (containing GCY), inoculated with a five mm agar plug of the test fungus

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was inverted and placed over the bacterial culture. The two plates were sealed together with parafilm and further incubated at 30±1 °C (Photograph 3.7). This ensured that both the organisms were growing in similar conditions though physically separated. As a control, a Petri plate containing agar medium without bacteria was placed over the GCY medium inoculated with the fungal pathogen. For each test, three replications were maintained. Reduction in radial growth (inhibition percentage) of mycelia over control was calculated.
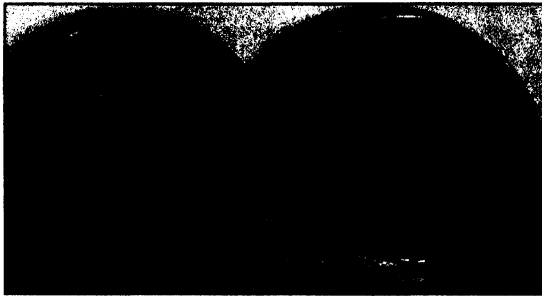
Photograph 3.7. Detection of bacterial volatiles by separation plate method. Lower plate inoculated with bacteria and upper plate with fungi (*M. phaseolina*). Control=uninoculated.

3.9.4 Effect of minerals on antifungal activity by two antagonistic bacteria

EB 13 and CDB 35 were tested for antifungal activity against *S. rolfsii* and *M. phaseolina* along with different mineral nutrients. PDA was autoclaved at 121 °C for 15 minutes and amended with four mineral solutions to give 1mM: $FeSO_4.H_2O$; $MgSO_4.4H_2O$; NaCl, $ZnSO_4.7H_2O$ and mixture of all these nutrients. Antifungal activity was detected by the dual plate method. Three replications were maintained for each and reduction in radial growth (inhibition percentage) of mycelia over control was calculated.

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3.10 PLANT GROWTH STUDIES

3.10.1 Plant growth in glasshouse conditions

Soil from BP2C field was used for plant growth studies. Based on *in vitro* studies twelve bacterial isolates were selected and evaluated for growth of pearl millet and sorghum using sterilized and unsterilized soil in glasshouse conditions. Soil was sterilized by autoclaving at 121° C for 15 minutes for three consecutive days. Vertisol soil: sand (2:1) was used as potting medium in 15 cm diameter plastic pots. After seed bacterization ten seeds were sown in each pot. *Azotobacter chroococcum* HT 54 was used as positive control. Seeds coated only with peat served as control. The treatments were arranged in a completely randomized block design with six replications. Thinning was done to five plants per pot at five days after emergence. Plants were irrigated once every two days with 20 mL sterilized distilled water. All pots were watered by weight, once a week, to achieve field capacity of the potting mix. Temperature in the glasshouse ranged from 22°-32°C (average 26°C) during the experiment period. After 30 days plant growth measurements were recorded.

3.10.2 Plant growth measurements

Plant growth measurements that were recorded in different glasshouse and field experiments:

    i.   Plant height (shoot length) (using scale)

    ii.   Spad meter reading (using chlorphyll meter)

    iii.   Leaf area (using Leaf Area meter Licor-model, Inc. Nebraska, USA)

    iv.   Root length density (using Rhizo Regent Win Mac instr. Inc USA)

    v.   Dry matter (biomass) of both root and shoot samples (oven dried at 70° C for 72 hrs to constant weight)

    vi.   Root volume (measured using water displacement)

3.10.3 Phase contrast microscopy

Selective bacterial isolates were observed under the phase contrast microscope for characterizing vegetative and sporulating conditions. A loopful of freshly grown (12–18 hrs) bacterial inoculum in LB was placed on a clean glass slide and observed under 100x.

3.11 INTERACTION STUDIES OF PLANT GROWTH PROMOTING BACTERIA

Interaction of PGPB with *Rhizobium* was studied *in vitro* and selected isolates were evaluated along with *Rhizobium* and Mycorrhizae under glasshouse conditions.

3.11.1 Compatibility of PSB with *Rhizobium* (in plate culture conditions)

Twelve bacterial isolates used for plant growth studies were characterized for their interaction with rhizobia. Five-rhizobial strains, two of chickpea (IC 59 and IC 76), two of pigeonpea (IC 3100 and IC 4060) and one of groundnut (IC 7114) were obtained from the microbial culture collection at ICRISAT (Rupela *et al.* 1991). All the potential isolates grew on yeast extract mannitol agar (YEMA) (Dalton 1980). Six different bacteria (one PGPB and five rhizobia were streaked on YEMA in 10 cm diameter plate in a specific pattern prepared as a template (Sriveni *et al.* 2004). Rhizobia were inoculated 24 h prior to the inoculation of potential bacteria because of the slow growth of rhizobia. The plates were further incubated for 24 h and observed for "interaction distance" and "spreading capacity" and were measured (Photograph 3.8). Cluster analysis was performed on the similar matrix employing UPGMA (unweighted pair-group method analysis) in the numerical taxanomy multivariate analysis system (NTSYS-pc) software version 2.0 (James 2000).

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Photograph 3.8. Interaction of plant growth promoting bacteria (Vertical streak) with rhizobia (horizontal streak).

3.11.2 Interaction of *Rhizobium* (IC 59) and two PSB (EB 67 and CDB 35) in glasshouse conditions

Based on the compatibility of the two PSB with *Rhizobium* (IC 59 and IC 76) they were evaluated on chickpea growth in glasshouse conditions. Priming was done by soaking 200 g chickpea ICCV2 in 200 mL of water for five hours and peat-based inoculum (10^8-10^9 CFU g^{-1} peat) of *Rhizobium* and PSB was coated. The seeds were allowed to dry and five seeds were sown in 15 cm diameter plastic pot using unsterilized soil of BR1D as potting mix. Thinning was done to three plants per pot at five days after emergence. Plants were irrigated once every two days with 50 mL deionized water. Temperature in the glasshouse ranged from 18°-24°C (average 24°C) during the experiment period. The treatments were arranged in a completely randomized block design with three replications. Harvesting was done after 45 days and the parameters measured were nodule number, dry matter yield and nitrogenase activity (Hardy *et al.* 1968).

3.11.3 Acetylene reduction assay

After harvesting, plant roots were carefully removed along with nodules and kept in airtight wide-mouthed glass bottles (jam bottles). Bottles were closed with a seal and screw cap. Acetylene gas was injected (10% of free volume of assay container) through the subaseal and incubated for 30 minutes. After incubation gas sample was collected from each container through the subaseal and stored in pre-evacuated 'Venoject' tubes. Gas chromatogram (GC) is fitted with a flame ionization detector and a column packed with a propak N. The GC unit was calibrated by injecting 0.1 mL of standard pure ethylene gas of known concentration. The area under known ethylene peak was estimated. Before using the sample for GC analysis gas sample vacuatainers were brought to normal atmospheric pressure by puncturing with a syringe needle. After thorough mixing, 0.5 mL gas sample was withdrawn with one mL syringe and injected onto a gas chromatograph (GC). Measurement of acetylene and ethylene concentration was used to calculate amount of ethylene produced in a given time.

3.11.4 Interaction of PGPB with Mycorrhizae

Three plant growth promoting bacteria (EB 35, EB 67 and CDB 35) based on plant growth studies were applied along with mycorrhizae to study their compatibility in glasshouse conditions. Unsterilized low P soil of BR 1D was used as potting medium in 21 cm diameter plastic pots. Nitrogen was applied at 40 kg N ha^{-1} and P was applied as RP @ 20 kg P ha^{-1} wherever specified. Mycorrhizal fungi, *Glomus* spp., was prepared as sand inoculum and applied at the rate of 1% and mixed evenly to the soil before filling up the pots. Seeds were coated with peat-based inoculum of the

three bacterial isolates. Sorghum (sweet stalk) ICSV 93046 was used as host plant. The treatments included combination of AM and bacteria with and without RP. Uninoculated plants served as controls. Three seeds were sown and thinning was done to one per pot within a week after germination. The plants were watered every alternate day. The temperature of the glasshouse ranged from 22°-32°C (average 26°C). The treatments were arranged in a completely randomized block design with five replications. Harvesting of the plants was done at vegetative growth period [45 days after sowing (DAS)] and flowering period (90 DAS). Plant growth measurements and mycorrhizal colonization in root was assessed.

3.11.5 Root sample collection and staining procedure for mycorriza

The quantification of (AM) fungi colonization in plant roots was carried out by the protocol of Phillips and Hayman (1970).

i. The terminal feeder roots were excavated from four or five different portions and subsample of one gram from the entire root system was collected. Then they were washed gently with tap water to remove the soil adhered particles.

ii. To preserve, the subsamples they were kept in tubes or vials with formalin: acetone: alcohol (FAA) 90:5:5 as fixation.

iii. The roots were cut into two cm segments and placed in the vials and covered with 10% potassium hydroxide (KOH) solution.

iv. The roots were digested in an autoclave at 15 psi for 10 minutes. The KOH solution clears the host cytoplasm and nuclei and readily allows stain penetration.

v. After autoclaving KOH solution was poured off and then the roots were washed with tap water until no brown color appears in rinse water.

vi. The root sample was covered with one per cent HCl and incubated for 10-15 minutes and HCl solution was poured off.

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vi. The roots were covered with 0.08% trypan blue stain in lactic acid glycerol solution (200 ml lactic acid, 400 mL glycerin, 400 mL tap water) (Harinikumar and Bagyaraj 1988).

vii. The stain was decanted and lactic acid glycerol solution (without trypan blue) was added and the roots were observed for mycorrhizal colonization.

viii. The roots were arranged on glass slide and observed using microscope for the presence of vesicles and arbascules and mycorrhizal colonization was calculated:

$$\% \text{ Mycorrhizal colonization} = \frac{(\text{No. of root segments with mycrorrhizal colonization})}{(\text{Total no. of root segments observed})} \times 100$$

3.12 EFFICACY OF SELECTED ANTAGONISTIC BACTERIA AGAINST SOIL BORNE PLANT PATHOGENIC FUNGI

Two-antagonistic bacteria *Bacillus licheniformis* EB 13 and *Pseudomonas* sp. CDB 35 were evaluated against soil borne plant pathogenic fungi *S. rolfsii* and *M. phaseolina* in glasshouse conditions.

3.12.1 Biocontrol for collar rot of chickpea

Efficacy of two antagonistic strains was tested in glasshouse conditions using chickpea (ICCV 2) as host. Soil from a collar rot sick plot at ICRISAT was used for this experiments and was infested with *S. rolfsii*. Biopriming was done by soaking 100 g of ICCV 2 seeds in 100 mL of water for five h and peat-based inoculum of *Rhizobium* IC 59 and antagonistic bacterial isolates (EB 13 and CDB 35) were added and one per cent CMC was used as adhesive. In the treatments for no priming, the seeds were surface coated with the peat based bacterial inoculants and CMC as adhesive. Seeds were allowed to dry and ten seeds were sown in 21 cm diameter

plastic pots for each treatment. Later on, thinning was done to five per pot. The treatments were control (infested), fungicide (captan: 0.3%), EB 13 and CDB 35 and arranged in a completely randomized block design with five replications and were harvested at 45 DAS and germination percentage and plant growth parameters were recorded.

3.12.2 Biocontrol of charcoal rot of sorghum

3.12.2.1 Soil inoculation method

Inoculum of *M. phaseolina* was prepared by using the method suggested by Avizohar Hershenzon and Shacked (1988). For this purpose, 300 grams of sorghum seeds were boiled for 15 minutes in 500 mL distilled water containing five percent glucose solution and then autoclaved in one litre conical flasks. The flasks were inoculated with five mm discs of young culture of *M. phaseolina* (seven discs per flask). The flasks were incubated for seven days at 30 ± 1 °C.

3.12.2.2 Efficacy of two antagonistic bacteria and influence of mineral and carbon sources against *M. phaseolina* in glasshouse conditions

Inoculum grown on sorghum seeds was added to the top five cm layer of soil in 10 cm diameter plastic pots at the rate of 10 g/kg of soil and mixed thoroughly and moistened with water and incubated for three days. After inoculating *M. phaseolina*, ten seeds of sorghum (CSV 15) were sown per pot. Treatments were arranged in a completely randomized block design with five replications. The experiment was terminated after 30 DAS and shoot dry weight and percent root

infection was recorded. There were thirteen treatments as follows:

I.	Control	II.	*Trichoderma viridae*
III.	Glucose	IV.	Zinc`
V.	Glucose+Zn	VI	*B. licheniformis* EB 13
VII.	EB 13 + Glucose	VIII.	EB13 + zinc
IX.	EB 13 + Glucose + zinc	X.	*Pseudomonas* sp. CDB 35
XI.	CDB 35 + Glucose	XII.	CDB 35 + zinc
XIII.	CDB 35 + Glucose + zinc		

3.12.2.3 Percent root infection caused by *M. phaseolina*

The root systems of five randomly chosen plants from three replications were cut into small (five mm long) segments after thorough washing and running tap water. After surface sterilization with three percent Na (OCl_2) for three minutes, the root segments were rinsed several times with distilled water and plated (five segments) onto CMRA medium. The plates were incubated at 30 ± 1 °C for five days and root infection (by *M.phaseolina*) percentage was calculated as:

Percentage infection in roots $= \dfrac{\text{(No. of plants infected by the fungus)} \times 100}{\text{(Total no. of plants)}}$

3.13 EVALUATION OF COMPOSTS

3.13.1 Effect of composts on growth of pearl millet

Farm waste compost (FWC), rice-straw compost (RSC) and *Gliricidia* vermicompost (GVC) were evaluated for plant growth promotion using pearl millet (ICMV 155) in glasshouse conditions using unsterilized soil of BP2C as potting mix in 15 cm diameter plastic pots. Compost was applied at the rate of 5 t ha^{-1}. Treatments were arranged in a completely randomized block design with six replications. Ten seeds

were sown in each pot and thinning was done to five plants per pot at five days after emergence. Plants were irrigated once every two days with 20 mL distilled water. Temperature in the glasshouse ranged from 22°-32°C (average 26°C) during the experiment period. After 30 days plant growth measurements were recorded.

Table 3.3. Chemical characterization of the compost samples

Treatment	pH	Total N mg g^{-1}	Total P mg g^{-1}	% OC
FWC	6.98	4650	1088	18.6
RSC	7.58	4800	1125	18.3
GVC	6.51	6188	1313	23.1

FWC = farm waste compost, RSC = rice straw compost, GVC = *Gliricidia* vermicompost

3.13.2 Co-inoculation of composts and selected bacterial isolates on growth of pearl millet

Three bacterial isolates (EB 35, EB 67 and CDB 35) were selected based on plant growth studies and were inoculated along with composts to see the dual effect on pearl millet growth. Unsterilized soil from BP2C field was used as potting medium. Compost was applied at the rate of five tones ha^{-1}. Treatments were arranged in a completely randomized block design with three replications and repeated twice. Ten seeds were sown in each pot and thinning was done to five plants per pot. Plants were irrigated once every two days with 20 mL distilled water. Temperature in the glasshouse ranged from 22°-32°C (average 26°C) during the experiment period. After 30 days plant growth measurements were recorded.

3.13.3 Effect of compost and vermicompost on growth of sorghum

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Rice straw vermicompost was prepared in cement cylinders (90 cm diameter x 50 cm height) using rice straw soaked in 1% cow dung slurry. After two weeks, about 500 earthworms (*Eisenia fetida*) were released into the same tank. Seven layers of rice straw of 10 cm thickness were added after decomposition of lower layer. Rice-straw compost (prepared in heaps) and rice straw vermicompost (prepared in cement cyliners) were evaluated for growth of sorghum (sweet stalk) (ICSV 93046). Unsterilized low P soil of BR1D was used as potting medium in 21 cm diameter plastic pots. Treatments were arranged in a completely randomized block design with six replications. Both the composts were applied @ 2.5, 5 and 10 tonnes ha^{-1} per pot. Harvesting of the plants was done 45 days after sowing. Plant growth parameters and mycorrhizal colonization in sorghum roots was assessed.

3.13.4 Effect of rice straw vermicompost enriched with PSB on growth of maize (Surabhi variety)

Vermicompost (VC) was prepared using rice straw, one percent cow dung slurry, 0 1% inoculum of EB 67 and CDB 35 (peat based) which was placed in a 11 inch pot of height (29 cm) and diameter (32.5 cm). Earthworms (*Eisenia foetida*) 500 in number were introduced, after two weeks. Thickness of the rice straw added at one time was restricted to about five cm depth. 70% moisture was maintained in the pots. After 90 days, the resultant compost was used as an amendment to test growth of maize using low P soil of BR1D as potting medium in glasshouse conditions. Compost enriched with PSB and control compost (prepared without inoculation of PSB) was added @ 5 t ha^{-1} to 8 inch pots. The experiment was set up with six replications for each of the following four treatments. T1 = Control (uninoculated); T2 = Compost

(control); T3=EB 67 compost; T4=CDB 35 compost. RP was applied @20kg P ha^{-1} wherever compost was added. Five seeds were sown in each pot and thinned to two plants per pot after one week of emergence. Irrigation was done once every two days with 100 mL water. Temperature in the glasshouse ranged from 22 ^{o}C to 32°C (avg. 26°C) during experiment period. After 60 days, the plants were harvested and growth parameters and mycorrhizal colonization in roots was studied.

3.13.5 Effect of vermicompost on biological control of charcoal rot fungus

Vermicompost prepared using *Gliricidia* foliage was applied at 10% (based on dry weight) to the pots infested with the *M.phaseolina*. There were three treatments vermicompost, positive control (untreated control) and vermicompost alone for comparison. The treatments were arranged in a completely randomized block design with five replications. The experiment was terminated after 30 DAS and shoot dry weight percent root infection with *M. phaseolina* was recorded.

Plant growth studies inoculated with plant growth promoting bacteria (PGPB) involved pearl millet and sorghum as test host (mandated crops of ICRISAT) in glasshouse conditions. But to carry out the field study of selected plant growth (phosphate solubilizing) promoting bacteria, we selected maize as test crop, as conditions were suitable for it.

3.14 EVALUATION OF SELECTED PSB *S. mareescens* EB 67 and *Pseudomonas* sp. CDB 35

3.14.1 Treatments involved in glasshouse and field conditions

Maize Surabhi variety was used as host plant to evaluate the performance of PSB in glasshouse and field conditions. Nitrogen was applied in the form of urea @ 80 kg

ha^{-1} in two-split dose (initially during sowing and during flowering stage). P was applied as 20 kg P ha^{-1} either as single super phosphate (SSP) or rock phosphate (RP) based on the treatments. Peat-based formulation of the bacteria @ 150 g ha^{-1} was applied as seed coat with one percent carboxymethylcellulose (CMC) as adhesive. Viable cell count as determined by dilution plating was 10^6-10^7 colony forming unit (CFU) per seed. *Enterobacter asburiae* PSI3 (Gyaneshwar *et al.* 1999) with P solubilizing ability in buffered conditions and *Bacillus coagulans* that did not perform well in buffered conditions (Gyaneshwar *et al.* 1998) were used as reference strains.

The experiments had the following treatments:

i.   Uninoculated (control)
ii. N_{80}+RP_{20}
iii. N_{80}+SSP_{20}
iv.   *E. asburiae* PSI3 + RP
v.   *B. coagulans* + RP
vi.   *S. marcescens* EB 67 + RP
vii. *Pseudomonas* sp. CDB 35 + RP

3.14.2 Performance of EB 67 and CDB 35 in glasshouse conditions

The experiment was conducted in glasshouse in 21 cm diameter plastic pots using unsterilized soil from BP2C field. The treatments were arranged in a completely randomized block design with five replications. Harvesting was done after the complete growth period of plants and plant growth measurements were recorded.

3.14.3 Performance of EB 67 and CDB 35 in field conditions

The experiment was conducted in field BP2C at ICRISAT, Patancheru, India during January-April, 2004. Each treatment was raised in eight rows, each of 9m x 6m

length, with an intra- and inter-row spacing of 10 and 60 cms. The treatments were arranged in a completely randomized block design with five replications. Sub samples of 10 plants from four replications were sacrificed and plant growth measurements were taken at day 24, 48, 72 and 96. After harvesting, the dry matter, grain and stover yield was calculated.

3.14.4 Rhizosphere population dynamics of EB 67 and CDB 35

The rhizosphere soil from three treatments uninoculated pots, S. *marcescens* EB 67 and *Pseudomonas* sp. CDB 35 inoculated pots was collected at 24 d, 48 d, 72 d and 96 days after inoculation (DAI) from both glasshouse and field treatments. Population dynamics of introduced bacteria was studied using the intrinsic antibiotics marker. The roots were washed to remove the adhering soil particles and the appropriate dilution was plated onto RP buffered antibiotic medium with benomyl (to inhibit the fungal growth) and incubated at 30 ± 1 °C and observed for the number of CFU after 96 h.

3.14.5 Survival of EB 67 and CDB 35 in peat

Viability of both the PSB in the peat formulation was determined at 30-day intervals up to 180 DAI. Ten g of the peat-based inoculum was diluted in 90 mL water and serially diluted. Appropriate dilutions were plated on LB agar and RP buffered medium. The plates were incubated at 30 ± 1 °C and the number of colonies after 36 h in case of LB agar and 96 h in case of RP agar media was expressed as log CFU g^{-1}. The experiment was repeated thrice with two replications in each treatment.

3.15 STRATEGIES AND DESIGN OF LONG-TERM FIELD EXPERIMENT TO DERIVE SUSTAINABILITY INDEX BASED ON SOIL QUALITY

3.15.1 Design of field experiment

The experiment was conducted on Vertisol field BW3, ICRISAT, Patancheru that is fully rainfed. The annual mean rainfall at Patancheru is 783 mm, which allows two crops to be grown in a year either as intercrops or sequential crops. Due to variability in the timing of rainfall, these crops have to be sown as intercrops during the rainy season, June or July, to be certain of production. Different crops were grown in each year of the experiment, but were the same across all four treatments (Table 3.4).

3.15.2 Soil sampling

Field experiment had four treatment plots T1, T2, T3 and T4. Each treatment was of 0.2 hectare, with a total area of 1.02 hectare. After a five-year experimental cycle, close to the period of harvesting stage, soil sampling was done twice during January 2004 and January 2005 from the four different crop husbandry treatments T1, T2, T3 and T4. During the first year (2004), sampling was done from 0-10 and 10-20 cm depth. In the second year for a more detailed study sampling was done up to 0-60 cm (0-10, 10-20, 20-30, 30-45 and 45-60) depth. Sampling was done with a 40 mm diameter soil core. Soil from each depth were pooled, mixed well and used for analysis. Samples were pooled to five replications from each treatment in first year, whereas in the second year they were pooled to three replications and rhizosphere soil of crops grown (maize and pigeonpea) was also studied.

Table 3.4. Four different crop husbandry systems in continuing long-term experiment of BW3 (Vertisol) field[1] at ICRISAT Patancheru.

Act/Inputs	T1: Low-cost system I	T2: Low-cost system II	T3: Conventional agriculture	T4: Conventional agriculture + biomass
Tillage	Zero-till	Zero-till	Conventional (bullock plough)	Conventional (bullock plough)
Sowing	Sd drill	Sd drill	Sd drill	Sd drill
Microbial inoculants[*]	+	+	-	-
Biomass (t ha^{-1} yr^{-1} first 3 years only)	10 Rice straw as surface mulch	10 Farm-waste, stubble and hedgerow foliage as surface mulch	None	10 Farm waste, stubble and hedgerow foliage incorporated
Compost (t ha^{-1})	1.5-1.7 (annually)	1.5-1.7 (annually)	1.8 (1 in 2 year)	1.8 (1 in 2 year)
Fertilizer (N) Urea (kg N ha^{-1})	0	0	80	80
Fertilizer (P) kg P ha^{-1} (1 in 2 year)	20 (RP)	20 (RP)	20 (SSP)	20 (SSP)
Pest management	Biopesticides[a]	Biopesticides	Chemical pesticides	Chemical Pesticides
Weeding	Manual, weeds retained	Manual, weeds retained	Manual, weeds discarded	Manual, weeds discarded

1 Same crops were grown in all treatments each year:
Year 1 pigeonpea-chickpea sequential (June 1999-May 2000); Year 2 sorghum/pigeonpea intercrop (June 2000-May 2001);
Year 3 cowpea/cotton intercrop (June 2001-May 2002); Year 4 maize/pigeonpea intercrop (June 2002-May 2003);
Year 5 cowpea/cotton intercrop (June 2003-May 2004); Year 6 maize/pigeonpea intercrop (June 2004-May 2005)
*Microbial inoculants applied were B. circulans EB 35, S. marcescens EB 67 and Pseudomonas sp. CDB 35 along with other inoculants like Rhizobium and Azotobacter. a Biopesticide (Microorganisms, herbal extracts and vermiwash) formulation developed at ICRISAT

3.15.3 Soil pH

Twenty g of soil was weighed and put in a 100 mL beaker. Add 40 mL of distilled water (1:2) and stir frequently for 30 min. The soil suspension was used to measure the pH of immediately after stirring.

3.15.4 Water holding capacity (WHC)

Water holding capacity is generally determined at three critical points i.e. saturation point, field capacity and wilting point. Available water capacity is defined as the water retained in the rooting zone of the soil at field capacity (drained upper limit: DUL) minus the permanent wilting point (PWP). Therefore to correlate the laboratory studies to the field conditions it is important to adjust the moisture content of soils to the field capacity. For microbial biomass studies the moisture content of water was adjusted at 40% of WHC at drained upper limit (DUL) before analysis. Moisture content at DUL was determined by using the pressure plate method. Fifty grams of soil (two mm) was taken in rubber or brass sample rings and completely saturated by wetting overnight. Soil was allowed to absorb moisture from below because adding water from top may disturb the natural porosity of the soil system. Next day the saturated soil samples were placed in the pressure chamber apparatus at 2.0×10^{-3} Mpa of applied air pressure and left for 24 h to attain the equilibrium when all the gravitational water was removed. Moisture content of soil was determined at this stage by using the gravimetric method. Similarly, 0.1 Mpa of air pressure was applied to determine the moisture content at lower limit of field capacity (PWP). The median value of the water contents at two limit i.e. drained upper limit (DUL) and PWP was used as WHC at field capacity.

3.15.5 Biological indicators

3.15.5.1 Mineral nitrogen

The mineral nitrogen present in the ammonical and nitrate forms was determined by the method described by Keeney and Nelson (1982). Twenty gram of dry soil was extracted with 100 mL of 2N KCl by shaking it for one hour. The supernatant solution was filtered through Whatman No. 1 filter paper. Twenty-five mL of filtrate was taken in a distillation flask with 0.2 g of MgO and Devarda's alloy each and attached to the distillation unit. About 25 mL of distillate was collected in the flask containing two mL of H_3BO_3 solution. The distillate containing ammonical, a nitrite and nitrate form of nitrogen was finally titrated against 0.025 N H_2SO_4 using the auto-titrator (Mettler-Titrator). The concentration of nitrogen in the soil was expressed as kg ha^{-1} and calculated as:

$$\text{Mineral nitrogen (kg ha}^{-1}\text{)} = \frac{\text{(mL of acid consumed – blank) x 784 x 100}}{\text{W x V}}$$

W = weight of soil in g, V = volume of extract taken for distillation,
1 mL of 0.025 N H_2SO_4 consumed is equivalent to 350 µg N.

3.15.5.2 Microbial Biomass

The moisture content of soil samples was adjusted to 55% water holding capacity (WHC) by adding distilled water. Twenty grams (dry weight equivalent) of soil was taken in 50 mL beakers in duplicate. One set was fumigated and the other set was non-fumigated. Soil samples were fumigated in desiccator (lined with moistened filter paper) with ethanol free $CHCl_3$ under vaccum and placed in the incubator at $25°$ C for 24 hours. Unfumigated samples were also kept in a desiccator and placed in the incubator at 25 °C to maintain similar conditions. After incubation, fumigated

soil samples were made free of vaccum by repeated evacuations. Later on, soil samples were incubated in closed airtight mason jars under standard conditions (at 25 °C in dark) for a period of 10 days before further analysis. A vial containing 20 mL of 1N NaOH was placed into the each jar in order to determine biomass C.

The amount of CO_2 released by soil had been used as indirect measure of microbial activity assuming that the amount of CO_2 released is only due to respiration by microorganisms present in known amount of soil. The amount of CO_2 evolved during the period of 10 days incubation was absorbed in known amount of 1N NaOH. To know the amount of CO_2 absorbed by the aliquot of alkali, two mL of this was titrated back against 0.5 M of HCl, in presence of five mL of $BaCl_2$ using auto-titrator (Metohm 785 DMP, Titrino). In this way, the amount of CO_2 evolved by each sample was determined for both fumigated and non-fumigated samples. The microbial biomass carbon (MBC) was calculated as given by Anderson and Domsch (1978) and was expressed as mg per kg of dry soil.

$$MBC = \frac{CO_2 \text{ evolved from fumigated soil - } CO_2 \text{ evolved from non fumigated soil}}{(0.411)}$$

$$CO_2 \text{ evolved (mg kg}^{-1}) = \frac{6 \times A \times (B-V) \times 1000}{U}$$

B   = ml of 0.5N HCl used for sample
V   = ml of 0.5N HCl used for sample
A   = Normality of HCl used for titration = 0.5
U   = ml of 1N NaOH used for titration = 2 mL
1000 = conversion factor (1mg = 1000 μ g)
0.411 = Kc, defined as the fraction of biomass C mineralized to CO_2
1 ml of 1N HCl is used for 6 μg of CO_2-C

3.15.5.3 Microbial Biomass Nitrogen

The fumigated and non-fumigated soil samples after 10 days incubation was extracted for inorganic nitrogen with 2M KCl in 5:1 ratio (extractant/soil). The amount of inorganic nitrogen was analyzed by the Kjeldahl distillation method. The incubated samples were extracted with 100 mL of 2M KCl by shaking on a shaker for one hour. Supernatant was filtered through Whatman No.1 filter paper. 25 mL aliquot of filtrate was analyzed for inorganic nitrogen following the Kjeldahl distillation process described by Keeney and Nelson (1982). The amount of nitrogen mineralized by microbial component was determined as difference of inorganic nitrogen released by the fumigated and non-fumigated samples. Microbial biomass nitrogen (BMN) was calculated as given by Jenkinson (1988) and was expressed as mg per kg of dry soil.

$$MBN = \frac{\text{Mineral nitrogen in fumigated soil - Mineral nitrogen in non fumigated}}{0.57}$$

$$\text{Mineral N (mg kg}^{-1}) = \frac{70 \times (V-B) \times 100}{25 \times 20}$$

V   =   mL of 0.005N H_2SO_4 for the sample
B   =   mL of 0.005N H_2SO_4 used for the blank
100 =   mL of 2M KCl used for extraction
25 =   mL of filtrate used for distillation
20 =   g of soil used for incubation
0.57 =   Kn, defined as the proportion of microbial N mineralized to NH_4^+
1mL of 0.005N H_2SO_4 is used for 70 µg of mineral N

3.15.5.4 Soil enzymes

After collecting the soil sample was air-dried, sieved to 2 mm and was used to estimate soil dehydrogenase and acid phosphatase and alkaline phosphatase.

3.15.6 Microbial indicators

Ten g of soil sample from all the four different crop husbandry systems T1, T2, T3 and T4 was added to 90 mL water. Appropriate dilutions were plated on different media. LB agar for bacteria, PDA+streptomycin (500 mg L^{-1}) for fungi, actinomycetes isolation agar for actinomycetes, PIA for *Pseudomonas*, P solubilizers on RP buffered medium. Chromeazurol S (CAS) agar for siderophore producers. Antagonistic microorganisms against *M. phaseolina* were enumerated on PDA by the two-layer method. Actively growing *M. phaseolina* in PDB medium was homogenized with a Tissumizer. The fungal suspension (0.1 mL) was spread plated on the previously prepared agar plates and was air-dried for four hours. A second layer of 15 mL PDA was poured on top of first layer and the plates were dried for 45 minutes (Rupela *et al.* 2003b). An appropriate dilution of soil sample was plated. All the plates were incubated at 30 ± 1 °C. Plates were observed everyday up to four days and the numbers of colonies were expressed as log CFU g^{-1} dry weight of soil.

3.15.7 Chemical (nutrient) indicators

Soil nutrient status was studied by estimating total N and P, available P & K and OC% from top 20 cm during fifth year and 0-20, 20-40 and 40-60 cm during sixth year of study.

3.15.8 Productivity indicators

Crop productivity was determined based on the grain yield and dry matter of cereal and legume crops. Crops raised were cotton and cowpea during 2003-04 and maize and pigeonpea during 2004-05.

3.15.9 Sustainability indicators

The choice of measureable parameters that were studied to define the sustainability index of soil was biological, microbial, nutrient and crop indicators. This approach is based on measurements of soil biological index [calculated from microbial biomass C, N, soil enzyme activities (acid phosphatase, alkaline phosphatase and dehydrogenase)], microbial index (calculated from microbial counts), soil nutrient index (calculated from total N and P, NaHCO₃ extractable- available P, available K and organic C) and crop index (calculated from dry matter and grain yield of crops). Means of two-year data of 0-20 cm depth of all the four different crop husbandry treatments T1, T2, T3 and T4 of BW3 field was used in the study to derive the sustainability index.

The index value of parameter (Iij) calculated by dividing the value by the respective threshold of a parameter is given as:

$$I_{ij} = \frac{A_{ij}}{T_{hj}}$$

where,

Iij is the index value for ith treatment corresponding to Jth parameter in an experiment, Aij is the actually measured value for the ith treatment and jth parameter in an experiment, and Thj is the threshold value for jth parameter.

Biological index (BIi) was calculated as an average of index values (I$_{ij}$) of all the 6 parameters studied in the four treatment plots of field experiment.

$$BI_i = \frac{1}{6} \sum_{j=1}^{6} I_{ij}$$

Microbial index (MIi) was calculated as an average of index values (I$_{ij}$) of all the eight parameters studied in the four treatment plots of field experiment.

$$MI_i = \frac{1}{8} \sum_{j=1}^{8} I_{ij}$$

Nutrient index (BI$_i$) was calculated as an average of index values (I$_{ij}$) of all the five parameters studied in the four treatment plots of field experiment.

$$NI_i = \frac{1}{5} \sum_{j=1}^{5} I_{ij}$$

Crop Index (CIi) was calculated as an average of index values (I$_{ij}$) of cereal and legume yield in field experiment based on average values of two-year data.

$$CI_i = \frac{1}{4} \sum_{j=1}^{4} I_{ij}$$

Sustainability index of the soil was measured as the area of polygon with biological index, microbial index, nutrient index and crop index of soil at four vertices; they were represented in radar graph as a, b, c and d respectively, which are the four lines of different lengths originating from a common point 'O'. By joining the tail ends of these four lines, polygon is formed as shown in Figure 3.1.
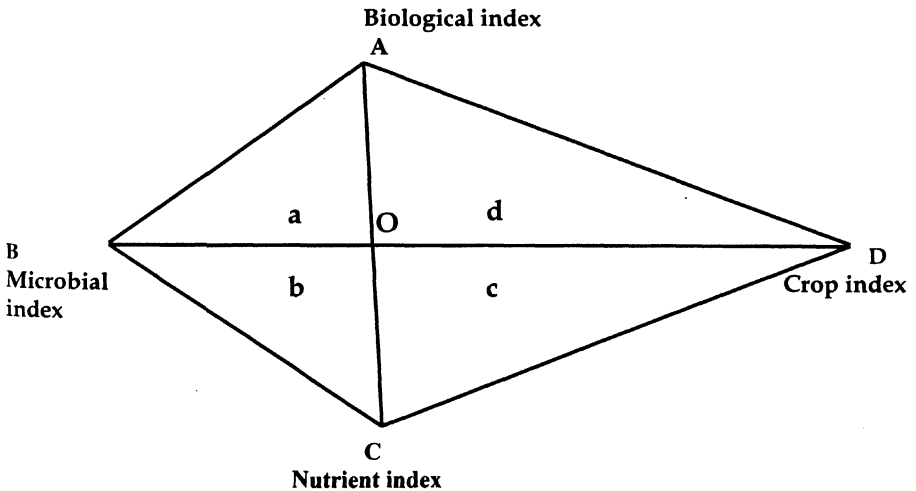
Figure 3.1 Measurement of Sustainability of cropping sytem from the polygon area

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The sustainability index of the system is given as

Sustainability of system = Area of polygon ABCD
$$= \text{Area } (\Delta \text{ AOB} + \Delta \text{ BOC} + \Delta \text{ COD} + \Delta \text{ DOA})$$
$$= \tfrac{1}{2} \text{ ab sin } 90 + \tfrac{1}{2} \text{ bd sin } 90 + \tfrac{1}{2} \text{ cd sin } 90 + \tfrac{1}{2} \text{ da sin } 90$$
$$= \tfrac{1}{2} \text{ sin } 90 \text{ (ab+bc+cd+da)}$$
$$= \frac{1}{2} \cdot 1 \text{ (ab+bc+cd+da)} \quad \ldots\ldots\ldots\ldots\ldots\text{(i)}$$

In order to have a sustainable system, the absolute value of each parameter should be equal or greater than the threshold value and the sustainability index should be always positive and greater than 2.00; the higher the value, the more sustainable the sytem. The least value of sustainability index (2.00) is calculated from equation (i), by putting the corresponding indices of biological activity (a) microbial activity (b), nutrient status (c) and productive potential (d) equal to 1.

Statistical analysis

All the glasshouse and field experiments were arranged in completely randomized block design. The data were subjected to analysis of variance (ANOVA) using Genstat 6.1 statistical package (Lawes Agricultural Trust, Rothamsted, UK).

CHAPTER - IV

RESULTS

RESULTS

Rhizospheric bacteria, termed plant growth-promoting rhizobacteria (PGPR), colonize the roots and promote growth and yield of crops in addition to disease control. Majority of these were isolated from rhizosphere and few have been classified as endophytes. Selection of plant growth promoting bacteria (PGPB) from diverse habitat such as composts can broaden the spectrum of PGPB. Bacteria that colonize the composts are exposed to cellulosic substrate, higher temperature, moisture fluctuation, organic matter etc. and have a better chance to survive and multiply in soil, compost and nutritionally rich buffered rhizosphere soil. To understand the beneficial effect of compost products for plant growth and pathosystems, it was aimed to study the microbiology of three different composts. Two hundred and seven bacteria isolated from three different compost samples were maintained as germplasm collection at ICRISAT. All the isolates were screened for different plant growth promoting traits and antagonistic ability against soil borne plant pathogenic fungi.

Based on plant growth promoting and antagonistic ability twenty-three isolates were selected to test germination per cent and seedling vigor index of pearl millet (ICMV 155) by paper towel method. Twelve out of 23 isolates showed significant seed vigor index and plant weight of pearl millet and were tested using sorghum as host. Selected potential plant growth promoting and antagonistic bacteria were identified. Detailed investigation of phosphate solubilizing bacteria (PSB) was done *in vitro* and two efficient isolates *Serratia marcescens* EB 67 and *Pseudomonas* sp. CDB

35 showed RP solubilization in presence of different carbon sources, crop residues and soil conditions. Eighteen of the 23 bacterial isolates suppressed four soil borne plant pathogenic fungi (*Sclerotium rolfsii, Macrophomina phaseolina, Fusarium solani and Fusarium oxysporum*) on PDA and were tested on GCY and KBM. All the 18 bacterial isolates were tested for volatile metabolites against *S. rolfsii* and *M. phaseolina*. Four bacterial isolates *Bacillus licheniformis* EB 13, *Bacillus alvei* CDB 15, *Pseudomonas* sp. CDB 35 and *Bacillus licheniformis* CDB 47 that suppressed four fungi in plate culture conditions and promoted plant growth were tested for biomass reduction of fungi in GCY broth. Two isolates *B. licheniformis* EB 13 and *Pseudomonas* sp. CDB 35 showed significant reduction in biomass of pathogenic fungi. Efficacy of two antagonistic bacteria EB 13 and CDB 35 along with mineral nutrients against *S. rolfsii* and *M. phaseolina* was also tested in plate culture conditions.

Twelve isolates that showed plant growth promotion *in vitro* (by paper towel method) were tested for growth of pearl millet and sorghum in glasshouse conditions. Interactions of PGPB with *Rhizobium* and Mycorrhizae were evaluated in glasshouse conditions. Efficacy of the two antagonistic isolates EB 13 and CDB 35 against *S. rolfsii* and *M. phaseolina* were evaluated at glasshouse. The two PSB *Serratia marcescens* EB 67 and *Pseudomonas* sp. CDB 35 were evaluated for growth enhancement of maize under glasshouse and field conditions. Soil quality indices in a long-term field experiment, where crop residues, composts and PGPB were applied from last four years were studied to derive the sustainability index of different cropping system. The results of these studies are presented in this chapter.

4.1 ISOLATION AND CHARACTERIZATION OF BACTERIAL ISOLATES FOR PLANT GROWTH PROMOTING AND ANTAGONISTIC ACTIVITY

Bacteria were isolated from farm waste compost (FWC), rice straw compost (RSC), *Gliricidia* vermicompost (GVC) and macrofauna (naturally present in FWC). All the three composts used in the study were rich in bacterial population compared to fungi (Table 4.1). The selection of PGPB was made after screening the bacteria isolated from composts for fluorescent pseudomonads, phosphate solubilization, phytase, IAA, ACC deaminase, siderophore, chitinase and HCN production and antagonism against *S. rolfsii, M. phaseolina, F. solani* and *F. oxysporum.*

Population of bacteria was log 7.4, 6.7 and 5.7 CFU g^{-1} in FWC, RSC and GVC respectively. Bacterial population on body surface of macrofauna was log 4.4 for centipedes, log 5.0 for earthworm and log 5.4 for slugs CFU macrofauna^{-1}. Bacteria in excreta of macrofauna on hourly basis were log 3.8 for centipedes, log 5.7 for earthworms, log 6.4 for slugs and log 5.1 for snails CFU macrofauna^{-1} h^{-1}. Population of fungi was low compared to bacteria in all the sources characterized and ranged from log 2.3 in GVC to 3.7 in RSC g^{-1} (Table 4.1). No fungi were detected on body-surface of centipede and slugs and excreta of centipedes, slugs and snails (at 10^{-1} dilution) in this study. We carried out only preliminary identification of these macrofauna and only three of them were identified, earthworm (*Dichigaster bolaui*), garden centipede (*Lithobius* sp.) and garden slug (*Limax* sp.).

Two hundred and seven bacteria were isolated from three different composts based on variation in colony morphology. Of 207 isolates, 24 were from FWC, 78 from

body surface or excreta of macrofauna, 55 from RSC and 50 from GVC. Proportion of plant growth promoting and antagonist bacteria were 54 and 19% from FWC, 56 and 38% from RSC, 64 and 39% from GVC and 41 and 23% from macrofauna associated with FWC respectively (Fig. 4.1).

Table 4.1. Population of microorganisms (bacteria and fungi) in composts and macrofauna[*]

Source	Bacteria ($Log_{10} g^{-1}$) [a]	Fungi ($Log_{10} g^{-1}$) [a]
Farm waste compost	7.4	2.3
Rice straw compost	6.7	3.7
Gliricidia vermicompost	5.7	2.3
Centipede body surface	4.4	ND
Centipede excreta	3.8	3.0
Earthworm body surface	5.0	2.7
Earthworm excreta	5.7	3.5
Slug body surface	5.4	ND
Slug excreta	6.4	ND
Snail excreta	5.1	ND
Mean	5.6	1.7

Population in composts is expressed as ($log_{10} g^{-1}$) data on all the macrofauna is expressed as macrofauna[-1] (body-surface) and macrofauna[-1] h^{-1} (excreta).

To collect sufficient excreta for dilutions from different macrofauna, it took about 6-24 hrs, so the population was calculated per macrofauna per hour.

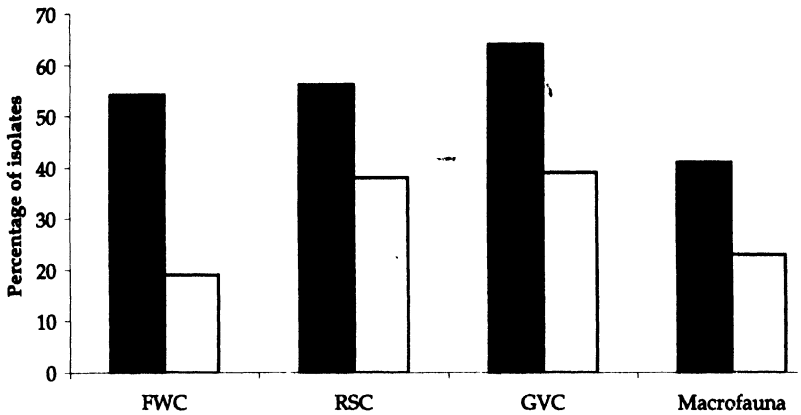ND = No fungi were detected (<10^{-1}).

Figure 4.1. Proportion (of total 207) of the different isolates found positive for plant growth promoting (■) and antagonistic activity (□) in the different types of compost and macrofauna. FWC = Farm waste compost, RSC = Rice-straw compost, GVC = *Gliricidia* vermicompost and data on all macrofauna (earthworms, centipedes, slugs and snails) was pooled as one source.

The bacterial isolates that showed zone of hydrolysis in cellulose agar medium after flooding the plates with Congo red (Teather and Wood 1982) were considered as cellulose degrading bacteria (Photograph 4.1). Bacterial isolates that showed deep red diffusion below the colonies due to acid production in RP buffered medium (Gyaneshwar *et al.* 1998) were recorded as P solubilizers (Photograph 4.2). The bacterial isolates that showed zone of hydrolysis (clearance) in phytic acid medium (Richardson and Hadobas 1997) were recorded as phytase producers (Photograph 4.3). The bacterial isolates that showed orange and yellow halo around the colonies on CAS agar plates were considered as siderophore producers (Photograph 4.4). Colonies that showed fluorescence with UV light and pigment production on the medium (Gould *et al.* 1985) were recorded as fluorescent *Pseudomonas* (Photograph

5) Indole acetic acid (IAA) production was detected by characteristic pink to urple color on the nitrocellulose membrane (NCM) disk within 30 min to 2 hrs 'hotograph 4 6) The bacterial isolates that could use ACC as nitrogen source and grew on the medium were recorded as ACC deaminase producers HCN production by bacterial isolates was recorded due to change in color of filter paper from yellow to orange-brown (Photograph 4 7) Chitinase production was observed by zone of hydrolysis on chitin-amended medium (Photograph 4 8)
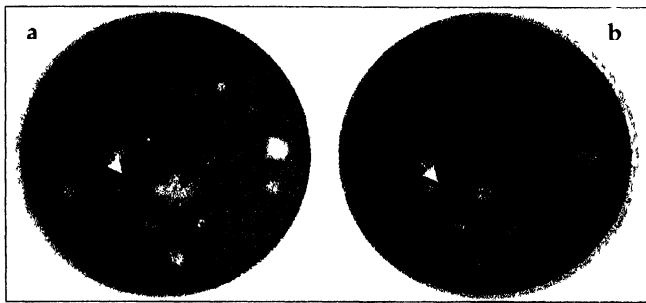
Photograph 4 1   Rice straw agar medium Cellulose degrading bacteria showing
                  zone of hydrolysis a = on rice straw agar medium b= alkali
   treated rice straw agar medium   FB 67 and ▼ CDB 35
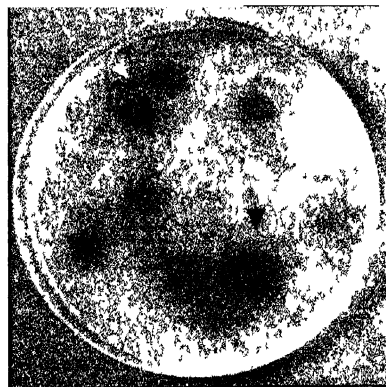
otograph 4 2   Rock phosphate buffered agar medium Phosphate solubilizing
                bacteria showing red zone due to acid production   EB 67 and
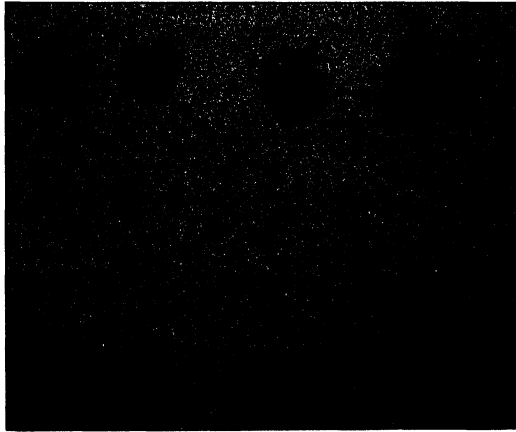   ▼ CDB 35

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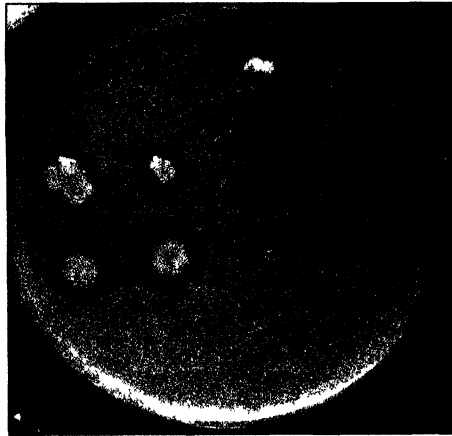
Photograph 4.3. Phytic acid agar medium: Phytase activity indicated by zone of hydrolysis around the colonies. ▼ CDB 35
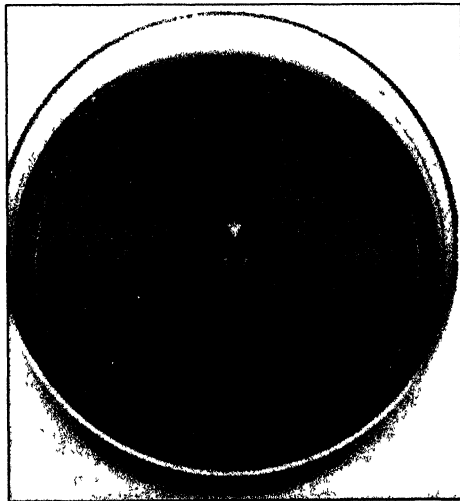
Photograph 4.4. Chromeazurol S agar medium: Orange or yellow halo around the colonies indicate siderophore production. ⸜ EB 67 and ▼ CDB 35
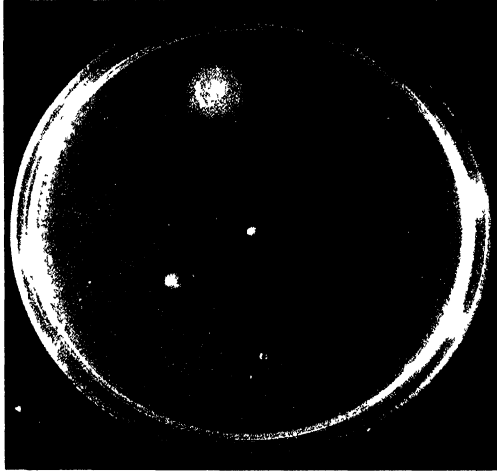
Photograph 4.5. *Pseudomonas* isolation agar: Colonies with yellow pigment and fluorescence with UV were considered as *Pseudomonas*. ▼ CDB 35

Photograph 4.6. Indole acetic acid production indicated by pink color on nitrocellulose membrane (NCM) disk that was overlaid on a filter paper saturated with Salkowski reagent ▼ CDB 35
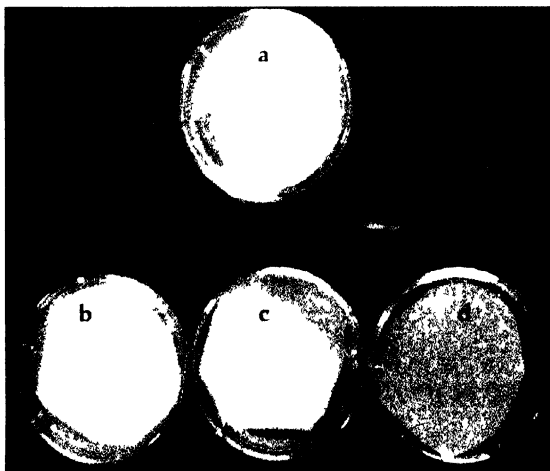
Photograph 4 7   Glycine LB agar medium for HCN production Brown color of Whatman filter paper No 2 impregnated with (0 5%) picric acid indicated HCN production (a) Control, (b) EB 27 (negative), (c) EB 77 (moderately positive), (d) CDB 35 (positive)
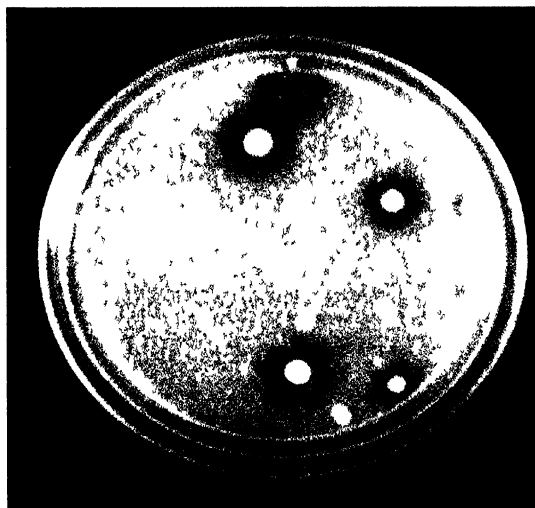
Photograph 4 8   Chitin amended minimal medium for chitinase enzyme production. Zone of hydrolysis around the colonies indicates chitinase activity   EB 67

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The abundance of Gram-negative rod shaped species was from macrofauna, whereas all three composts had Gram-positive bacteria as predominant species (Table 4.2). Gram–ve bacteria were 36% in RSC and 54% in macrofauna and Gram+ve were 46% in macrofauna and 64% in RSC. Most of the bacterial isolates from RSC were cellulose-degrading (91%) while in other three sources their population was low (24 to 31%). Per cent of fluorescent pseudomonads were 9 and 16% in RSC and GVC respectively and were not present in FWC and macrofauna. Per cent of phosphate solubilizing bacterial population was meager of all the traits studied and phytic acid mineralizing bacteria (phytase activity) ranged from 16-23% from all the four sources studied. Siderophore producers were marginally similar in RSC (46%) and GVC (43%) low in FWC (13%) and macrofauna had 32%. IAA producers were more in GVC (57%) followed by RSC (33%) macrofauna (26%) and FWC (4%). Percentage of ACC deaminase was maximum in RSC (53%) followed by GVC (50%), macrofauna (22%) and FWC (13%). Cyanogens were 22% in RSC, 15% in GVC and 2% in macrofauna. Chitinase producers were only in GVC (7%) and macrofauna (6%).

Per cent of bacterial isolates that had antagonistic activity against *S. rolfsii* and *M. phaseolina* was 17% in FWC, 28-34% in RSC, 38% in GVC and 21% in macrofauna. Antagonistic activity against *F. solani* and *F. oxysporum* was 20% in FWC, 44% in RSC, 40% in GVC and 25% in macrofauna. Bacterial isolates with highest antagonistic ability were present in RSC and GVC (Table 4.2).

Table 4.2. Percentage* of bacterial isolates from composts characterized for selected plant growth promoting traits

Traits	FWC n = 24	RSC n = 55	GVC n = 50	Macrofauna[a] n = 78
Gram-ve	42	36	48	54
Gram+ve	58	64	52	46
Cellulose utilization	29	91	24	31
Plant growth promoting traits				
Phosphate-solubilization	0	2	2	3
Phytase production	21	16	21	23
Siderophore production	13	46	43	32
Flourescent pseudomonads	0	9	16	0
IAA[b] production	4	33	57	26
ACC deaminase production[c]	13	53	50	22
HCN[d] production	0	22	14	2
Chitinase production	0	0	7	6
Antagonistic against				
Sclerotium rolfsii	17	28	38	21
Macrophomina phaseolina	17	34	38	21
Fusarium solani	20	44	40	25
Fusarium oxysporum	20	44	40	25

* per cent value predicted using the total number of isolates, and calculated source wise.
n number of bacterial isolates picked, purified for further studies.
[a] total isolates from macrofauna pooled as one group.
[b] Indole Acetic Acid; [c] 1- aminocyclopropane 1-carboxylic acid, [d] Hydrocyanic acid

4.2 SELECTION AND IDENTIFICATION OF PLANT GROWTH PROMOTING BACTERIA (PGPB)

4.2.1 Selection of PGPB

Twenty-three of 207 isolates with plant growth promoting and antagonistic ability were selected to test on pearl millet (ICMV 155) as host for germination and seedling vigor using paper (roll) towel. All the isolates (except BWB 32) significantly improved the seedling emergence, root length, germination percentage and seed vigor index (Table 4.3). Isolate BWB 32 that inhibited seedling emergence and growth parameters were not used in the future studies. CDB 35 showed maximum seed vigor index (4178) followed by EB 67 (4067) and improved plant growth (13-19% increase) (Photograph 4.9). Twelve of the twenty-three isolates showed significant increase in plant biomass and was used to test the germination per cent and seed vigor of sorghum (CSV 15) and all twelve isolates significantly improved the seedling emergence, shoot and root length and plant biomass (Table 4.4). Increase in biomass ranged between 17-27% by bacterial isolates and maximum increase was by EB 67 followed by CDB 35 (Photograph 4.10). Seed vigor index ranged between 3569 (BWB 36) to 4218 (EB 67) when peat-based inoculum of bacteria was applied as seed coat.

4.2.2 Biochemical identification of potential bacterial isolates

Twenty-two bacterial isolates with plant growth promoting ability *in vitro* were selected for identification study. They were identified based on staining, morphological, cultural and biochemical tests. The genera included *Bacillus*, *Pseudomonas*, *Serratia*, *Enterobacter* and *Klebsiella* (Table 4.5-4.6).

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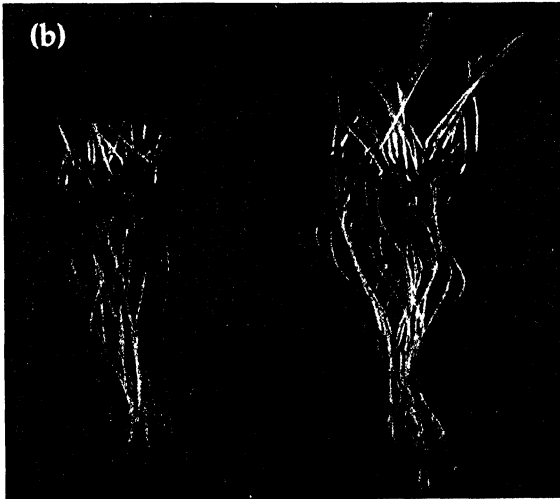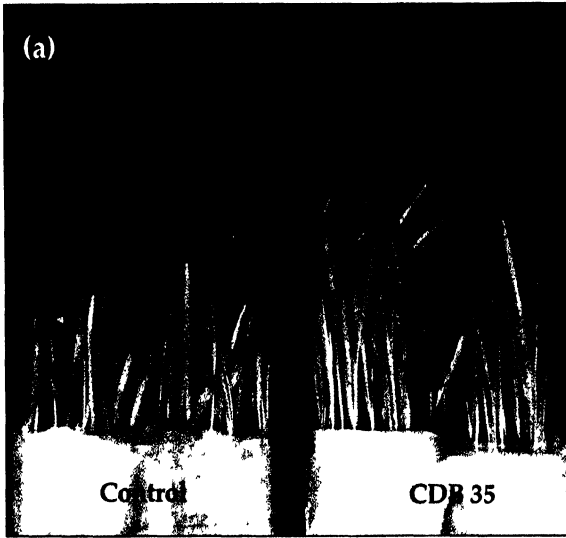
Photograph 4.9. Growth of pearl millet (ICMV 155) inoculated with (a) CDB 35 and (b) EB 67 by paper towel method. Control=uninoculated.
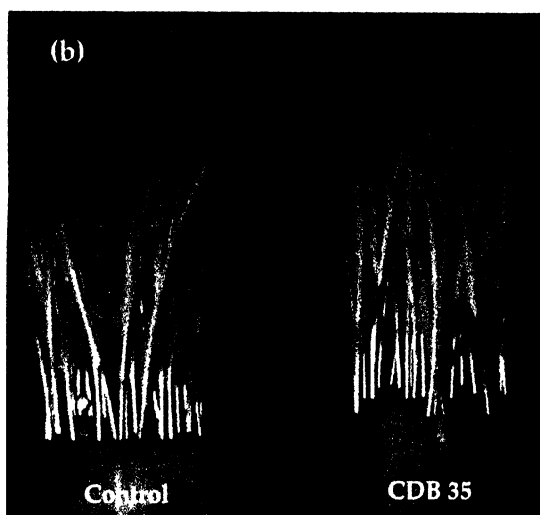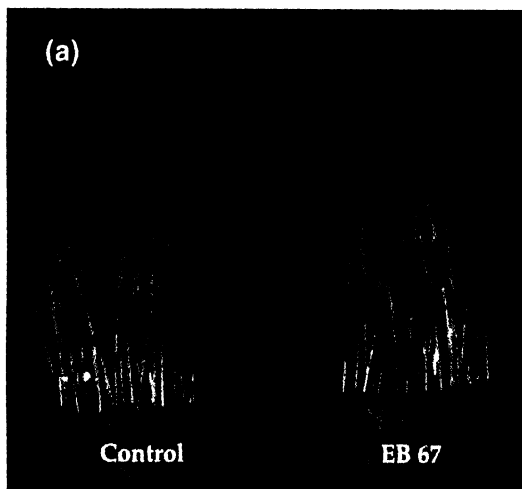
Photograph 4.10. Growth of sorghum (CSV 15) inoculated with (a) EB 67 and (b) CDB 35 by paper towel method. Control= uninoculated

Table 4.3. Effect of PGPB on growth of pearl millet (ICMV 155) by paper (roll) towel method in sterilized conditions

Isolates	Source of isolation	Shoot length (cm)	Root length (cm)	Plant wet weight (mg)	Germi- Nation (%)	Seed vigor index
Control	(Uninoculated)	5.8	29	103	79	2731
A chroococcum HT 54 (+ve control)		6.2	32 [a]	108	93	3508 [a]
EB 3	Earthworm body-surface	6.1	31	100	91	3399 [a]
EB 10	Earthworm body-surface	6.0	31	92	90	3335 [a]
EB 13	Earthworm body-surface	6.4	34 [a]	121 (17) [a]	98	4038 [a]
EB 15	Earthworm excreta	5.3	32 [a]	105	88	3260 [a]
EB 27	Centipede excreta	6.4	33 [a]	117 (13) [a]	94	3705 [a]
EB 35	Centipede excreta	6.4	33 [a]	117 (13) [a]	95	3782 [a]
EB 48	Farm waste compost	5.8	29	102	96	3402 [a]
EB 67	**Slug body-surface**	**6.7** [a]	**35** [a]	**123 (19)** [a]	**98**	**4067** [a]
EB 75	Slug body-surface	6.5	33 [a]	119 (15) [a]	96	3728 [a]
EB 77	Slug body-surface	6.3	32 [a]	117 (13) [a]	98	3761 [a]
CDB 6	Rice straw compost	5.7	32 [a]	94	97	3708 [a]
CDB 15	Rice straw compost	5.4	32 [a]	101	97	3603 [a]
CDB 22	Rice straw compost	5.9	32 [a]	88	98	3691 [a]
CDB 31	Rice straw compost	5.7	30	101	94	3408 [a]
CDB 35	**Rice straw compost**	**6.9** [a]	**38** [a]	**119 (15)** [a]	**93**	**4178** [a]
CDB 36	Rice straw compost	6.9 [a]	38 [a]	118 (14) [a]	96	3761 [a]
CDB4 7	Rice straw compost	6.3	33 [a]	117 (13) [a]	98	3688 [a]
BWB 21	*Gliricidia* vermicompost	7.0 [a]	33 [a]	117 (13) [a]	96	3806 [a]
BWB 32*	*Gliricidia* vermicompost	4.9	27	84	79	2540
BWB 36	*Gliricidia* vermicompost	6.4	32 [a]	118 (14) [a]	95	3632 [a]
BWB 40	*Gliricidia* vermicompost	6.7 [a]	32 [a]	118 (14) [a]	94	3684 [a]
BWB 41	*Gliricidia* vermicompost	5.3	29	97	87	3008
Mean		6.1	31.9	108	93	3559
LSD (P=0.05)		0.8	2.5	1.34	8.1	385.4
CV %		9	5	9	6	8

Data is means of 50 seeds (in each roll) with four replications, [a] Significant than control; values in parenthesis are per cent increase over control. *Isolate inhibited plant growth.

Table 4.4. Effect of selected PGPB on growth of sorghum (CSV 15) by paper (roll) towel method in sterilized conditions

Isolates	Shoot length (cm)	Root length (cm)	Plant wet weight (mg)	Germination (%)	Seed vigor index
Control	9	29	150	89	3381
A. chr HT54	11	31	178 (17)	90 [b]	3833
EB13	11	33	170 (12)	92 [b]	4026
EB27	11	32	170 (12)	83 [b]	3585 [b]
EB35	11	34	166 (10)	92 [b]	4208
EB67	12	33	190 (25)	95	4218
EB75	11	33	165 (9)	92 [b]	4097
EB77	11	31	169 (11)	93 [b]	3978
CDB35	11	33	187 (23)	87 [b]	4136
CDB36	11	34	171 (13)	92 [b]	3812
CDB47	11	33	172 (13)	93 [b]	4086
BWB21	11	32	171 (13)	96	4181
BWB36	11	32	162 (7)	90 [b]	3569 [b]
BWB40	11	31	173 (14)	85 [b]	3869
Mean	11	32	171	91	3927
LSD (P=0.05)	0.8	1.2	12	6	274
CV %	5	3	5	4	5

Data is means of 50 seeds in four replications
[b] Non-significant than control, values in parentheses are per cent increase over control.
A. chr HT54=*A. chroococcum* HT 54 (+ve control)

Table 4.5. Morphological, cultural and microscopic characteristics of potential bacteria

Name of the test	EB 3	EB 10	EB 13	EB 15	EB 27	EB 35	EB 48	EB 67	EB 75	EB 77	CDB 6	CDB 15	CDB 22	CDB 31	CDB 35	CDB 36	CDB 47	BWB 21	BWB 34	BWB 36	BWB 40	BWB 41
GS	+	+	+	+	-	+	+	-	-	-	+	+	+	+	-	-	+	-	+	-	-	+
SS	S	S	S	S	NS	S	S	NS	NS	NS	S	S	S	S	NS	NS	S	NS	S	NS	NS	S
Cell shape	R	R	LR	R	LR	SR	R	SR	SR	SR	R	R	R	R	SR	SR	R	SR	R	SR	SR	R
Density	O	O	Tl	O	O	Tl	O	O	O	O	O	O	O	O	Tl	Tl	O	Tl	O	Tl	Tl	O
Elevation	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	Ra	C	Ra	C	C	Ra
Surface texture	Sm	Sm	Sm	Sm	Sm	Sm	Sm	Sm	Sm	Sm	Sm	Sm	Sm	Sm	Sm	Sm	Sin	Sm	Sm	Sm	Sm	Sm
Margin	W	W	W	W	W	Ro	I	E	E	Ro	W	I	I	W	Ro	Ro	Ro	Ro	Ro	Ro	Ro	Ro
Pigments	NP	NP	NP	NP	NP	NP	NP	Pink	Light pink	Light yellow	NP	NP	NP	NP	GY	GY	NP	GY	NP	GY	GY	NP

GS = Gram stain, +Gram positive rods, -Gram negative rods
SS = spore stain, S=sporulating, NS=non-sporulating,
R = rods, LR = long rods, SR = short rods, O = opacity, Tl = transluscent,
C = convex, Ra = raised, Sm = smooth, W = wavy, Ro = round, E = entire, I = irregular, NP=no pigment, GY = greenish yellow on Luria B agar medium.

Table 4.6. Identification of the potential bacterial isolates based on biochemical tests

Name of the test	EB 3	EB 10	EB 13	EB 15	EB 48	CDB 6	CDB 15	CDB 22	CDB 31	CDB 47	BWB 34	BWB 4)
Motility	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	+
Vogues Proskeur	+	+	+	D	+	+	+	+	D	+	D	-
Citrate utilization	+	+	D	-	+	+	+	+	-	+	-	-
Starch hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+
Casein hydrolysis	+	+	+	+	+	+	+	+	+	+	+	-
Gelatin hydrolysis	+	-	+	-	+	-	-	+	-	+	-	-
Nitrate reduction	-	+	D	-	-	+	-	-	-	+	-	D
Anaerobic growth	-	+	-	+	-	+	+	-	+	+	+	+
Growth at different pH, Nacl concentration, temperature												
pH 5	+	+	+	+	+	+	-	+	+	+	+	+
pH 8	-	-	+	-	+	+	+	-	-	+	-	-
pH 9	-	-	+	-	-	-	-	-	-	+	-	-
2%NaCl	+	+	+	-	+	+	+	+	-	+	-	+
5% NaCl	+	+	+	-	+	+	+	+	-	+	-	-
7% NaCl	+	+	+	-	+	+	-	+	-	+	-	-
4 °C	-	-	-	-	-	-	-	+	-	-	-	-
15 °C	+	+	-	+	+	+	-	+	+	+	+	+
25 °C	+	+	+	+	+	+	+	+	+	+	+	+
37 °C	+	+	+	+	+	+	+	+	+	+	+	+
42 °C	+	+	+	+	+	+	+	+	+	+	+	+
55 °C	-	-	-	+	+	-	-	-	+	+	+	-
Acid production from different carbon substrates												
Glucose	+	+	+	+	+	+	+	+	+	+	+	+
Arabinose	+	-	+	+	+	-	-	+	+	+	+	+
Mannitol	+	-	+	+	+	-	-	+	+	+	+	+
Xylose	+	+	+	-	+	-	-	+	+	+	-	+
Identification of the isolate	Bacillus pumilus	Bacillus cereus	Bacillus licheniformis	Bacillus coagulans	Bacillus subtilis	Bacillus cereus	Bacillus alvei	Bacillus pumilus	Bacillus coagulans	Bacillus licheniformis	Bacillus coagulans	Bacillus s

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Table 4.6 contd.....

Name of the test	EB 27	EB 35	EB 67	EB 75	EB 77	CDB 35	CDB 36	BWB 21	BWB 36	BWB 40
Mac conkey	LF	+	NLF	NLF	LF	NLF	NLF	NLF	NLF	NLF
Motility	+	+	+	+	-	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	+	+	+	+	+
Methyl red	+	-	-	-	D	-	-	-	-	-
Vogues Proskeur	+	-	-	-	+	-	-	-	-	-
Indole	-	-	+	+	+	-	-	-	-	-
Citrate utilization	+	-	+	+	+	+	+	+	+	+
Starch hydrolysis	-	+	-	+	-	-	-	-	-	-
Casein hydrolysis	-	+	+	-	-	+	+	+	+	+
Gelatin hydrolysis	-	+	+	-	-	+	+	+	+	+
Oxidative fermentation	F	-	-	-	F	O	O	O	O	O
Anaerobic growth	+	-	+	-	+	-	-	-	-	-
Urease	-	-	+	+	+	+	+	+	+	+
Growth at: pH 5	+	+	+	+	+	+	+	+	+	+
pH 8	+	+	+	+	+	+	+	+	+	+
pH 9	+	+	+	+	+	+	+	+	+	+
2%NaCl	+	+	+	+	+	+	+	+	+	+
5% NaCl	+	-	-	-	+	+	+	+	+	+
4 °C	-	-	+	+	-	-	-	-	-	-
15 °C	+	+	+	+	+	+	-	+	-	-
25 °C	+	-	+	+	+	+	+	+	+	+
37 °C	+	+	+	+	+	+	+	+	+	+
42 °C	+	-	+	+	-	-	-	-	-	-
55 °C	-	+	+	+	+	-	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+
Mannitol	-	+	+	+	+	-	-	-	-	-
Xylose	+	D	+	+	+	+	+	+	+	+
Meso-Inositol	-	-	-	-	-	-	-	-	-	-
Galactose	+	-	+	+	+	+	+	+	+	+
Identification of the isolate	Enterobacter cloacae	Bacillus circulans	Serratia marcescens	Serratia sp.	Klebsiella oxytoca	Pseudomonas sp.	Pseudomonas sp.	Pseudomonas sp.	Pseudomonas sp.	Pseudomonas sp.

LF=lactose fermenting, NLF=non-lactose fermenting, D=differential, O=oxidative, F=Fermentative,

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4.2.3 Correlation analysis of PGPB

The bacteria were rated as 0, 1, 2 and 3 for production of enzyme, metabolite or solubilization of nutrients based on visual observation of zone size or color development (Table 4.7 a). Antagonistic trait was rated based on the suppression of the four soil borne plant pathogenic fungi.

Table 4.7a. Ranking of plant growth promoting bacteria for different traits used in the study

Isolates	PSB	Phytase	Sidero-phore	Chitin	ACC deaminase	Indole	HCN	Plant weight	Root length	Antagonistic
EB 13	0	0	0	0	0	0	0	3	2	++++
EB 27	3	3	2	0	3	0	0	3	3	++
EB 35	0	0	0	2	0	0	0	3	3	++
EB 67	3	0	3	3	3	0	0	3	3	++
EB 75	2	0	3	3	2	0	0	3	2	++
EB 77	0	3	2	0	2	1	2	3	3	++
CDB 35	3	3	3	0	3	3	3	3	3	++++
CDB 36	0	0	2	0	3	3	3	3	3	++++
CDB 47	0	3	0	0	0	0	0	3	2	++++
BWB 21	3	2	3	0	2	3	3	3	2	++++
BWB 36	0	0	2	0	2	3	3	3	2	++++
BWB 40	0	0	2	0	2	3	3	3	2	++++
EB 3	0	0	0	0	0	1	0	1	1	++++
EB 10	0	0	0	0	0	1	0	1	1	++++
EB 15	0	0	0	0	0	1	0	1	1	++++
EB 48	0	0	0	0	0	1	0	1	1	++++
CDB 6	0	2	0	0	0	1	0	1	1	++++
CDB 15	0	0	0	0	2	1	0	1	2	++++
CDB 22	0	2	0	0	0	1	0	1	1	++++
CDB 31	0	2	0	0	0	1	0	1	1	++++
BWB 34	0	0	0	3	0	1	0	1	1	++++
BWB 41	0	0	0	3	0	1	0	1	1	++++

0 = No activity, 1 = Minimal activity, 2 = Medium activity 3 = Maximum activity,
PSB=phosphate solubilizing bacteria. ++++Suppression of all four soil borne plant pathogenic fungi used in the study, ++Suppression of any two soil borne plant pathogenic fungi used in the study.

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A correlation was run between plant growth of pearl millet (by paper towel method) and plant growth promoting traits characterized *invitro* at 5% level (Table 4.7 b). Plant growth promoting traits were significantly correlated (P=0.05) with plant biomass (wet weight) and root length of pearl millet *in vitro* (paper towel) conditions. Siderophore producers showed significant correlation with ACC deaminase producers (r=0.89) followed by P solubilizers (r=0.77), biomass (r=0.73), root length (r=0.67) and HCN producers (0.66). Significant correlation was also observed between indole and HCN producers (r=0.89), PSB and ACC deaminase producers (r=0.67), root length and ACC deaminase producers (r=0.78) and root length and plant biomass (r=0.86).

Table 4.7 b. Correlation between plant growth promoting traits of bacteria

	ACC	Antg	Chit	HCN	Ind	Phy	PW	PSB	RL	Sid
ACC	1.00									
Antg	-0.39	1.00								
Chit	-0.01	-0.44	1.00							
HCN	0.62	0.16	-0.32	1.00						
Ind	0.40	0.50	-0.38	0.89	1.00					
Phy	0.18	-0.13	-0.39	0.16	0.01	1.00				
PW	0.66 *	-0.50	0.03	0.55	0.16	0.23	1.00			
PSB	0.67 *	-0.44	0.20	0.22	0.05	0.35	0.49	1.00		
RL	0.78 *	-0.62	0.04	0.45	0.08	0.26	0.86	0.51	1.00	
Sid	0.89 *	0.44	0.12	0.66 *	0.41	0.21	0.73 *	0.77 *	0.67 *	1.00
	ACC	Antg	Chit	HCN	Ind	Phy	PW	PSB	RL	Sid

*Statistically significant, ACC = ACC deaminase, Antg=antagonistic, Chit = chitinase, Ind = IAA, Phy = phytase, PSB = Phosphate solubilizing bacteria, PW = plant weight (biomass), RL = root length, Sid = siderophore.

4.3 STUDIES ON PHOSPHATE SOLUBILIZING BACTERIA (PSB)

Out of twelve bacterial isolates that showed improvement in plant growth of pearl millet and sorghum, five isolates were positive for RP solubilization in plate culture conditions and were characterized further in RP broth medium. All the isolates solubilized RP in presence of glucose and two isolates EB 67 and CDB 35 were studied further in presence of different carbon sources and soil conditions.

4.3.1 Selection of efficient PSB in RP buffered medium

Five bacterial strains *E. cloacae* EB 27, *S. marcescence* EB 67, *Serratia* sp. EB 75, *Pseudomonas* sp. CDB 35 and *Pseudomonas* sp. BWB 21 were inoculated in RP broth medium to monitor viability, drop in pH, production of organic acid, glucose dehydrogenase activity and RP solubilized (Table 4.8). All PSB showed drop in pH within 96 hours in presence of glucose and solubilized P. All isolates except CDB 35 showed maximum growth at 48 hours and then declined (Fig. 4.2a). Drop in pH ranged from 4.7 with CDB 35 to 3.3 with EB 67 after 96 hours (Fig. 4.2b). Gluconic acid produced was highest with EB 67 (67 mM) followed by EB 27 (35 mM), CDB 35 (27 mM), BWB 21 (22 mM) and EB 75 (20 mM). RP solubilization depended on amount of gluconic acid produced with the bacterial strains. RP solubilized was highest with EB 67 (1036 µM), followed by EB 27 (748 µM), CDB 35 (560 µM), EB 75 (535 µM) and BWB 21 (430 µM) (Table 4.8).
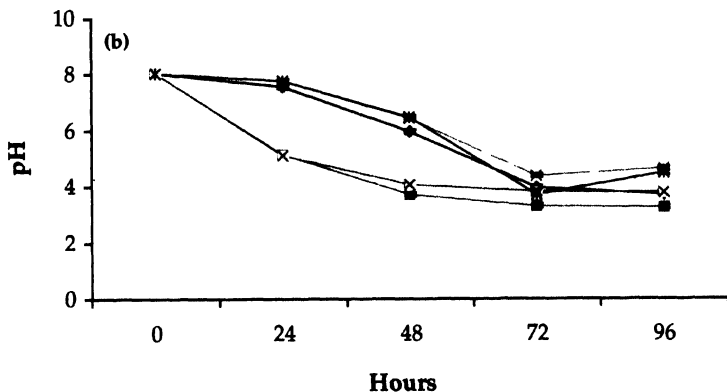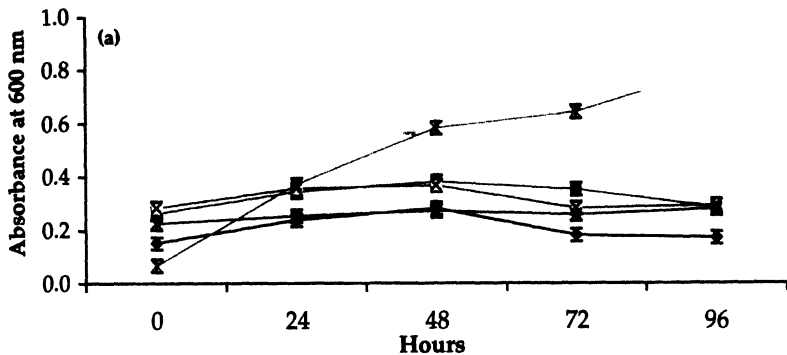
Figure·4.2. Growth (a) and change in pH (b) with phosphate solubilizing bacteria in RP buffered medium. Data points are mean of three replicates from three independent experiments and the standard error of means are shown as vertical lines.

Table 4.8. Gluconic acid production and P released by selected bacterial isolates in RP buffered medium

Isolates	Gluconic acid (mM)	P released (µM)
E. cloacae EB 27	35	748
S. marcescens EB 67	67	1036
Serratia sp. EB 75	20	535
Pseudomonas sp. CDB 35	27	560
Pseudomonas sp. BWB 21	22	430
Enterobacter asburiae PSI3 *	55	500
Mean	34	662
LSD	3.9	13.7
CV%	6	11

* *Enterobacter asburiae* PSI3 (reference strain)

4.3.2 Growth, drop in pH, glucose dehydrogenase (Gcd) activity and RP solubilization by EB 67 and CDB 35 in presence of different carbon sources

Detailed investigation of two PSB *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 was done in RP broth medium. Growth, drop in pH, Gcd activity, solubilization of P and organic acid produced using different carbon sources were studied. EB 67 showed optimum growth at 48 hours in presence of glucose and mixture of sugars. Optimum growth with other carbon sources was at 72-96 hours (Fig. 4.3a). However, with mannose there was no decline in growth of EB 67 even at 120 hours. Drop in pH by EB 67 ranged from 6 with cellobiose to 3.2 with glucose (Fig. 4.3b). CDB 35 showed optimum growth in presence of glucose and mannose at 48 hours and with

rest of the carbon sources it was at 72 hours (Fig. 4.4a). Drop in pH by CDB 35 ranged from 5.0 with cellobiose to 4.1 with maltose (Fig. 4.4b).
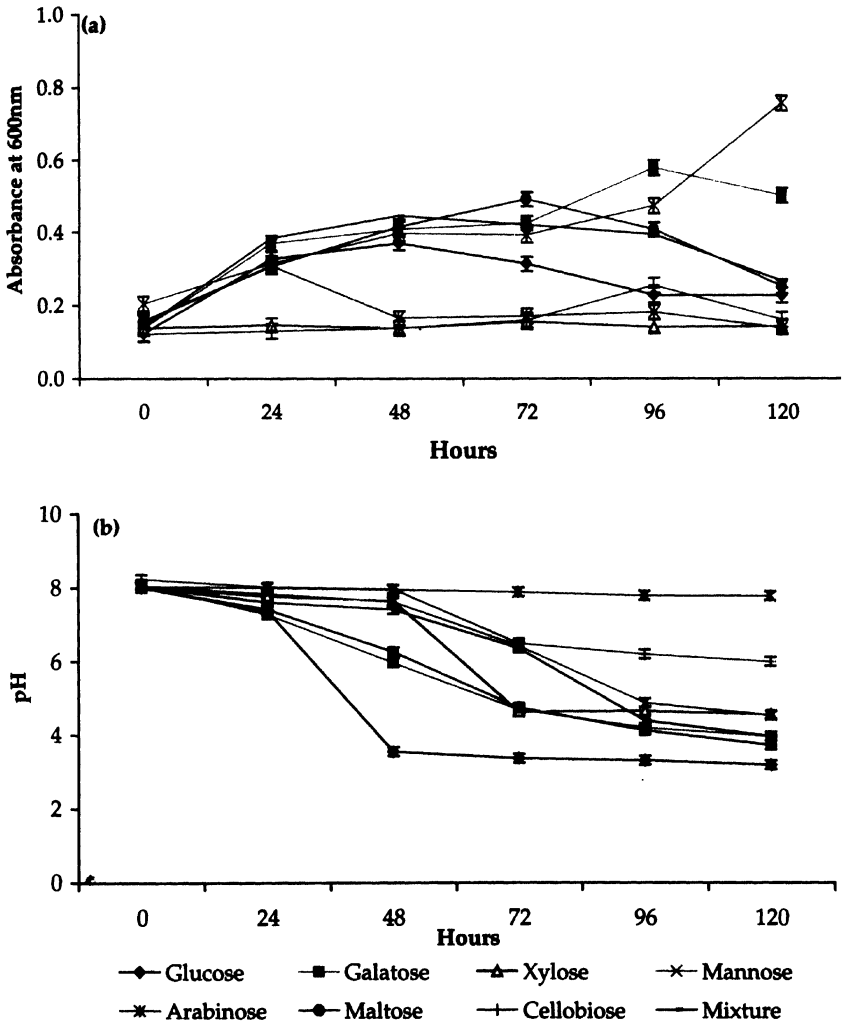
Figure 4.3. Growth (a) and change in pH (b) of *S. marcescens* EB 67 RP buffered medium in presence of different carbon (sugar) sources. Data points are mean of 3 replicates from three independent experiments and the standard error of means are shown as vertical lines ·
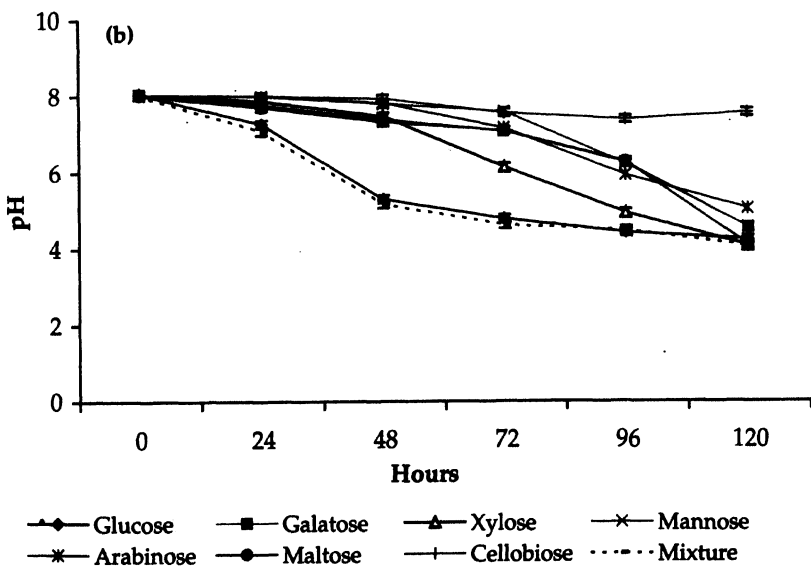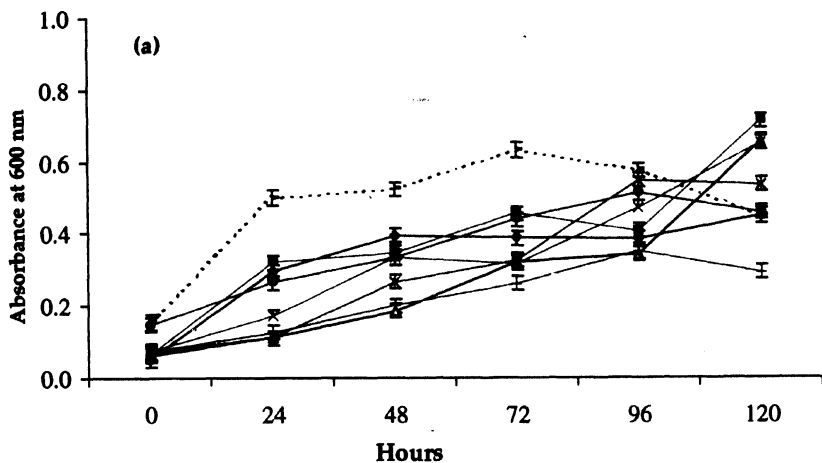
Figure 4.4. Growth (a) and change in pH (b) of *Pseudomonas* sp. CDB 35 in RP buffered medium in presence of different carbon (sugar) sources. Data points are mean of three replications from three experiments and the standard error of means are shown as vertical lines.

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(ells were grown on glucose a sole carbon source and different sugars were used to measure the direct oxidation using Gcd activity. Based on direct oxidation of glucose, the Gcd activity of EB 67 was 63% with galactose and 59% with xylose when compared with that of glucose (100%) (Fig. 4.5). Gcd activity was low with mannose (46%), maltose (44%) and cellobiose (35%). When a mixture of seven sugars was used (15mM) as substrate, Gcd activity was 41%. Gcd activity with CDB 35 was highest with galactose (77%) and least with cellobiose (46%) when compared to that of glucose (100%) (Fig. 4.5).
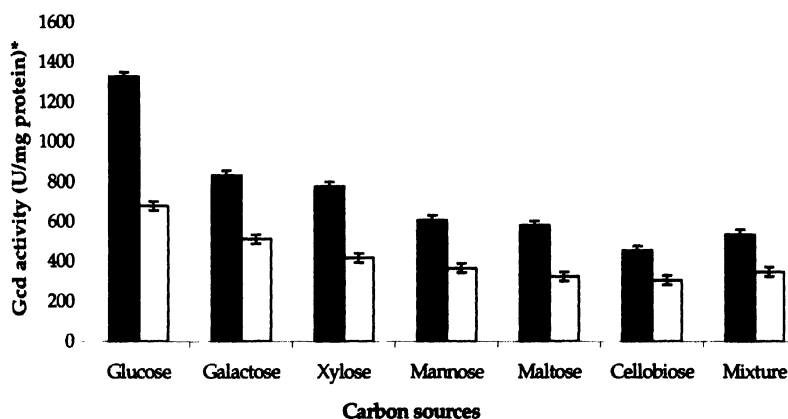
Figure 4.5.   Glucose dehydrogenase (Gcd) activity by *S. marcescens* EB 67 (■) and *Pseudomonas* sp. CDB 35 (□). *Enzyme activity is given as units (mg protein[-1]). Values are given as means of three independent experiments. Cells were grown on glucose a sole carbon source and different sugars were used to measure the direct oxidation using Gcd activity.

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Solubilization of RP with EB 67 was maximum in presence of glucose 1200 μM followed by galactose (836 μM), xylose (775 μM), mixture of sugars (642 μM), maltose (540 μM) and cellobiose (455 μM) (Table 4.9). RP solubilized with CDB 35 was maximum with glucose 522 μM followed by mixture of sugars (411 μM), galactose (449 μM), maltose (366μM), xylose (356 μM) and cellobiose (306 μM) (Table 4.9). Both the isolates did not solubilize P in presence of arabinose as carbon source. Organic acid analysis by HPLC in presence of different carbon sources showed change in retention time of the peak unlike glucose, which showed 2.6-2.8 minutes.

Table 4.9. P released by *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 in RP medium in presence of different carbon sources

Carbon source	P released (μM)	
	S. marcescens EB 67	*Pseudomonas* sp. CDB 35
Glucose	1212	522 .
Galactose	836	449
Xylose	775	356
Maltose	540	366
Cellobiose	455	306
Arabinose	0	0
Mixture	642	411
Mean	637	344
LSD (P = 0.05)	39.5	
CV%	5	

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4.3.3 Effect of nutrient supplementation on growth and P release by native and inoculated microorganisms in different soils (Vertisols)

Native microorganisms were assessed for their ability to solubilize P from Vertisol soil when sufficient amount of carbon (C) and nitrogen (N) sources were added. Native microorganisms showed distinct growth over a period of 48 h but failed to lower pH of soil and did not release P in solution (Fig. 4.6 a-b). Similar results were obtained with both soils of fields BR1D (low P, Fig. 4.6a) and BP2C (medium P, Fig. 4.6b) used in study.

4.3.4 Growth, drop in pH, RP-solubilization abilities of EB 67 and CDB 35

S marcescens EB 67 and *Pseudomonas* sp. CDB 35 could grow utilizing RP as sole P source when supplemented with C and N source in soil suspension. *E. asburiae* PSI3 was used as reference strain to compare the growth (Gyaneshwar *et al.* 1999). On inoculation EB 67, CDB 35 and PSI3 isolates separately in Vertisol soil solution, each isolate showed significant increase in their viable count (Fig 4.6 a-b) with concomitant lowering of the soil pH. In RP medium plates buffered at 100 mM Tris-HCl pH-8.0. PSI3, EB 67 and CDB 35 showed acid secretion turning the methyl red color from yellow to red, whereas the native microorganisms did not show any acid production, which indicated that the native microorganisms present in soil were not able to solubilze P. Drop in pH ranged from 3.3 with EB 67 to 3.7 with CDB 35 within 24 hours. P released by CDB 35 ranged from 337– 537 µM and 547-765 µM by EB 67 after 24 hours of incubation (Table 4.10 & 4.11).
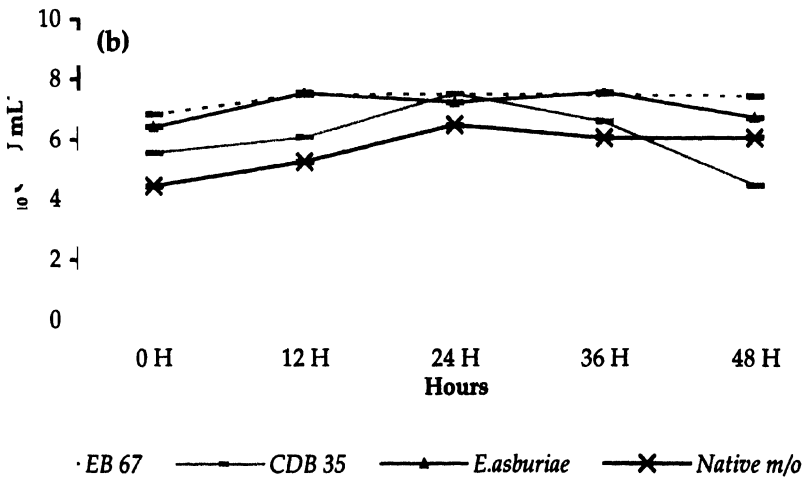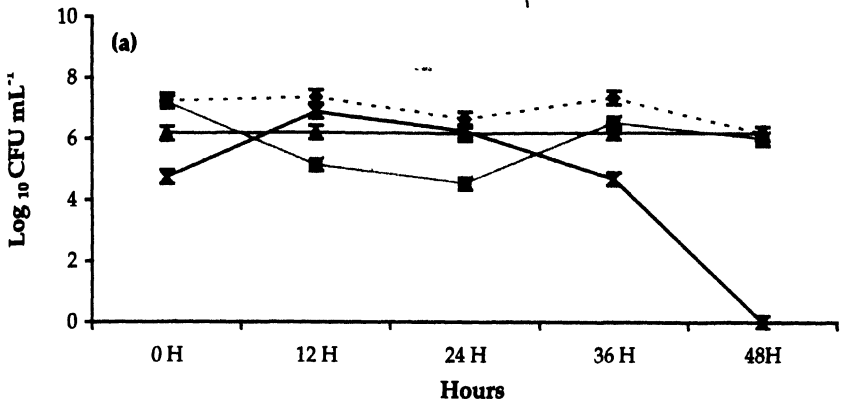
Figure 4.6. Viability of native and inoculated PSB *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 in Vertisol from BR1D (a) and BP2C (b) field. Data points are means of three replicates from two independent experiments and the standard error of means are shown as vertical lines. Growth in control flask (that was uninoculated and unsterilized) was monitored to study the P solubilizing ability of native m/o (microorganisms).

Table 4.10. Drop in pH and release of P in soil from BR1D field by native and inoculated PSB *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35

Isolates	pH					P (µM)				
	0 H	12 H	24 H	36 H	48 H	0 H	12 H	24 H	36 H	48 H
EB 67	7.9	4.5	3.3	6.4	6.6	UD	222	547	237	UD
CDB 35	7.9	4	3.7	5.8	6.8	UD	137	337	137	UD
E. asb PSI3	7.9	3.9	3.3	6.8	6.7	UD	132	473	127	UD
Native m/o	7.9	7.2	6.9	7.2	7.2	UD	UD	53	UD	UD
Mean	7.5	4.9	4.3	6.5	6.8	-	163	353	167	-
LSD (P=0.05)		0.53						35.3		
CV%		4						13		

UD=undetected; m/o=microorganisms; *E. asb* PSI3= *E. asburiae*

Table 4.11. Drop in pH and release of P in soil from BP2C field by native and inoculated PSB *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35

Isolates	pH					P (µM)				
	0 H	12 H	24 H	36 H	48 H	0 H	12 H	24 H	36 H	48 H
EB 67	7.6	4.1	3.6	5.1	5.9	UD	234	765	337	UD
CDB 35	7.6	4.9	3.6	5.3	4.7	UD	179	537	274	UD
E. asb PSI3	7.6	4.9	3.1	5.1	6.0	UD	96	575	257	UD
Native m/o	7.6	7.5	6.4	6.4	6.5	UD	UD	63	20	UD
Mean	7.6	5.4	4.2	5.5	5.8	-	170	485.0	222	-
LSD (P=0.05)		0.43						70.8		
CV%		4						20		

UD=undetected; m/o=microorganisms; *E. asb* PSI3= *E. asburiae*

4.4 CELLULASE ACTIVITY AND RP SOLUBILIZATION BY EB 67 AND CDB 35 IN PRESENCE OF DIFFERENT CROP RESIDUES

4.4.1 Re-cycling of crop residues by *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 EB 67 and CDB 35 in submerged conditions

Both the isolates could utilize cellulose and rice straw as sole carbon source and were evaluated for the cellulase activity in presence of different crop residues. Cellulase activity was detected in submerged conditions using RP media amended with 1% crop residue viz., rice straw (RS), rice root (RR), rice straw and root (RS+RR), pigeonpea root (PR), grass (GS) and commercially available cellulose (CL) (Table 4.12 & 4.13). Cellulase activity on day 4 by EB 67 and CDB 35 was maximum with RS+RR (0.034 and 0.024 units) and minimum with CL and GS (0.017-0.018 units). Cellulase enzyme activity was maximum on day 6 by both the isolates. Maximum activity by EB 67 was with RS (0.030 units), followed by RS+RR (0.027 units), PR and GS (0.024 units), CL (0.020 units) and RR (0.019 units). CDB 35 showed maximum activity with RS+RR (0.036 units) followed by RR (0.034 units), RS (0.031), CL (0.023 units) and GS (0.022 units). Cellulase activity decreased after 8 days of incubation by both the isolates. Cellulase activity by EB 67 ranged between 0.015 units using PR to 0.024 units with RS on 8th day and using grass (0.006 units) and RS (0.025 units) on 12th day. CDB 35 showed cellulase activity between 0.016 with GS and 0.029 units with RS+RR on day 8 and using GS (0.005 units) and RS (0.023 units) (Table 4.12).

In submerged conditions both the isolates presented similar behavior towards reducing sugar liberation and consumption, as a peak of sugar concentration was observed on day 6 after inoculation, followed by sharp decline, which indicated carbon source consumption. On day 4, with EB 67, sugar liberated with different

crop residues ranged between 86-159 µg and CDB 35 69-144 µg. On 6th day, sugar liberated was maximum in presence of CL (155 µg mL⁻¹) with EB 67 and PR (208 µg mL⁻¹) with CDB 35. On day 8 and 12, sugars liberated decreased and ranged from 83-155 µg mL⁻¹ with EB 67 and 57-126 µg mL⁻¹ with CDB 35 (Fig 4.7 a-b). RP solubilized by EB 67 ranged between 24 µM (GS) to 112 µM (PR) and CDB 35 ranged between 23 µM (GS) to 103 µM (RS) on 4th day. P solubilized after 6 days, by EB 67 and CDB 35 was minimum using grass (77 µM) and cellulose (64 µM), maximum using PR (132-137 µM). EB 67 showed 73 µM with cellulose and 189 µM with RR, CDB 35 showed 59 µM with cellulose and 176 µM with PR. After 12 days, RP solubilized using PR by EB 67 was 151 µM and CDB 35 was 169 µM after 8 days (Table 4.13).

Table 4.12. Cellulase enzyme activity by *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 in presence of different crop residues in submerged conditions

| (Carbon source 1%) | Cellulase Uml⁻¹min⁻¹* | | | | | | | | |
| | EB 67 | | | | CDB 35 | | | | |
	4 D	6 D	8 D	12 D	4 D	6 D	8 D	12 D	Mean
Cellulose (CL)	0.018	0.020	0.016	0.019	0.018	0.023	0.018	0.015	0.018
Rice straw (RS)	0.027	0.030	0.024	0.025	0.022	0.031	0.021	0.023	0.025
Rice root (RR)	0.027	0.019	0.016	0.020	0.021	0.034	0.022	0.016	0.022
RS+RR	0.034	0.027	0.022	0.021	0.024	0.036	0.029	0.020	0.027
Pigeonpea root (PR)	0.031	0.024	0.015	0.017	0.021	0.036	0.019	0.021	0.023
Grass (GS)	0.018	0.024	0.020	0.006	0.017	0.022	0.016	0.005	0.016
Mean (Strains)	0.022				0.022				
Mean (Days)	0.023	0.027	0.019	0.017					
LSD (P = 0.05)	(S*C)	0.019			(S*D)	0.0020			
LSD (P = 0.05) CV %	S*C*D	0.029							

*One unit of cellulase activity is equivalent to 1µ mole of reducing sugar equivalents per minute. D = days, S = strains, C = carbon source.
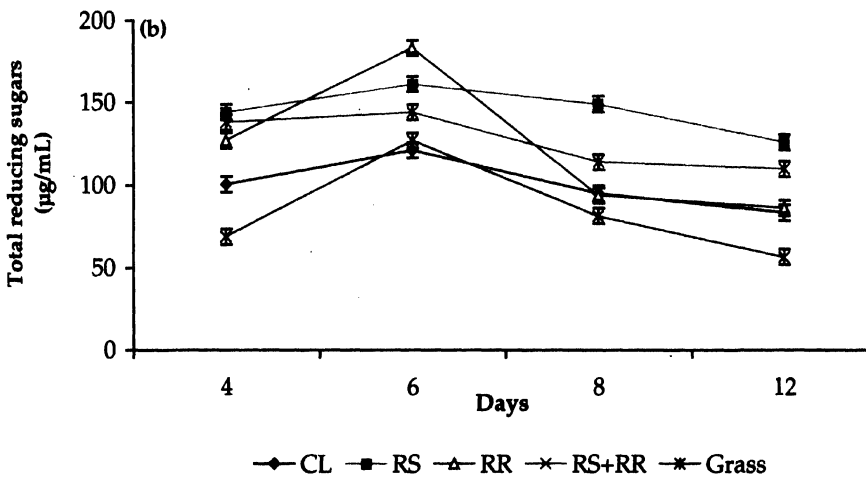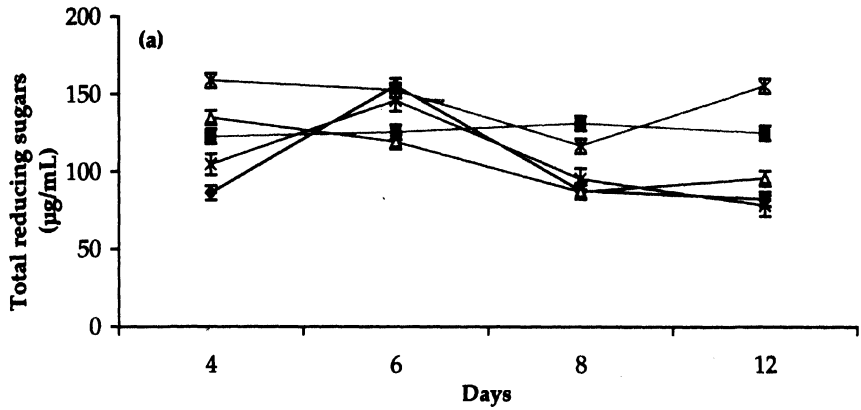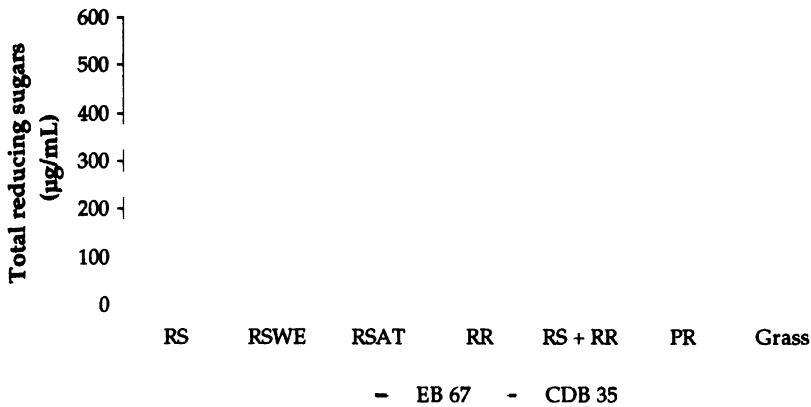
Figure 4.7. Total reducing sugars released by (a) *S. marcescens* EB 67 and (b) *Pseudomonas* sp. CDB 35 in presence of different crop residues in submerged conditions. CL = cellulose, RS = rice straw, RR = rice root.

Table 4.13. Solubilization of RP by *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 using different crop residues in submerged conditions

Carbon (C) source	P released (µM) EB 67				CDB 35				Mean
	4 D	6 D	8 D	12 D	4 D	6 D	8 D	12 D	
Cellulose (CL)	57	85	73	68	59	64	59	63	66
Rice straw (RS)	76	126	98	89	103	147	122	92	107
Rice root (RR)	83	125	189	108	84	127	113	97	116
RS+RR	92	124	139	135	100	110	143	139	123
Pigeonpea root (PR)	112	137	136	151	81	132	176	169	137
Grass (GS)	24	77	97	94	23	73	85	87	70
Mean (Strains)	102				104				
Means (Days)	74	110	119	108					
LSD (P = 0.05)		(S*C)	6.1			(S*D) 9.4			
LSD (P = 0.05)					(S*C*D)	13.3			
CV%					7				

S=strains, D=days, C=carbon source

4.4.2 Re-cycling of crop residues by EB 67 and CDB 35 in solid-state conditions

In submerged conditions both the isolates showed cellulase activity and RP solubilization. But to simulate soil and field conditions for applying these isolates along with crop-residues a modified protocol of solid-state cultivation (SSC) was used. Except rice straw, all other crop residues were used without any pre-treatment. Rice straw was pretreated with water and alkali (NaOH). Cellulase activity was higher with EB 67 in presence of GS (0.072) and CDB 35 (0.073) in presence of RS and RR. Least cellulase activity was in presence of RS by EB 67 (0.043) and CDB 35 (0.047)

(Table 4.14). Reducing sugars released at day 6 was maximum with EB 67 in presence of alkali treated RS (455 µg mL^{-1}) followed by grass (451 mL^{-1}), water extracted RS (389 µg mL^{-1}), RS+RR (365 µg mL^{-1}), PR (345 µg mL^{-1}), RS (341 µg mL^{-1}), RR (320 µg mL^{-1}). CDB 35 showed reducing sugars, maximum in presence of alkali treated RS (507 µg mL^{-1}) followed by water extracted RS (490 µg mL^{-1}), RS (458 µg mL^{-1}), grass (437 µg mL^{-1}), RS+RR (402 µg mL^{-1}), PR (341 µg mL^{-1}), RR (338 µg mL^{-1}) at day 6 (Fig. 4.8). P released was higher with alkali pre-treated RS with EB 67 (343µM) and CDB 35 (362µM) (Table 4.15).

Table 4.14. Cellulase enzyme activity by *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 in presence of different crop residues in solid-state conditions after eight days of growth

Carbon (C) source (1%)	Cellulase (U mL^{-1}min^{-1}) *	
	EB 67	CDB 35
RS (untreated)	0.043	0.047
RS (water extract)	0.049	0.055
RS (alkali treated)	0.056	0.059
RR (untreated)	0.049	0.054
RS + RR (untreated)	0.055	0.073
PR (untreated)	0.063	0.067
Grass (untreated)	0.072	0.053
LSD (Strains) (P = 0.05)	0.009	0.006
Mean	0.055	0.058
LSD (P=0.05) S*C	0.0066	
CV %	6.8	

*One unit of cellulase activity is equivalent to 1µ mole of reducing sugar equivalents per minute.

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Figure 4.8. Total reducing sugars released by *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 in presence of different crop residues in solid-state conditions. RS = rice straw, RSWE = water extracted (pretreated) rice straw, RSAT = alkali extracted (pretreated) rice straw, RR = rice root, PR = pigeonpea root

Table 4.15. RP solubilization by *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 in presence of different crop residues in solid-state conditions after eight days of growth

Carbon (C) source (1%)	P released (μM)	
	EB 67	CDB 35
RS (untreated)	270	274
RSWE (water extract)	284	301
RSAT (alkali treated)	343	362
RR (untreated)	264	249
RS + RR (untreated)	274	259
PR (untreated)	290	258
Grass (untreated)	243	200
LSD (Strains) (P = 0.05)	28.7	18.6
Mean (P = 0.05)	281	272
LSD (S*C)	20.9	
CV %	4	

C = carbon sources, S = strains, RS=rice straw, RR=rice root, PR=pigeonpea root, G=grass

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4.5   PERFORMANCE OF TWO PSB IN SOIL CONDITIONS IN PRESENCE OF DIFFERENT ORGANIC CARBON SOURCES.

Soil quality was evaluated after addition of different organic carbon sources viz., glucose (GL), organic acids (OA), rice-straw (RS), rice root (RR), rice straw and root (RS+RR), grass (GS), pigeonpea root (PR) applied along with PSB *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 in soil containing RP. Both the inoculated isolates survived in unsterilized soil up to 48 days and enhanced activity of few parameters involved in study.

4.5.1 Intrinsic antibiotic markers of EB 67 and CDB 35

Antibiotic resistance pattern of both the isolates was determined and the marker developed was used to study the population densities and rhizosphere competence of the isolates with the native microorganisms. Both the isolates were tested for their intrinsic tolerance to various antibiotics alone and in combination (Table 4.16). Both the isolates showed tolerance of 50 µg mL^{-1} of erythromycin, vancomycin, trimethoprim and 2.5µg mL^{-1} of streptomycin and rifamycin when used in combination with LB agar and RP agar medium.

4.5.2 Effect of carbon sources on survival of PSB in soil

Survival of the inoculated bacteria was traced back on RP antibiotic medium amended with benomyl (to inhibit fungal growth). The introduced population of bacteria (10^7 to 10^8) declined significantly (P=0.05) with time for all organic carbon sources added. Survival of EB 67 and CDB 35 was two to three times more in presence of GL compared to other treatments at days 12, 24 and 48. However, when soil was not amended with any additional organic carbon source, population of CDB

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35 was log 4.2 g^{-1} soil and EB 67 declined after 48 days (Table 4.17). Uninoculated soil did not show any PSB and in presence of RS, RR, RS+RR and PR, the inoculated strains EB 67 and CDB 35 survived for 48 days (Fig. 4.9).

4.5.3 Effect of carbon source on acid and alkaline phosphatase activity in soil

Acid phosphatase at day 12 was more significant (P=0.05) with all carbon sources than at day 24 or 48. It showed an increase at day 12 and then a progressive decrease was observed with time (Table 4.18). Maximum and significant activity on day 24 was with EB 67 (266 µg g^{-1} soil) followed by uninoculated control (249 µg g^{-1} soil) and CDB 35 (246 µg g^{-1} soil). Alkaline phosphatase activity was greater at day 12 and then on declined. Activity with CDB 35 was 484 µg g^{-1} soil followed by EB 67 483 µg g^{-1} soil and uninoculated control 353 µg g^{-1} soil (Table 4.19 and Fig. 4.11). Mean values at different days showed maximum acid phosphatase and alkaline phosphatase activity with glucose followed by RS, RR, GS, PR and OA (Fig. 4.11).

4.5.4 Effect of carbon source on soil CO$_2$-C and biomass carbon in soil

Soil CO$_2$-C was maximum at day 6 with all the treatments inoculated with PSB and in control treatment except where GL was applied it was maximum at day 6. CO$_2$-C evolved was highest with CDB 35 (201 µg g^{-1} soil) followed by EB 67 (185 µg g^{-1} soil) and uninoculated control (103 µg g^{-1} soil) at day 12 (Table 4.20). Soil microbial biomass C at day 6 was significantly greater (P=0.05) than at 12, 24 and 48 days. Biomass C at day 6 was highest with EB 67 (303 µg g^{-1} soil) followed by CDB 35 (297 µg g^{-1} soil) and control (208 µg g^{-1} soil) (Table 4.21). In presence of organic carbon source soil CO$_2$-C and biomass C throughout the experimental period was maximum with glucose followed by OA, GS, RS, PR and RR (Fig. 4.12).

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4.5.5 Effect of carbon source on soil pH and phosphorous concentration

Soil used in the study had alkaline pH (7.8). In presence of GL and OA, where PSB was inoculated drop in pH was observed 6.9 with EB 67 and 6.6 with CDB 35 up to 24 days. In control, drop in pH was observed only upto day 6 in presence of GL and OA. Soil pH in presence of other carbon sources ranged between 7.1-7.8 (Table 4.22) in control treatment and where EB 67 was applied. CDB 35 showed slight drop in pH at day 6 in presence of RS. Concentration of P increased with GL till 24 days, irrespective of inoculation. In all other treatments, it was maximum at day 6 using OA, and at day 12 with RR, GS, PR (Fig. 4.10). Maximum P liberated with EB 67 (368 μM) and CDB 35 (326 μM) was in presence of GL at day 24 and in control it was 213 μM at day 6. (Table 4.23).

Table 4.16. Intrinsic antibiotic resistance of *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35

Treatments (conc. μg mL^{-1})*	EB 67	CDB 35
Erythromycin (E)$_{100\,\mu g}$	+	+
Vancomycin (V)$_{100\mu g}$	+	+
Trimethoprim (T)$_{100\mu g}$	+	+
Streptomycin (S)$_{2.5\mu g}$	+	+
Rifamycin (R) $_{3\mu g}$	+	+
E+V+T $_{50\mu g}$	+	+
S+R$_{2.5\mu g}$	+	+
E+V+T $_{(50\mu g)}$ +S+R$_{(2.5\mu)}$[a]	+	+

*LA and RP buffered medium amended with antibiotics was used to test.
[a]marker used for tracing back the strains EB 67 and CDB 35.
Antibiotic solutions were prepared in sterilized distilled water, except Erythromycin which was prepared in ethanol.

Table 4.17. Effect of different organic carbon sources on survival of inoculated PSB S. *marcescens* EB 67 and *Pseudomonas* sp. CDB 35 (population log $_{10}$ CFU g^{-1} soil) in unsterilized soil containing RP

Treatment (T)	6 D Control	EB 67	CDB 35	12 D Control	EB 67	CDB 35	24 D Control	EB 67	CDB 35	48 D Control	EB 67	CDB 35	Mean
Soil +RP	0.0	6.1	6.2	0.0	4.2	5.3	0.0	4.2	4.2	0.0	0.0	4.2	2.9
Soil + Glu + N +RP	0.0	7.9	7.8	0.0	7.9	7.3	0.0	7.3	7.2	0.0	6.1	6.1	4.8
Soil + OA + RP	0.0	7.3	7.5	0.0	6.6	7.2	0.0	5.4	6.2	0.0	5.3	5.3	4.2
Soil + RS+RP	0.0	6.9	7.1	0.0	6.2	6.4	0.0	6.1	5.4	0.0	4.4	5.2	4.0
Soil + RR+RP	0.0	6.7	7.0	0.0	6.2	6.0	0.0	6.2	5.6	0.0	4.7	5.4	4.0
Soil + PR+RP	0.0	6.7	7.3	0.0	6.4	6.1	0.0	6.1	5.7	0.0	5.1	5.1	4.0
Soil + GS+RP	0.0	6.3	7.8	0.0	6.2	6.2	0.0	6.2	6.2	0.0	5.3	5.6	4.1
Mean (Strains)	0.0	5.9	6.1		4.2			3.9			3.2		
LSD (P=0.05) S*T		0.11											
Mean (Days)		4.7											
LSD (P=0.05) (S*D) (T*D)		0.08	0.13										
LSD (P=0.05) (S*T*D)			0.22										
CV%			3										

RP = rock phosphate, Glu = glucose, N = NH$_4$Cl, OA = organic acid mixture (gluconic, citric and tartaric), RS = rice straw, RR=rice root, GS = Grass, PR = pigeonpea root; S = strains, D = days

Table 4.18. Effect of different organic carbon sources on acid phosphatase activity (μg pNP g^{-1} soil h^{-1}) in unsterilized soil containing RP inoculated with the two PSB *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35

Treatments (T)	6 D			12 D			24 D			48 D			Mean
	Control	EB 67	CDB 35	Control	EB 67	CDB 35	Control	EB 67	CDB 35	Control	EB 67	CDB 35	
Soil + RP	149	166	153	147	172	147	134	141	135	125	126	116	143
Soil + Glu + N + RP	290	312	297	356	357	321	403	428	361	344	424	316	351
Soil + OA + RP	213	216	222	228	235	230	213	238	180	205	320	152	213
Soil + RS + RP	159	169	166	216	195	209	247	277	275	257	251	270	224
Soil + RR + RP	182	197	180	227	228	216	256	268	285	254	273	279	237
Soil + PR + RP	213	212	223	232	238	207	271	270	240	228	235	221	223
Soil + GS + RP	174	167	181	240	225	238	219	239	243	218	221	218	225
Mean (Strains)	229	239	224										
LSD (P=0.05) S*T		13.2											
Mean (Days)		202			232			253			236		
LSD (P=0.05) (S*D) (T*D)		9.9		15.2									
LSD (P=0.05) (S*T*D)		26.3											
CV%		7.1											

RP = rock phosphate, Glu = glucose, N = NH$_4$Cl, OA = organic acid mixture (gluconic, citric and tartaric), RS = rice straw, RR=rice root, GS = Grass, PR = pigeonpea root, S = strains, D = days

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Table 4.19. Effect of different organic carbon sources on alkaline phosphatase (µg pNP g^{-1} soil h^{-1}) activity in unsterilized soil containing RP inoculated with the two PSB *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35

Treatment (T)	6 D			12 D			24 D			48 D			Mean
	Control	EB 67	CDB 35	Control	EB 67	CDB 35	Control	EB 67	CDB 35	Control	EB 67	CDB 35	
Soil +RP	277	357	343	256	327	327	227	301	303	209	295	234	288
Soil + Glu + N+RP	524	781	735	539	750	727	521	690	628	312	651	537	616
Soil + OA + RP	382	375	419	376	423	414	359	402	421	303	384	354	383
Soil + RS+RP	299	337	477	313	375	417	302	455	317	312	464	364	369
Soil + RR+RP	358	552	536	355	512	496	330	465	475	292	427	467	439
Soil + PR+RP	324	542	560	299	474	537	285	443	486	244	419	445	421
Soil + GS+RP	333	567	527	336	522	471	341	445	438	312	431	403	427
Mean (Strains)	333	470	459										
LSD (P=0.05)	S*T	19.5											
Mean (Days)	457			440			410			374			
LSD (P=0.05)	(S*D)	(T*D)		22.5	14.7								
LSD (P=0.05)	(S*T*D)				39.1								
CV%				6									

RP = rock phosphate, Glu = glucose, N = NH$_4$Cl, OA = organic acid mixture (gluconic, citric and tartaric), RS = rice straw, RR=rice root, GS = Grass, PR = pigeonpea root; S = strains, D = days
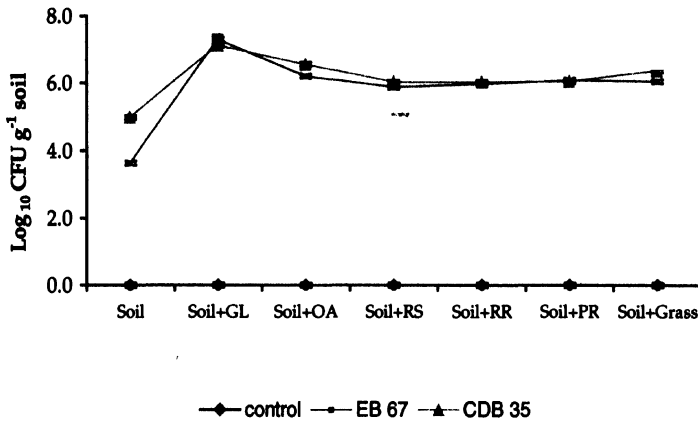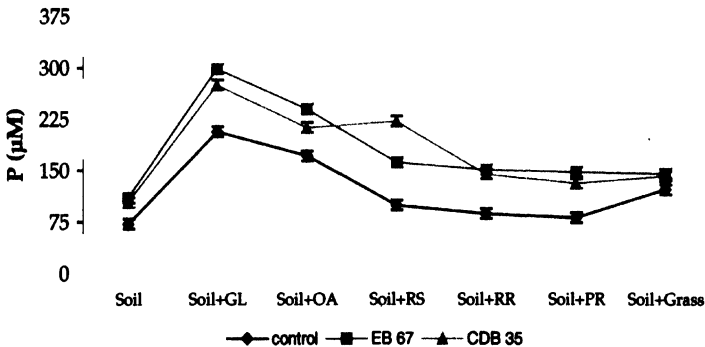
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Table 4.20. Effect of different organic carbon sources on soil respiration (µg CO_2-C g⁻¹ soil) in unsterilized soil containing RP inoculated with the two PSB S. marcescens EB 67 and Pseudomonas sp. CDB 35

Treatment (T)	6 D			12 D			24 D			48 D			Mean
	Control	EB 67	CDB 35	Control	EB 67	CDB 35	Control	EB 67	CDB 35	Control	EB 67	CDB 35	
Soil +RP	64	124	133	58	111	119	52	104	96	44	75	87	89
Soil+Glu+N+RP	179	245	215	192	247	207	211	230	192	147	217	162	205
Soil+OA+RP	165	254	246	121	237	230	122	208	169	102	199	172	184
Soil + RS+RP	75	125	191	77	154	183	72	143	181	64	138	114	126
Soil + RR+RP	73	157	184	87	174	184	69	146	189	64	139	182	137
Soil + PR+RP	73	200	191	77	189	188	75	163	96	59	132	153	133
Soil + GS+RP	90	187	246	92	191	199	77	153	196	59	138	171	150
Mean (Strains)	94	171	174		158			140			124		
LSD (P=0.05) S*T			6.1										
Mean (Days)		163											
LSD (P=0.05) (S*D) (T*D)		4.6		7.0									
LSD (P=0.05) (S*T*D)				12.1									
CV%				5.2									

RP = rock phosphate, Glu = glucose, N = NH₄Cl, OA = organic acid mixture (gluconic, citric and tartaric), RS = rice straw, RR=rice root, GS = Grass, PR = pigeonpea root; S = strains, D = days
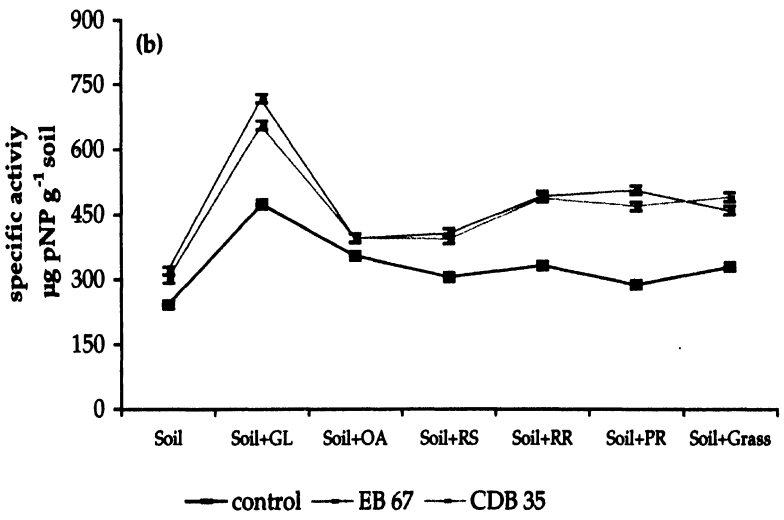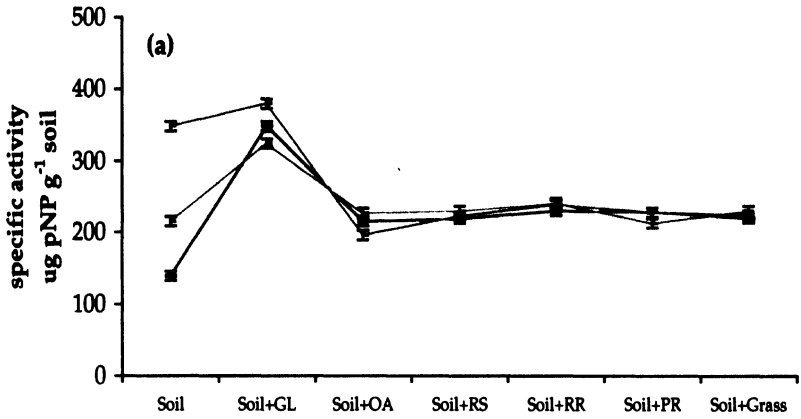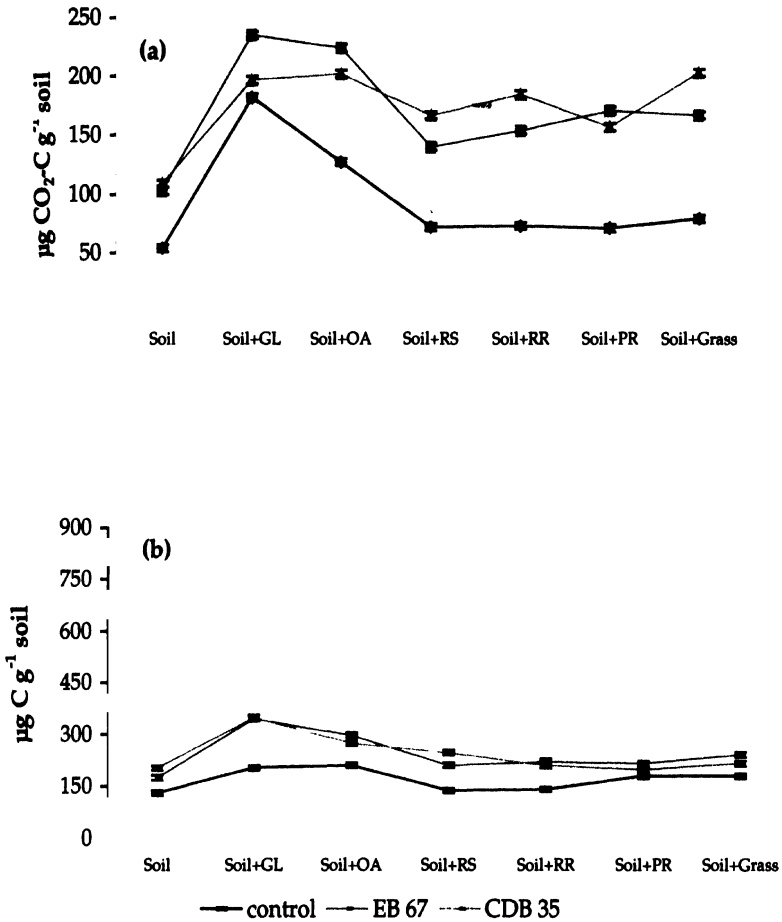
Table 4.21. Effect of different organic carbon sources on soil microbial biomass carbon (µg C g⁻¹ soil) in unsterilized soil containing RP inoculated with two PSB *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35

Treatment (T)	6 D			12 D			24 D			48 D			Mean
	Control	EB 67	CDB 35	Control	EB 67	CDB 35	Control	EB 67	CDB 35	Control	EB 67	CDB 35	
Soil +RP	185	276	277	145	186	216	105	127	183	85	104	133	168
Soil+Glu+N+RP	331	433	474	219	412	382	140	286	318	124	249	221	299
Soil+OA+RP	291	375	377	199	316	310	182	255	240	169	245	169	261
Soil + RS+RP	106	238	264	138	215	255	162	198	257	142	188	213	198
Soil + RR+RP	143	253	222	154	256	208	136	197	205	128	177	200	190
Soil + PR+RP	184	258	218	192	233	217	179	216	187	157	156	168	197
Soil + GS+RP	216	291	246	218	284	218	145	212	208	134	179	167	211
Mean (Strains)	168	243	242										
LSD (P=0.05)	S*T	8.1											
Mean (Days)		269			237			197			168		
LSD (P=0.05)	(S*D)	6.2	(T*D)	9.4									
LSD (P=0.05)	(S*T*D)			16.2									
CV%				5									

RP = rock phosphate, Glu = glucose, N = NH₄Cl, OA = organic acid mixture (gluconic, citric and tartaric), RS = rice straw, RR=rice root, GS = Grass, PR = pigeonpea root; S = strains, D = days

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Table 4.22. Effect of different organic carbon sources on soil pH in unsterilized soil containing RP inoculated with the two PSB
S. marcescens EB 67 and Pseudomonas sp. CDB 35

Treatment (T)	6 D			12 D			24 D			48 D			Mean
	Control	EB 67	CDB 35	Control	EB 67	CDB 35	Control	EB 67	CDB 35	Control	EB 67	CDB 35	
Soil +RP	7.8	7.8	7.8	7.6	7.4	7.1	7.8	7.4	7.7	7.8	7.6	7.8	7.6
Soil+Glu+N+RP	6.9	6.6	7.1	7.4	7.2	6.4	7.4	6.6	6.9	7.1	7.2	7.0	6.9
Soil+OA+RP	6.2	6.5	6.7	6.9	7.2	6.8	7.3	6.8	7.0	7.5	7.2	7.1	6.9
Soil + RS+RP	7.9	7.2	7.8	7.7	7.4	7.1	7.8	7.4	6.8	7.6	7.5	7.0	7.4
Soil + RR+RP	7.8	7.4	6.9	7.3	7.3	7.2	7.3	7.1	7.5	7.8	7.6	7.6	7.4
Soil + PR+RP	7.7	7.8	7.4	7.3	7.3	7.3	7.3	7.3	7.5	7.9	7.2	7.2	7.4
Soil + GS+RP	7.8	7.5	7.4	7.5	7.1	7.2	7.5	7.3	7.4	7.2	7.5	7.4	7..4
Mean (Strains)	7.5	7.3	7.2										
LSD (P=0.05) S*T	0.05												
Mean (Days)													
LSD (P=0.05) (S*D) 0.04 (T*D)	0.05			0.05									
LSD (P=0.05) (S*T*D)	0.09												
CV%	1.6												

RP = rock phosphate, Glu = glucose, N = NH₄Cl, OA = organic acid mixture (gluconic, citric and tartaric), RS = rice straw, RR=rice root, GS = Grass, PR = pigeonpea root; S = strains, D = days

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Table 4.23. Effect of different organic carbon sources on P (µM) released in unsterilized soil containing RP inoculated with two PSB S. marcescens EB 67 and Pseudomonas sp. CDB 35

Treatment (T)	6 D			12 D			24 D			48 D			Mean
	Control	EB 67	CDB 35	Control	EB 67	CDB 35	Control	EB 67	CDB 35	Control	EB 67	CDB 35	
Soil +RP	35	97	104	64	136	116	110	112	101	79	95	90	95
Soil+Glu+N+RP	213	252	220	193	340	313	213	368	326	212	236	239	260
Soil+OA+RP	200	324	260	190	276	270	153	239	175	144	121	146	208
Soil + RS+RP	101	177	211	112	155	217	106	189	275	75	126	186	161
Soil + RR+RP	74	78	88	96	136	194	106	247	151	71	147	148	128
Soil + PR+RP	72	74	86	111	177	130	55	173	158	84	171	151	120
Soil + GS+RP	75	81	79	117	151	193	143	189	151	154	169	146	137
Mean (Strains)	120	180	176										
LSD (P=0.05)	S*T	14.1											
Mean (Days)			138		175			177			142		
LSD (P=0.05)	(S*D)	10.7	(T*D)	16.3									
LSD (P=0.05)	(S*T*D)			28.3									
CV%				11									

RP = rock phosphate, Glu = glucose, N = NH_4Cl, OA = organic acid mixture (gluconic, citric and tartaric), RS = rice straw, RR=rice root, GS = Grass, PR = pigeonpea root; S = strains ,D = days

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Figure 4.9. Effect of different carbon sources on survival of S. *marcescens* EB 67 and *Pseudomonas* sp. CDB 35 in unsterilized soil, containing RP. Data presented are mean values of different days from three replicates and vertical lines are standard error of means. Gl = glucose, OA = organic acid mixture (gluconic, citric and tartaric), RS = rice straw, RR = rice root, G = Grass, PR = pigeonpea root.

Figure 4.10. Effect of different organic sources on phosphorous concentration in unsterilized soil containing RP inoculated with S. *marcescens* EB 67 and *Pseudomonas* sp. CDB 35. Data presented are mean values of different days in three replicated and vertical lines are standard error of means. Gl = glucose, OA = organic acid mixture (gluconic, citric and tartaric), RS = rice straw, RR = rice root, G = Grass, PR = pigeonpea root.

Figure 4.11. Effect of different organic sources on (a) acid phosphatase and (b) alkaline phosphatase in unsterilized soil containing RP inoculated *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35.Data presented are mean values of different days from three replicates and vertical lines are standard error of means. Gl = glucose, OA = organic acid mixture (gluconic, citric and tartaric), RS = rice straw, RR = rice root, G = Grass, PR = pigeonpea root.

Figure 4. 12. Effect of organic carbon sources on (a) soil respiration and (b) microbial biomass C in unsterilized soil containing RP inoculated with *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35. Data presented are mean values of different days from three replicates and vertical lines are standard error of means. Gl = glucose, OA = organic acid mixture (gluconic, citric and tartaric), RS = rice straw, RR = rice root, G = Grass, PR = pigeonpea root.

4.6 ANTAGONISTIC ACTIVITY OF SELECTED BACTERIAL ISOLATES

4.6.1 Selection of antagonistic bacteria

The results revealed that 18 of the 207 bacterial isolates reduced the mycelial growth of *S. rolfsii*, *M. phaseolina*, *F. solani* and *F. oxysporum* on three different media used in the study. Inhibition of the four fungi by the 18 strains was maximum on KB agar (63%) followed by GCY (49%) and PDA (46%) (Fig. 4.13). These experiments show varied fungal growth inhibition according to the tested strain and to the medium onto which it was grown (Photograph 4.11). CDB 35 showed green-yellow crystalline structures in plate culture conditions (Photograph 4.12). All the 18 bacterial isolates significantly (P=0.05) reduced the four soil-borne pathogenic fungi. Inhibition per cent on PDA<GCY<KB agar medium.
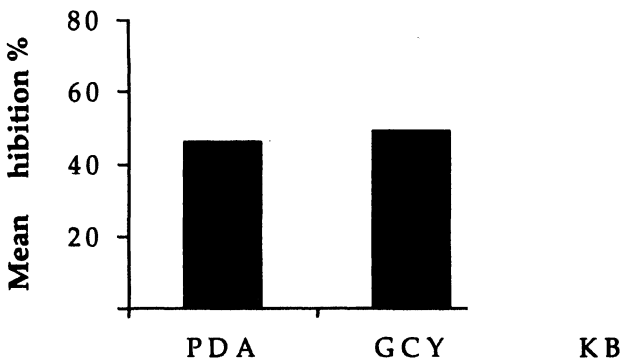
Figure 4.13. Mean inhibition per cent of four fungi by the eighteen bacterial isolates evaluated using PDA, GCY and KB agar medium

On PDA and GCY maximum inhibition against *S. rolfsii* (86 and 73%) was by isolate EB 13, against *M. phaseolina* (60 and 62%) (Photograph 4.13 and 4.14). , *F. solani* (66

On PDA and GCY maximum inhibition against *S. rolfsii* (86 and 73%) was by isolate EB 13, against *M. phaseolina* (60 and 62%) (Photograph 4.13 and 4.14). , *F. solani* (66 and 65%), *F. oxysporum* (71 and 70%) was by isolate CDB 35 (Table 4.24-4.25). On KB agar medium CDB 35 showed maximum inhibition against all four fungi (Table 4.26). On the overall mean basis, maximum suppression of fungi on three media was by the two bacterial isolates EB 13 and CDB 35. Per cent inhibition by EB 13 and CDB35 against *S. rolfsii* was 79 and 66%, *M. phaseolina* was 60 and 61%, *F. solani* was 63 and 70% and *F. oxysporum* was 58 and 76% respectively (Fig. 4.14). Interactions between the four fungi and three media were also significantly different.

4.6.2 Anti-fungal activity in GCY broth media

Of the four isolates EB 13, CDB 15, CDB 35 and CDB 47 used for testing antifungal activity by dual liquid culture method, CDB 35 showed significant (P=0.05) reduction of mycelium (dry biomass) over control. Biomass reduction against *S. rolfsii* with CDB 35 was 72% followed by EB 13 (64%), CDB 15 (58%) and CDB 47 (43%). Against *M. phaseolina* also CDB 35 showed maximum inhibition of 65%, followed by EB 13 (62%), CDB 15 (53%) and CDB 47 (45%). Biomass reduction against *F. solani* and *F. oxysporum* with CDB 35 (85 and 80%), EB 13 (68 and 65%), CDB 15 (62 and 55%) and CDB 47 (54 and 51%) (Fig. 4.15). In GCY medium, in presence of antagonistic strain *Pseudomonas* sp. CDB 35, the hyphal growth of *S. rolfsii* was observed to be backward rather than progressive. *B. licheniformis* EB 13 showed granulation in cytoplasm and vacuolization of cytoplasm of *M. phaseolina*. CDB 35 showed rupturing of sclerotia and EB 13 inhibited the sclerotial formation of *M. phaseolina* (Photograph 4.15).

Photograph 4 11 Antagonistic effect of *Pseudomonas* sp CDB 35 against *S rolfsii* on (a) PDA (b) GCY (c) KB agar media and (d) control (*S rolfsii*) on KBM

tograph 4.12. Needle shaped crystalline (antibiotic chlororaphin like) structures produced by CDB 35 on PDA medium
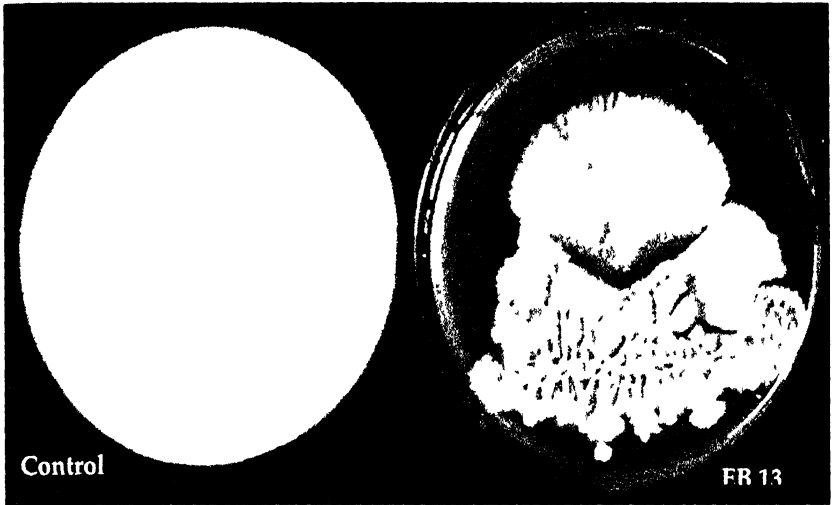
Photograph 4 13 Antagonistic effect of *Bacillus licheniformis* EB 13 against *S rolfsii* on KB agar medium
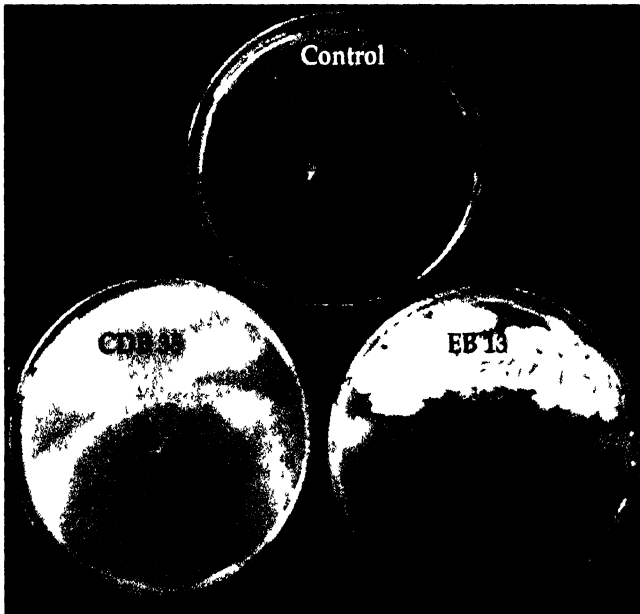
otograph 4 14. Antagonistic effect of *Pseudomonas* sp. CDB 35 and *Bacillus licheniformis* EB 13 against *M phaseolina* on KB agar medium

Table 4.24. Antagonistic activity of selected bacterial isolates against soil borne plant pathogenic fungi on potato dextrose agar (PDA) medium

Isolates	Per cent inhibition of			
	S. rolfsii	*M. phaseolina..*	*F. solani*	*F. oxysporum*
EB 3	53 (47)	45 (42)	42 (40)	58 (49)
EB 10	62 (52)	51 (45)	48 (44)	37 (38)
EB 13	**86 (68)**	**59 (50)**	**62 (52)**	**57 (49)**
EB 15	47 (43)	29 (36)	20 (27)	14 (22)
EB 48	76 (61)	41 (40)	29 (32)	66 (54)
CDB 6	38 (42)	45 (46)	52 (44)	48 (44)
CDB15	53 (47)	50 (45)	50 (45)	57 (49)
CDB 22	50 (45)	50 (45)	50 (45)	47 (43)
CDB 31	43 (41)	48 (44)	28 (32)	37 (37)
CDB 35	**54 (47)**	**60 (51)**	**66 (55)**	**71(58)**
CDB 36	36 (37)	33 (35)	52 (46)	54 (47)
CDB 47	47 (43)	42 (40)	40 (39)	57 (49)
BWB 21	22 (28)	38 (38)	44 (41)	46 (42)
BWB 32	54 (47)	35 (36)	32 (35)	32 (35)
BWB 34	62 (52)	35 (33)	28 (32)	20 (26)
BWB 36	37 (38)	46 (43)	46 (43)	45 (42)
BWB 40	38 (38)	50 (45)	39 (38)	34 (36)
BWB 41	53 (47)	35 (36)	50 (45)	50 (45)
LSD (5%)			3.7	
Mean	51	44	43	46
LSD (5%)			1.8	
CV%			5.1	

Data are means of three replicates from two independent experiments. Figures in parantheses are angular transformed values.

Table 4.25. Antagonistic activity of selective bacterial isolates against soil borne plant pathogenic fungi on Glucose casamino yeast extract (GCY) agar medium

Isolates	Per cent inhibition of			
	S. rolfsii	*M. phaseolina*	*F. solani*	*F. oxysporum*
EB 3	65 (54)	51 (45)	48 (44)	63 (52)
EB 10	70 (57)	62 (52)	50 (45)	52 (46)
EB 13	**73 (59)**	**58 (41)**	**58 (49)**	**63 (52)**
EB 15	37 (38)	53 (47)	50 (45)	55 (48)
EB 48	50 (45)	47 (43)	65 (53)	52 (46)
CDB 6	41 (40)	55 (48)	50 (45)	50 (45)
CDB15	62 (52)	53 (47)	65 (54)	55 (48)
CDB 22	58 (49)	56 (49)	52 (46)	57 (49)
CDB 31	37 (37)	47 (43)	50 (45)	50 (45)
CDB 35	**61 (52)**	**62 (52)**	**65 (54)**	**70 (57)**
CDB 36	42 (40)	41 (40)	50 (45)	45 (42)
CDB 47	37 (38)	41 (40)	50 (45)	50 (45)
BWB 21	36 (37)	27 (31)	45 (42)	48 (44)
BWB 32	59 (50)	37 (38)	40 (39)	34 (35)
BWB 34	37 (38)	16 (24)	50 (45)	45 (42)
BWB 36	31 (34)	18 (25)	48 (44)	43 (41)
BWB 40	37 (38)	23 (29)	50 (45)	48 (44)
BWB 41	51 (45)	39 (38)	43 (41)	52 (46)
LSD (P = 0.05)			1.9	
Mean	49	44	52	52
LSD (P = 0.05)			0.95	
CV%			2.3	

Data are means of three replicates from two independent experiments. Figures in parenthesis are angular transformed values.

Table 4.26. Antagonistic activity of selective bacterial isolates againstsoil borne plant pathogenic fungi on Kings B (KB) agar medium

Isolates	Per cent inhibition of			
	S. rolfsii	*M. phaseolina*	*F. solani*	*F. oxysporum*
EB 3	74 (51)	56 (49)	49 (44)	65 (54)
EB 10	75 (60)	61 (53)	57 (49)	57 (49)
EB 13	**81 (64)**	**62 (52)**	**70 (57)**	**79 (62)**
EB 15	75 (60)	35 (36)	35 (36)	30 (33)
EB 48	75 (60) .	53 (47)	37 (38)	75 (60)
CDB 6	57 (49)	58 (49)	60 (51)	65 (54)
CDB15	71 (57)	56 (49)	57 (49)	57 (49)
CDB 22	66 (54)	62 (52)	57 (49)	57 (49)
CDB 31	44 (42)	41 (40)	45 (42)	65 (54)
CDB 35	**84 (65)**	**65 (51)**	**79 (63)**	**87 (69)**
CDB 36	81 (64)	49 (44)	74 (59)	81 (64)
CDB 47	72 (58)	52 (46)	53 (47)	70 (57)
BWB 21	76 (60)	47 (43)	79 (63)	54 (47)
BWB 34	81 (64)	47 (43)	75 (60)	70 (57)
BWB 36	78 (64)	50 (45)	73 (59)	55 (47)
BWB 40	61 (52)	43 (41)	63 (53)	78 (62)
BWB 41	75 (60)	41 (40)	38 (38)	57 (49)
LSD (P=0.05)			5.7	
Mean	72	52	59	65
LSD (P=0.05)			1.4	
CV%			5.8	

Data are means of three replicates from two independent experiments. Figures in parenthesis are angular transformed values.
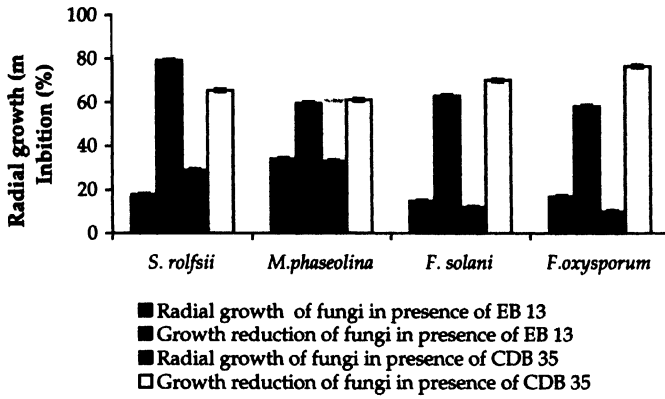
Figure 4.14. Effect of antagonistic bacterial isolates EB 13 and CDB 35 on radial growth and per cent inhibition (growth reduction over control) of *S. rolfsii, M. phaseolina, F. solani* and *F. oxysporum*. Data presented are means of three media.
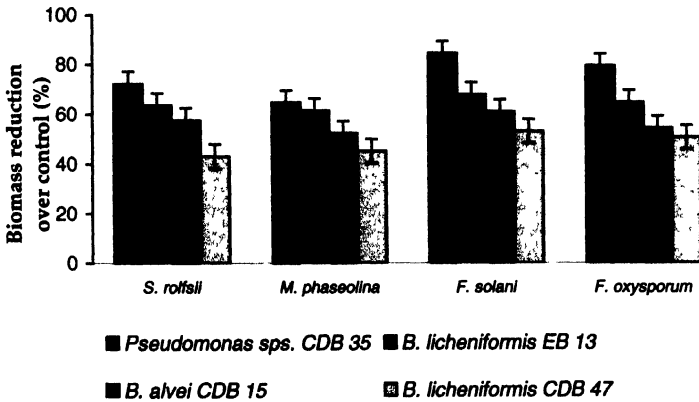
Figure 4.15. Biomass reduction (percentage) of four soil borne plant pathogenic fungi by selective bacterial isolates (EB 13, CDB 15, CDB 35 and CDB 47) in GCY broth medium
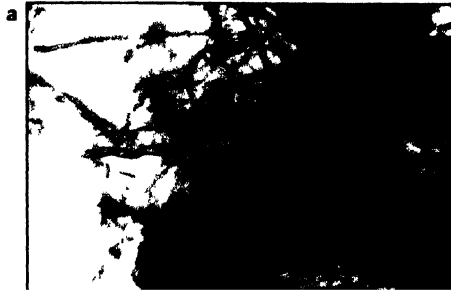
Photograph 4.15. Microscopic observation: Sclerotia of *M. phaseolina* (a) control (b) *Bacillus licheniformis* EB 13 inhibiting sclerotial development and (c) *Pseudomonas* sp. CDB 35 rupturing sclerotia in GCY broth medium

4.6.3 Effect of volatile compounds of bacteria against *S. rolfsii* and *M. phaseolina*

The results of experiment on effect of volatile metabolites produced by bacteria against *S. rolfsii* and *M. phaseolina* on GCY agar medium showed that only six (EB 13, EB 15, CDB 15, CDB 35, CDB 36 and BWB 21) of the eighteen bacterial isolates suppressed the growth of *S. rolfsii* significantly. Reduction in mycelial growth of *S. rolfsi* was highest with isolate BWB 21 (73%) followed by CDB 35 and CDB 36 (53%), CDB 15 (52%), EB 13 (51%) and EB 15 (49%) (Fig. 4.16). None of the bacterial isolates reduced the mycelial growth of *M. phaseolina* due to volatile compounds, however there was variation in pigment production. Reduction in mycelium due to volatile metabolites of two potential antagonistic bacteria EB 13 and CDB 35 is shown in Photograph (4.16).
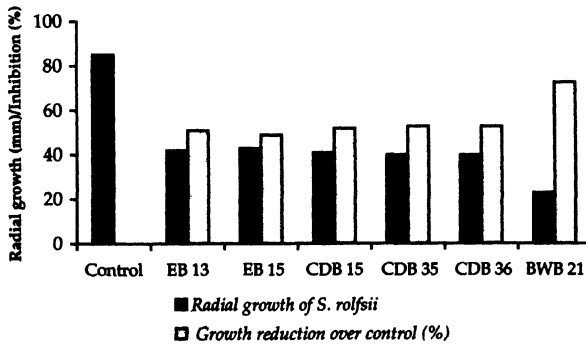
Figure 4.16. Radial growth and inhibition per cent of bacterial volatile compounds against *S. rolfsii* in GCY media

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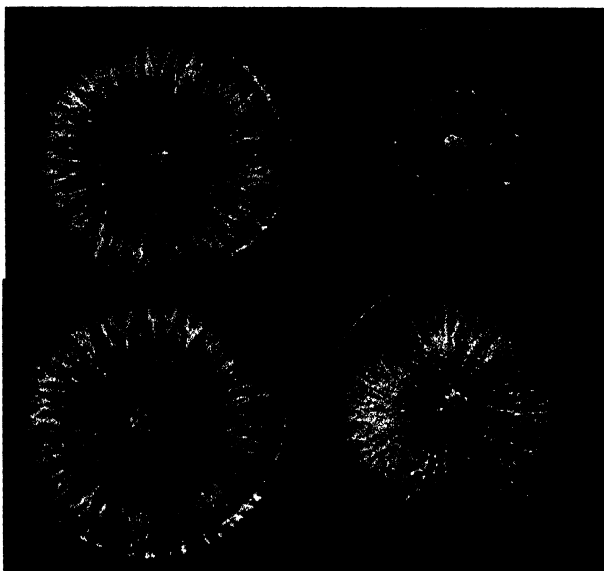
Photograph 4.16. Volatile metabolites produced by *B. licheniformis* EB 13 and *Pseudomonas* sp. CDB 35 that suppressed the growth of *S. rolfsii* in GCY medium.

4.6.4 Inhibition of fungi in presence of mineral nutrients

Both the isolates EB 13 and CDB 35 were tested in presence of mineral nutrients for suppression of *S. rolfsii* and *M. phaseolina*. Addition of zinc sulphate to PDA reduced the mycelial growth of test fungi comparatively more than PDA alone. Per cent inhibition of *M. phaseolina* with *B. licheniformis* EB 13 was maximum when PDA was amended with Zn (67%) followed by mixture of salts (65%), Mg (47%), NaCl (42%) and Fe (41%) (Fig. 4.17). *Pseudomonas* sp. CDB 35 showed maximum suppression when PDA was amended with Zn (72%) followed by Fe (53%), Mg (43%) and NaCl and mixture (41%) (Fig. 4.18). Inhibition rate of fungi was increased 5-7% more with I B 13 and 8-12% with CDB 35 in presence of zinc (Fig. 4.17 & 4.18).

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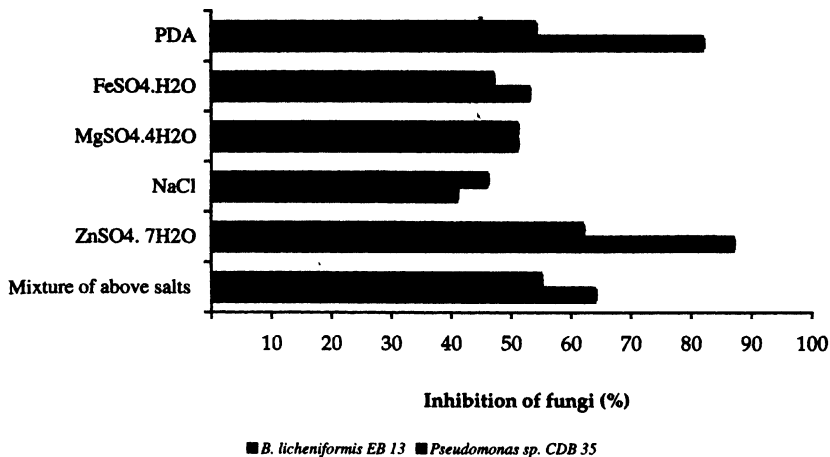
Figure 4.17. Influence of mineral nutrients amended onto PDA as measured by percent inhibition (growth reduction over control) of *S. rolfsii* with antagonistic strains *B. licheniformis* EB 13 and *Pseudomonas* sp. CDB 35. Standard errors of means are shown as vertical lines.
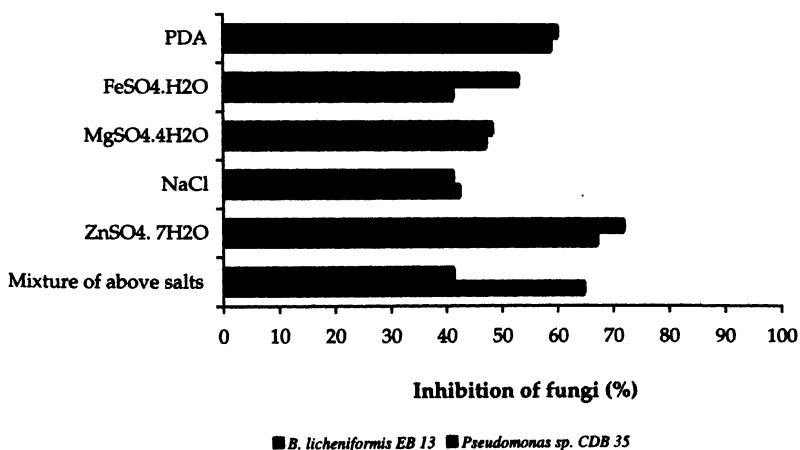
Figure 4.18. Influence of mineral nutrients amended onto PDA as measured by percent inhibition (growth reduction over control) of *M. phaseolina* with antagonistic strains *B. licheniformis* EB 13 and *Pseudomonas* sp. CDB 35. Standard errors of means are shown as vertical lines.

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4.7 PLANT GROWTH PROMOTING ACTIVITY OF SELECTED BACTERIAL ISOLATES IN GLASSHOUSE CONDITIONS

Twelve isolates that promoted seedling emergence and germination per cent of pearl millet and sorghum in *in vitro* (paper towel) conditions were evaluated in natural (soil) conditions for plant growth activity. When tested in sterilized soil conditions, significant improvement in plant growth was with six bacterial isolates on pearl millet and two bacterial isolates on sorghum growth in repeated experiments. In unsterilized soil conditions, ten isolates showed significant improvement in plant growth of pearl millet and sorghum in repeated experiments.

4.7.1 Effect of seed treatment with bacteria on plant growth of pearl millet

Twelve bacterial isolates were selected for plant growth studies and were evaluated in a pot experiment using sterilized and unsterilized soil under glasshouse conditions (Table 4.27 and 4.28). Six of the 12 isolates significantly ($P=0.05$) increased the plant dry weight (biomass), leaf area, shoot and root length in repeated experiments using sterilized soil. Maximum increase in root length (61% over control) and dry biomass (52% over control) was observed with *Pseudomonas* sp. CDB 35 isolated from RSC followed by *Serratia marcescens* EB 67 (60% increase in root length and 50% in plant dry biomass) isolated from macrofauna (Table 4.27). Out of the twelve bacterial isolates evaluated using unsterilized soil, ten isolates significantly ($P=0.05$) improved the shoot length, root length and leaf area following seed treatment in repeated experiments. All the bacterial isolates producing ACC deaminase (except EB 75 and BWB 36) showed significant increase in root length and increase in root length density ranged from 22-71% over control. Ten bacterial

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isolates significantly improved the plant dry weight (biomass). The maximum increase in biomass was observed with *S. marcescens* EB 67 (56%), followed by *Pseudomonas* sp. CDB 35 (52%) and *B. circulans* EB 35 (42%) (Table 4.28; Photograph 4.17). Most of the bacterial isolates tested for plant growth activities were at par or greater than *A. chroococcum* HT 54 (reference strain) used in the study. Few of the bacterial isolates (*B. circulans* EB 35, *S. marcescens* EB 67 and *B. licheniformis* CDB 47) that did not produce auxin *in vitro* were as effective as auxin producers in promotion of root growth. Three bacterial isolates *B. licheniformis* EB 13, *Pseudomonas* CDB 36 and *Pseudomonas* BWB 40 showed increase in plant dry weight over control comparatively better in unsterilized soil conditions. Growth performance by rest of the isolates was more or less similar in both sterilized and unsterilized soil conditions. Interestingly, all the inoculated treatments except *Serratia* sp. EB 75 and *Pseudomonas* sp. BWB 36, showed significant increase in plant dry biomass.

4.7.2 Effect of seed treatment with bacteria on plant growth of sorghum

Seed treatment with peat-based inoculum of 12 bacterial isolates increased seedling emergence, shoot and root length and dry weight of sorghum. Plant biomass was significantly greater with *S. marcescens* EB 67 (49%) and *Pseudomonas* sp. CDB 35 (43%) using sterilized soil (Table 4.29). Using unsterilized soil, eleven bacterial isolates significantly improved growth parameters involved in the study (Table 4.30). *S. marcescens* EB 67 showed 74% and *Pseudomonas* sp. CDB 35 showed 70% increase in plant dry biomass of sorghum (Photograph 4.18) when compared to control.
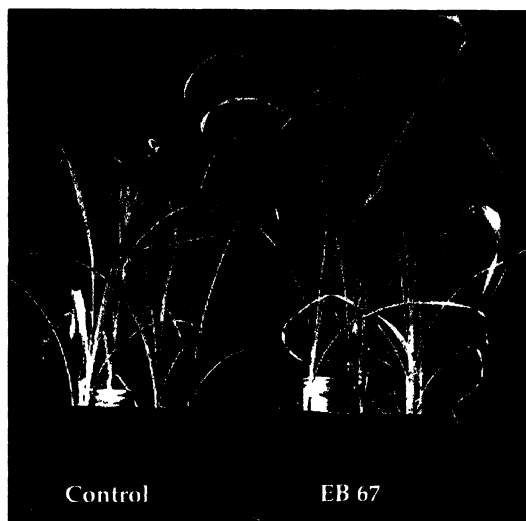
Photograph 4.17. Growth of pearl millet (ICMV 155) inoculated with *Serratia marcescens* EB 67 and *Pseudomonas* sp. CDB 35 in pots having unsterilized soil. Control=uninoculated.

Photograph 4.18.   Growth of sorghum (CSV 15) inoculated with *Serratia marcescens* EB 67 and *Pseudomonas* sp. CDB 35 in pots having unsterilized soil. Control=uninoculated.

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Photograph 4.18.   Growth of sorghum (CSV 15) inoculated with *Serratia marcescens*
EB 67 and *Pseudomonas* sp. CDB 35 in pots having unsterilized
soil. Control=uninoculated.

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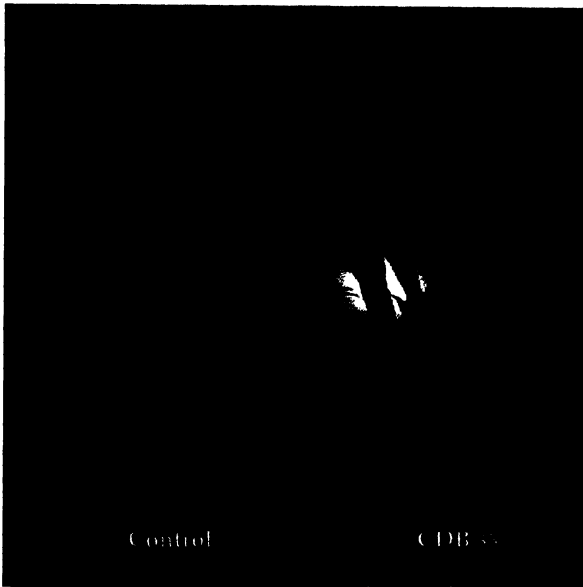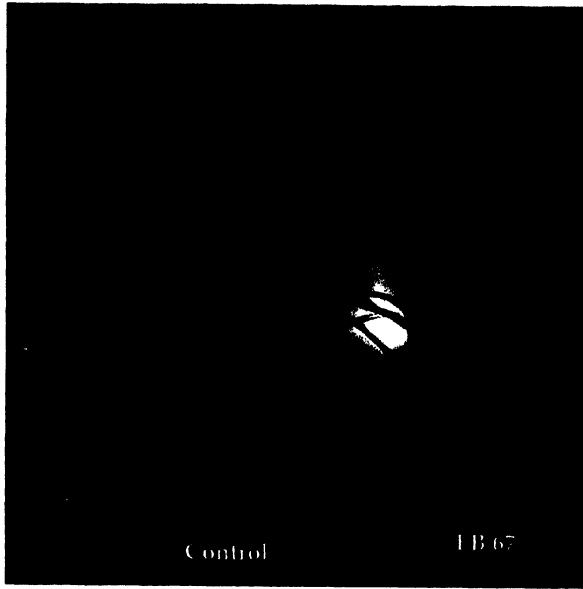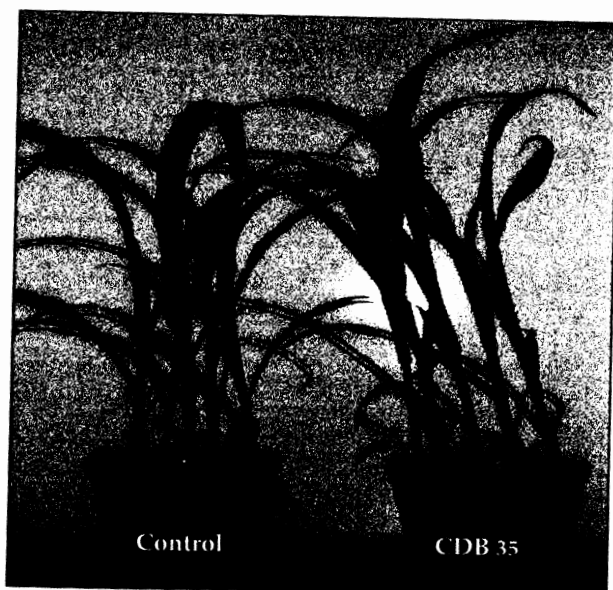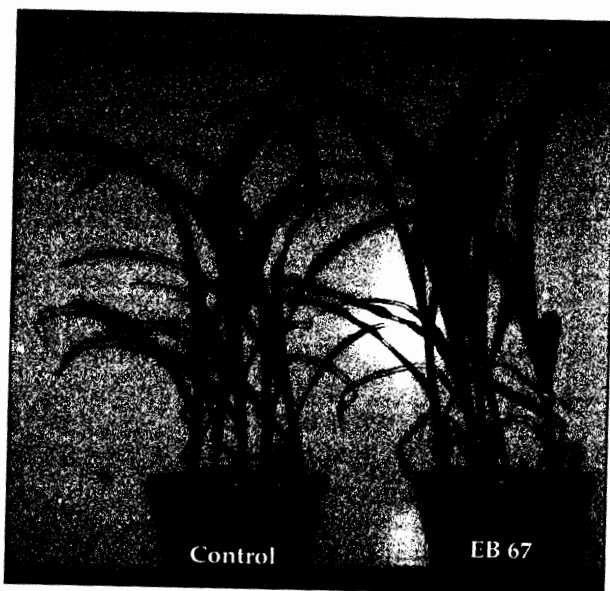
Table 4.27. Effect of seed treatment with bacteria from composts and macrofauna on growth of pearl millet (ICMV 155) in pots having sterilized soil

| Isolate*	Shoot length (cm)	Leaf area (cm²)	Root length density (cm cm⁻³ soil)	Dry weight (mg)
Control (uninoculated)	45 (0)	50 (0)	0.94 (0)	681 (0)
A.chroococcum HT-54	48	52	1.27 (35) [a]	795
B. licheniformis EB 13	47	55	1.23 (31) [a]	762
E. cloacae EB 27	52 (14) [a]	52	1.15 (22) [a]	862
B.circulans EB 35	54 (19) [a]	55	1.33 (41) [a]	975 (43) [a]
S. marcescens EB 67	51 (12) [a]	52	1.51 (60) [a]	1018 (50) [ac]
Serratia sp.EB 75	49	55	1.12	874 (28) [a]
K. oxytoca EB 77	53 (17) [ac]	52	1.46 (55) [a]	894 (31) [a]
Pseudomonas sp. CDB 35	53 (17) [ac]	53	1.52 (61) [ac]	1035 (52) [ac]
Pseudomonas sp. CDB 36	52 (12) [a]	52	1.13	818
B.licheniformis CDB 47	54 (19) [ac]	50	1.25 (33) [a]	881 (30) [a]
Pseudomonas sp. BWB 21	42	52	1.30 (38) [a]	871
Pseudomonas sp. BWB 36	54 (19) [ac]	50	1.01	691
Pseudomonas sp. BWB 40	45	52	1.11	819
Mean	50	52	1.24	855
LSD (P = 0.05)	5.2	10.6	0.25	193.3
CV (%)	6.3	12	11.9	13

Values are means of two experiments with three replications and data calculated per plant.
[a] Values are significant than control (uninoculated).
[ac] Values are significant than control (uninoculated) and positive strain (A.chroococcum HT54).
Values in parentheses are per cent increase over control.

Table 4.28. Effect of seed treatment with bacteria from composts and macrofauna on growth of pearl millet (ICMV 155) in pots having unsterilized soil

Isolate*	Shoot length (cm)	Leaf area (cm²)	Root length density (cm cm⁻³ soil)	Plant dry weight (mg)
Control (uninoculated)	39 (0)	33 (0)	0.99 (0)	709 (0)
A.chroococcum HT-54	45 (15) [a]	42 [a]	1.23 (24) [a]	965 (36) [a]
B. licheniformis EB 13	47 (20) [a]	45 (36) [a]	1.30 (31) [a]	913 (29) [a]
E. cloacae EB 27	43	41	1.24 (25) [a]	908 (28) [a]
B.circulans EB 35	49 (25) [a]	52 (58) [a]	1.42 (43) [ac]	**1008 (42)** [a]
S. marcescens EB 67	47 (20) [a]	51 (55) [a]	1.70 (71) [ac]	**1112 (56)** [a]
Serratia sp.EB 75	44	48 (45) [a]	1.05	770
K. oxytoca EB 77	41	46 (39) [a]	1.59 (61) [ac]	910 (28) [a]
Pseudomonas sp. CDB 35	49 (25) [a]	48 (45) [a]	1.60 (62) [ac]	**1082 (52)** [a]
Pseudomonas sp. CDB 36	46 (18) [a]	51 (54) [a]	1.48 (49) [ac]	941 (32) [a]
B.licheniformis CDB 47	52 (33) [ac]	50 (51) [a]	1.34 (35)	939 (32) [a]
Pseudomonas sp. BWB 21	48 (23) [a]	53 (61) [ac]	1.50 (51) [ac]	925 (30) [a]
Pseudomonas sp. BWB 36	47 (20) [a]	34	1.01	710
Pseudomonas sp. BWB 40	52 (33) [ac]	49 (48) [a]	1.21 (22) [a]	940 (32) [a]
Mean	46	46	1.334	921
LSD (P = 0.05)	5.5	11.4	0.174	159.8
CV%	7.1	14.8	7.8	10

Values are means of two experiments with three replications and data calculated per plant.
[a] Values are significant than control (uninoculated).
[ac] Values are significant than control (uninoculated) and positive strain (*A.chroococcum* HT54). Values in parentheses are per cent increase over control.

Table 4.29. Effect of seed treatment with bacteria from composts and macrofauna on growth of sorghum cultivar CSV 15 in pots having sterilized soil

Isolate*	Shoot length (cm)	Leaf area (cm²)	Root length density (cm cm⁻³)	Dry weight (mg)
Control	53	58	0.96	732
A.chroococcum HT-54	57	88 (51) a	1.43 (49) a	1034
B. licheniformis EB 13	56	88 (51) a	1.48 (53) a	981
E. cloacae EB 27	56	86 (48)	1.26 (31) a	937
B. circulans EB 35	58	79 (36)	1.45 (51) a	1017
S. marcescens EB 67	61	86 (48) a	1.61 (67) a	1094 (49) a
Serratia sp. EB 75	52	60	1.05	842
K. oxytoca EB 77	60	76	1.60 (67) a	949
Pseudomonas sp. CDB 35	57	80 (38)	1.58 (64) a	1045 (43) a
Pseudomonas sp. CDB 36	57	73	1.06	903
B.licheniformis CDB 47	56	72	1.56 (62) a	979
Pseudomonas sp. BWB 21	58	74	1.33 (38) a	1007
Pseudomonas sp. BWB 36	55	60	1.04	757
Pseudomonas sp. BWB 40	51	53	1.06	857
Mean	56	74	1.32	938
LSD (P = 0.05)	10.1	23.7	0.20	312
CV%	11	19	9.2	20

Values are means of two experiments with three replications and data calculated per plant.
a Values are significant than the control treatment. Values in parentheses are per cent increase over control.

Table 4.30. Effect of seed treatment with bacteria from composts and macrofauna on growth of sorghum cultivar CSV 15 in pots having unsterilized soil

Isolate*	oot length (cm)	Leaf area (cm²)	Root length density (cm cm⁻³)	Dry weigh (mg)
Control	52	58	0.99	635
A.chroococcum HT-54	57 (9) [a]	73 (26) [a]	1.47 (48) [a]	1039 (63) [a]
B. licheniformis EB 13	55	72 (24) [a]	1.37 (38) [a]	1051 (65) [a]
E. cloacae EB 27	55	74 (27) [a]	1.53 (55) [a]	962 (51) [a]
B.circulans EB 35	59 (13) [a]	80 (38) [a]	1.61 (63) [ac]	**1062 (66)** [a]
S. marcescens EB 67	61 (17) [a]	83 (43) [ac]	1.63 (64) [ac]	**1111 (74)** [a]
Serratia sp.EB 75	54	57	1.49 (50) [a]	859 (35) [a]
K. oxytoca EB 77	58 (11) [a]	76 (31) [a]	1.61 (62) [ac]	993 (56) [a]
Pseudomonas sp. CDB 35	58 (11) [a]	81 (39) [ac]	1.62 (63) [ac]	**1083 (70)** [a]
Pseudomonas sp. CDB 36	56	71 (22) [a]	1.10	883 (39) [a]
B.licheniformis CDB 47	56	73 (26) [a]	1.62 (63) [ac]	997 (57) [a]
Pseudomonas sp. BWB 21	57 (9) [a]	72 (24) [a]	1.63 (65) [ac]	987 (55) [a]
Pseudomonas sp. BWB 36	48	50	0.98	688
Pseudomonas sp. BWB 40	50	59	1.08	791 (24) [a]
Mean	56	70	1.41	939
LSD (P = 0.05)	5.4	8.5	0.14	110.3
CV%	6	7	6	7

Values are means of two experiments with three replications and data calculated per plant.
[a] Values are significant than control (uninoculated).
[ac] Values are significant than control (uninoculated) and positive strain (*A.chroococcum* HT54). Values in parentheses are per cent increase over control.

1.7.3 Phase contrast microscopic observations

Selective bacterial isolates were observed under phase contrast microscopy for microscopic characterization. Gram positive isolates EB 13 and EB 35 showed both vegetative and sporulating stage. Gram-negative isolates EB 67 and CDB 35 were short rods (Photograph 4.19).
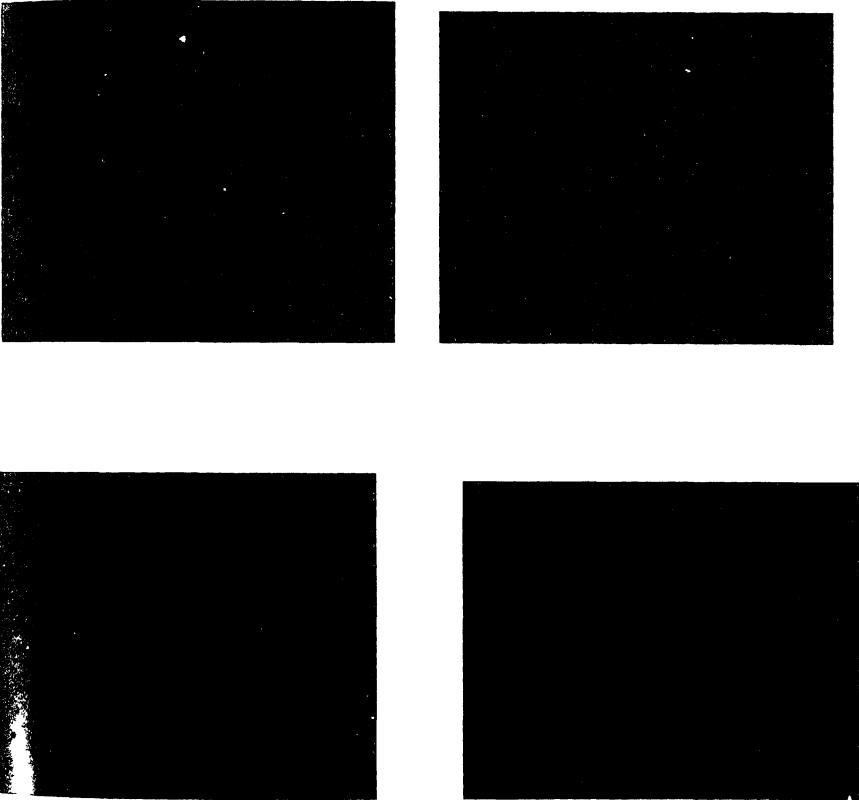
Photograph 4.19. Phase contrast microscopic image (1000x) (a) *B. licheniformis* EB 13 (b) *B. circulans* EB 35, (c) *S. marcescens* EB 67 (d)*Pseudomonas* sp. CDB 35

4.8 INTERACTION STUDIES

4.8.1 Compatibility of PSB with *Rhizobium* (plate culture conditions)

All the twelve bacterial isolates studied for plant growth (Table 4.27-4.30) were tested for their interaction with the rhizobial strains of chickpea, groundnut and pigeonpea. Their interaction with *Rhizobium* was measured as interacting distance and their growth away from *Rhizobium* was measured as spreading capacity (Table 4.31).

Table 4.31. Interaction between the different plant growth promoting bacteria and *Rhizobium* strains

Isolates	Spreading capacity (mm) of plant growth promoting bacteria (away from rhizobia)						Interaction distance (mm) of plant growth promoting bacteria with rhizobia					
	IC 59	IC 76	IC 3100	IC 4060	IC 7114	Mean	IC 59	IC 76	IC 3100	IC 4060	IC 7114	Mean
EB 13	11	11	9	9	11	10	8	8	5	8	14	9
EB 27	5	4	3	3	4	4	5	5	4	4	3	4
EB 35	14	11	7	11	11	11	12	8	11	9	9	10
EB 67	11	11	9	14	9	10	8	8	7	7	10	8
EB 75	6	6	6	6	7	6	4	4	5	3	6	4
EB 77	5	5	6	5	4	5	4	4	5	3	6	4
CDB 35	23	19	20	19	20	20	26	25	11	14	14	18
CDB 36	11	10	10	11	11	11	8	4	5	5	13	7
CDB 47	4	4	4	4	4	4	4	5	3	3	4	4
BWB 21	19	19	18	19	13	17	24	27	23	20	15	21
BWB 36	6	6	6	6	6	6	5	4	3	3	7	4
BWB 40	7	7	7	7	5	6	5	5	4	5	6	5
Mean	10	9	9	9	9	9	9					

LSD (P=0.05)   1.4
CV%   3

IC (Strain number given for *Rhizobium* isolates from ICRISAT)

Analysis of the data on "interaction distance" and "spreading capacity" indicated existence of three clusters (Fig. 4.19). A dendogram based on UPGMA is obtained for the 12 isolates with Euclidean measure as a criteria for obtaining the distance matric. Isolates CDB 35 and BWB 21 with their maximum interaction with the tested rhizobial isolates were grouped into one cluster, followed by isolates EB 13, EB 35 and CDB 47 with their medium interaction were grouped into the second cluster. Rest of the isolates (EB 27, EB 67, EB 75, EB 77. CDB 36, BWB 36 and BWB 40) formed the third cluster. However, there was no apparent sign of suppression of rhizobia by any of the twelve PGPB that were studied.
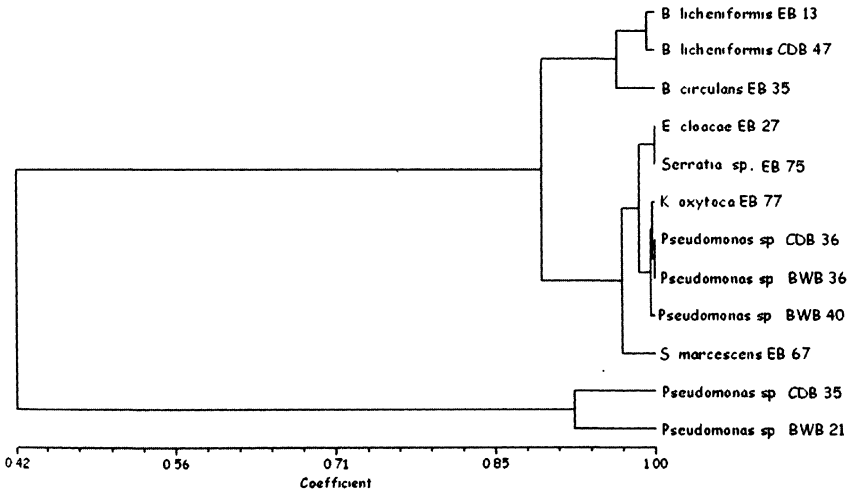
Figure 4.19. Clustering of potential plant growth promoting bacterial isolates based on interaction with *Rhizobium* spp. (IC 59, IC 76, IC 3100, IC 4060 and IC 7114).

4.8.2 Dual-inoculation of potential bacteria with *Rhizobium* and Mycorrhizae

The two PSB EB 67 and CDB 35 were compatible with the rhizobial strains (IC 59, IC 76, IC 3100, IC 4160 and IC 7114) in plate culture conditions. *Rhizobium* IC 59 (that

nodulates chickpea) was selected and evaluated along with two PSB EB 67 and CDB 35 to see their dual effect on plant growth under glasshouse conditions with chickpea as host plant (Table 4.32). Dry matter of the plant was significant in chemical treatment and wherever bacteria were applied.

Table 4.32. Effect of dual inoculation of PSB with *Rhizobium* on chickpea (ICCV 2) growth, nodulation and nitrogenase activity

Treatments	No. of nodules	Dry weight (mg)	Nodule wt (mg)	Nitrogenase activity μM of C_2H_4 plant^{-1}h^{-1}
Control + SC	7	529	15	1.3
Control + SP	7	741	15	1.5
SSP + SC	15	577 (9)	27	3.4
SSP + SP	13	692	39	3.8
IC 59 + SC	14	817 (54)[a]	35	4.7
IC 59 + SP	16	889 (20) [a]	34	5.3
RP + SC	14	721 (36)	26	3.4
RP + SP	14	769 (4) [a]	37	3.9
IC 59 RP + SC	19	867 (64) [a]	35	3.9
IC 59 RP + SP	20	907 (22) [a]	34	4.4
IC 59 + EB 67 RP + SC	20	984 (86) [ac]	37	5.6
IC 59 + EB 67 RP + SP	21	1048 (41) [a]	38	6.8
IC 59 + CDB 35 RP + SC	25	939 (78) [a]	45	4.8
IC 59 + CDB 35 RP + SP	28	989 (33) [ac]	51	4.9
Mean	17	819	33	4.1
LSD	8.5	216	12.4	3.45
CV%	30	16	22	40

IC = Strain name of rhizobia used in the study.
SC = Seed coating, SP = Seed priming, SSP = Single super phosphate, RP = Rock phosphate,
Values are means of three replications and data are per plant
Values in parentheses are per cent increase over control by the respective treatments SC/SP.
[a]Values are significant than control by SC and method
[ac]Values are significant than control by SC and SP method

Biopriming with *Rhizobium* and PSB increased the dry matter (plant biomass) and significant difference was with EB 67 (41% over control) followed by CDB 35 (33% over control). Nodule number and weight was significant with CDB 35. Nitrogenase activity was significantly greater with biopriming of EB 67 (6.8 µM of C_2H_4 plant^{-1}h^{-1}) followed with EB 67 applied as seed coat (5.6 µM of C_2H_4 plant^{-1}h^{-1}) (Table 4.32). Nitrogenase activity did not vary much with CDB 35 by seed coating and priming methods.

4.8.3 Effect of dual inoculation PGPB with Mycorrhizae (*Glomus* spp.) on growth and AM colonization in sorghum root in glasshouse conditions

Compatibility of potential plant growth promoting bacteria (*B. circulans* EB 35, *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35) with mycorrhizae (*Glomus* spp.) was studied on sorghum (ICSV 93046) in pots using unsterilizied soil of BR1D field. Seed treatment with all three isolates showed significant increase in growth parameters at first harvest (45 DAS) when compared to the uninoculated control. *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 applied along with AM and RP showed significant difference in shoot length (36-39%), leaf area (10-14%), dry weight (18-20%), root volume (53-70%) compared to AM+RP application. AM colonization in sorghum root with CDB 35 and EB 67 was 27 to 35 % more (Table 4.33) compared AM+RP treatment (Photograph 4.20 to 4.22). *B. circulans* EB 35 applied alone did not show improvement in plant biomass AM colonization in sorghum root.

Photograph 4.20. Mycorrhizal colonization in sorghum root where only AM (*Glom* spp.) was inoculated in pots having unsterilized soil.
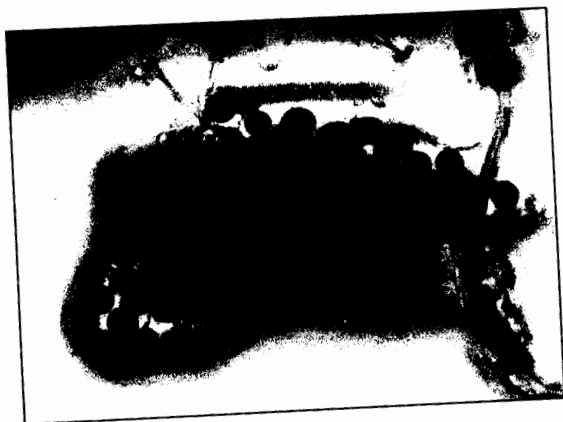
Photograph 4.21. Mycorrhizal colonization in sorghum root where AM (*Glomus* spp.) + *S. marcescens* EB 67 was inoculated in pots having unsterilized soil.

Photograph 4.22. Mycorrhizal colonization in sorghum root where AM (*Glomus* spp.) + *Pseudomonas* sp. CDB 35 was inoculated in pots having unsterilized soil.

A further increment in most of the growth parameters studied was also noticed after second harvest (90 days) and was significantly different than control and AM+RP treatment. Increase in shoot length was 2-3%, leaf area 19-25%, dry weight 11-19%, root volume 85-129% by *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35. Both the PGPB applied in presence of RP increased AM colonization by 19 to 32 % more than the treatment where AM and RP was applied. Application of *B. circulans* EB 35 alone and in combination with AM did not show significant increase in plant dry weight, root volume and AM colonization (Table 4.34).

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Table 4.33. Effect of dual inoculation of bacteria and mycrorrhizae on growth of sorghum ICSV (93046) (sweet stalk) and mycorrhizal association in sorghum roots (45 days after sowing)

Treatments	Spad reading	Shoot length (cm)	Leaf area (cm^2)	Dry weight (g)	Root volume (mL)	AM colonization (%)
Control	27	64	122	1.2	0.6	8
AM	34	84 [a]	413 [a]	5.2 [a]	2.8 [a]	15 [a]
RP	31	78 [a]	392 [a]	4.7 [a]	2.7 [a]	14
AM + RP	34	64 [a]	418 [a]	5.4 [a]	3.0 [a]	21 [a]
B. circulans EB 35	32	84 (31) [a]	431 (3) [a]	5.8 (7) [a]	3.4 (13) [a]	17 [a]
B. circulans EB 35 + AM RP	33	87 (36) [a]	460 (10) [a]	6.3 (17) [ac]	3.8 (27) [a]	46 [ac]
S. marcescens EB 67	33	87 (36) [a]	456 (9) [a]	6.1 (13) [a]	4.2 (40) [ac]	44 [ac]
S. marcescens EB 67 + AM RP	32	89 (39) [ac]	478 (14) [a]	6.4 (18) [ac]	5.1 (70) [ac]	56 [ac]
Pseudomonas sp. CDB 35	33	83 (30) [a]	453 (8) [a]	6 (11) [a]	4.1 (37) [ac]	42 [ac]
Pseudomonas sp. CDB 35 + AM RP	33	87 (36) [a]	461 (10) [a]	6.5 (20) [a]	4.6 (53) [ac]	48 [ac]
Mean	32	83	406	5.3	3.2	29
LSD	3.2	3.5	93.1	1.03	1.0	6.7
CV%	6	11	13	11	19	14

Values are means of three replications and data calculated per plant.
AM = arbascular mycorrhizae (*Glomus* spp.)
N = 40 kg h^{-1} (applied twice, during sowing and 45 days after sowing) for all the treatment except control), P = 20 kg h^{-1} RP-rock phosphate wherever mentioned).
[a]Values are significant than uninoculated control
[ac]Values are significant than AM+RP treatment.
Values in parentheses are per cent increase over AM+RP treatment.

Table 4.34. Effect of dual inoculation of bacteria and mycrorrhizae on growth of sorghum (ICSV 93046) (sweet stalk) and mycorrhizal association in sorghum roots (90 days after sowing)

Treatments	SPAD reading	Leaf area (cm^2)	Shoot length (cm)	Dry weight (g)	Root volume (mL)	AM coloniza (%)
Control	28	85	269	8	4.9	13
RP	28	120	403 [a]	17 [a]	7	23
AM	27	115	453 [a]	17 [a]	8.3	27 [a]
AM RP	28	124	481 [a]	18 [a]	8.2	41 [ac]
B.circulans EB 35	34	109	516 (7) [ac]	19 (4) [a]	11.7 (43) [a]	31 [a]
B.circulans EB35 + AM RP	36	122	567 (18) [ac]	19(5) [a]	14 (71) [a]	50 [a]
S. marcescens EB 67	38	111	561 (17) [ac]	20 (9) [ac]	14.6 (78) [a]	55 [ac]
S. marcescens EB67 + AM RP	41	128 (3)	600 (25) [ac]	22 (19) [ac]	18.8 (129) [a]	74 [ac]
Pseudomonas sp. CDB 35	36	112	555 (15) [a]	19 (5) [a]	14 (71) [a]	53 [ac]
Pseudomonas sp. CDB35 + AM RP	40	127 (2)	573 (19) [ac]	20 (11) [ac]	15.2 (85) [a]	60 [ac]
Mean	33	490	115	18	11	41
LSD	3.7	57.6	16.2	2.3	4.8	11.6
CV%	6	7	8	8	25	17

Values are means of three replications and data calculated per plant after 90 days of sowing.
AM = arbascular mycorrhizae (*Glomus* spp.).
N = 40 kg h^{-1} (applied twice, during sowing and 45 days after sowing) for all the treatments except control), P = 20kg h^{-1} RP-rock phosphate wherever mentioned).
[a]Values are significant than uninoculated control
[ac]Values are significant than AM+RP treatment.
Values in parentheses are per cent increase over AM+RP treatment.

4.9 BIOCONTROL ABILITY OF TWO SELECTED ANTAGONISTIC BACTERIA

4.9.1 Efficacy of *B. licheniformis* EB 13 and *Pseudomonas* sp. CDB 35 against *S. rolfsii*

The two antagonistic isolates EB 13 and CDB 3 were evaluated against *S.rolfsii* in glasshouse conditions by seed biopriming and seed coating method. It was observed that inoculant applied by seed priming was better than seed coating for few parameters characterized. Per cent germination observed was similar with captan (53%) and CDB 35 (53%) application. EB 13 did not show significant difference either in germination per cent (33%) or plant biomass. Captan and CDB 35 showed significant (P=0.05) difference of plant biomass applied as seed priming. EB 13 could reduce the disease incidence up to 47% and CDB 35 up to 60% (Table 4.35). However, after harvesting, nodule formation in chickpea root and nodule weight was better in CDB 35 and EB 13 when applied by seed priming method (Photograph 4.23).

4.9.2 Effect of zinc and/or glucose on the efficacy of *B. licheniformis* EB 13 and *Pseudomonas* sp. CDB 35 against *M. phaseolina* in sorghum roots

Soil amendment with zinc sulphate or glucose without the bacterial inoculants did not affect root infection caused by *M.phaseolina*. When amendments were applied along with the bacteria, there was reduction in pathogen infection (P=0.05) (Table 4.36). Shoot weight of sorghum increased when both the isolates were applied along with zinc (Photograph 4.24). Zinc applied along with the *Pseudomonas* sp. CDB 35 and *B. licheniformis* EB 13 separately gave the best control of *M.phaseolina* sorghum root infection (Photograph 4.25). Zinc and glucose applied without the bacterial

inoculants also markedly reduced the *M.phaseolina* infection. When the efficacy of two antagonistic bacteria was compared, *Pseudomonas* sp. CDB 35 caused greater suppression of *M. phaseolina* sorghum root infection than *B. licheniformis* EB 13. Soil amendments with zinc and glucose showed reduction in shoot weight similar to the infected control. CDB 35 and EB 13 applied alone or in combination with glucose and glucose with zinc did not show any influence on plant growth (Table 4.36).
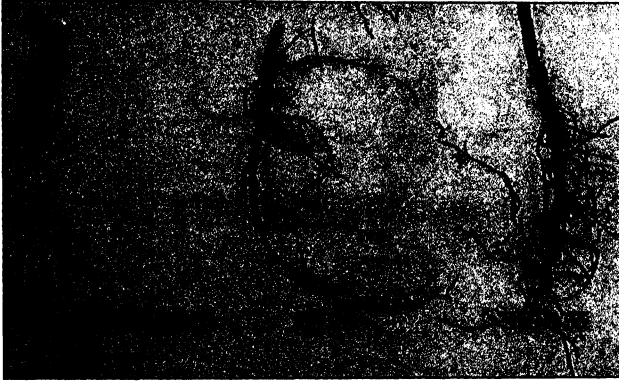
Photograph 4.23. Nodule formation in chickpea (ICCV 2) roots infected with *S. rolfsii* in presence of antagonistic bacteria *B. licheniformis* EB 13 and *Pseudomonas* sp. CDB 35 in glasshouse conditions.
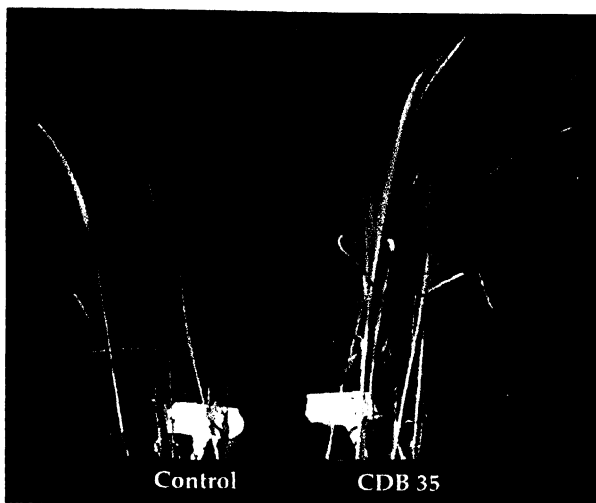
Photograph 4.24.   Plant growth of sorghum in control (uninoculated) and CDB 35 (in pots infested with *M. phaseolina*). Inhibition of plant growth (in control) due to infection of *M. phaseolina* in roots and CDB 35 inoculated plants showed less root infection and improved plant growth.

Photograph 4.25.   Plates showing growth of *M. phaseolina* from sorghum root infected with the fungi. All the root bits (five) from the control (uninoculated) treatment showed fungal infection, while with *Pseudomonas* sp. CDB 35 only two of five root bits showed fungal growth. CMRA specific medium was used for isolating *M. phaseolina*

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Table 4.35. Evaluation of antagonistic bacteria (*B. licheniformis* EB 13 and *Pseudomonas* sp. CDB 35) and *Rhizobium* on germination, growth and nodulation of chickpea (ICCV2) infected with *S. rolfsii*

Treatments	Germi-nation (%)	Shoot weight (mg)	Root weight (mg)	Number of nodules	Nodule weight (mg)
Control + SC	27	396	145	2	2
Control + SP	20	286	12	2	1
Captan + SC+ IC 59	33	688	285	11 [a]	4
Captan + SP+ IC 59	53	1038[a]	293 [a]	13 [a]	7
B. licheniformis EB 13 + IC 59 + SC	33	758	150	6	4
B. licheniformis EB 13 + IC 59 + SP	40	958	187	12 [a]	22
Pseudomonas sp. CDB 35 + IC 59 + SC	40	939	207	7	5
Pseudomonas sp. CDB 35+ IC 59 + SP	53	1070 [a]	261	20 [a]	34
Mean	37	767	193	9	10
LSD		631.5	145.5	7.56	30.9
CV%		63.6	58.4	64	352

Values are means of five replications and data calculated per plant.
SC = seed coating, SP = seed priming.
[a] Values are statistically significantly different than uninoculated control.
IC 59=*Rhizobium* strain.

Table 4.36. Influence of zinc and/or glucose on the efficacy of *B. licheniformis* EB 13 *Pseudomonas* sp. CDB 35 on root infection caused by *M. phaseolina* and growth of sorghum

Treatments	Shoot weight (g)	*M. phaseolina* infection %
Control	0.5	80 (64)
Trichoderma viridae	1.8	34 (35)
Glucose	0.6	72 (58)
Zinc	1.8	62 (46)
Glucose+Zinc	1.5	61 (46)
B. licheniformis EB 13	1.7	45 (42)
EB 13 + Glucose	1.1	42 (40)
EB13 + zinc	2.1	36 (37)
EB 13 + Glucose + zinc	1.9	42 (40)
Pseudomonas CDB 35	1.6	34 (35)
CDB 35 + Glucose	1.6	46 (43)
CDB 35 + zinc	2.3	20 (27)
CDB 35 + Glucose + zinc	1.7	27 (31)
Mean	1.6	45 (43)
LSD (P=0.05)	0.44	14.2 (8.6)
CV %	22	25 (16.3)

Values are means of five replications and data calculated per plant.
Values in parentheses are angular transformation values.

4.10 PLANT GROWTH PROMOTING & BIOCONTROL ACTIVITY OF COMPOSTS

4.10.1 Effect of composts on growth of pearl millet in glasshouse conditions

Microbial characterization of composts revealed that all three composts used in this study had good number of PGPB. Unsterilized soil of BP2C field was used as potting medium and composts were applied @ five tons ha^{-1}. Plant growth showed significant difference in dry matter (plant biomass) with all the three composts when compared to control. Growth parameters like shoot and root length, leaf area, were significantly enhanced due to application of RSC. Increase in plant weight was (81%) when RSC was applied followed by GVC (54%) and FWC (39%) (Table 4.37).

Table 4.37. Effect of different composts on growth of pearl millet (ICMV 155) in pots having unsterilized soil

Treatments	Shoot length (cm)	Leaf area (cm^2)	Root length density cm cm^{-3} soil	Plant weight (mg)
Control	59	56	0.96	896
FWC	63 (6)	65 (16)	1.31 (37)	1248 (39) [a]
RSC	65 (10) [a]	87 (55) [a]	1.71 (77)	1628 (81) [a]
GVC	62 (5)	67 (19)	1.49 (55)	1388 (54) [a]
Mean	62	69	1.3 7	1290
LSD	5.11	12.7	295.5	295.5
CV%	7	15	19	19

Values are means of six replications and data calculated per plant
FWC=Farm waste compost, RSC=Rice-straw compost, GVC=*Gliricidia* vermicompost and values in parantheses indicate per cent increase over control
[a] Statistically significantly different than the control treatment.

4.10.2 Co-inoculation of composts and bacteria on plant growth of pearl millet

Composts and selective bacterial strains *B. circulans* EB 35, *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 were evaluated to study their dual effect on pearl millet growth. Seed treatment with peat-based formulation of selected bacterial isolates applied along with compost (5 t ha^{-1}) significantly increased the shoot length, leaf area, plant dry weight and root length density. Per cent increase of growth parameters over uninoculated control is shown (Fig. 4.20). Increase in root length density ranged from 17% in FWC to 82% when EB 67 was co-inoculated with FWC (Photograph 4.26). Maximum increase in plant biomass occurred on application of RSC with EB 67 (88%), followed by GVC with EB 67 (83%) and GVC with CDB 35 (82%). However, there was no significant change on application of RSC with bacteria.
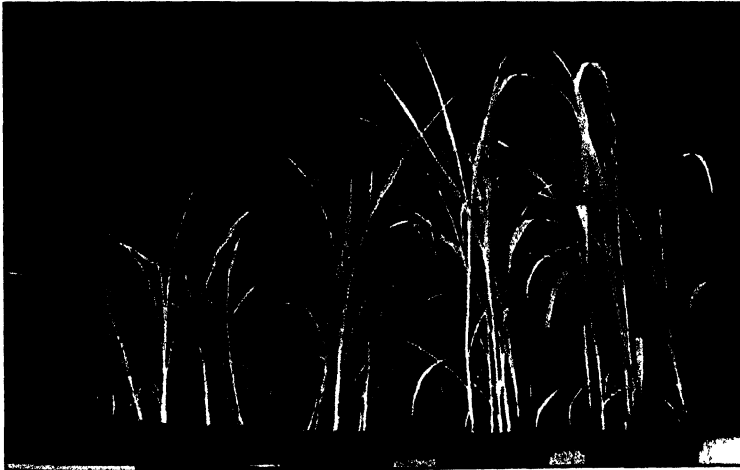
Photograph 4.26. Effect of farm waste compost (FWC) and bacteria on growth of pearl millet (ICMV 155) in pots having unsterilized soil. a) Control b) FWC c) FWC+ *S. marcescens* EB 67 d) FWC+ *Pseudomonas* sp. CDB 35. Compost was applied @ 5 t ha^{-1}

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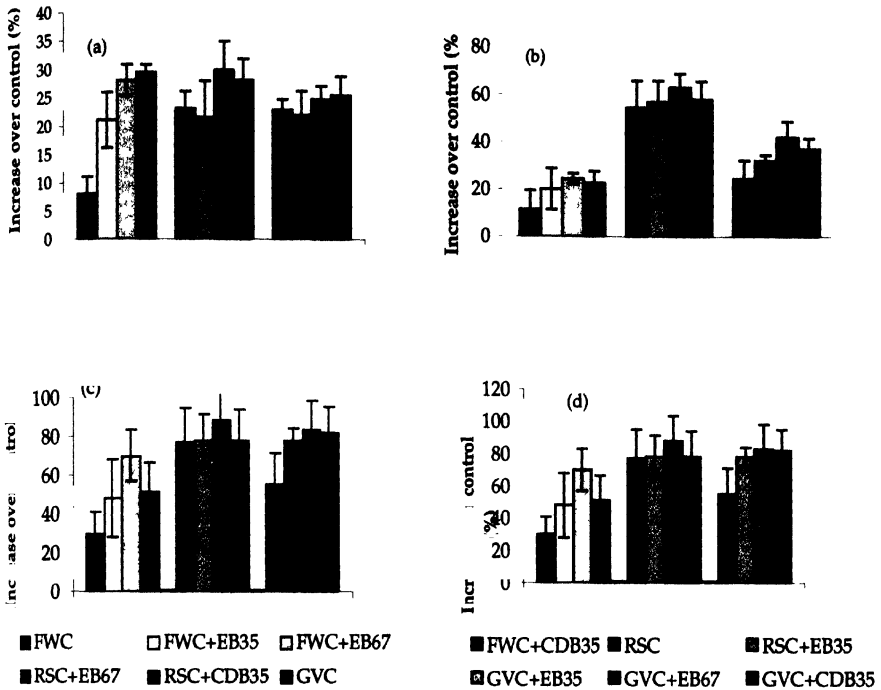
Figure 4.20. Effect of three composts and selected bacteria (*B.circulans* EB 35, *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35) on growth of pearl millet ICMV 155 in pots having unsterilized soil. (a) Shoot length, (b) leaf area, (c) root length density and (d) plant dry weight. FWC = Farm waste compost, RSC = Rice straw compost, GVC = *Gliricidia* vermicompost. Compost was applied @ 5 tones ha^{-1} and bacteria were applied as seed coat. Data (per cent increase over uninoculated control) are means of three replications from two experiments. The standard errors of means are shown as vertical lines.

4.10.3 Effect of compost and vermicompost on growth and mycorrhizal colonization in root of sorghum

Rice straw compost (RSC) and rice straw vermicompost (RSVC) along with AM was

applied to evaluate plant growth of sorghum in glasshouse conditions. Increase in

plant biomass with RSC and RSVC at 2.5 t ha⁻¹ alone and in combination with AM was marginally similar. RSC (at 5 t ha⁻¹) applied with and without AM inoculation was better than the RSVC application. Both the composts showed significant difference in plant growth parameters studied at 5 t ha⁻¹. However, application of RSVC at 10 t ha⁻¹ did not show any inhibitory effect on mycrorrhizal colonization but did decrease the dry matter of plant. RSC did not have any inhibitory effect on growth and AM colonization of sorghum when applied at 10 t ha⁻¹ and showed significant difference when compared to other treatments (Table 4.38).

4.10.4 Effect of enriched vermicompost on growth of maize

Enriched vermicompost prepared using the two PSB EB 67 and CDB 35 showed significant increase in growth parameters studied when compared to the uninoculated control and control compost. Per cent increase of leaf area was from 15-24%, plant biomass from 10-17% and the colonization of AM from 54-61% (Table 4.39).

4.10.5 Efficacy of *Gliricidia* vermicompost (GVC) on control of *M. phaseolina* infection

In a separate experiment to evaluate the efficacy *Gliricidia* vermicompost (GVC) on reduction of *M.phaseolina* infection, GVC was applied alone and in combination with the two bacterial isolates. GVC added to the soil at 5 t ha⁻¹ inhibited the pathogen infection (49%) but when applied along with seed treatment of bacteria separately produced better biocontrol affect (Table 4.40).

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Table 4.38. Effect of composts applied along with arbascular mycorrhizae (AM) on growth of sorghum cultivar ICSV (93046) (sweet stalk) and mycorrhizal association in roots

Treatments	SPAD reading	Shoot length (cm)	Leaf area (cm²)	Dry weight (g)	Root volume (mL)	AM colonization (%)
Control	26	72	254	5.5	4.3	15
AM	26	72	266	5.8	4.3	37
RSC 2.5 t	29	73	307	6.0	5.1	33
RSVC 2.5 t	32	74	311	6.0	4.4	31
RSC 2.5 t + AM	31	81	341	6.8	5.3	53
RSCV 2.5 t + AM	32	71	344	7.0	6.6	35
RSC 5 t	34	96	488	12.4	9.5	61
RSVC 5 t	37	77	341	8.6	7.9	67
RSC 5 t + AM	36	84	491	14.4	12.0	69
RSVC 5 t + AM	38	83	401	10.5	9.1	76
RSC 10 t	35	98	586	21.6	19.1	62
RSVC 10 t	35	81	419	15.0	13.8	67
RSC 10 t + AM	43	108	600	23.8	21.5	72
RSVC 10 t + AM	41	110	539	20.6	17.9	77
Mean	34	84	406	11.7	10.1	54
LSD (P=0.05)	3.60	10.88	95.62	2.14	2.28	13.82
CV%	9.20	11.20	20.40	15.90	19.70	21.60

Means of six replicates are given and data calculated per plant. RSC = Rice straw compost RSVC=Rice straw vermicompost. Composts applied at the rate of 2.5, 5, 10 t ha⁻¹ (t = tones). AM = arbascular mycorrhizae (*Glomus*) spp. was used.

Table 4.39. Effect of vermicompost prepared using selected bacteria on growth of maize and mycorrhizal association

Treatments	SPAD reading	Shoot length (cm)	Leaf area (cm^2)	Dry weight (g)	AM colonization (%)
Control	10	58	178	1.9	27
Compost	18	98[a]	519[a]	5.2[a]	52[a]
S. marcescens EB 67compost	24	103 (5)[a]	645 (24)[ac]	6.1 (17)[ac]	80 (54)[ac]
Pseudomonas sp. CDB 35 compost	20	100 (2)[a]	597 (15)[ac]	5.7 (10)[ac]	84 (61)[ac]
Mean	18	90	485	4.7	61
LSD (P=0.05)	3.9	7.2	75.2	0.76	18.5
CV%	18	6	13	13	16

Values are means of six replications and data calculated per plant. RP (applied at 20 kg ha^{-1} wherever composts were applied. Values [a] significant than control [ac] control compost.

Table 4.40. Influence of vermicompost and antagonistic bacteria on growth of sorghum and root infection caused by *M. phaseolina*

Treatments	Shoot weight (g)	M. phaseolina infection (%)
Control	0.5	80 (64)
Trichoderma viridae	1.8	34 (35)
GVC	1.4	49 (44)
B. licheniformis EB 13 + GVC	1.2	45 (44)
Pseudomonas CDB 35 + GVC	1.9	38 (37)
Mean	1.8	30 (28)
LSD (P=0.05)	0.48	16.6 (10.7)
CV %	20	44 (30.2)

GVC = *Gliricidia* vermicompost. Values are means of five replications. Angular transformation values are given in parantheses.

4.11 EVALUATION OF P-SOLUBILIZING BACTERIA IN GLASSHOUSE AND FIELD CONDITIONS

Based on *in vitro* studies, two PSB, *S. marcescens* EB 67 *Pseudomonas* sp. CDB 35, were evaluated for plant growth of maize (Surabhi variety) in glasshouse and field conditions. *S. marcescens* EB 67 performed better than the reference strain *E. asburiae* PSI3 used in the study.

4.11.1 Rhizosphere colonization of EB 67 and CDB 35 (peat-based) in glasshouse and field conditions

Seed treatment with peat-based formulation (10^7-10^8 cells of each PSB per seed) of *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 increased the seedling emergence, shoot length and dry biomass and grain yield of maize. Rhizosphere soil from the control (uninoculated) treatment was also used to compare and study for any PSB present that could grow on RP buffered antibiotic media. Survival of inoculated bacteria (EB 67 and CDB 35) in the rhizotic zones of maize was detected on RP buffered antibiotic media at 12 days, 24 days, 48 days and 96 days after sowing (DAS).

In glasshouse conditions, there was no growth of any PSB from control pot on RP antibiotic buffered plates. Population of EB 67 ranged from 6.4-$\log_{10}$ g^{-1} soil 12 DAS to 4.1 $\log_{10}$ g^{-1} of soil DAS. Population of CDB 35 ranged from 6.0 $\log_{10}$ g^{-1} soil 12 DAS to 5.5 $\log_{10}$ g^{-1} 96 DAS (Fig. 4.21). In field conditions, there was no count of any PSB from control plots till 24 days. But after 48 DAS control plot, showed population of 3.8 $\log_{10}$ g^{-1} soil and it was 3.2 $\log_{10}$ g^{-1} at 72 and remained same upto 96 DAS. In inoculated plots, population of EB 67 was 6.0 $\log_{10}$ g^{-1} soil at 12 DAS to 4.7

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log$_{10}$ g^{-1} soil at 96 days and population of CDB 35 was 6.9 log$_{10}$ g^{-1} soil at 12 DAS to

5.1 log$_{10}$ g^{-1} soil at 96 days (Fig. 4.22).
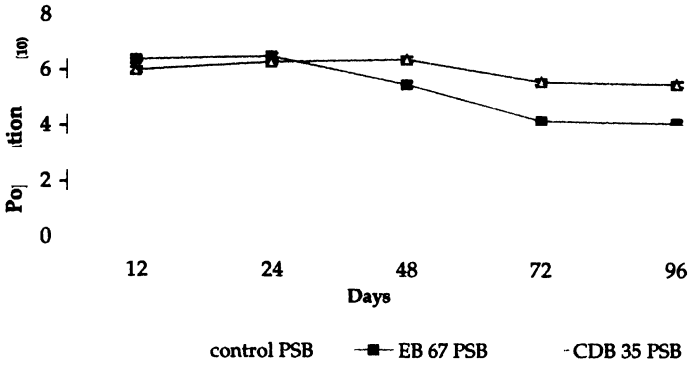
Figure 4.21. Population of *S. marcescens* 67 and *Pseudomonas* sp. CDB 35 in rhizosphere of maize in glasshouse conditions. Bacteria were applied as seed treatment and their rhizosphere populations were monitored by dilution plating on RP agar medium with a marker. Data points are means of four replicates.
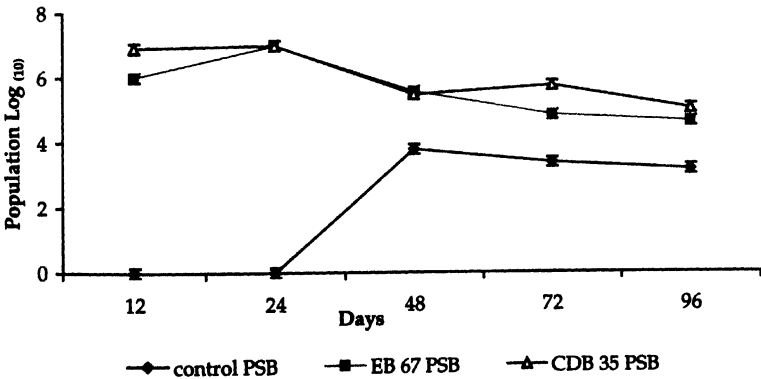
Figure 4.22. Population of *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 in rhizosphere of maize in field conditions. Bacteria were applied as seed treatment and their rhizosphere populations were monitored by dilution plating on RP agar medium with a marker. Data points are means of four replicates.

4.11.2 Growth parameters of maize in pots having unsterilized soil

PSB, EB 67 and CDB 35 were evaluated for growth of maize in glasshouse conditions. In addition to this, two reference strains *E. asburiae* PSI 3 and *B. coagulans* were also used in the study (Table 4.41). Control treatments included uninoculated plot and uninoculated with singlesuperphosphate (SSP) and rock phosphate (RP) application separately. Growth parameters in glasshouse conditions showed significant difference in shoot length, leaf area and dry weight (except where RP was applied) compared to control.

Table 4.41. Effect of PSB on growth of maize in pots having unsterilized soil

Treatments*	Spad reading	Shoot length (cm)	Leaf area (cm²)	Dry weight (g)
Control	18	130	578	44
N_{80}+ RP_{20}	22	189 (45)	788 (36)	61 (38)
N_{80}+ SSP_{20}	19	192 (48)	720	73 (65)
E.asburiae PSI-3	23	204 (57)	1092 (89)	87 (96)
B.coagulans	16	186 (43)	1082 (87)	67 (52)
S. marcescens EB 67	26	221 (70)	1148 (99)	88 (99)
Pseudomonas sp. CDB 35	25	192 (48)	1105 (91)	86 (94)
Mean	21	130	578	44
LSD (P=0.05)	3.9	16.5	202.3	9.6
CV %	15	7	16	10

Values are means of four replications and data calculated per plant. N = urea at 80 kg ha⁻¹. SSP = single super phosphate and rock phosphate (RP) at 20 kg ha⁻¹. *Wherever microorganisms were applied, RP was also added. Values in parentheses are per cent increase over control and calculated for significantly different values. b non significant than control treatment.

Maximum increase in dry weight was by isolate EB 67 (99%) followed by CDB 35 (94%) and was similar to the reference strain *E. asburiae* used in the study (96%) (Photograph 4.27 and 4.28). Both the isolates EB 67 and CDB 35 and the reference isolate *E. asburiae* showed 42-47% increase over *B. coagulans* (which showed P solubilization in unbuffered conditions).
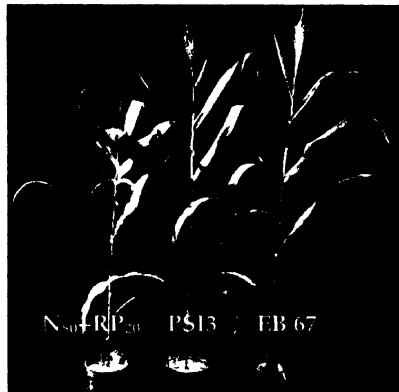
Photograph 4.27. Growth of maize inoculated with *S. marcescens* EB 67 in pots having unsterilized soil from BP2C field. $N_{80}+RP_{20}$ (urea+rockphosphate). PSI3=*E. asburiae* PSI3 (reference strain).

Photograph 4.28. Growth of maize inoculated with *Pseudomonas* spp. CDB 35 having unsterilized soil from BP2C field. $N_{80}+RP_{20}$ (urea+rockphosphate). PSI3=*E. asburiae* PSI3 (reference strain).

4.11.3 Growth parameters of maize in field conditions

Seed treatment with peat-based formulation of two PSB, *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 in field conditions improved the growth parameters and yield of maize. Both the isolates increased the shoot length ranged between 8 to 12 per cent and leaf area 8 to 22 per cent more than the uninoculated control (Table 4.42). Increase in plant dry weight (biomass) was significantly different (36%) with *S. marcescens* EB 67 at 24 days after sowing (DAS). After 48 DAS, except the treatments where SSP was applied, all the other treatments showed significant difference in plant dry weight than the uninoculated control (Table 4.43). Maximum increase in plant dry weight at 48 DAS was with *S. marcescens* EB 67 (66%) followed by *B. coagulans* (57%) and *Pseudomonas* sp. CDB 35 (51%).

After 72 days, increase in corn weight (42%) and plant dry weight (25%) was by *S. marcescens* EB 67, compared to uninoculated control (Table 4.44). After 96 days, increase in dry weight (50%) was significant and maximum with isolate *S. marcescens* EB 67 and was similar to *E. asburiae* used in the study (Table 4.45). Throughout the study, *S. marcescens* EB 67 was better than both the reference strains *B. coagulans*, *E. asburiae* in most of the growth parameters observed. *Pseudomonas* sp. CDB 35 was better than *B. coagulans* but was similar or less than *E. asburiae* used in the study.

Seed treatment with *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 increased the grain yield of field-grown maize by 85 and 64% compared to the uninoculated control (Table 4.46). *S. marcescens* EB 67 showed 19% increase over *E. asburiae*, 33%

over *B. coagulans*, 35% over urea+ rockphosphate (N80+RP20) treatment and 43% over urea+single superphoshpate (N80+ SSP20). *Pseudomonas* sp. CDB 35 showed 12% increase over *B. coagulans*, 14% over N80+RP20, and 19% over N80+ SSP20. Increase in P uptake of shoot and grain tissue with *S. marcescens* EB 67 was 13 and 27 kg ha-1, CDB 35 was 9 and 22 kg ha-1 and control 5 and 11 kg ha-1 (Table 4.47). N uptake in shoot with *S. marcescens* EB 67 was 54 kg ha-1, CDB 35 48 kg ha-1 and control was 30 kg ha-1.

Table 4.42. Effect of PSB on growth parameters of maize, BP2C field, January-April 2004 (24 days after sowing)

Treatments*	SPAD reading	Shoot length (cm)	Leaf area (cm^2)	Dry weig (g)
Control	35	48	364	2.5
E. asburiae PSI3	38	50	383 (5)	2.9 (16)
B. coagulans	40	51	388 (7)	3.0 (20)
N$_{80}$+ RP$_{20}$	40	53 (10)	414 (14)	3.0 (20)
N$_{80}$+ SSP$_{20}$	33	48	368	2.4
S. marcescens EB 67	40	54 (12) [a]	442 (22)	3.4 (36)
Pseudomonas sp. CDB 35	40	52	391 (8)	2.6 (4)
Mean	40	51	393	2.8
LSD (P=0.05)	6.6	6.2	122.5	0.96
CV%	12	8	22	23

Values are means of four replications and data calculated per plant.
SSP = single super phosphate and rock phosphate (RP) at 20 kg ha-1,
* wherever microorganisms were applied, RP was also added.
Values in parentheses are per cent increase over control.
[a] Statistically significantly different than uninoculated control.

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Table 4.43. Effect of PSB on growth parameters of maize, BP2C field, January-April 2004 (48 days after sowing)

Treatments*	SPAD reading	Shoot length (cm)	Leaf area (cm^2)	Dry weight (g)
Control	26	122	2927	35
E.asburiae PSI3	28	142 (16)	3782 (29)	51 (46) [a]
B.congulans	30 [a]	149 (22) [a]	3951 (35) [a]	55 (57) [a]
N_{80}+ RP_{20}	29 [a]	140 (15)	3959 (35) [a]	53 (51) [a]
N_{80}+ SSP_{20}	25	117	3142 (7)	37
S. marcescens EB 67	29 [a]	143 (17)	4097 (40) [a]	58 (66) [a]
Pseudomonas sp. CDB 35	29 [a]	143 (17)	3790 (29)	53 (51) [a]
Mean	28	137	3664	49
LSD (P=0.05)	3.4	22.6	992.7	14.0
CV%	8	11	19	20

Values are means of four replications and data calculated per plant.
SSP=single super phosphate and rock phosphate (RP) at 20 kg ha^{-1},
* wherever microorganisms were applied, RP was also added.
Values in parentheses are per cent increase over control.
[a]Statistically significantly different than uninoculated control.

Table 4.44. Effect of PSB on growth parameters of maize, BP2C field, January-April (72 days after sowing)

Treatments*	SPAD reading	Leaf area (cm^2)	Shoot length (cm)	Corn weight (g)	Dry weight (g)
Control	37	2584	152	36	276
E.asburiae PSI3	39	2946 (14)	164 (8)	50 (39)	304 (10)
B.coagulans	40	3103 (20)	167 (10)	49 (36)	322 (17)
N$_{80}$+ RP$_{20}$	37	3114 (21)	168 (11)	40 (11)	288 (4)
N$_{80}$+ SSP$_{20}$	38	2695 (4)	151	45 (25)	265
S. marcescens EB 67	39	3788 (47) [a]	170 (12) [a]	51 (42)	345 (24)
Pseudomonas sp. CDB 35	38	3236 (25)	170 (12) [a]	47 (31)	306 (11)
Mean	38	3067	163	45	301
LSD (P=0.05)	4.8	812	17.8	17.9	71.6
CV%	9	18	8	27	16

Values are means of four replications and data calculated per plant.
SSP = single super phosphate and rock phosphate (RP) at 20 kg ha^{-1},
* wherever microorganisms were applied, RP was also added. Values in parentheses are per cent increase over control.
[a] Statistically significantly different than uninoculated control.

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Table 4.45. Effect of PSB on growth parameters of maize, BP2C field, January-April 2004 (96 days after sowing)

Treatments*	SPAD reading	Leaf area (cm^2)	Shoot length (cm)	Corn weight (g)	Dry weight (g)
Control	20	1604	157	68	127
E.asburiae PSI3	24	2073 (29)	181 (15) [a]	105 (85) [a]	191 (50) [a]
B.coagulans	25 [a]	1331	174 (11)	90 (33)	168 (31)
N$_{80}$+ RP$_{20}$	25 [a]	1727	166 (6)	70 (4)	135 (5)
N$_{80}$+ SSP$_{20}$	23	1817 (7)	161 (3)	85 (26)	147 (14)
S. marcescens EB 67	26	1910 (13)	174 (11)	104 (54) [a]	192 (50) [a]
Pseudomonas sp. CDB 35	28	2004 (24)	183 (17) [a]	85 (26)	152 (18)
Mean	24	1781	171	86	127
LSD (P=0.05)	5.1	768.4	20.4	33.9	47.7
CV%	14	29	8	27	21

Values are means of four replications and data calculated per plant.

SSP = single super phosphate and rock phosphate (RP) at 20 kg ha^{-1},

* wherever microorganisms were applied, RP was also added.

Values in parentheses are per cent increase over control.

[a] Statistically significantly different than uninoculated control.

Table 4.46. Effect of PSB on total dry matter, grain and stover yield of maize, BP2C field, January-April 2004

Treatments*	Grain yield (t ha^{-1})	Total dry matter (t ha^{-1})	Stover yield (t ha^{-1})
Control	2.8	7.8	5.0
E.asburiae PSI3	4.8 (71) [a]	11.6 (48) [a]	6.7 (21)
B.coagulans	4.2 (52)	10.6 (36)	6.4 (14)
N$_{80}$+ RP$_{20}$	4.2 (50)	10.6 (35)	6.4 (14)
N$_{80}$+ SSP$_{20}$	4.0 (42)	9.5 (22)	5.5(11)
S. marcescens EB 67	5.2 (85) [a]	12.2 (57) [a]	7.0 (27) [a]
Pseudomonas sp. CDB 35	4.6 (61) [a]	11.4 (46) [a]	6.8 (23) [a]
Mean	4.3	10.5	6.3
LSD (P=0.05)	1.4	2.88	1.81
CV%	26	21	22

Values are means of four replications and data calculated per plant.
SSP = single super phosphate and rock phosphate (RP) at 20 kg ha^{-1}, t=tones.
* where microorganisms were applied, RP was also added.
Values in parentheses are per cent increase over control.
[a] Statistically significant than uninoculated control.

Table 4.47. Effect of PSB on nutrient uptake by maize shoot and grain in field experiment, BP2C field, January-April 2004

Treatments	P uptake* (kg ha^{-1})	P uptake** (kg ha^{-1})	N uptake* (kg ha^{-1})
Control	5	11	30
E.asburiae PSI3	9	23	50
B.coagulans	7	19	42
N$_{80}$+ RP$_{20}$	7	19	42
N$_{80}$+ SSP$_{20}$	6	18	35
S. marcescens EB 67	13	27	54
Pseudomonas sp. CDB 35	9	22	48
Mean	8	20	43
LSD	3.9	6.8	18.7
CV (%)	37	26	33

*N and P uptake in shoot, ** P uptake in the grain tissue
SSP = single super phosphate and rock phosphate (RP) at 20 kg ha^{-1}

4.11.4 Shelf life of EB 67 and CDB 35 in peat formulation

Population of *S. marcescens* EB 67 ranged between 9.6 (6 DAI) to 7.1 CFU g^{-1} (180

DAI) on LB agar and 8.6 (6 DAI) to 6.2 CFU g^{-1} (180 DAI) on RP agar. Viability of

Pseudomonas sp · CDB 35 was 9.7 (6 DAI) to 7.9 CFU g^{-1} (180 DAI) on LB agar and 9.3

(6 DAI) to 7.3 CFU g^{-1} (180 DAI) on RP agar medium (Fig. 4.23).
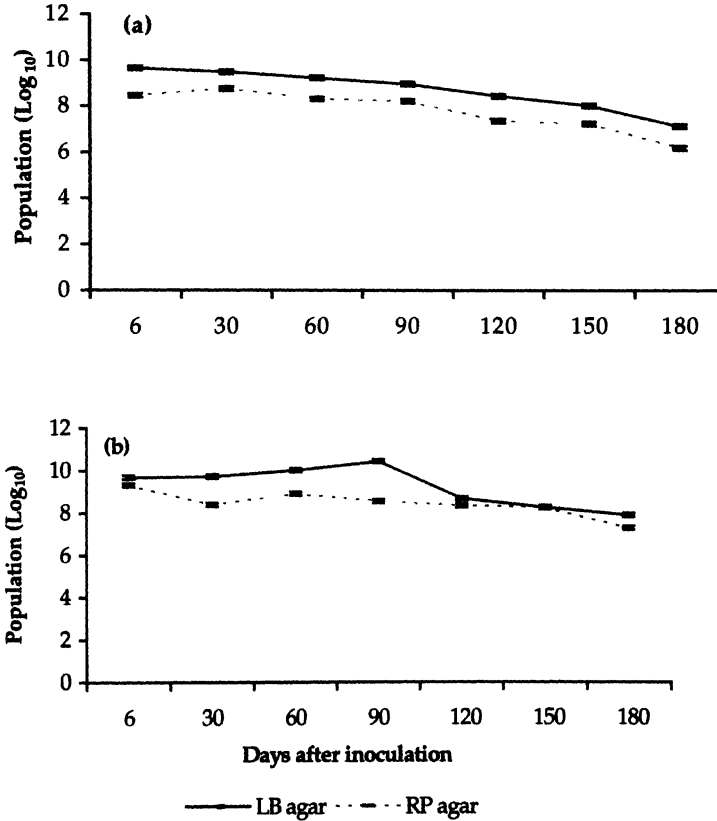
Figure 4.23. Survival of selected PSB (a) *S. marcescens* 67 and (b) *Pseudomonas* sp. CDB 35 in peat based formulation. Data presented are mean values and standard errors of three replications of each treatment in a repeated experiment. Peat packets were stored at 30 ± 2 °C.

4.12 CALCULATION OF INDICES TO DETERMINE THE SUSTAINABILITY INDEX OF DIFFERENT CROPPING SYSTEMS

4.12.1 Studies on soil biological, microbiological, nutrient and crop yield during year 2003-04, in different treatment plots (T1, T2, T3 and T4)

The long-term experiment set up at ICRISAT for managing crop residues and pests using biological approaches and conventional system was evaluated for soil quality twice (during 2004 and 2005), when the crop was close to the harvesting period. Six different crops (soybean, pigeonpea, maize, sorghum, cowpea and cotton) were grown in the last six years. The pH of soil solution from BW3 field was 7.8 and the bulk density was similar across the four treatment plots: T1 (1.02-1.38 g cm^{-3}), T2 (1.19-1.44 g cm^{-3}), T3 ((1.20-1.49 g cm^{-3}) and T4 (1.27-1.40 g cm^{-3}). Gravimetric water content of four treatment plots was T1=0.103, T2=0.115, T3=0.098 and T4=0.102 g/g.

Of the different parameters measured to assess the biological activity in soil samples from the four different crop husbandry systems, more activity was noted in year 1 (2003-04) in treatment plots T1, T2 and T4 compared to T3. Soil respiration was more by 18-52% in T1, T2 and T4 plots compared to T3 (which had a rate of 94 mg C kg^{-1} soil per 10 days). Microbial biomass carbon in T1, T2 and T4 was 21-30% higher compared to T3). Microbial biomass nitrogen was 27-42% more compared to T3. Acid and alkaline phosphatases activities were 5-21% higher in T1, T2 and T4 compared to T3. Dehydrogenase was more or less similar among the four treatments. Different parameters are based on point-in-time measurements of microbial activity in lab conditions, but they depict treatment differences (Table 4.48).

The overall results on the different functional groups of bacteria characterized strongly suggested that soils from plots T1, T2 and T4 were more active microbiologically than that of T3. Bacterial and fungal population (5.3-5.7 and 3.1-3.5 $\log_{10}$ g^{-1}) did not vary much among the four treatment plots. Population of actinomycetes and *Pseudomonas* sp. an indicator of suppressive soils to manage disease-causing fungi and nematodes, was about 5-10 times more in T1 and T2 than in T3 and T4 (4.2-4.6 $\log_{10}$ g^{-1} soil vs. 3.2-3.4 $\log_{10}$ g^{-1} soil) (Table 4.49). Other groups of PGPB characterized siderophore and phytase producers (organic P mineralizing bacteria) and antagonisitic bacteria which was about 10 times more in T2 and T4.

It was important to note that soil from both the treatment plots T1 and T2 characterized for soil nutrient status showed greater increases (rather than decreases) in their concentrations of soil nutrients (total N and available P and K) relative to those in T3 that received chemical fertilizers (Table 4.50). At the same time they produced yields and dry matter (biomass) comparable to T3 (Table 4.51). Biomass obtained by cotton and cowpea was higher with T4>T2>T3>T1.

4.12.2 Studies on soil biological, microbiological, nutrient and crop yield during year 2004-05, in different treatment plots (T1, T2, T3 and T4)

A repetition of all parameters was done, of six different depths and the rhizosphere soils of the crops (maize and pigeonpea) raised during that year close to the harvesting period. Data given is pool of three different depths (0-20 cm, 20-40 cm and 40-60 cm). Mean of the depths and the rhizosphere soils compared among the four treatments showed that soil respiration was more by 22-49% in T1, T2 and T4

compared to T3 (which had a rate of 129 mg C kg^{-1} soil per 10 days). Microbial biomass carbon was 10-32% higher in T1, T2 and T4 compared in T3. Potentially mineralizable nitrogen was 36-53% more in T1, T2 and T4 compared to T3. Microbial biomass nitrogen was 14-35% more in T1, T2 and T4 (compared with 21 mg N kg^{-1} soil in T3). Acid and alkaline phosphatases were 3-19% higher in T1, T2 and T4 (over 307 units for acid and 871 units for alkaline phosphatase in T3) (Table 4.52).

Plant growth promoting bacteria characterized from four different crop husbandry systems of treatment plots showed that bacterial and fungal population (5.2-5.4 and 2.8-3.2 log$_{10}$ g^{-1}) did not vary much among the four treatment plots (photograph 4.29). Population of actinomycetes (Photograph 4.30) and *Pseudomonas* sp. was about 5-10 times more in T1 and T2 than in T3 and T4 (Table 4.53). P solubilizers (Photograph 4.31) siderophore producers (Photograph 4.32) antagonistic bacteria were also present in more number. However, the population of all the groups studied decreased with depth.

Nutrient status of soil also showed that the treatments T1 had 9-16% increase, T2 had 2-21% increase in total N, P and available K over control (Table 4.54). On comparison of crop yield of maize and pigeonpea after year 6, among the four treatment plots (T1, T2, T3 and T4), maximum yield and biomass was obtained in T4. Biomass obtained in T1, where locally available biological inputs were applied was comparable with T3, where chemical fertilizers were applied (Table 4.55).
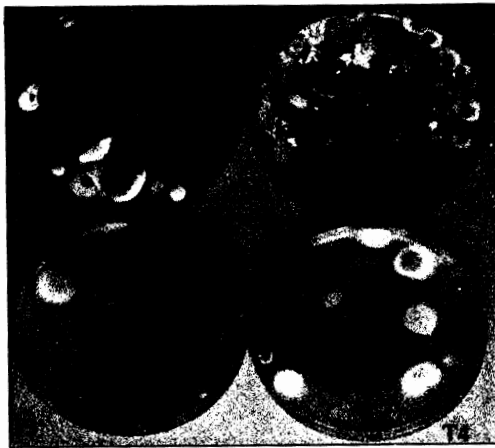
Photograph 4.29. Fungal population in soil sample from four crop husbandry systems T1, T2, T3 and T4 of BW3 field. Fungi were counted on PDA with antibiotic. *Trichoderma* sp. were in high number in T2 plot
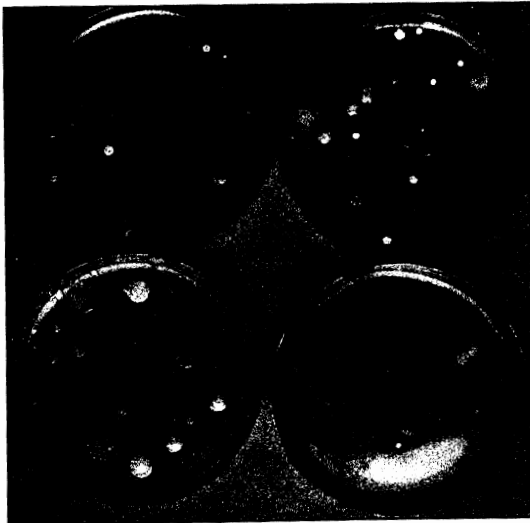
Photograph 4.30. Actinomycetes population in soil sample from four crop husbandry systems T1, T2, T3 and T4 of BW3 field. Actinomycetes were counted in AIA. T1 plot had more variable actinomycetes
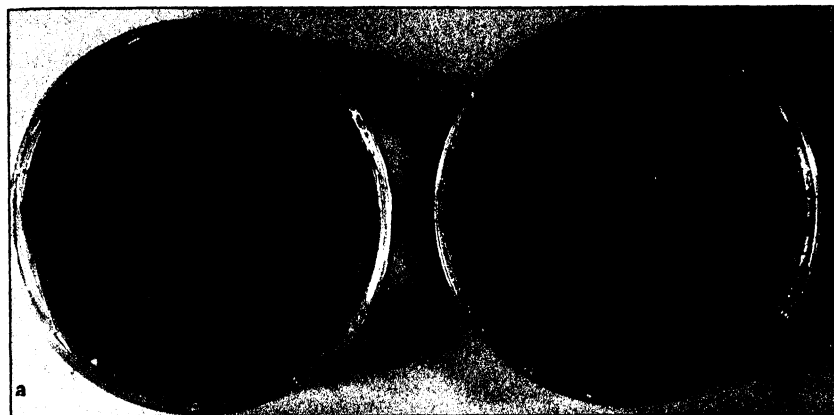
Photograph 4.31. Phosphate solubilizing bacteria from soil sample of BW3 field on RP buffered medium. (a) T3 (less population) and (b) T2 (high population), red color due to acid production of PSB
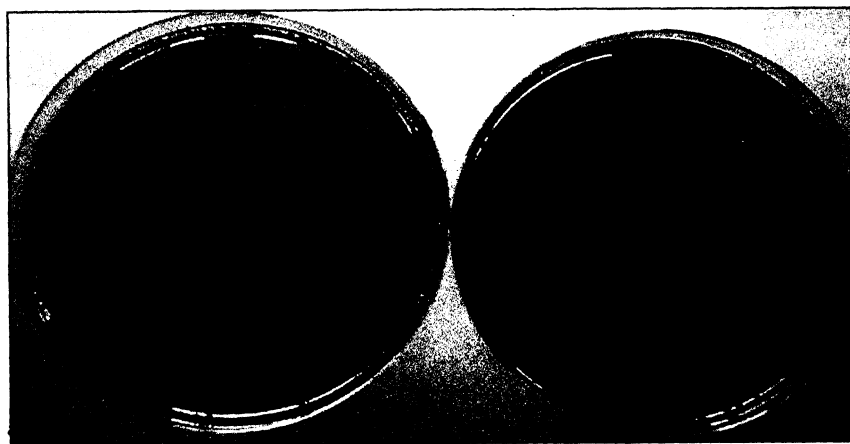
Photograph 4.32. Siderophore producing bacteria from soil sample at 20-40 cm depth of BW3 field on CAS agar medium (a) T3 (absent) and (b) T2 plot (Colonies with orange halo)

Table 4.48. Biological properties in top 20 cm soil from four crop husbandry systems (T1 to T4) of a long-term field experiment collected in January 2004

Properties	Soil depth (cm)	T1	T2	T3	T4	Mean	LSD (P=0.05)	CV %
Soil respiration (mg C kg^{-1})	0-20	111	126	94	143	119	41.9	39
Microbial biomass (mg C kg^{-1})	0-20	524	522	402	506	488	211.2	47
Organic carbon (kg C ha^{-1})	0-20	11	10	8	11	10	3.1	34
Mineral N (mg N kg^{-1})	0-20	10	11	9	13	11	4.5	45
Net N mineralization (mg N kg^{-1})	0-20	1.92	0.0	2.78	0.36	1.3	2.52	219
Microbial biomass (mg N kg^{-1})	0-20	33	37	26	33	32	14.5	49
Acid phosphatase (μg p-NPg^{-1}h^{-1})[a]	0-20	310	332	294	357	323	18.9	9
Alkaline phosphatase (μg p-NPg^{-1}h^{-1}) [a]	0-20	937	1008	890	1011	962	43.3	7
Dehydrogenase (μg TPFg^{-1}24h^{-1})[b]	0-20	133	137	130	142	136	10.7	12
Mean		230	243	206	246	231		

[a] p-NP- para nitro phenol; b.TPF- Triphenylformazan

T1=Low-cost system 1, no tillage, rice straw as surface mulch + Biological inputs for plant growth and pest management.

T2= Low-cost system 2, no tillage, farm waste as surface mulch+ Biological inputs for plant growth and pest management.

T3=Conventional system, tillage, integrated nutrient management and chemicals for plant growth and pest management.

T4=Same as T3 + Farm waste as biomass as in T2

Table 4.49. Microbial population (Log $_{10}$ gm^{-1}dry soil) in top 20 cm soil from four crop husbandry systems (T1 to T4) of a long-term field experiment collected in January 2004

Functional groups*	T1	T2	T3	T4	Mean	LSD (5%)	CV%
	Population Log $_{10}$ (CFU g^{-1} soil)						
Bacteria	5.6	5.7	5.3	5.7	5.6	0.39	7.7
Fungi	3.1	3.5	3.2	3.2	3.3	0.21	7.2
Actinomycetes	4.6	4.4	3.5	4	4.1	0.22	17
Pseudomonas (fluorescent colonies)	4.2	4.6	3.4	3.2	3.9	0.84	24
Siderophore producers	3.7	4.5	3.4	4.3	4.0	0.44	12
PSB	1.4	1.7	1.2	1.9	1.6	0.75	54
Phytase producers	3.2	4.2	2.8	3.8	3.5	1.14	37
Antagonistic bacteria (against *M. phaseolina*)	4	4	3	4.2	3.8	0.58	17
Mean	3.7	4.1	3.2	3.8	3.7		

PSB = phosphate solubilizing bacteria used (RP as insoluble P-source).

T1=Low-cost system 1, no tillage, rice straw as surface mulch + Biological inputs for plant growth and pest management.

T2= Low-cost system 2, no tillage, farm waste as surface mulch+ Biological inputs for plant growth and pest management.

T3=Conventional system, tillage, integrated nutrient management and chemicals for plant growth and pest management.

T4=Same as T3 + Farm waste as biomass as in T2

*=Functional groups: Plant growth promoting bacteria

Table 4.50. Soil nutrient data in top 20 cm soil from four crop husbandry systems (T1 to T4) of a long-term field experiment collected in January 2004

Treatments	T1	T2	T3	T4
Total N (ppm)	737(47.6)	798(43.3)	585(30.7)	642(21.7)
Total P (ppm)	356(39.6)	417(40.1)	347(68.6)	340(69.0)
Available K (ppm)	223(19.8)	243(16.1)	180(6.3)	189(6.5)
Available P (ppm)	2.6 (0.59)	2.4 (0.47)	1.2 (0.08)	2 (0.29)

Data in parentheses are ± SEs.

T1=Low-cost system 1, no tillage, rice straw as surface mulch + Biological inputs for plant growth and pest management.
T2= Low-cost system 2, no tillage, farm waste as surface mulch+ Biological inputs for plant growth and pest management.
T3=Conventional system, tillage, integrated nutrient management and chemicals for plant growth and pest management.
T4=Same as T3 + Farm waste as biomass as in T2

Table 4.51. Crop and dry matter yield of cotton and cowpea in the four different crop husbandry systems (T1 to T4) of a long-term field experiment

Treatments/ Yield (t ha^{-1})	T1	T2	T3	T4
Cotton yield	1.1 (0.039)	1.03 (0.033)	1.06 (0.033)	1.38 (0.033)
Cotton dry matter	3.83 (0.095)	4.04 (0.103)	4.32 (0.134)	4.38 (0.091)
Cowpea grain yield	0.46 (0.016)	0.52 (0.015)	0.34 (0.017)	0.38 (0.015)
Cowpea dry matter	2.38 (0.065)	2.78 (0.066)	2.10 (0.061)	2.97 (0.086)

Data in parentheses are ± SEs.

T1=Low-cost system 1, no tillage, rice straw as surface mulch + Biological inputs for plant growth and pest management.
T2= Low-cost system 2, no tillage, farm waste as surface mulch+ Biological inputs for plant growth and pest management.
T3=Conventional system, tillage, integrated nutrient management and chemicals for plant growth and pest management.
T4=Same as T3 + Farm waste as biomass as in T2

Table 4.52. Biological properties in top in top 60 cm profile and rhizosphere soil (of maize and pigeonpea) from four crop husbandry systems (T1 to T4) of a long-term field experiment collected in January 2005

Properties	Soil depth (cm)	T1	T2	T3	T4	Mean	LSD (P=0.05)	CV%
Soil respiration	0-20	134	147	105	150	134	41.4	15
(mg C kg^{-1})	20-40	77	92	41	78	71		
	40-60	22	66	16	55	40		
	Maize rhizo	357	341	315	340	340		
	Pigeonpea rhizo	376	206	168	228	228		
	Mean	192	170	129	159			
Microbial biomass	0-20	534	525	406	478	486	208.2	30
(mg C kg^{-1})	20-40	298	316	177	245	259		
	40-60	225	233	138	135	183		
	Maize rhizo	474	520	417	408	454		
	Pigeonpea rhizo	715	674	549	637	644		
	Mean	449	453	338	380			
Mineral N	0-20	12	11	9	15	12	2.5	12
(mg N kg^{-1})	20-40	5	4	3	10	5		
	40-60	2	3	1	6	3		
	Maize rhizo	22	25	17	22	22		
	Pigeonpea rhizo	22	22	15	16	19		
	Mean	12	13	9	14			
Net Mineral N	0-20	1.7	-0.6	0.7	-1.3	0.1	3.5	148
(mg N kg^{-1})	20-40	-0.1	0.4	2.0	-0.8	0.4		
	40-60	0.5	-1.6	2.4	2.6	1.0		
	Maize rhizo	1.7	8.8	7.8	2.6	5.2		
	Pigeonpea rhizo	-4.0	0.8	5.5	-0.8	0.4		
	Mean	-0.06	1.5	3.7	0.5			

Contd…

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Table 4.52 Contd…

Properties	Soil depth cm)	T1	T2	T3	T4	Mean	LSD (P=0.05)	CV%
Microbial biomass N	0-20	35	34	25	31	32	8.1	19
(mg N kg^{-1})	20-40	17	16	11	16	15		
	40-60	14	13	10	8	11		
	Maize rhizo	33	37	26	23	30		
	Pigeonpea rhizo	36	39	32	40	37		
	Mean	27	28	21	24			
Organic carbon	0-20	13	15	12	13	13	0.78	12
(kg C ha^{-1})	20-40	9	14	9	8	8		
	40-60	9	8	8	8	8		
	Maize rhizosphere	17	14	12	12	14		
	Pigeonpea rhizo	15	11	11	12	12		
	Mean	13	12	10	11			
Acid phosphatase	0-20	308	392	283	309	323	55.9	10
(μg p-NPg^{-1}h^{-1})[a]	20-40	162	147	117	136	140		
	40-60	137	108	97	110	113		
	Maize rhizo	573	591	550	540	563		
	Pigeonpea rhizo	525	615	486	517	536		
	Mean	341	371	307	322			
Alkaline phosphatase	0-20	957	1053	829	1037	969	185	11
(μg p-NPg^{-1} h^{-1})[a]	20-40	476	516	419	510	480		
	40-60	386	329	302	428	361		
	Maize rhizo	1582	1608	1500	1528	1555		
	Pigeonpea rhizo	1720	1700	1304	1468	1548		
	Mean	1024	1041	871	994			
Dehydrogenase	0-20	144	137	120	134	134	13.3	6
(μg TPFg^{-1} 24h^{-1})[b]	20-40	29	39	23	47	35		
	40-60	24	32	16	38	28		
	Maize rhizo	230	293	226	225	244		
	Pigeonpea rhizo	193	207	164	216	195		
	Mean	124	142	110	132			

rhizo=rhizosphere, a p-NP- para nitro phenol; b.TPF- Triphenylformazan

Table 4.53. Microbial population Log $_{10}$ (CFU g^{-1} dry soil) in top in top 60 cm profile and rhizosphere soil (of maize and pigeonpea) from four crop husbandry systems (T1 to T4) of a long-term field experiment collected in January 2005

Treatments	Soil depth (cm)	T1	T2	T3	T4	Mean	LSD (P=0.05)	CV%
Bacteria	0-20	5.5	5.7	5.4	5.7	5.6	0.25	3
	20-40	4.8	5.0	4.6	4.9	4.8		
	40-60	4.3	4.7	4.5	4.6	4.6		
	Maize rhizosphere	6.3	5.9	5.9	6.1	6.0		
	Pigeonpea rhizosphere	5.8	6.1	5.7	5.8	5.8		
	Mean	5.3	5.5	5.2	5.4			
Fungi	0-20	3.2	3.3	3.0	3.0	3.2	0.19	3
	20-40	3	3	2.5	2.8	2.8		
	40-60	2.5	3	2.1	2.2	2.4		
	Maize rhizosphere	3.4	3.6	3.3	3.3	3.5		
	Pigeonpea rhizosphere	3.4	3.4	3.3	3.5	3.5		
	Mean	3.1	3.3	2.9	3.0			
Actinomycetes	0-20	4.7	4.4	4.1	4.3	4.4	0.12	2
	20-40	4.0	3.7	3.6	3.6	3.8		
	40-60	3.2	3.1	3.1	3.1	3.2		
	Maize rhizosphere	4.7	4.3	4.2	4.3	4.4		
	Pigeonpea rhizosphere	4.7	4.5	4.2	4.4	4.5		
	Mean	4.2	4.1	3.9	4.0			

Table 4.53 Contd...

Treatments	Soil depth (cm)	T1	T2	T3	T4	Mean	LSD (P=0.05)	CV%
Pseudomonas (fluorescent)	0-20	3.9	3.6	3.2	3.3	3.5	0.28	5
	20-40	3.4	3.0	2.8	2.8	3.0		
	40-60	2.6	2.5	2.0	2.1	2.3		
	Maize rhizosphere	4.3	4.2	3.5	3.3	4.2		
	Pigeonpea rhizosphere	4.3	4.2	4.0	4.1	3.9		
	Mean	3.7	3.5	3.1	3.1			
Siderophore producers	0-20	3.5	3.8	2.4	3.1	3.2	0.23	6
	20-40	2.5	2.2	0.0	1.1	1.4		
	40-60	0	0	0	0	0.0		
	Maize rhizosphere	3.3	3.4	3.1	3.3	3.3		
	Pigeonpea rhizosphere	3.1	3.0	2.6	2.9	3		
	Mean	2.5	2.5	1.6	2.1			
Antagonistic Bacteria (against M. phaseolina)	0-20	4	3.8	3.8	3.7	3.9	0.27	5
	20-40	3.4	3	2.9	3.6	3.3		
	40-60	2.8	3	2.4	3	2.8		
	Maize rhizosphere	4.3	4.5	4.1	4.2	4.3		
	Pigeonpea rhizosphere	4.4	4.4	4.2	4.2	4.3		
	Mean	3.8	3.8	3.5	3.8			

Table 4.53 contd.

Treatments	Soil depth (cm)	T1	T2	T3	T4	Mean	LSD (P=0.05)	CV%
Phytase producers	0-20	4.3	4.4	3.6	4.0	4.1	0.22	3
	20-40	4.4	4.3	4.1	4.2	4.3		
	40-60	4.3	4.2	3.7	4.2	4.1		
	Maize rhizosphere	5.3	5.2	5.0	3.2	4.7		
	Pigeonpea rhizosphere	5.3	5.8	5.1	5.3	5.4		
	Mean	4.8	4.8	4.4	4.2			
P-solubilizers	0-20	1.3	2.8	0	0.4	1.13	0.37	11
	20-40	1.2	2.4	1.0	2.4	1.8		
	40-60	0	0	0	2.1	0.5		
	Maize rhizosphere	3.2	3.2	2.1	2.3	2.7		
	Pigeonpea rhizosphere	3.5	3.9	2.8	3.9	3.6		
	Mean	1.9	2.5	1.2	2.2			
Cellulose degraders	0-20	4.4	4.7	3.6	4	4.2	0.18	3
	20-40	3.6	3.9	3.4	3.6	3.7		
	40-60	3.1	2.8	2.4	3.1	3		
	Maize rhizosphere	5.1	5.0	5.0	4.9	5.0		
	Pigeonpea rhizosphere	5.1	5.1	4.8	5.0	5.0		
	Mean	4.3	4.3	3.9	4.2			

Table 4.54. Soil nutrient data in top 60 cm profile from four crop husbandry systems (T1 to T4) of a long-term field experiment collected in January 2005

Treatments	T1	T2	T3	T4	Mean
Total N (ppm) at different depths					
0-20 cm	768 (20.9)	693 (26.0)	682 (18.9)	701 (18.1)	711
20-40 cm	484 (35.1)	468 (19.9)	446 (17.3)	493 (30.0)	473
40-60 cm	362 (16.0)	369 (5.6)	356 (8.7)	367 (5.1)	363
Mean	538	510	494	520	
Total P (ppm) at different depths					
0-20 cm	333 (24.8)	310 (16.3)	247 (6.5)	232 (4.7)	281
20-40 cm	202(12.3)	208(13.1)	195(5.0)	188(6.5)	198
40-60 cm	160(6.1)	160(3.3)	164(2.9)	161(3.8)	161
Mean	232	226	202	194	
Available (K) ppm at different depths					
0-20 cm	216 (12.3)	224 (8.5)	170 (6.0)	195 (5.9)	201
20-40 cm	161 (6.9)	167 (1.6)	146 (4.1)	168 (4.6)	161
40-60 cm	154 (7.8)	169 (2.7)	144 (3.2)	168 (3.9)	159
Mean	177	187	153	177	

Data in parentheses are ± SEs.

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Table 4.55. Crop and dry matter yield of maize and pigeonpea in the four treatment plots of BW3 in 2004-05

Treatments/ Yield (t ha^{-1})	T1	T2	T3	T4
Maize grain yield	5.1 (0.158)	4.9 (0.17)	5.3 (0.13)	6.1 (0.13)
Maize dry matter	11.3 (0.29)	10.6 (0.34)	11 (0.22)	13.1 (0.32)
Pigeonpea grain yield	0.95 (0.02)	0.93 (0.014)	0.89 (0.014)	0.85 (0.016)
Pigeonpea dry matter	2.59 (0.08)	2.6 (0.066)	2.82 (0.071)	2.00 (0.041)

Data in parentheses are ± SEs.

T1 = Low-cost system 1, no tillage, rice straw as surface mulch + Biological inputs for plant growth and pest management.

T2 = Low-cost system 2, no tillage, farm waste as surface mulch+ Biological inputs for plant growth and pest management.

T3 = Conventional system, tillage, integrated nutrient management and chemicals for plant growth and pest management.

T4 = Same as T3 + Farm waste as biomass as in T2.

4.12.3 Indicators of soil-quality

The application of crop residues increased the microbial biomass C, microbial biomass N, mineralizable N and basal respiration. Means of 0–20 cm depths of two-year data was used to evaluate the soil quality indices based on four parameters, biological (Table 4.56, Fig. 4.24), microbial (Table 4.57, Fig. 4.25), nutrient (Table 4.58, Fjg. 4.26) and crop index (Table 4.59, Fig. 4.27). The treatment plot T2 where farm-waste was applied along with compost and plant growth promoting bacteria and biological agents for four years was the most sustainable for different cropping systems studied. Least value of sustainability calculated from the formula was 2.00. Of the four different crop husbandry treatments T2 plot had sustainability index of 2.29. The nutrient index of T4 plot was low (0.95) and the sustainability index was 2.10, whereas T1 plot had lower crop index (0.98) and the sustainability index was

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2.07. In T3 plot, biological, microbial and nutrient indices were low (0.85, 0.83 and

0.86) and, therefore, the sustainability index calculated was also low (1.56). (Table

4.60). Hence, the sustainability index derived was T2>T4>T1>T3 (Fig. 4.28).

Table 4.56.   Biological indicators of soil quality (means of 0-20 cm of two-year 2003-
05 data) from four crop husbandry systems (T1 to T4) of a long-term
field experiment

Treatments	ACP	ALP	DH	SR	MBC	MBN	Biological index
T1	0.96	0.98	1.03	0.97	1.09	1.07	1.01
T2	1.12	1.07	1.02	1.07	1.08	1.08	1.07
T3	0.89	0.89	0.93	0.79	0.84	0.81	0.85
T4	1.03	1.06	1.03	1.16	0.99	1.01	1.08

ACP = Acid phosphatase, ALP = Alkaline phosphatase, DH = Dehydrogenase, SR = Soil
respiration, MBC = Microbial biomass C, MBN = Microbial biomass N.

Table 4.57. Microbial indicators of soil quality (means of 0-20 cm of two-year 2003-05
data) from four crop husbandry systems (T1 to T4) of a long-term field
experiment

	Bacteria	Fungi	Actino	Pseudo	Sidero	PSB	Phytase	Antag	MI
T1	1.00	0.98	1.09	1.09	1.02	0.92	1.00	1.04	1.02
T2	1.02	1.06	1.04	1.20	1.09	1.55	1.16	1.02	1.14
T3	0.95	0.97	0.90	0.82	0.89	0.39	0.86	0.88	0.83
T4	1.02	0.96	0.98	0.91	1.02	0.76	1.05	1.04	0.97

Actino = Actinomycetes, Pseudo = *Pseudomonas* (fluorescent type), Sidero = sidherophore
producers, PSB = phosphate solubilzing bacteria, Anta = antagonistic bacteria, MI-
microbiological index.

T1 = Low-cost system 1, no tillage, rice straw as surface mulch + Biological inputs for plant
growth and pest management. T2 = Low-cost system 2, no tillage, farm waste as surface
mulch+ Biological inputs for plant growth and pest management. T3 = Conventional
system, tillage, integrated nutrient management and chemicals for plant growth and pest
management. T4 = Same as T3 + Farm waste as biomass as in T2.

Table 4.58. Soil nutrient indicators of soil quality (means of 0-20 cm of two-year 2003-05 data) from four crop husbandry systems (T1 to T4) of a long-term field experiment

Treatments	Total N	Total P	Available K	OC%	Available P	Nutrient index
T1	0.92	1.08	1.07	1.17	1.05	1.06
T2	0.98	1.12	1.07	1.15	0.93	1.05
T3	0.77	0.91	0.93	1.05	0.73	0.88
T4	0.78	0.89	0.92	1.10	0.99	0.94

Table 4.59. Crop yield and productive potential as indicator of soil quality (means of two-year 2003-05 yield data) from four crop husbandry systems (T1 to T4) of a long-term field experiment

Treatments	Cereal yield	Legume yield	Cereal dry matter	Legume dry matter	Crop index
T1	0.95	1.10	0.96	0.91	0.98
T2	0.90	1.17	0.96	0.99	1.00
T3	1.00	0.92	1.01	0.94	0.97
T4	1.15	0.97	1.10	1.12	1.09

T1 = Low-cost system 1, no tillage, rice straw as surface mulch + Biological inputs for plant growth and pest management.

T2 = Low-cost system 2, no tillage, farm waste as surface mulch+ Biological inputs for plant growth and pest management.

T3 = Conventional system, tillage, integrated nutrient management and chemicals for plant growth and pest management.

T4 = Same as T3 + Farm waste as biomass as in T2
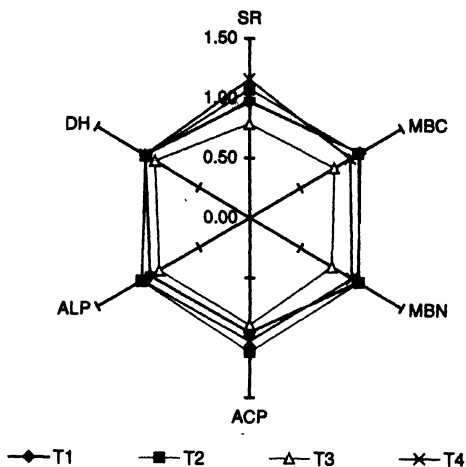
Figure 4.24. Biological indicators of soil quality from four crop husbandry systems (T1 to T4) of a long-term field experiment. ACP = Acid phosphatase ALP = Alkaline phosphatase, DH = Dehydrogenase SR = Soil respiration, MBC = Microbial biomass C, MBN = Microbial biomass N.
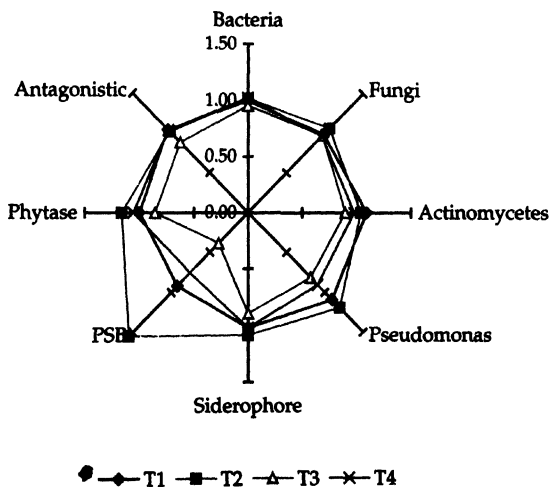
Figure 4.25. Microbial indicators of soil quality from four crop husbandry systems (T1 to T4) of a long-term field experiment.
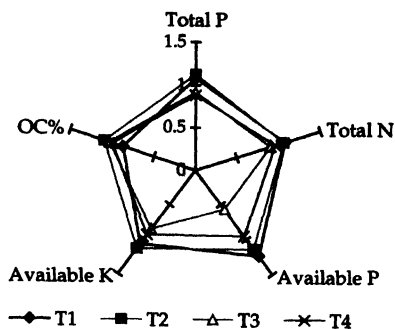
Figure 4.26. Soil nutrient indicators of soil quality from four crop husbandry systems (T1 to T4) of a long-term field experiment.
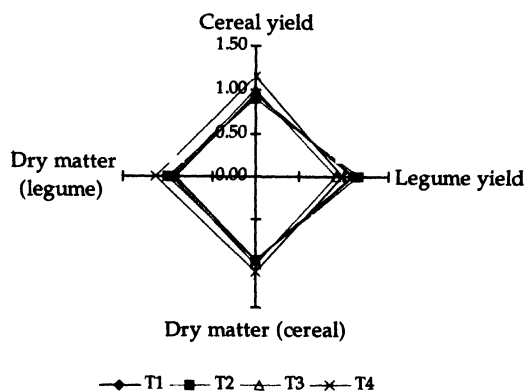
Figure 4.27. Productive crop indicators of soil quality from four crop husbandry systems (T1 to T4) of a long-term field experiment

Table 4.60. Sustainability indicators of soil quality from four different crop husbandry systems (T1 to T4) of a long-term field experiment

	Biological index	Crop index	Microbial index	Nutrient index	Sustainability index	System
T1	1.01	0.98	1.02	1.07	2.07	Sustainable
T2	1.07	1.00	1.09	1.11	2.29	Sustainable
T3	0.85	0.97	0.85	0.86	1.56	Unsustainable
T4	1.08	1.09	0.98	0.95	2.10	Sustainable

Figure 4.28. Sustainability indicators of soil quality from four crop husbandry systems of a long-term field experiment.

T1 = Low-cost system 1, no tillage, rice straw as surface mulch + Biological inputs for plant growth and pest management.

T2 = Low-cost system 2, no tillage, farm waste as surface mulch + Biological inputs for plant growth and pest management.

T3 = Conventional system, tillage, integrated nutrient management and chemicals for plant growth and pest management.

T4 = Same as T3 + Farm waste as biomass as in T2.

CHAPTER - V

DISCUSSION

DISCUSSION

Bacteria associated with farm waste compost (FWC), rice straw compost (RSC) and *Gliricidia* vermicompost (GVC) and macrofauna (earthworms, centipedes, slugs and snails) present in farm waste compost have been studied in this thesis work. Bacteria that can enhance growth of plant and productivity have been known from past (Brown 1974). Prominent among these organisms are species of genus *Rhizobium* (Eagleshman 1989) whose potential and practical use in agriculture is beyond doubt. Mutualistic bacteria used as inoculants are promising agents for sustainable agriculture since they contribute to decrease the application of mineral fertilizers and chemical pesticides. They form loose associations with plants, living near, on, or even inside roots (Larcher *et al.* 2003). Among mutualistic bacteria, plant growth promoting bacteria (PGPB), stimulate plant growth through direct mechanisms while biocontrol PGPB increase plant growth indirectly, through preventing deleterious effects of phytopathogenic microorganisms (Bashan and Holguin 2002; Gamalero *et al.* 2002).

Composts have positive effects on soil fertility and crop productivity as they increase organic matter and nutrient levels (Atiyeh *et al.* 2000a; Pascual *et al.* 2002). In addition, they activate the autochthonous microorganisms of the soil and indirectly, the biogeochemical cycles therein increase (Pascual et al. 1997) Composts and vermicomposts when used as soil amendments, stimulate the proliferation of antagonistic bacteria, protects plants against soil-borne pathogens. (Grappelli *et al.* 1987; Ros *et al.* 2005).

Many researchers have studied microbial inoculants for composting the wastes, especially for minimizing and eliminating the lag time and have focused on the changes in physio-chemical parameters, primarily in an effort to find a simple and reliable indicator of compost maturity, and to improve the efficiency of the composting process. However, very little information is available on the microbiota, which determine the rate of composting and affect the quality of end product (Epstein 1997). The role of compost-inhabiting microbes in inhibiting the growth of pathogens is reported (Schueter 1989) although microbial characterization of the composts (end products) and the faunal population (such as slugs, snails, millipedes and centipedes) and assessing their role in promoting plant growth has received little attention.

In light of this, efforts have been made to screen plant growth promoting and antagonistic bacteria from three different composts. Characterization of selective bacterial isolates, their effect on plant growth, cellulase activity, phosphate solubilization, biocontrol ability and their interaction with the existing potential microorganisms (*Rhizobium* and Mycorrhizae) have been studied. The impact of a low-input technology (crop residue application) combined with biological strategy and/or biotechnological practice (use of microbial inoculants for soil fertility and pest management) has also been discussed with regard to investigate how these agro-technological applications affect the sustainability index and crop productivity.

5.1 SCREENING OF BACTERIA FROM COMPOSTS FOR PLANT GROWTH AND ANTAGONISM TO PATHOGENIC FUNGI

Microbial enumeration revealed that population of bacteria in all the sources studied was high ($3.8 - 7.4$ $\log_{10}$) but propagules of fungi were low ($2.3-3.7$ $\log_{10}$) (Table 4.1). The feeding activities of soil invertebrates can cause gross shifts from fungal to bacterial activity in litter and soils (Hanlon and Anderson 1980) not only through the sensitivity of fungal thallus to disruption and the formation of finely particulate faecal materials which favor bacteria, but also as a result of bacterial growth in animal gut. Previous reports also support the faeces of invertebrates such as millipedes (Anderson and Bignell 1980), woodlice (Reyes and Tiedje 1976) and earthworms (Satchell 1983) contain 500 times more bacteria, in terms of total counts and viable cells, than the food materials and form sites of intense bacterial activity. Thus, invertebrates can act as vectors of beneficial soil bacteria and can influence their population dynamics and impact of microorganisms on soil and plants (Doube et al. 1994; Lavelle 1997).

A total of 207 bacterial isolates were screened for different plant growth promoting traits (Table 4.2). Of the 207, 24 were from farm waste compost (FWC), 78 from excreta or body surface of macrofauna, 55 from rice straw compost (RSC) and 50 from *Gliricidia* vermicompost (GVC). Potential plant growth promoting and biocontrol bacteria were present in high number in GVC followed by RSC (Fig. 4.1). Predominant plant growth promoting and biocontrol agents of genera *Bacillus*, *Pseudomonas*, *Pantoea* etc. recolonize composts during curing stage. Compost is a product rich in disease suppressive microorganisms and when applied to the soil,

enrich variety of nutrients that are available for the indigenous microflora and hence plants (Hoitink and Boehm 1999).

Population of Gram-negative rods was slightly lower than Gram-positive bacteria, except for bacterial isolates from macrofauna (Table 4.2). Thermophilic bacteria, particularly Gram-positive bacteria appear to dominate the early phase of high activity and later on actinomycetes predominate (Finstein and Morris 1975). Population of *Pseudomonas* in composts was low in our observation (Table 4.2) though they are reported to be predominant group in rhizosphere and rhizoplane as plant growth promoting bacteria (Sharma and Johri, 2003a). *Pseudomonas* spp. are rapid colonizers of organic matter and are widely distributed in diverse agricultural ecosystems (Garbeva *et al.* 2004). Only a small percentage of bacteria (2-3%) from each source studied were P solubilizers (Table 4.2). Most media used earlier for the isolation of phosphate solubilizing microorganisms (PSMs) lacked a buffering component. Such PSMs, isolated using conventional unbuffered medium, secrete 10-20 fold less organic acids than that required to solubilize soil phosphate (Gyaneshwar *et al.* 1998). We used Tris HCl buffer of pH 8, which allows only efficient and excess organic acid producing bacteria to show P solubilization. The population of phytase producing bacteria was more or less similar (16-23%) in all sources studied and is comparable with earlier studies of Richardson and Hadobas (1997).

Significant population of siderophore producers (46% in RSC and 43% in GVC) supports that composts have the potential to provide a conducive environment for

proliferation of antagonists that aid in plant growth (Sharma and Johri 2003b). Indole producers were present in all sources studied and this group of bacteria is known to promote root elongation and plant growth (Pattern and Glick 2002). The population of ACC deaminase producers was 53% in RSC and 50% in GVC. Bacteria that possess ACC deaminase promote root and plant growth by hydrolyzing ACC and decrease ethylene biosynthesis in roots (Burd *et al.* 1998).

Low populations of HCN producers in RSC and GVC and chitinase producers in GVC and macrofauna were observed (Table 4.2). Significant numbers of bacterial isolates were antagonistic to all the four soil borne plant pathogenic fungi (Table 4.2). Cyanide production, chitinase activity and pathogen antagonism, are considered indirect PGP properties (Elo *et al.* 2000). Hydrogen cyanide (HCN) a volatile compound is produced by certain fluorescent pseudomonads in presence of glycine and Fe (III), both *in vitro* and in soil conditions (Gaffney *et al.* 1994; Keel *et al.* 1989). HCN production has been shown as a beneficial and harmful property for plants (Cattelan *et al.* 1999). Although chitinase has been proposed as a mechanism of fungal antagonism, but this has been tested *in vitro* only (Renwick *et al.* 1991; Kurek and Jaroszuk 1997). In this study, bacterial isolates that produced HCN and chitinase suppressed soil borne plant pathogenic fungi (Table 4.7). These studies show the association of PGPB with all the macrofauna (earthworms, centipedes, snails and slugs - data on individual macrofauna not shown separately). More studies with increased sample size are suggested to compare microorganisms from each of the four macrofauna to differentiate them for their relative values. Their

identification may also help to differentiate the beneficial and harmful macrofauna. Of the four macrofauna studied, slugs and snails are considered harmful as they damage the seedlings and feed on foliage of crop plants. In our studies, we observed that macrofauna harbor beneficial bacteria on their body surface and in excreta that can promote plant growth (Table 4.3). Soil fauna are known to have an important function in regulating rhizosphere microbial processes and significantly improve plant growth (Bonkowski *et al.* 2000). These macrofauna when present in soil or/and compost can act as source of inoculants for plant growth promoting bacteria.

5.2 SELECTION, CHARACTERIZATION AND IDENTIFICATION OF PLANT GROWTH PROMOTING BACTERIA

Twenty-three isolates with more than one plant growth promoting and antagonistic trait were tested for plant growth studies *in vitro*. Twelve out of twenty-three showed significant seedling vigor index of pearl millet (ICMV 155) and sorghum (CSV 15) by paper towel method (Table 4.3 and 4.4). Many of these 12 isolates shared common characteristics. Five were positive for P-solubilization, five for phytase activity, nine were positive for siderophore and ACC deaminase, six for indole and HCN production and three showed chitinase activity (Table 4.7). Previous studies indicate, that bacteria possessing these traits promote plant growth (Correa *et al.* 2004).

Results of bacterial identification indicated that a restricted group of culturable genera inhabited the compost. The major genera were *Bacillus, Pseudomonas, Serratia, Enterobacter* and *Klebsiella* (Table 4.5 and 4.6). Most of these bacteria are non-symbiotic diazotrophs that play a significant role in plant growth promotion

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(Kennedy *et al.* 2004). Microbial groups *Bacillus, Flavobacterium* and *Pseudomonas* are found not only in soils but also during composting (Tiquia *et al.* 2002). Of the 12 potential strains, *Bacillus licheniformis* EB 13 and *Serratia marcescens* EB 67 enhanced germination and seedling vigor index of pearl millet significantly different than *A. chroococcum* HT 54 (reference strain) used in the studies. Seedling vigor index of sorghum was significantly different with *S. marcescens* EB 67 than reference strain used in the studies (Table 4.4). *P. fluorescens* was reported to enhance seed germination and seedling vigor of sorghum and pearl millet (Umesha *et al.* 1998; Raju *et al.* 1999).

5.3   PERFORMANCE OF PHOSPHATE SOLUBILIZING BACTERIA (PSB)

Out of five strains that showed P-solubilization ability in buffered conditions (Fig. 4.2) with glucose as carbon source, two strains *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 were picked up for future studies. Mineral phosphate solubilizing (MPS) bacteria utilize the direct oxidation pathway to produce gluconic acid and 2 keto-gluconic acid. *S. marcescens* EB 67 secreted 64 mM gluconic acid and *Pseudomonas* sp. CDB 35 secreted 27 mM of gluconic acid (Table 4.8). *E. asburiae* PSI3 (reference strain) showed 55 mM of gluconic acid.

Both the strains EB 67 and CDB 35 showed drop in pH in presence of all the carbon sources used (except arabinose) (Fig. 4.3 and 4.4). P released by *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 in presence of glucose RP buffered medium was 1212 µM and 522 µM respectively (Table 4.9). Previous studies by Gyaneshwar *et al.* (1999) showed P released by *E. asburiae* in presence of glucose and RP medium was 500 µM.

Both the strains showed RP solubilization in presence of nutrients (carbon and nitrogen source) in soil conditions (Table 4.10 and 4.11). Plants secrete various root exudates such as carbohydrates, organic acids, aminoacids etc. to varying extents (Lugtenberg 1999). These strains can use root exudates as nutrient source and supply P to plants. Such strains, which can utilize varied carbon sources and solubilize P, may play a better role in soil conditions/rhizosphere. However, the concentration of carbon sources employed for P-solubilization in our studies is much higher than found in rhizosphere and some of these carbon sources also may not be present in rhizosphere.

Gcd activity of two strains *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 varied depending on nature of sugars used as carbon source and was highest with glucose, galactose, xylose than maltose. *Pseudomonas* and *Serratia* sp. show MPS by the non-phosphorylating oxidation pathway for aldose sugars. The enzymes for direct oxidation pathway are oriented in the cytoplasmic membrane such that glucose (or other aldose sugars) undergoes oxidation leading to gluconic or 2-keto gluconic acid in periplasmic space with the resultant acidification of this region and the medium (Goldstein 1995; Krishnaraj and Goldstein 2003). Ability of these two strains to secrete organic acids is expected to not only depend upon efficiency with which the Gcd enzyme converts different sugars to corresponding acids but also on the amount of Gcd enzyme present when grown in presence of a particular sugar (Fig. 4.5). Gcd has been characterized from bacteria such as *Acetobacter calcoaceticus, Escherichia coli, Glucanobacter oxydans* from the point of view of understanding its role in basic carbohydrate metabolism (Cleton Jansen *et al.* 1989). However, organic acid

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produced by both the strains EB 67 and CDB 35 varied with respect to the sugar. Organic acid estimated by HPLC showed that the acid formed in presence of galatose, xylose, maltose, mannose and cellobiose showed two different retention peaks, unlike glucose where peak was only due to gluconic acid.

5.4 EVALUATION OF SELECTED PSB EB 67 AND CDB 35 FOR RECYCLING CROP-RESIDUES.

S. marcescens EB 67 and *Pseudomonas* sp. CDB 35 could utilize cellulose and crop residues as carbon source. Nutrients when added as biomass (crop residues) are not in readily available form for crops and need to be mineralized by microbial activity. Also, when biomass is added as surface mulch, microbial activity of the soil surface might not be sufficient for its decomposition. To enhance mineralization and decomposition of crop residues, screening plant growth promoting bacteria for their cellulose utilization should be given criteria of selection, for their survivability in soil/field conditions.

Both the PSB EB 67 and CDB 35 showed cellulase activity and solubilized RP in presence of different crop residues in submerged and solid state media conditions. In submerged conditions, cellulase activity was maximum using rice root, which was after day 4 with EB 67 and day 6 with CDB 35 (Table 4.12). Studies with cellulolytic bacteria suggest that prokaryotic cellulases vary differently from those of fungal cellulases, based on temperature and pH. Therefore, comparision in terms of enzyme activity is difficult to establish (Heck *et al.* 2002). In solid-state conditions (SSC), there was enhancement in cellulase activity by both the strains in presence of grass and

rice root (Table 4.14). The action of cellulases is synergistic over substrate, especially for microorganisms isolated from environments where agro-residues are biodegraded (Pandey and Soccol 1998). In our work, both the strains were from composts, confirming their ability to use crop residues as carbon source. Gessesse and Mamo (1999) postulate that the great advantages of using SSC over submerged conditions for enzyme production are the lower water content of enzymatic extract, easy to control, lower probability of contamination. However, purpose of our study, in solid-state conditions was to see the performance of these strains when applied along with crop residues in compost or soil conditions.

S. marcescens EB 67 and *Pseudomonas* sp. CDB 35 were introduced in unsterilized soil along with different organic carbon sources (glucose, organic acids, crop residues such as rice straw, rice root, pigeonpea root and grass) and RP. Survival of PSB was detected on RP buffered agar medium with intrinsic levels of antibiotics. Both the strains survived for 48 days in soil (Table 4.17) and the population was less in soil where crop residues were applied when compared to glucose. Kim *et al.* (1998a) reported survival of PSB *Enterobacter agglomerans* on hydroxyapatite medium in soil amended with carbon sources upto 55 days. Kundu and Gaur (1981) reported that peat was the best carrier when compared to farm soil for PSB. In this study, there was absolutely, no growth of PSB on RP buffered antibiotic medium from control (uninoculated) treatment (Table 4.17). It may be due to the absence of native PSB that could solubilize P in buffered conditions. Therefore, the activity of added PSB, EB 67 and CDB 35, in other treatments was completely attributable to inoculation. Survival

of both the strains EB 67 and CDB 35 in soil conditions can be studied for prolonged periods and formulations using soil as carrier can be developed.

Acid phosphatase activity in soil due to application of glucose or crop residues did not vary significantly (Table 4.18). It suggests that there was no effect of inoculated phosphate solubilizing bacterial strains on acid phosphatase activity in soil. At the range of soil pH found in the soils used in this study (pH 7.8-8.0) acid phosphatases are unlikely to be active (Table 4.18 and Fig. 4.11). Eivazai and Tabatabai (1977) and Rojo *et al.* (1990) reported that alkaline soil contained predominantly alkaline phosphatase and acid soil predominantly acid phosphatase. In view of the pH of the soil studied, Trasar-Cepeda and Gill-Stores (1987) interpreted that enzyme production adapts to the condition of soils. In this study, alkaline phosphatase activity decreased with time, with all organic carbon sources applied (Table 4.19 and Fig. 4.11). Effect of pH on sorption of enzymes has only been realized as being important in measuring phosphatase activity. The consensus in literature is that acid and alkaline phosphatase are differentiated by their source of production. Acid phosphatase is exclusively produced by plants and alkaline phosphatase does not have plant origin and is produced by microorganisms (George *et al.* 2002).

In all treatments, soil respiration and soil microbial biomass C at day six was significantly different and decreased at 12, 24 and 48 days (Table 4.20 and 4.21). Mean microbial activity (CO_2 released) in this study followed a pattern similar to the classic exponential curve of microorganism growth. After inoculation of bacteria based on the nutrient availability in soil different phases can be observed. Initial

phase refers to intensive mineralization during the first week of incubation, and decrease in its intensity was observed after twelve days of incubation (Table 4.20). Second phase corresponds to maintenance of an average mineralization activity and the third phase of is represented by decrease in activity dependant on number of microorganisms and limiting factors such as nutrients (Valarini 2002). Mineralization of organic carbon sources in non-rhizosphere soil is rapidly degraded depending on soil type (Jones and Darrah 1994; Jones 1998). Most of the carbon sources added along with the bacterial inoculants may be responsible for increase soil microbial biomass at day six (Table 4.21). Biomass C with both the isolates was significantly different on 6th day, which might be due to population of inoculated bacteria. The incorporated glucose as well as organic acid, was rapidly used to increase the soil microbial biomass when compared to crop residues.

Organic acid application showed pH drop of 6.2 at day six. None of the crop residues used as carbon source showed pH drop (Table 4.22). Hence it cannot be concluded that organic acids were involved in P solubilization and it might be due to alkaline phosphatases produced by PSB strains. During stress conditions PSB are known to produce alkaline phosphatase and solubilize P (Thamodharan *et al.* 2003). Phosphorous concentrations inoculated with EB 67 was significantly different at day 6 where soil was amended with glucose (Table 4.23), while with the crop residue application it was significantly different at day 24. CDB 35 also showed similar pattern of P release in case of readily available carbon source (glucose), but with crop-residues it was maximum at day 12 (rice root and grass) and at day 24 (rice straw and pigeonpea root) (Table 4.23). Their activity in soil in presence of crop

residues could be due to microbial breakdown of cellulose into cellobiose and glucose which can act as nutrient source and might have improved biomass C and available P.

5.5 PLANT GROWTH PROMOTING BACTERIA (PGPB)

Twelve strains were selected based on plant growth promoting traits for growth promoting studies of pearl millet and sorghum (Table 4.3 and 4.4). High diversity of microorganisms is reported from macrofauna and insects, but very few reports of bacterial identification exist (Thimm *et al.* 1998). *Serratia marcescens* EB 67 isolated from slug (body surface) present in FWC and *Pseudomonas* sp. CDB 35 isolated from RSC exhibited multiple PGPR traits like P solubilization, ACC deaminase, siderophore and increased root length and plant dry weight of pearl millet under glasshouse conditions (Table 4.27 and 4.28). Bacteria possessing these traits are known to increase plant growth (Kloepper 1993). Various species of *Serratia* are diazotrophs that enhance plant growth and have been used as potential biocontrol agents (Kalbe *et al.* 1996; Gyaneshwar *et al.* 2001). Fluorescent pseudomonads that produce siderophores and auxins are known to colonize roots and promote plant growth (Dey *et al.* 2004).

All five bacterial strains that showed P solubilization were positive for siderophore and ACC deaminase production. Two strains that showed P solubilization increased plant dry weight significantly (Table 4.27 to 4.30). Considering the P availability is a limiting step in plant nutrition (Goldstein 1986), the contribution of these bacteria to plant growth promotion can be supported. PSB are also reported to produce

metabolites such as phytohormones, antibiotics and siderophores (Kloepper *et al.* 1989). Four of the five strains having phytase activity (important in P-acquisition by plant) showed significant increase in plant dry weight of pearl millet (Table 4.28 and 4.30). However, responses to inoculation with phytate mineralizing bacteria have generally been observed only when a large amount of phytate is provided (Richardson *et al.* 2001b). Seven of the nine strains possessing ACC deaminase activity significantly improved root length. This was in consistence with the observations in canola and other crops, which showed root elongation when ACC deaminase producing bacteria were applied (Penrose *et al.* 2001).

Two strains *Bacillus circulans* EB 35 and *Bacillus licheniformis* CDB 47 were effective in promotion of root growth though they did not produce IAA. Recent report by Kishore *et al.* (2005a) showed that there was no correlation between IAA production and root length. The difference in performance could be due to the availability of L-tryptophan to the bacteria in root exudates for IAA production. The optimal concentration of IAA required for plant growth promotion is extremely low and doses of IAA above the threshold levels are deleterious for root growth (Xie *et al.* 1996) as observed with isolate BWB 36 in our studies. Root length density was increased with few strains (table 4.27 to 4.30) and is considered as a better estimate of root system to acquire water and nutrients (Ali *et al.* 2005). In this study, beneficial effects of PGPB on plants can be due to production of phytohormones, metabolites and enzymes that can promote root development and proliferation resulting in efficient uptake of water and nutrients.

Assessment of growth by the strains under glasshouse conditions using sterilized soil on growth of pearl millet and sorghum showed that three strains *S. marcescens* EB 67, *Pseudomonas* sp. CDB 35 and *B. circulans* EB 35 showed significant difference in dry matter (Table 4.27 and 4.29). In unsterilized soil conditions, ten strains, *B. licheniformis* EB 13, *E. cloaceae* EB 27, *B. circulans* EB 35, *S. marcescens* EB 67, *K. oxytoca* EB 77, *Pseudomonas* sp. CDB 35, *Pseudomonas* sp. CDB 36, *B. licheniformis* CDB 47 and *Pseudomonas* sp. BWB 21 and *Pseudomonas* sp. BWB 40 showed significant increase in the plant growth parameters studied and dry matter (Table 4.27 and 4.30). In sterilized conditions improvement in plant growth can be attributed purely due to the inoculated PGPB. In unsterilized conditions, PGPB with different plant growth traits might have enhanced plant growth or they can also provided a niche to the other beneficial bacteria that may aid in plant growth.

5.6   INTERACTION STUDIES OF PLANT GROWTH PROMOTING BACTERIA

S. marcescens EB 67, *Pseudomonas* sp. CDB 35 were compatible with the rhizobial strains in plate culture conditions (Table 4.31). Both the strains inoculated along with *Rhizobium* showed growth promotion of chickpea in pots using unsterilized soil (Table 4.32). The plants inoculated by seed priming method showed significant difference in dry matter yield and nitrogenase activity of chickpea than seed coating method (Table 4.32). The increase in nitrogenase activity was more with EB 67 and CDB 35 than the control treatment. On-farm participatory trials in Western India, showed that seed priming increased yields of chickpea, maize, wheat and upland rice (Harris *et al.* 1999). Seed priming is also done to alleviate stress conditions for *in*

vitro tissue-propagated plants (Nowak and Shulaev 2003). Co-inoculation of fluorescent *Pseudomonas* and *Rhizobium* improved plant growth of *Pisum sativum* (Dileep Kumar *et al.* 2001). Studies by Parmar and Dadarwal (1999) showed that *Pseudomonas* sp. that produce siderophores could increase the level of flavonoid-like compounds in the root which, increased total plant nitrogen in chickpea. In this study, both the strains were siderophore producers. It is also known that plant root flavonoids are inducers of nodulation gene (nod genes) expression in *Rhizobium*.

Dual inoculation of PSB *S. marcescens* EB 67, *Pseudomonas* sp. CDB 35 along with AM (*Glomus* spp.) showed significant difference in dry matter yield of sorghum. Per cent infection due to application of bacteria along with AM within 45 days was same as that of AM inoculation alone at 90 days (Table 4.33 and 4.34). This suggests the possible reduction of AM culturing period by 45 days compared to its usual three-month pot culture method. Previous reports (Bhowmik and Singh 2004) due to the inoculation of *Azospirillum* with *Glomus* spp. also showed significant improvement in AM colonization. PGPB are known to synthesize biologically active substances (plant growth hormones) that affect AM spore germination, hypal elongation that in turn increased root biomass which accelerates the AM root colonization (Azcon 1987). However, studies of Bending *et al.* (2002) showed that *Pseudomonas, Serratia* and *Burkholderia* isolates inhibited mycorrhizal colonization while *Bacillus* doubled ectomycorrhizal colonization in roots. Kucey (1987) reported maximum plant growth and P uptake in sterilized soils where both AM and *Penicillium bilaii* were inoculated. 'Mycorrhizal helper bacteria' mainly of *Pseudomonas* spp., has ability to

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enhance mycorrhizal formations significantly in plants (Garbaye and Bowen 1989; Duponnois and Garbaye 1991).

5.7   EFFICACY OF ANTAGONISTIC BACTERIA EB 13 AND CDB 35

Frequency of anti-fungal strains was high among RSC followed by GVC, macrofauna and FWC (Table 4.2). Selected strains also varied in their activity spectrum on the three media used and eighteen were effective against all the four fungi in our studies (Fig. 4.13). Bacteria isolated from composts showed antagonistic activity in this study (Table 4.24 to 4.26). The antagonistic effect towards the pathogenic fungi may be due to lytic enzymes, antibiotics, siderophores and secondary metabolites produced by the microorganisms (Hamdan *et al.* 1991). Of the 18 strains that showed inhibition against all the four soil borne pathogenic fungi on PDA, GCY and KBM. Mean per cent inhibition against *S. rolfsii* by *B. licheniformis* EB 13 was maximum and *B. coagulans* CDB 31 was minimum, *M. phaseolina* by *Pseudomonas* sp. CDB 35 was maximum and B. *coagulans* BWB 34 was minimum, *F. solani* and *F. oxysporum* by CDB 35 was maximum and *B. coagulans* EB 15 was minimum (Table 4.24 and 4.26; Fig. 4.14).

In order to avoid the unpredictability due to competition for nutrients, provide a suitable niche simulating the conditions existing in composts/soil, obtain consistency in results; antagonistic nature of strains against different fungi was studied using three different media (Table 4.24 to 4.26). The variability of inhibition by a given bacterium on the three different media illustrates the importance of nutrition on the efficacy of biological control microorganisms (Photograph 4.11).

Similar results of varied performance on two media were observed when *Burkholderia cepacia* strains were tested against the selected soil borne fungal pathogens (Baligh *et al.* 1999). Variation in the spectrum of antifungal activity of microorganisms or their metabolites is not uncommon (Leifert *et al.* 1995). Of the three media used PDA is known to support fungal growth. Though in our studies, PDA supported both the growth of bacteria and fungi. However, suppression of fungal growth was less when compared to the other two media. It could be due to lack of nitrogen or nutrient source that promotes anti-fungal compounds. King's B medium enhances pigment production by *Pseudomonas* sp., favors the siderophore (pyoverdins and pseudobactins) (Buyer and Leong 1986) production. *P. aeruginosa* produced two antibiotic compounds phenazine 1-carboxylic acid and phenazine-1-carboxamide (oxychlororaphin), in GCY medium that suppressed *Pythium debaaryanum* and *Fusarium oxysporum* f.sp. ciceris (FOC), (Anjaiah 1998).

In this study *Pseudomonas* sp. CDB 35 produced needle shaped greenish yellow crystalline structures in plate culture conditions (Photograph 4.1 *l*). *P. aeruginosa* are known to produce bundles of bright green needle-shaped crystals (chlororaphin) of several mm in size in glucose salt minimal medium (Kanner *et al.* 1978). *Bacillus licheniformis* produces phenyl acetic acid (Kim *et al.* 2004), which is related to anti-fungal activity. *B. cereus* produces lytic enzymes and *B. subtilis* produces different antibiotics such as subtilin, bacitracin, bacillin (Muhammad and Amusa, 2003). All these varied substances produced by bacterial isolates might have acted in concert to inhibit the growth of the pathogens used in this study.

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Both the strains *S. marcescens* EB 13 and *Pseudomonas* sp. CDB 35 reduced the biomass of all four fungi significantly, when inoculated in GCY broth media (Fig. 4.15). In the present study, the maximum inhibition against four pathogenic fungi was observed in Kings B medium, which indicates that siderophore production is involved in antagonism, followed by GCY medium, which indicates the role of antibiotic and volatile compounds involved in antagonism (Fig. 4.16). Microscopic studies revealed that in presence of antagonistic isolates there was backward growth of *S. rolfsii* hyphae, cytoplasmic granulation in hyphae of *M. phaseolina*. Rupturing of *sclerotia* formation of *M. phaseolina* with CDB 35 and poor development of *sclerotia* with *B. licheniformis* EB 13 was also observed (Photograph 4.15). Cell-free culture filtratres of *Pseudomonas* sp. GRS 175 showed inhibition of soil-borne necrotrophic fungal pathogens of groundnut *in vitro* conditions (Kishore et al, 2005b). Similarly, studies of Saleem and Ulanganathan (2002) showed lysis of *Curvularia lunata* mycelium in presence of *Bacillus* spp (strain BC 121). Additional nutrient sources (FeSO$_4$.H$_2$O; MgSO$_4$.4H$_2$O; NaCl, ZnSO$_4$. 7H$_2$O and mixture of salts) amended to PDA medium showed zinc sulphate aided in improving the antagonistic ability of both the strains EB 13 and CDB 35 against *S. rolfsii* and *M. phaseolina invitro* conditions (Fig. 4.17 and 4.18).

Two strains *B. licheniformis* EB 13 and *Pseudomonas* sp. CDB 35 that suppressed the two soil borne plant pathogenic fungi *M. phaseolina* and *S.rolfsii* in laboratory studies were evaluated for disease control in glasshouse conditions. *B. licheniformis* EB 13 and *Pseudomonas* sp. CDB 35, suppressed *S. rofsii* and reduced disease level (Table 4.35). Bio priming with *Rhizobium* and biocontrol bacteria showed enhancement in

growth of chickpea and reduced the collar rot disease. Seed bio priming is reported to induce resistance against downy mildew and enhance growth of pearl millet (Niranjan *et al.* 2004). *Serratia marcescens* suppressed *S. rolfsii* in sterilized soil conditions and due to lytic enzymes produced by bacteria (Ordentlich *et al.* 1988). Fungicide and biocontrol bacteria, *Pseudomonas fluorescence* in combination reduced the incidence of collar rot of chickpea (Singh *et al.* 2003).

Soil amended with zinc alone did not inhibit infection of *M. phaseolina* in sorghum roots but did significantly reduce in combination with the antagonistic bacteria (Table 4.36). The results of effect of mineral nutrients on fungal suppression obtained under laboratory conditions correlate well with those obtained under glasshouse conditions. Mineral nutrients and carbon sources reduced the infection of *M. phaseolina* in tomato roots. Increased production of antifungal compounds is presumably the primary mechanism involved in suppression of pathogen (Shaukat and Siddiqui 2003). The other mechanisms may be altering the physiological aspects of plant following amendment with zinc sulphate and biocontrol bacteria or it might be due to the stimulation of soil microorganism antagonistic to *M. phaseolina* that could reduce colonization of fungal pathogen in the roots. Zinc and other elements are known to stimulate *in vitro* production of 2,4-diacetylphloroglucinol; pyoluteorin, phenazine type antibiotics and stabilize regulatory genes critical for antibiotic production in fluorescent pseudomonads (Slininger and Jackson 1992 Duffy and Defago 1995; Duffy and Defago 1997).

From a practical perception, effect of minerals on the biocontrol effectiveness may explain the involvement between soil chemical and physical properties and the erratic performance of biocontrol strains in field conditions. A previous study by Siddiqui and Shoukat (2002) reports the coexistence of *Pseudomonas aeruginosa* and *P. fluoroscens* exhibited greater suppression towards tomato root pathogens compared to their individual application. Coalescence of antagonists in the milieu of niche differentiation provides an effective means for improving biocontrol of soil-borne plant pathogens (Wilson and Lindow 1994). This can be an inexpensive way to improve the biocontrol activity of certain bacterial strains. Formulations with additional mineral nutrients can improve the biocontrol ability.

5.8   EVALUATION OF COMPOSTS FOR PLANT GROWTH

Composts amendment to soil stimulated growth of pearl millet in glasshouse conditions (Table 4.37). Fertilizer effect of composts is due to release of nutrients to plants directly or indirectly by cation-exchange capacity (Mbagwu and Piccolo 1990). Composts and vermicomposts are known to promote plant growth when added to soil (Atiyeh *et al.* 2000; Arancon *et al.* 2004b), enhance enzymatic activity and phytohormones (Tomati and Galli 1995). In our studies, there was not much difference between the nutrient content among the three composts (Table 3.1) making it hard to predict plant growth responses to these materials based solely on such chemical analyses. GVC had a pH of 6.5, FWC had pH 6.9 and RSC had pH 7.6. Goh and Haynes (1977) reported that plant growth is generally optimized when pH is between 5.0 and 6.5. The improvement in plant growth observed in our studies

might be due to the presence of beneficial bacteria such as siderophore (46% in RSC and 43% in GVC), ACC deaminase (53% in RSC, 50% in GVC) and IAA producers (33% in RSC and 52% in GVC). FWC also showed enhancement in plant growth, but was less than RSC and GVC (Table 4.37), which might be due to low per cent of siderophore, IAA and ACC deaminase producers (Table 4.2). Composts do not stimulate the growth of microorganisms in the rhizosphere. They do impinge on the species composition in rhizosphere thus causing a shift in species groups of microorganisms, such as antagonists to pathogens in suppressive composts (Chen *et al.* 1988) and/or functional groups of rhizobacteria and may result in stimulation of plant growth (Pera *et al.* 1983).

Growth of millet amended with the three composts and three-plant growth promoting bacteria *B. circulans* EB 35, *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 enhanced plant biomass (dry matter) and root length density (Fig. 4.20). This showed the synergistic effect of compost plus bacterial inoculation. Previous studies of Bacilio *et al.* (2003) where *Azospirillum* sp. was added along with composts showed improvement in germination and alleviated the noxious effect of cattle ranch composts.

Dual inoculation with plant growth-promoting bacteria (PGPB) and compost amendment is an uncommon practice, albeit these practices, separately, are commonly used in modern agriculture (van Elsas and Hiejnen 1990). Few types of composts have phenolic compounds and humic acids produced during the composting processes (Valdrighi *et al.* 1995). Nevertheless, humic acids in proper

concentration can enhance plant and root growth (Hartwigsen and Evans 2000). Indigenous soil microorganisms are known to use humic acids to degrade, transform, mineralize and in general over long periods of time, significantly reduce in soil (Filip and Kubat 2001). Though we did not carry out studies to see the metabolism of humic acids present in composts by bacteria (as the composts did not have any negative effect on plant growth), enhancement in plant growth speculates that these bacteria survived well for prolonged periods in organic matter.

Our experiments have shown that composts and vermicomposts have considerable potential for improving plant growth significantly when used as amendment to soil (Table 4.38). However, RSVC application was not so stimulatory for plant growth when applied at 10 t ha^{-1}, when compared to RSC. However, both composts and vermicomposts provided a suitable environment for mycorrhizal colonization. It seems more likely that the nutrient content of vermicompost stimulated fungal development but not growth of plant. Previous work by Cavender *et al.* (2003) showed that nutrients present in vermicomposts stimulated the fungal colonization, but at the expense of plant growth. Thus, application of vermicompost (*Gliricidia* vermicompost) used in the studies at higher concentrations was antagonistic to plant growth. Studies of Atiyeh *et al.* (2000a) showed major difference in the effect of compost and vermicompost that depended on the source of material used for compost preparation. It might be due to the microbial communities with composting result in release of mineral nitrogen in ammonium form and vermicompost released most nutrients in nitrate form readily available for plant.

Vermicomposts enriched with PSB showed significant difference in dry matter of maize and mycorrhizal colonization (Table 4.39). Harinikumar and Bagyaraj (1989) observed that farmyard manure application stimulated VAM colonization. Utilization of low-grade phosphate resources could be achieved by microbes or partial acidulation or alkalization or else exploiting these resources by *in situ* leaching and developing management strategies to increase phosphorus efficiency Bio-phospho-composts will also ensure supply of other associated nutrients to the crops. This increases the potential applicability of microbial inoculants to be used for preparation of enriched composts in agriculture (Zayed and Motaal 2005). Our results have shown that *Gliricidia* vermicompost along with the two-biocontrol bacteria EB 13 and CDB 35 were suppressive to *M. phaseolina* root infection (Table 4.40). Composts applied as soil amendments are known to suppress soil borne plant pathogens (Phae *et al.* 1990).

5.9 PHOSPHATE SOLUBULIZING BACTERIA-PLANT INTERACTIONS

S. marcescens EB 67 and *Pseudomonas* sp. CDB 35 known to solubilize P were evaluated in glasshouse and field conditions. *B. coagulans* and *E. asburiae* known to solubilize P in unbuffered and buffered conditions respectively were used as reference strains. Survival of inoculated bacteria on RP buffered antibiotic media showed similar patterns of inocula survival in glasshouse and field conditions. Both the strains survived and were able to colonize the rhizosphere of maize (Fig. 4.21 - 4.22) and showed significant difference in plant growth parameters studied in glasshouse conditions (Table 4.41). Root colonization is critical in plant growth

promotion and biological control (Kloepper and Beauchamp 1992). Determining the dynamics of root colonization by the introduced bacteria is essential for their effective use. There was no growth of any PSB from control (uninoculated) pots. It may be possible that the native bacteria present in rhizosphere of control pots were not able to grow on RP buffered antibiotic medium plates, which might be due to their inability to grow and solubilize P in buffered conditions (Gyaneshwar *et al.* 2002) or their sensitivity to the antibiotics used in the study. Success of tracking back the introduced *Rhizobium* strains in field trails using serological and antibiotic resistant markers have been reported (Schwinger and Dudman 1973).

Generally in unsterilized soil, introduced microorganisms will not survive in high numbers than those in natural population, or in soils other than their natural habitats (Wessendorf and Lingens 1989). *Pseudomonas fluorescens* strain isolates from *Lupinus hispanicus* colonized pepper roots (Lucas Garcia *et al.* 2003) and *Pseudomonas aeruginosa* isolated from phylloplane colonized the groundnut ecto and endorhizosphere (Kishore *et al* 2005a). The relationship between source of isolation (compost and macrofauna) and colonization of rhizoshere environment will need to be investigated further to know the factors involved in root colonization capacity of both the strains EB 67 and CDB 35.

Plant biomass (dry matter) recorded at different days after sowing (DAS) by EB 67 was significant than the chemical control (single superphosphate: SSP) and was at par the reference strain *E. asburiae* used in the studies (Table 4.42 to 4.45). *S. marcescens* EB 67 showed significant difference in grain and stover yield of maize

when compared to control and where urea and RP was applied. (Table 4.46). P uptake by shoot and grain was also more with *S. marcescens* EB67 (Table 4.47). However, there are no previous reports of performance of the reference strain in field conditions. These results also support the addition of PSB along with rock phosphate might replace the addition of SSP. Phosphate solubilizing and phytohormone producing *Azotobacter chroococcum* showed increase in grain and straw yield (Kumar *et al.* 2001). Though the survival of *Pseudomonas* spp. CDB 35 was equal to *S. marcescens* EB 67 and comparatively higher at few times of sampling, it did not enhance plant growth or uptake of P as shown by EB 67. It might be due to high P solubilizing ability of EB 67 as observed in the presence of different carbon sources and soil conditions (Table 4.9 to 4.11). Interactions occurring in the rhizosphere between plant, soil and microorganisms are important on soil nutrient level and on the microbial effect in rhizosphere (van Veen *et al.* 1997). Banik and Dey (1982) measured increased levels of available P in soils to which farmyard manure, RP and PSB *Bacillus, Streptomyces, Penicillium* and *Aspergillus* spp. were added. Kundu and Gaur (1984) reported increased P uptake and plant growth in various crops inoculated with PSMs. In these cases, the uptake of P from RP by inoculated plants was equal to or greater than that from superphosphate.

S. marcescens EB 67 and *Pseudomonas* sp. CDB 35 inoculated in peat had good shelf life of log 7.1 and 7.9 CFU g^{-1} on LB agar and log 6.2 and 7.3 CFU g^{-1} on RP agar, up to 180 DAI. Population of both the strains was comparatively lower on RP antibiotic buffered medium (Fig. 4.23). Population of *Pseudomonas* sp. CDB 35 was comparatively higher than *S. marcescens* EB 67. Viewing their viability in peat upto

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180 days further indicates that these strains can be tested and used with other low cost carriers. It further indicates the possibility of developing formulations of *Pseudomonas* with long shelf-life without decreasing its effectiveness (Vidhyasekaran and Muthamilan 1995).

5.10. INDICES TO ASSESS SOIL QUALITY AND SUSTAINABILITY OF CROP PRODUCTION BY APPLYING BIOLOGICAL AND CONVENTIONAL TREATMENTS

Soil characterization studies were carried out from four different field treatments of an ongoing long-term experiment of field BW3 at ICRISAT. Soil was collected from four treatment plots T1, T2, T3 and T4 during 2004 and 2005 January, close to the harvesting period. Treatment plots T1 and T2 received locally available, low-cost and eco-friendly materials such as biomass and compost, along with microbial inoculants (*Rhizobium, Azotobacter, B. circulans* EB 35, *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 as required at the time of sowing). Plot T3 received chemical fertilizers similar to integrated nutrient management (conventional agriculture) and plot T4 received inputs of both T3 and biomass (farm-waste, similar to T2) without microbial inoculants at time of sowing. Plots T1, T2 and T4 showed significant enzyme activities and biomass carbon and nitrogen compared to T3. Soil microbial biomass (source and sink of nutrients) will be able to release nutrients more rapidly and with an increase in microbial biomass, availability of N and P in soils is also increased. This is reflected with improved productivity and soil quality. Microbial biomass is one of the most labile pools of organic matter and thus serves as an important reservoir of plant nutrients (Powlson and Jenkinson 1981; Marumato *et al.* 1982).

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Biomass C and N of plots T2 and T4 was significantly higher than T3 (Table 4.48 and 4.52). Increased biomass C and N which resulted in improved production and carbon sequestration was reported by Wani *et al.* (2003) in legume based cropping of an improved system that received integrated nutrient management application (El-swaify *et al.* 1985). With an increase in biomass C (10-32%), there was an increase in mineral N (36-53%), increase in microbial biomass N (14-35%) and about 10-36% increase in total N in the treatment plots T1, T2 and T4, when compared to T3. Net nitrogen mineralized in T2 (0-60 cm depth) was found to be negative (-1.8), indicating immobilization of N and the availability of organic matter for microbial growth. Biomass C, as a proportion of total soil C, serves as a proxy for soil quality (Jenkinson and Ladd 1981). In year six (2005), data was collected from 0-60 cm depth (Table 4.52) and at deeper soil depths the microbial biomass was less which might be due to less metabolic activity than in surface layers, because of reduced availability of oxygen as a result of poor aeration under field conditions.

Acid phosphatase and alkaline phosphatase of treatment plots T1, T2 and T4 were 3-19% higher than T3 (Table 4.48 and 4.52). The physio-chemical properties of soil can influence the respiration rate and enzyme activities such as saccharase, urease, phosphatase and protease activity. Martinez and Tabatabai (2001) described the correlation between phosphatase activity and pH. Throughout the studies soil used was alkaline Vertisol and alkaline phosphatase activity was more than acid phosphatase activity. In addition, acid phosphatase is produced by bacteria, fungi, yeasts and protozoa, so that enhanced acid phosphatase activity in the rhizosphere

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may be produced directly by plant roots or indirectly via stimulation of the microbial biomass. Alkaline phosphatase does not have plant origin and is produced by bacteria, fungi and earthworms (Herbien and Neal 1990). Phosphatase activity decreases with soil depth and corresponds to the distribution of microorganisms in the soil profiles. It is also related to soil organic matter, total N (Aon and Colaneri 2001), soil pH and organic phosphorous content (Chhonkar and Tarafdar 1984). Application of organic manures significantly increased phosphatase activity and mineral fertilizers reduced phosphatase activity (Sarapatka 2003). Dehydrogenase activity of treatment plots T1, T2 and T4 was 13-29% more when compared to T3 only during 2004-05 and was similar among the treatments during 2003-04. Application of farmyard manure enhanced dehydrogenase activity and fertilizer or chemicals doesn't inhibit the same (Lee *et al.* 2000).

The distribution of microorganisms in no-till soils appears to be closely related to distribution of organic matter. In conventionally managed fields, there is not much difference in the microbial counts such as bacteria and fungi (Table 4.49 and 4.53). However, there appeared significant difference in the population of microorganisms that have role in plant growth promotion such as siderophore producers, pseudomonads and antagonistic bacteria. It might be due to the inoculation of such bacteria to the fields at the time of sowing or the nutrients provided by the application of composts and crop-residues might have provided an environment for enhancement of beneficial groups of bacteria. Unfortunately, limited information exists as to the extent and activity of microorganisms on surface exposed residues themselves. However, it would be expected that significant transformations of

nutrients might take place, including mobilization and immobilization as well as gaseous evolution (Schoenau and Campbell 1996) due to application of crop residues. Changes in microbial community in soils take time to develop, since they are a result of complex process and interactions in soil and three to six years are often needed to effect significant, visible changes. Three successive years of organic matter addition were needed to achieve significant disease suppression (Davis *et al.* 1996). Fortunately, just as it takes time to change the soil structure and community, the benefits are long-lived, potentially lasting for years. It may be noted that much less than 20% of the microbial population that may live in the soil can be cultured in laboratory media (Ward *et al.* 1990). This suggests that soil respiration and microbial biomass carbon and nitrogen are more dependable parameters of soil biological activity, reflecting the total microbial community.

The application of inorganic and organic nutrients improved soil health and thereby increased the yield of T1 and T2 was similar to T3 and T4 that received chemical fertilizers and pesticides. T1 and T2 that received plant biomass, compost and microorganisms as source of nutrients showed substantially more N (27-52%) and P (50-58%), than that was added to T3, largely as chemical fertilizers (Rupela *et al.* 2005). In all four treatments, nutrient, biological, microbial and crop indices were calculated and the least sustainability index (2.00). The nutrient, biological, microbial and crop indices were 1.07, 1.11, 1.14 and 1.00 (Table 4.56 to 4.59) respectively for treatment T2, with a sustainability index of 2.29 (Table 4.60). In T3, the sustainability index was 1.56 and all four indices were lower than the least sustainability index (Fig. 4.28). Recent report by Kang *et al.* (2005) showed sustainability index of 2.2,

where farmyard manure and green manure was used in rice-wheat system, where indices were calculated using three parameters biological (that included biological and microbial), nutrient and crop indices unlike our studies, where biological and microbial indices were used separately for calculating the sustainability index. Abiotic characteristics such as pH, cation exchange capacity, moisture content, temperatures etc. allows a better understanding of the physical and biochemical data obtained and support the final evaluation of soil quality (Filip 1998). Though in this study we characterized few physical parameters they were not used to derive the sustainability values. As soils vary widely in biological activity, the parameters studied here can be used as components of a universal composed index for determining soil quality in relation to plant growth.

Agricultural practices such as incorporation of crop residues have a direct impact on plant health and crop productivity. These practices influence nutrient availability, release of biologically active substances from both crop residues and soil microorganims and suppress root diseases and pests (van Bruggen 1995, Bailey and Lazarovits, 2003). Although the development of alternative agricultural systems is generally considered important, it is not clear which practices will improve sustainability and maintain adequate productivity. Field studies of the four different crop husbandry systems showed the great potential of application of microbial inoculants (technology) in low input agricultural pratice and environmental pollution abatement for non-use of chemical fertilizers and pesticides.

CHAPTER – VI

SUMMARY AND CONCLUSIONS

SUMMARY

Modern green revolution aimed at increased food grain production and was largely dependent on chemical fertilizers, pesticides and fertilizer responsive crop varieties. Such crop production systems requiring chemicals, machinery for tillage and water for irrigation are expensive and led to substantial decrease in soil organic matter (SOM) levels. In addition, they threaten water security for future generations due to pollution, particularly when synthetic pesticides are not used properly. Agriculture as practiced 100 years ago, without modern inputs, certainly had lower productivity than present systems of production. However, many 'pre-modern' practices such as no-till and the addition of organic manure and compost to soil can be made more efficient by value-addition through the scientific knowledge that has been gained over the past five decades. This can make crop production more sustainable without sacrificing productivity.

Microrganisms from three different types of composts farm waste compost (FWC), rice straw compost (RSC), *Gliricidia* vermicompost (GVC) and those associated with body surface and excreta of macrofauna (earthworms, centipedes, slugs and snails) in farm waste compost were studied. Bacteria efficient for different plant growth promoting and antagonistic traits were identified. Selected strains were evaluated in glasshouse conditions for ability to enhance plant growth. Efficacy of two antagonistic strains EB 13 and CDB 35 was studied in pot experiment. Intrinsic antibiotic markers were developed for two strains EB 67 and CDB 35 that showed RP solubilization. Their ability to survive in soil in pot and field experiments was

studied. Inoculation with these strains formed a part of two of the four different crop husbandry treatment plots in a long-term field experiment on Vertisol. Salient results of these studies follow.

Two hundred and seven bacteria were isolated from farm waste compost (FWC), rice straw compost (RSC), *Gliricidia* vermicompost (GVC) and macrofauna present in FWC. All the isolates were screened for phosphate solubilization, phytase activity, siderophore, chitinase, IAA, ACC deaminase and HCN production. Biocontrol ability of all the isolates was tested against four soil borne plant pathogenic fungi (*Sclerotium rolfsii, Macrophomina phaseolina, Fusarium solani* and *Fusarium oxysporum*). Proportion (of total 207 isolates) of isolates having plant growth promoting traits were 54% from FWC, 56% from RSC, 64% from GVC and 41% from macrofauna. Biocontrol bacteria were 19% from FWC, 38% from RSC, 39% from GVC and 23% from macrofauna.

Twenty three of 207 isolates had more than one plant growth-promoting trait and also inhibited the plant pathogenic fungi. All these bacteria were identified by biochemical tests. Most of these strains belong to genera *Bacillus, Enterobacter, Klebsiella, Pseudomonas* and *Serratia*. Bacteria antagonistic to all four fungi were generally *Bacillus* and *Pseudomonas* spp. All the twenty-three were tested for ability to promote plant growth of pearl millet (ICMV 155) using paper towel method and twelve of 23 significantly increased the plant growth parameters and seedling vigor index. In another similar evaluation using sorghum (CSV 15) as test crop, these 12 strains performed similar to that when pearl millet was used as test crop.

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Siderophore producers showed significant correlation with ACC deaminase producers (r=0.89) followed by P solubilizers (r=0.77), biomass (r=0.73), root length (r=0.67) and HCN producers (0.66) and ACC deaminase producers with root elongation (0.78).

Of the twelve strains that were used for plant growth studies, five (*Enterobacter cloacae* EB 27, *Serratia marcescens* EB 67, *Serratia* sp. EB 75, *Pseudomonas* sp. CDB 35 and *Pseudomonas* sp. BWB 21) were phosphate-solubilizing bacteria (PSB). Of them, *S. marcescens* EB 67 showed highest gluconic acid production (67 mM) and P solubilization (1036 μM). *Pseudomonas* sp. CDB 35 produced 27 mM of gluconic acid and 560 μM of P in liquid culture RP buffered medium. Both the strains solubilized rock-phosphate (RP) in presence of six different carbon sources (glucose, galactose, xylose, mannose, maltose and mixture of all these carbon sources). Maximum glucose dehydrogenase activity (Gcd) with EB 67 was in presence of glucose (100%) followed by galactose (63%) and xylose (59%). CDB 35 showed 77% Gcd activity with galactose when compared to Glucose (100%).

RP solubilization was tested using Vertisol soil of low P from field BR1D and field BP2C with medium P and ranging pH 7.6-7.8. Additional carbon, nitrogen and RP sources were added to the soil in flasks and inoculated with the PSB, EB 67 and CDB 35 in these lab studies. Both the strains solubilized P and it was observed that soil from neither of the fields had native bacteria to solubilize P, which might be due to the inability of the microorganisms present to overcome the buffering capacity of the

soil. Both the bacteria were able to degrade cellulose in plate culture conditions and were evaluated in broth medium for their cellulase activity and RP solubilization ability in presence of different crop residues under submerged and solid state conditions. Cellulase activity in submerged conditions was maximum in presence of rice straw and rice root (powdered form). In solid-state conditions cellulase activity was higher in presence of grass with EB 67 (0.072 units) and in presence of rice straw and root with CDB 35 (0.073). Both the strains also showed significant difference in alkaline phosphatase activity, biomass C and solubilization of P when added with different organic carbon sources in soil compared to uninoculated soil.

All the twelve strains were evaluated for their effect on growth of pearl millet and sorghum in glasshouse conditions. Increase in growth of pearl millet was significantly different from control (uninoculated) and ranged between 28-52% in pots having sterilized soil and 28-56% in pots having unsterilized soil. Similarly, increase in plant growth of sorghum ranged between 43-49% in pots having sterilized soil and 35-74% in pots having unsterilized soil when compared to control (uninoculated). Out of 12, six strains significantly improved growth of pearl millet and only two strains significantly improved growth of sorghum in sterilized soil in repeated experiments. Ten strains significantly improved growth of pearl millet and sorghum using unsterilized soil in repeated experiments.

Interaction of the twelve PGPB with *Rhizobium* strains *in vitro* showed the clustering of strains into three different groups. None of the bacteria inhibited the rhizobial strains in plate culture conditions. Two strains *S. marcescens* EB 67 and *Pseudomonas*

sp. CDB 35 were applied along with *Rhizobium* IC 59 to see their interaction on growth of chickpea (ICCV 2) in pots having unsterilized soil. Growth of chickpea ICCV 2 by seed priming method was better than seed coating method. Increase in plant biomass by priming with control was (40%), followed by Rhizobium IC 59 (8%), *S. marcescens* EB 67 (6%) and *Pseudomonas* CDB 35 (5%) when compared with seed coating method. Increase in plant growth recorded with seed biopriming was significantly different when compared to seed coating. In another experiment three strains *B. circulans* EB 35, *S. marcescens* EB 67 and *Pseudomonas* CDB 35 were co-inoculated with mycorrhizae (*Glomus* spp.) for growth of sorghum in glasshouse conditions. Increase in biomass 18-20% and root colonization by AM was 19 to 32 % more with bacteria and AM when compared to treatment where AM alone was applied.

Eighteen strains suppressed all the four plant pathogenic fungi in plate culture. Inhibition percentage of the 18 strains was higher on Kings B medium (63%) followed by glycerol casaminoacid yeast extract medium (49%) and potato dextrose agar medium (46%). Two of the 18 strains (*B. licheniformis* EB 13 and *Pseudomonas* sp. CDB 35) showed maximum inhibition of the mycelial growth and per cent inhibition which was 79 and 66% against *S. rolfsii,* 60 and 61% against *M. phaseolina,* 63 and 70% against *F. solani* and 58 and 76% against *F. oxysporum.* Both the strains reduced the biomass of all the four fungi in broth culture condition when studied on GCY medium. Volatile compounds produced by the strains inhibited *S. rolfsii* in plate culture conditions. Additional nutrients such as zinc sulphate added to PDA

medium improved the antagonistic activity of both the strains EB 13 and CDB 35. *B. licheniformis* EB 13 and *Pseudomonas* sp. CDB 35, were also evaluated in glasshouse conditions against *M. phaseolina* and *S. rolfsii* infection of chickpea and sorghum respectively. Both the strains lowered the disease incidence. Collar rot disease (caused by *S. rolfsii*) in chickpea was inhibited upto 46-60% by both the strains in glasshouse conditions. Sorghum root infection with *M. phaseolina* was about 45% in presence of EB 13 and 35% in presence of CDB 35, as compared to control infection (80%). Both the strains also, reduced the root infection with *M. phaseolina* in range of 27-42% when they were applied with additional nutrients glucose and zinc.

Composts applied at five t ha^{-1} were evaluated for growth of pearl millet using unsterilized soil in glasshouse conditions. RSC increased the plant biomass up to 81% when compared to uninoculated control. Dual application of compost along with few selective strains *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 as seed treatment significantly improved the growth of pearl millet in glasshouse conditions. RSC and rice straw vermicompost (RSVC) were applied separately along with AM (*Glomus* sp.) to see the growth promotion of sorghum and mycorrhizal colonization in roots. RSC and RSVC applied at five t ha^{-1} proved to be better to promote plant growth. RSVC applied at 10 t ha^{-1} showed improvement in AM colonization at the expense of plant growth. Enriched vermicompost prepared using the two PSB, EB 67 and CDB 35, improved growth of maize in glasshouse conditions. Root infection of sorgum with *M. phaseolina* was reduced to 49% when GVC was applied when compared to control (80%).

Performance of the two PSB, were evaluated in glasshouse and field conditions. Both the PSB strains significantly increased leaf area, plant dry weight (biomass) and colonized maize rhizosphere. Population of EB 67 and CDB 35 at 96 days after sowing was log 4.1 to 5.5 CFU g^{-1} when traced back on antibiotic buffered RP medium. EB 67 had 19 – 33% more plant biomass than the reference strains *E. asburiae* PSI3 and *B. coagulans*. Seed treatment with *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 increased the grain yield of field-grown maize by 85 and 64% compared to the uninoculated control. Increase in P uptake of shoot and grain tissue with *S. marcescens* EB 67 was 13 and 27 kg ha^{-1}, CDB 35 was 9 and 22 kg ha^{-1} and control 5 and 11 kg ha^{-1}. N uptake in shoot with *S. marcescens* EB 67 was 54 kg ha^{-1}, CDB 35 48 kg ha^{-1} and control was 30 kg ha^{-1}. Both the strains survived in peat upto 180 days after inoculation (DAI). Population of EB 67 ranged between 9.6 (6 DAI) to 7.1 CFU g^{-1} (180 DAI) on LB agar and 8.6 (6 DAI) to 6.2 CFU g^{-1} (180 DAI) on RP agar. Viability of CDB 35 was 9.7 (6 DAI) to 7.9 CFU g^{-1} (180 DAI) on LB agar and 9.3 (6 DAI) to 7.3 CFU g^{-1} (180 DAI) on RP agar medium.

An ongoing long-term field experiment with four different crop husbandry systems (T1 = Low-cost system 1, no tillage, rice straw as surface mulch + Biological inputs for plant growth and pest management. T2 = Low-cost system 2, no tillage, farm waste as surface mulch+ Biological inputs for plant growth and pest management. T3 = Conventional system, tillage, integrated nutrient management and chemicals for plant growth and pest management. T4 = Same as T3 + Farm waste as biomass as in T2) was studied to derive indicators of soil quality. T1 and T2 had substantially more N (27-52%) and P (50-58%), than T3 plot that received compost and chemical

fertilizers. Soil sampled during the two years (2003-2005) from 0-20 cm depth showed microbial biomass C of T1 (31%), T2 (30%) and T4 (22%) was greater than T3 in this order. Similarly biomass N of T2 (39%), T1 (33%) and T4 (25%) was greater than T3 in this order. Bacterial populations characterized for plant growth promoting traits were more in T2, T1, when compared to T3. In all four-treatment plots nutrient, biological, microbial and crop indices were calculated. The least sustainability index calculated based on threshold value was 2.00. The nutrient, biological, microbial and crop indices were 1.07, 1.11, 1.14 and 1.00 respectively for treatment plot T2, with a sustainability index of 2.29. In T3, the sustainability index was 1.56 and was lower than the least sustainability index (2.00).

Most of the strains that enhanced plant growth were high for phosphate solubilization, siderophore, indole, phytase, ACC deaminase and chitinase activity. Biocontrol activity of two strains, *B. licheniformis* EB 13 and *Pseudomonas* sp. CDB 35, may be due to lytic enzymes and antibiotic production. Both the strains inhibited the fungi in plate culture and in glasshouse conditions. *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 showed plant growth promoting activity and P solubilizing ability in buffered medium conditions. Ability of these strains to enhance plant growth and suppress the plant pathogenic fungi makes them potential strains for enhancing crop yields. Their ability to produce cellulase and enhance soil phosphatase activity, from crop residues and survival in soil might have enhanced their performance in field conditions. Their application in field treatments (T1 and T2, from past four years) showed an impact on sustainability. .

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Farming practices involving inoculation of PGPB along with plant biomass (crop residues) and increasing their population in the rhizosphere, aiding in plant growth is a tool for bio prospecting. It was apparent that plant biomass was the engine of crop-productivity mediated by biological processes that enhanced soil fertility. This technology can be easily learnt or followed by farmers for obtaining crop yields in an environmental safe manner.

6.2 CONCLUSIONS

- Overall, 207 bacterial cultures were isolated from three different composts Farm waste compost (FWC), rice straw compost, *Gliricidia* vermicompost and macrofauna present in FWC were evaluated for eight plant growth promoting traits (phosphate solubilization, phytase activity, siderophore, IAA, ACC deaminase, chitinase and HCN prodcuction) and antagonistic activity against four soil-borne plant pathogenic fungi, *S. rolfsii*, *M. phaseolina*, *F. solani* and *F. oxysporum*.

- Twenty-two strains were selected based on their plant growth promoting and biocontrol ability. Twelve of these increased the seed vigor index of pearl millet and sorghum in *in vitro* conditions.

- Two plant growth promoting - phosphate solubilizing strains *Serratia marcescens* EB 67 and *Pseudomonas* sp. CDB 35 performed best for their P solubilization ability in presence of different carbon sources and crop residues in soil conditions. EB 67 and CDB 35 were also efficient for plant-growth promotion and cellulolytic activity.

- Seven strains *B. licheniformis* EB 13, *B. circulans* EB 35, *S. marcescens* EB 67, *K. oxytoca* EB 77, *Pseudomonas* sp. CDB 35, *Pseudomonas* sp. CDB 36, *B. licheniformis* CDB 47 and *Pseudomonas* sp. BWB 21 enhanced growth of pearl millet and sorghum in glasshouse conditions. Strains that promoted plant growth had than one plant growth-promoting trait and inhibited atleast two plant pathogenic fungi.

- Both *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 were compatible with *Rhizobium* of chickpea. These were also compatible with arbascular mycorrhizal (AM) fungi (*Glomus* spp.) and increased colonization by 19-32 % more than the treatment where only AM and RP was applied.

- *Bacillus liceniformis* EB 13 and *Pseudomonas* sp. CDB 35 were efficient antagonistic agents and suppressed four plant pathogenic fungi *in-vitro* and suppressed the two fungal pathogens (*S. rolfsii, M. phaseolina*) and reduced the disease level in host plants of sorghum and chickpea in glasshouse conditions.

- *Serratia marcescens* EB 67 and *Pseudomonas* sp. CDB 35 increased the grain yield of maize, plant biomass and N and P uptake in field conditions.

- Field studies where bacteria (*B. circulans* EB 35, *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35) were used as microbial inoculants improved soil health for crop-productivity. The field treatment (T2), which received crop residues, composts and microorganisms, showed sustainability index of 2.29, whereas it was 1.56 with conventional treatment (T3) receiving recommended level of fertilizers, pesticides and compost.

- The two strains *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 could be useful for preparation of improved composts for crop productivity.

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Harini, G, **Hameeda Bee**, Rupela, OP, Manikya Reddy and Gopal Reddy (2005). Phosphate solubilizing bacteria (PSB) – enriched vermicompost and its effect on maize growth. 8-10th December, AMI conference. Osmania University, Hyderabad.

WORKSHOPS AND CONFERENCES ATTENDED

1. Attended International Conference on " **Microbial Biotechnology, Trade and Public Policy**" from 15 - 17 July 2000 held at Osmania University, Hyderabad, India.

2. Attended "**Bioinformatics and Molecular modeling**" from 18-20 Oct 2001 held at Nizam College, Hyderabad, India

3. Attended "**Genetic Diversity and Assessment**" from 15-17 July 2001, held at ICRISAT, Patancheru 502 324, and Andhra Pradesh, India.

4. Attended workshop on "**Mineral Phosphate Solubilization**"16th November 2002, University of Agricultural sciences, Dharwad, Karnataka, India.

5. Attended "**Biopesticides Meeting**" on 15-17 March 2005, ICRISAT, Patancheru 502 324, and Andhra Pradesh, India.

PAPERS PUBLISHED/COMMUNICATED

1. Rupela, O.P., Gowda, C.L.L., Wani, S.P. and **Hameeda Bee**. 2005. Evaluation of crop-production systems based on locally available biological inputs. Chapter-35 In: Biological approaches to sustainable soil systems (N. Uphoff ed.). CRC Press, Boca Raton, Florida, USA.

2. **Hameeda Bee**, Rupela O.P., Gopal Reddy and Satyavani, K. 2005. Application of plant growth-promoting bacteria associated with composts and macrofauna for growth promotion of Pearl millet (*Pennisetum glaucum* L.). Biology and Fertility of Soils. (Under Review).

3. **Hameeda Bee**, Rupela O.P. and Gopal Reddy. 2005. Evaluation of bacterial isolates from composts and macrofauna for their biocontrol activity against soil-borne plant pathogenic fungi. Indian Journal of Microbiology. (Under Review).

4. **Hameeda Bee**, Harish Kumar Reddy, Y., Rupela O.P., Naresh Kumar, G. and Gopal Reddy. 2005. Effect of carbon substrates on rock phosphate solubilization by bacteria from composts and macrofauna. Current Microbiolgy (Communicated).

5. **Hameeda Bee**, Harini, G., Rupela O.P. and Gopal Reddy. 2005. Growth promotion of maize by phosphate solubilizing bacteria isolated from composts and macrofauna. Microbiological Research (Communicated).

6. **Hameeda Bee**, Rupela O.P., Wani S.P. and Gopal Reddy. 2005. Indices to assess soil quality and sustainability in systems involving low cost biological inputs and conventional system. (Under preparation).

7. **Hameeda Bee**, Harini, G., Rupela O.P., and Gopal Reddy. 2005. Interaction of plant growth promoting bacteria with mycorrhizae (*Glomus* spp.) (Under preparation).

8. **Hameeda Bee**, Rupela O.P., Wani S.P. and Gopal Reddy. 2005. Effect of compost and vermicomposts on sorghum growth and mycorrhizal colonization. (Under preparation).

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Synopsis of Ph.D. Thesis

INTRODUCTION

In agriculture, sustainability is defined as meeting current production goals without compromising the future. Sustainable agriculture should involve the successful management of agriculture resources to satisfy changing human needs while maintaining or enhancing the natural resource base, and avoiding environmental degradation (Mahaffe and Kloepper 1994). Sustainable agriculture encompasses, but is not limited to, farming systems known as biological, ecologically clean, low-input, organic and alternative agriculture.

Furthermore, a broad goal for the present agricultural management of soil is to sustain and/or increase productivity for an increasing world population. The challenge, therefore, is to increase production while avoiding the most serious of the negative consequences. Development of agricultural systems that integrate features of traditional agricultural knowledge and new ecological knowledge into the intensification process can contribute to meeting this challenge. Indeed, integrated nutrient-organic matter management approaches are receiving attention as pathways to sustainable high-production agriculture and reduction of off-site problems. Strategies that help synchronize nutrient release from organic matter and nutrient supply from inputs with plant demand require more information than conventional high-input management strategies. These improved strategies will require better integration of organic matter inputs such as crop residues, green manures, composts, inclusion of legumes in cropping systems, intercropping and biocontrol of insect pests and diseases. These approaches influence the soil quality and enhance biotic community (soil fauna and microorganisms) that influence nutrient availability to meet plant demands. Soil-plant-microbe interactions are complex and there are many ways in which the outcome can influence the plant health and productivity. These interactions may be detrimental, beneficial and neutral to the plants. However, the focus is to

exploit the beneficial bacteria that enhance plant growth. These are known as plant growth promoting bacteria (PGPB) (Bashan and Holguin 1998) and represent a wide variety of soil bacteria which, when grown in association with a host plant, result in stimulation of plant growth. Until now, majority of the beneficial bacteria are isolated from rhizosphere, known as plant growth promoting rhizobacteria (PGPR) colonize the roots and promote growth and yield of crops in addition to disease control (Vessey 2003). Selection of PGPB from diverse habitat such as composts can broaden the spectrum of PGPB. Bacteria that colonize the composts are exposed to cellulosic substrate, higher temperature, moisture fluctuation, organic matter etc. and have a better chance to survive and multiply in soil, compost and rhizosphere soil. This study is targeted to isolate bacteria from different composts and characterize for plant growth promoting and antagonistic activity and to assess the soil quality in different crop husbandry systems where selected bacterial strains were applied.

OBJECTIVES

- Isolation and screening of bacteria from composts for plant growth promoting traits and antagonism to pathogenic fungi (*in-vitro*).

- Selection of plant growth promoting bacteria and their identification.

- Studies on selected phosphate solubilizing bacteria.

- Evaluation of plant growth promoting bacteria and their interactions with *Rhizobium* and *Mycorrhizae*.

- Characterization and evaluation of antagonistic bacteria (EB 13 and CDB 35) and plant growth promoting (phosphate solubilizing EB 67 and CDB 35) bacteria.

- Biological indicators and derivation of indices for sustainable crop production and soil quality.

METHODOLOGY

Preparation of composts and isolation of microorganisms

In view of this, studies were carried out to re-cycle crop residues and prepare composts. To understand the beneficial effect of compost products for plant growth and pathosystems, it was aimed to study the microbiology of composts. Population of microrganisms from different types of composts farm waste compost (FWC), rice straw compost (RSC), *Gliricidia* vermicompost (GVC) and those associated with body surface and excreta of macrofauna (earthworms, centipedes, slugs and snails) in FWC were studied.

Screening of bacterial isolates for plant growth promoting and antagonistic activity and identification of selective isolates

Two hundred and seven bacteria were isolated, purified, preserved and screened for cellulolytic activity and different plant growth promoting traits such as phosphate solubilization, phytase and siderophore production, fluorescent *Pseudomonas*, indole, 1-amino-cyclopropane-1-carboxylate (ACC) deaminase, chitin and hydrocyanic acid (HCN) production. They were also tested for antagonistic activity against four soil-borne plant pathogenic fungi (*Sclerotium rolfsii, Macrophomina phaseolina, Fusarium solani* and *Fusarium oxysprorum*) Twenty-three isolates with plant growth promoting and antagonistic activity were evaluated for germination percent and seed vigor index of pearl millet (*Pennisetum glaucum* L.) by paper towel method. Twelve isolates that improved seed vigor index of pearl millet was evaluated using sorghum (*Sorghum bicolor* L. Moench) as test crop. Potential bacterial isolates were identified by staining, morphology, cultural, growth and biochemical characters based on Bergeys manual of determinative bacteriology (Krieg and Holt 1984).

Characterization of selected phosphate solubilizing bacteria (PSB)

Five (EB 27, EB 67, EB 75, CDB 35 and BWB 21) of the twelve strains that showed improvement in seed vigor index of pearl millet showed rock phosphate (RP) solubilization (Gyaneshwar et al. 1998) in plate culture. All these five isolates were tested in RP broth for growth, drop in pH, organic acid produced (by HPLC) and solubilization of P. Two (EB 67 and CDB 35) out of the five strains were selected for further investigation. Both the strains EB 67 and CDB 35 were tested in presence of different carbon sources (100mM each) such as glucose, galactose, xylose, mannose, maltose, cellobiose, arabinose and their mixture (at 15mM each) for growth, drop in pH, organic acid and P.

Both the PSB strains EB 67 and CDB 35 were tested in alkaline Vertisol collected from two different fields BR1D (low P soil, 1.8 ppm) and BP2C (medium P soil, 5 ppm) at ICRISAT. Growth of the inoculated strains, drop in pH and solubilization of RP in presence of nutrient sources such as glucose and ammonium chloride was detected. Both the strains were tested in presence of different cellulosic substrates for cellulase activity and RP solubilization in submerged medium. Cellulose (CL), crop residues such as rice straw (RS), rice root (RR), RS + RR, pigeonpea root (PR) and grass (GS) were used as carbon source. Both the strains were also tested in solid-state conditions for cellulase activity and RP solubilization in presence of different crop residues as carbon source. Microbial activity [survival of bacteria (using intrinsic antibiotic marker), soil respiration, biomass C, acid phosphatase, alkaline phosphatase, pH, and P] was studied in soils inoculated with the PSB EB 67 and CDB 35 in presence of different organic carbon sources.

Evaluation of selected plant growth promoting and antagonistic bacteria

Based on *in vitro* plant growth studies, twelve bacteria were selected and evaluated for growth of pearl millet and sorghum in pots using sterilized and unsterilized soil. All the 12 isolates were tested for their interaction with *Rhizobium* strains in plate culture. Two plant growth promoting, phosphate solubilizing bacteria EB 67 and CDB 35 were evaluated with *Rhizobium* (IC 59) on growth, nodulation and nitrogenase activity of chickpea (ICCV 2) in pots having unsterilized soil. Three strains EB 35, EB 67 and CDB 35 were also evaluated along with arbascular mycorrhizae (AM) (*Glomus* spp.) in pots having unsterilized soil. Growth parameters of sorghum (ICSV 93046) and AM colonization in root were studied twice during the crop growth period.

Of the 207 isolates tested for antagonistic activity, eighteen isolates suppressed the four soil borne plant pathogenic fungi (*S. rolfsi, M. phaseolina, F. solani and F. oxysporum*) on potato dextrose agar (PDA). They were tested further on glycerol casamino yeast extract (GCY) and Kings B (KB) medium. Four strains EB 13, CDB 15, CDB 47, CDB 35, which showed antagonism and also plant growth *in vitro*, were tested for reduction in biomass of the four fungal pathogens in GCY broth Microscopic observation of GCY broth that was inoculated with both the antagonistic bacteria (EB 13 and CDB 35) and fungi *S. rolfsii* and *M. phaseolina* was made for morphological changes of inhibited fungi. All the 18 antagonistic strains were evaluated for volatile metabolites against *S. rolfsi* and *M. phaseolina* in plate culture. EB 13 and CDB 35 were tested for influence of mineral nutrient amendment on growth reduction of *S. rolfsii* and *M. phaseolina*. Two antagonistic strains EB 13 and CDB 35 were evaluated activity against collar rot disease of chickpea and charcoal rot of sorghum in glasshouse conditions.

Effect of composts on plant growth activity

Three composts (FWC, RSC and GVC) and three bacterial strains were co-inoculated along with the composts and were evaluated separately for growth of pearl millet in unsterilized soil. Effect of rice straw compost and rice straw vermicompost applied along with Mycorrhizae (*Glomus* spp.) was evaluated on growth of sorghum (ICSV 93046) and AM colonization in roots. Enriched vermicompost prepared using the two PSB strains EB 67 and CDB 35 was tested on growth of maize and AM colonization in root in glasshouse conditions.

Evaluation of efficient phosphate solubilizing bacteria

Performance of the two PSB, EB 67 and CDB 35 applied along with RP was evaluated to colonize maize rhizosphere, growth and yield of maize in glasshouse and field conditions. Survival of both the strains in peat-based formulation was studied upto 180 days after inoculation (DAI) into peat.

Characterization of soil quality using bio-indicators

Soil characterization of four different crop husbandry systems (T1, T2, T3 and T4) of an on-going long-term field experiment, BW3 field at ICRISAT was done. The methods used in the long-term field experiment are explained in Table 1. Further, an effort was made to identify and select suitable indicators, which can act as early signals of changes in soil quality of the four different crop-husbandry systems. Emphasis was given to biological, microbial, nutrient and productive potentials as indicators of soil quality and the sustainability index of the four different crop husbandary systems T1, T2, T3 and T4 was determined using the polygonal approach (Kang *et al.* 2005). Salient results of these studies follow.

Table 1 Four different crop husbandry systems in continuing long-term experiment of BW3 (Vertisol) field[1] at ICRISAT, Patancheru

Act/Inputs	T1: Low-cost system I	T2: Low-cost system II	T3: Conventional agriculture	T4: Conventional agriculture + biomass
Tillage	Zero-till	Zero-till	Conventional (bullock plough)	Conventional (bullock plough)
Sowing	Sd drill	Sd drill	Sd drill	Sd drill
Microbial inoculants*	+	+	-	-
Biomass (t ha^{-1} yr^{-1} first 3 years only)	10 Rice straw as surface mulch	10 Farm-waste as surface mulch	None	10 Farm waste, incorporated
Compost (t ha^{-1})	1.5-1.7 annual	1.5-1.7 annual	1.8 (1 in 2 year)	1.8 (1 in 2 year)
Fertilizer (N) Urea (kg N ha^{-1})	0	0	80	80
Fertilizer (P)kg P ha^{-1} (1 in 2 year)	20 (RP)	20 (RP)	20 (SSP)	20 (SSP)
Pest management	Biopesticides[a]	Biopesticides	Chemicals	Chemicals
Weeding	Manual, weeds retained	Manual, weeds retained	Manual, weeds discarded	Manual, weeds discarded

1 Same crops were grown in all treatments each year:

Year 1 pigeonpea-chickpea sequential (June 1999-May 2000); Year 2 sorghum/pigeonpea intercrop (June 2000-May 2001); Year 3 cowpea/cotton intercrop (June 2001-May 2002); Year 4 maize/pigeonpea intercrop (June 2002-May 2003); Year 5 cowpea/cotton intercrop (June 2003-May 2004); Year 6 maize/pigeonpea intercrop (June 2004-May 2005).

*Microbial inoculants applied were *B. circulans* EB 35, *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 along with other inoculants like *Rhizobium* and *Azotobacter*.

a Biopesticide (Microorganisms, herbal extracts and vermiwash) formulation developed at ICRISAT.

RESULTS AND DISCUSSION

Isolation, characterization and identification of bacteria for plant growth promoting and antagonistic bacteria

Microbial enumeration revealed that population of bacteria in all the sources studied was high (3.8 - 7.4 $\log_{10}$) but propagules of fungi were low (2.3-3.7 $\log_{10}$). Two hundred and seven bacteria were isolated from farm waste compost (FWC), rice straw compost (RSC), *Gliricidia* vermicompost (GVC) and macrofauna present in FWC. Proportion (of total 207 isolates) of isolates having plant growth promoting traits were 54% from FWC, 56% from RSC, 64% from GVC and 41% from macrofauna. Antagonistic bacteria were 19% from FWC, 38% from RSC, 39% from GVC and 23% from macrofauna.

Twenty-three of 207 isolates had more than one plant growth-promoting trait and also inhibited pathogenic fungi. Most of the strains belong to genera *Bacillus, Enterobacter, Klebsiella, Pseudomonas* and *Serratia*. Most of these bacteria are non-symbiotic diazotrophs that play a significant role in plant growth promotion (Kennedy *et al.* 2004). Predominant plant growth promoting and biocontrol agents of genera *Bacillus, Pseudomonas, Pantoea* etc. recolonize composts during curing stage. Compost is a product rich in disease suppressive microorganisms and when applied to the soil, enrich variety of nutrients that are available for the indigenous microflora and hence plants (Hoitink and Boehm 1999). All the twenty-three were tested for ability to promote plant growth of pearl millet (ICMV 155) using paper towel method. Twelve isolates significantly increased the plant growth parameters and seedling vigor of pearl millet and sorghum. Many of these 12 isolates shared common characteristics. Five were positive for P-solubilization, five for phytase activity, nine were positive for siderophore and ACC deaminase, six for indole and HCN production and two showed chitinase activity. Previous studies indicate, that bacteria possessing these traits promote plant growth (Correa *et al.* 2004).

Studies on phosphate solubilizing bacteria (PSB)

Of the twelve strains that were used for plant growth studies, five (*Enterobacter cloacae* EB 27, *Serratia marcescens* EB 67, *Serratia* sp. EB 75, *Pseudomonas* sp. CDB 35 and *Pseudomonas* sp. BWB 21) were phosphate-solubilizing bacteria (PSB). Of them, *S. marcescens* EB 67 showed highest gluconic acid production (67 mM) and P solubilization (1212 µM). *Pseudomonas* sp. CDB 35 produced 27 mM of gluconic acid and 522 µM of P. Both the strains solubilized rock-phosphate (RP) in presence of all the carbon sources used except arabinose. Gcd activity of two strains *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 varied depending on nature of sugars used as carbon source and was highest with glucose followed by galactose, xylose, mannose, maltose, mixture and cellobiose. Both the strains EB 67 and CDB 35, solubilized RP and it was observed that soil from neither of the fields used in this study had native bacteria to solubilize RP in buffered medium. Plants secrete various root exudates such as carbohydrates, organic acids, aminoacids etc. to varying extents (Lugtenberg 1999). These strains can use root exudates as nutrient source and supply P to plants. Such strains, which can utilize varied carbon sources and solubilize P, may play a better role in soil conditions/rhizosphere.

Both the bacteria were able to degrade cellulose in plate culture and were evaluated in broth medium for their cellulase activity and RP solubilization ability in presence of different crop residues. Cellulase activity in submerged conditions was maximum in presence of rice straw and rice root (powdered form). In solid-state conditions cellulase activity was higher in presence of grass with EB 67 (0.072 units) and in presence of rice straw and root with CDB 35 (0.073). In our work, both the strains were from composts, confirming their ability to use crop residues as carbon source. However, purpose of our study, in solid-state conditions was to see the performance of these strains when applied along with crop residues in compost or soil conditions. In presence of different organic carbon sources both the strains also showed significant

difference in alkaline phosphatase activity, biomass C and solubilization of P compared to control. Kim *et al.* (1998) reported survival of PSB *Enterobacter agglomerans* and enhanced microbial activity in soil amended with different carbon sources up to 55 days.

Effect of seed bacterization on plant growth and their interaction with other microbial inoculants

Twelve strains were selected based on plant growth promoting traits for growth promoting studies of pearl millet and sorghum. High diversity of microorganisms is reported from macrofauna and insects, but very few reports of bacterial identification exist. *Serratia marcescens* EB 67 isolated from slug (body surface) present in FWC and *Pseudomcnas* sp. CDB 35 isolated from RSC exhibited multiple PGPR traits like P solubilization, ACC deaminase, siderophore and increased root length and plant dry weight of pearl millet under glasshouse conditions. Bacteria possessing these traits are known to increase plant growth. Various species of *Serratia* are diazotrophs that enhance plant growth and have been used as potential biocontrol agents (Gyaneshwar *et al.* 2001). Fluorescent pseudomonads that produce siderophores and auxins are known to colonize roots and promote plant growth (Dey *et al.* 2004). Increase in growth of pearl millet was significantly different from control (uninoculated) and ranged between 28-52% in pots having sterilized soil and 28-56% in pots having unsterilized soil. Similarly, increase in plant growth of sorghum ranged between 43-49% in pots having sterilized soil and 35-74% in pots having unsterilized soil when compared to control (uninoculated). PGPB that solubilize nutrients and produce phytohormones increase the surface area of aerial plant parts, shoot length, number of leaves.

Interaction of the twelve PGPB with *Rhizobium* strains *in vitro* showed the clustering of strains into three different groups. None of the bacteria inhibited the rhizobial strains in plate culture conditions. Growth of chickpea ICCV 2

by seed priming method was better than seed coating method. Increase in plant biomass by priming with control was (40%), followed by *Rhizobium* IC 59 (8%), *S. marcescens* EB 67 (6%) and *Pseudomonas* CDB 35 (5%) when compared with seed coating method. Increase in plant growth recorded with seed biopriming was significantly different when compared to seed coating. Co-inoculation of fluorescent *Pseudomonas* and *Rhizobium* improved plant growth of *Pisum sativum* (Dileep Kumar *et al.* 2001). In another experiment three strains *B. circulans* EB 35, *S. marcescens* EB 67 and *Pseudomonas* CDB 35 were co-inoculated with mycorrhizae (*Glomus* spp.) for growth of sorghum in glasshouse conditions. Increase in biomass 18-20% and root colonization by AM was 19 to 32 % more with bacteria and AM when compared to treatment where AM alone was applied. This suggests the possible reduction of AM culturing period by 45 days compared to its usual three-month pot culture method. PGPB are known to synthesize biologically active substances (plant growth hormones) that affect AM spore germination, hypal elongation that in turn increased root biomass which accelerates the AM root colonization (Bhowmik and Singh 2004)

Efficacy of antagonistic bacteria

Eighteen strains suppressed all the four plant pathogenic fungi in *in vitro* conditions. Inhibition per cent of the 18 strains was higher on KB (63%) followed by GCY (49%) and PDA medium (46%). Two of the 18 strains (*B. licheniformis* EB 13 and *Pseudomonas* sp. CDB 35) showed maximum inhibition of the mycelial growth and per cent inhibition which was 79 and 66% against *S. rolfsii*, 60 and 61% against *M. phaseolina*, 63 and 70% against *F. solani* and 58 and 76% against *F. oxysporum*. Both the strains reduced the biomass of all the four fungi in broth culture condition when studied on GCY medium. Volatile compounds produced by the strains inhibited *S. rolfsii* in plate culture conditions. Additional nutrients such as zinc sulphate added to PDA medium improved the antagonistic activity of both the strains EB 13 and CDB 35. *B. licheniformis* EB 13 and *Pseudomonas* sp. CDB 35, were also evaluated in

glasshouse conditions against *S. rolfsii* and *M. phaseolina* infection of chickpea and sorghum respectively. Both the strains lowered the disease incidence. Collar rot disease (caused by *S. rolfsii*) in chickpea was inhibited upto 46-60% by both the strains in glasshouse conditions. Sorghum root infection with *M. phaseolina* was about 45% in presence of EB 13 and 34% in presence of CDB 35, as compared to control infection (80%). Both the strains also, reduced the root infection with *M. phaseolina* in range of 27-42% when they were applied with additional nutrients glucose and zinc. Antagonistic effect towards the pathogenic fungi may be due to lytic enzymes, antibiotics, siderophores and secondary metabolites produced by the microorganisms (Hamdan *et al.* 1991).

Effect of composts on plant growth

Composts applied at five t ha^{-1} were evaluated for growth of pearl millet using unsterilized soil in glasshouse conditions. RSC increased the plant biomass up to 81% when compared to uninoculated control. Dual application of compost along with few selective strains *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 as seed treatment significantly improved the growth of pearl millet in glasshouse conditions. RSC and rice straw vermicompost (RSVC) were applied separately along with AM (*Glomus* sp.) to see the growth promotion of sorghum and mycorrhizal colonization in roots. RSC and RSVC applied at five t ha^{-1} improvement in plant growth and AM colonization. RSVC applied at 10 t ha^{-1} showed improvement in AM colonization at the expense of plant growth. Enriched vermicompost prepared using the two PSB, EB 67 and CDB 35, enhanced maize growth in glasshouse conditions. Composts and vermicomposts are known to promote plant growth and suppress the soil borne pathogenic fungi (Atiyeh *et al.* 2000).

Performance of Phosphate solubilizing bacteria (PSB)

PSB, EB 67 and CDB 35 were evaluated in glasshouse and field conditions. Both the PSB strains significantly increased leaf area, plant dry weight

(biomass) and colonized maize rhizosphere. Population of EB 67 and CDB 35 at 96 days after sowing was log 4.1 to 5.5 CFU g^{-1} when traced back on antibiotic buffered RP medium. Root colonization is critical in plant growth promotion and biological control (Kloepper and Beauchamp 1992). EB 67 had 19 – 33% more plant biomass than the reference strains *E. asburiae* PSI3 and *B. coagulans*. Seed treatment with *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35 increased the grain yield of field-grown maize by 85 and 64% compared to the uninoculated control. Generally in unsterilized soil, introduced microorganisms will not survive in high numbers than those in natural population, or in soils other than their natural habitats. *Pseudomonas fluorescens* strain isolates from *Lupinus hispanicus* colonized pepper roots (Lucas Garcia *et al.* 2003) and *Pseudomonas aeruginosa* isolated from phylloplane colonized the groundnut ecto and endorhizosphere (Kishore *et al* 2005). The relationship between source of isolation (compost and macrofauna) and colonization of rhizoshere environment will need to be investigated further to know the factors involved in root colonization capacity of both the strains EB 67 and CDB 35. Population of EB 67 and CDB 35 in peat up to 180 DAI was log 6.2 and 7.3 CFU g^{-1} on buffered RP agar medium It further indicates the possibility of developing peat formulations of bacteria with long shelf-life without decreasing its effectiveness (Vidhyasekaran and Muthamilan 1995).

Bio-indicators and sustainability of different crop husbandry systems

Studies on nutrients showed that soil from T1 and T2 had substantially more N (27-52%) and P (50-58%), than T3 plot that received compost and chemical fertilizers. Soil sampled during the two years (2003-2005) from 0-20 cm depth showed microbial biomass C of T1 (31%), T2 (30%) and T4 (22%) was greater than T3 in this order. Similarly biomass N of T2 (39%), T1 (33%) and T4 (25%) was greater than T3 in this order. Bacterial populations characterized for plant growth promoting traits were more in T2, T1, when compared to T3. In all four-treatment plots nutrient, biological, microbial and crop indices were

calculated. The least sustainability index calculated based on threshold value was 2.00. The nutrient, biological, microbial and crop indices were 1.07, 1.11, 1.14 and 1.00 respectively for treatment plot T2, with a sustainability index of 2.29. In T3, the sustainability index was 1.56 and was lower than the least sustainability index (2.00) (Table 2). Biomass C and N of plots T2 and T4 was significantly higher than T3. Increased biomass C and N which resulted in improved production and carbon sequestration was reported by Wani *et al.* (2003) in legume based cropping of an improved system that received integrated nutrient management application. Finally it can be stated that site and crop specific organic matter management utilizing appropriate qualities of organic substances and clearly aiming at soil improvement aspects can be considered as a contribution to sustainability.

Table 2. Sustainability indicators of soil quality from four different crop husbandry systems (T1 to T4) of a long-term field experiment

	Biological index	Crop index	Microbial index	Nutrient index	Sustainability index	System
T1	1.01	0.98	1.02	1.07	2.07	Sustainable
T2	1.07	1.00	1.09	1.11	2.29	Sustainable
T3	0.85	0.97	0.85	0.86	1.56	Unsustainable
T4	1.08	1.09	0.98	0.95	2.10	Sustainable

CONCLUSIONS

Overall, 207 bacterial cultures were isolated from three different composts (FWC, RSC and GVC) and evaluated for eight plant-growth promoting traits.

Twelve were selected, identified and studied for plant growth-promoting character.

Of these two efficient phosphate solubilizing isolates *Serratia marcescens* EB 67 and *Pseudomonas* sp. CDB 35 were selected and detailed investigation was done.

EB 67 and CDB 35 are efficient for P-solubilization, plant-growth promotion and cellulolytic activity.

Both the strains are compatible with *Rhizobium* spp. (chickpea) in nitrogen-fixation and plant-growth promotion. These isolates are also compatible with VAM fungi (*Glomus* spp.) for plant-growth promotion.

Bacillus licheniformis EB 13 and *Pseudomonas* sp. CDB 35 are efficient as biocontrol agents on soil-borne plant pathogenic fungi (*S.rolfsi* and *M.phaseolina*).

Field studies where these bacteria were used as microbial inoculants improved soil health for crop-productivity.

The studied two bacterial isolates may be useful for preparation of improved composts for crop productivity.

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35

Evaluation of Crop Production Systems Based on Locally Available Biological Inputs

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CONTENTS

Crop production systems that require chemical fertilizers, pesticides, machinery for tillage, and irrigation water are expensive. In countries such as India, they have started to undermine the water security of future generations, contributing to soil and water pollution particularly when synthetic pesticides are not used properly. It is true that agriculture as practiced 100 years ago without modern inputs had lower productivity than present systems of production. However, many premodern practices, such as the use of organic manures to enhance soil fertility and of herbal extracts to protect crops, can be made more efficient by the scientific knowledge that has been gained over the past century, making crop production more sustainable while still achieving high productivity.

This is becoming more evident from the published literature on practices such as the use of organic manures and biopesticides (e.g., Carpenter-Boggs et al., 2000; Stockdale et al., 2001; Kough, 2003) and experience with conservation tillage (discussed in Chapters 22 and 24). This chapter reports the results from an ongoing, long-term experiment started at ICRISAT in June 1999 on a rainfed Vertisol at Patancheru, Andhra Pradesh, India. It examines the possibility of achieving high yields using low-cost inputs, plant biomass in particular, that are available within the vicinity of the farm or that could be produced *in situ*. The field trials utilized biological approaches reported in the published literature and from traditional knowledge.

While some of these methods require considerable labor, more than many large farmers might be able or willing to invest, they could be relevant to a large number of small and marginal farm households in the semiarid tropics that have family labor available but very

little cash. The methods reviewed here are proving to be profitable in terms of their returns to labor as well as to the other factors of production.

35.1 Designing Crop Production Systems for Sustainability

Production practices, such as putting on crop residues or other biomass as surface mulch, using compost and green manures, intercropping of legumes in cropping systems, and biocontrol of insect pests and diseases, all help to enhance yields and sustain soil fertility and health (e.g., Willey, 1990; Reganold et al., 1993; Fettell and Gill, 1995; van Keulen, 1995; Mäder et al., 2002; Delate and Cambardella, 2004;). Appropriate use of such biologically-based approaches has been reported to enhance soil microorganisms and macrofauna (e.g., Kukreja et al., 1991; Fatondji, 2002), thereby enhancing microbial transformations of different nutrients from bound to available form. These various approaches can be combined into an integrated soil–plant–animal cropping system for attaining sustainable high yields. Such a system, depicted in Figure 35.1 below, has been tested since 1999 and is explained below.

While a variety of crops and practices are known to be able to contribute to farming system success, it is not known to what extent they can be used jointly in ways that are sufficiently productive and profitable, as well as sustainable, to improve the lives of farmers. It is not necessary that any system be advantageous for all farmers, since no single
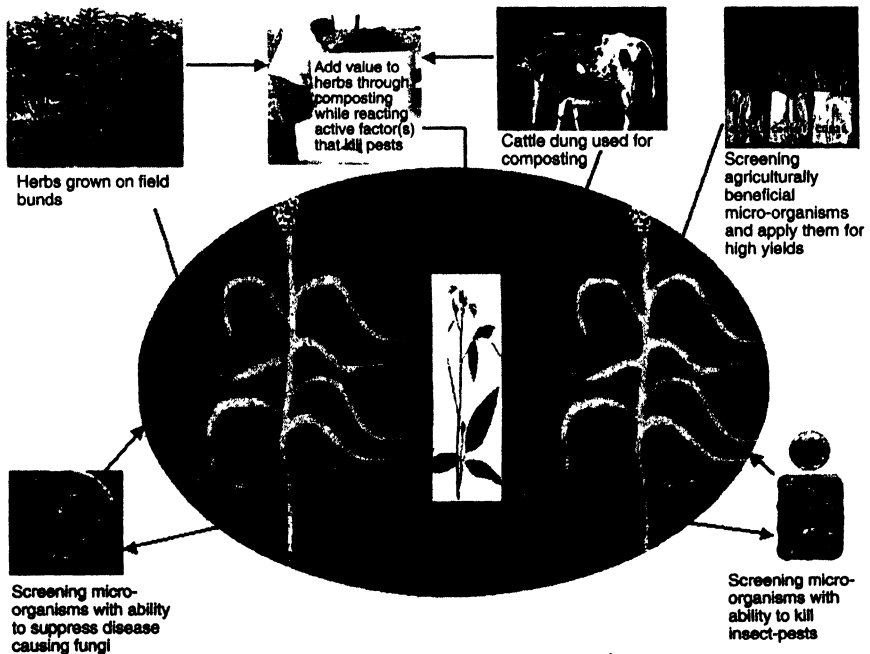

FIGURE 35.1
Elements of a biologically-based, integrated soil-plant-animal cropping system.

farming system should be expected to be optimal for everyone. Our effort was to design a crop production system that could be particularly beneficial for small landholdings. It drew on existing knowledge that:

- Legume and nonlegume crops can improve soil fertility when grown as intercrops (as examined further in Chapter 39).
- Crop residues produced *in situ* can improve the soil's physical and biological properties when retained as surface mulch, without tillage.
- Selected weeds can promote crop growth when grown under the main crop, i.e., not all weeds are deleterious.
- Where relevant or required, some amount of external inputs, preferably low cost, can be applied to the soil or crop on an as-needed basis to good effect.
- Certain soil microorganisms have beneficial traits, e.g., biological nitrogen fixation, plant growth promotion, or antagonism to disease-causing soil organisms (fungi, nematodes) or to insect pests. These can be effectively applied either as soil inoculants or sprayed on plants.
- Certain plant extracts sprayed on crops in a timely way, according to traditional knowledge, can protect crops from many if not all insect pests.
- Compost can be more than a source of nutrients for the soil, being also a soil-building substance and a source of beneficial microorganisms (Chapter 31).

As seen from our results, these practices are indeed quite compatible with one another, and as discussed in Chapter 17, cattle should be regarded as an important component of such systems. In the system that we designed and tested, only the grain produced is exported from the system. Crop stover is retained as surface mulch. Where stover is needed for economic purposes, e.g., as cattle feed, an equivalent quantity of biomass having no such economic value is returned to the field, i.e., foliage or loppings from shrubs or trees grown on field bunds or from outside the farm. The system is understood to function as a single entity, within which all of the functions in the soil, among plants, and at the soil–plant interface are highly interactive for producing yield.

Such a system is relevant to millions of small and marginal farmers in developing countries of the humid, subhumid, and semiarid tropics. About three-quarters of farmers in India have either small holdings (0.4 to 1.4 ha) or marginal holdings (<0.4 ha). They have little scope to benefit from technologies or implements designed for larger farms. This does not mean, however, that these small holdings are less productive. Actually, on a per-hectare basis they usually outperform larger farms, even by orders of magnitude (Feder, 1985; Rosset, 1999). Larger farms operate extensively rather than intensively and amass their higher total returns from their size of operation rather than from greater factor productivity or efficiency. The model presented in Figure 35.1 assumes that small and marginal farmers can and will mobilize family labor, their major asset, to undertake intensive crop and animal management if this is productive and profitable enough, i.e., if they can get higher returns per hour or per day of labor invested.

35.2   Design of the Long-Term Experiment

To examine whether yields comparable to conventional agriculture can be attained using the kinds of strategies and inputs reviewed in the preceding section, a multiyear

experiment was designed to compare and evaluate four different systems of crop husbandry (T1 to T4). Since it was assumed that very small farmers would own few animals and therefore would not have enough manure, the use of other organic matter was planned for. However, the systems being tested would benefit from the addition and incorporation of animal production and the use of animal wastes, whatever the availability. The results reported can quite certainly be improved upon to the extent that animals are incorporated into the farming system. We did not want our findings to be limited to a better-case scenario.

The major objective of the experiment was to learn whether plant biomass, added to three of the four systems evaluated, could be used profitably as surface mulch (serving as a source of crop nutrients) instead of being burned, which is common practice in South Asia (Sidhu et al., 1988). Details of these four systems are given in Table 35.1. Note that T3 is the treatment most similar to conventional current cropping systems, i.e., relying for its nutrient inputs on inorganic fertilizers, while T1 and T2 represent low-cost systems where crop nutrients are provided from biomass inputs, in addition to what can be mobilized from the soil through biotic activity. T4 is a combination of conventional and alternative systems as it receives the same organic inputs that are provided for T2 plus the T3 chemical fertilizer applications.

The experiment is being conducted on a 1.5 m deep Vertisol, with pH in the top 15 cm ranging from 8 to 8.2 and with electrical conductivity 0.16 to 0.22 dSm^{-1}. The area is fully rainfed, with annual mean rainfall at Patancheru of 783 mm. This allows two crops to be grown in a year, either as intercrops (in all years) or as sequential crops, with a probability of success in 6 of 10 years, given the possibility that the rains can fail. To be certain of some production, given the variability in timing of rainfall, second crops have to be sown as intercrops during the rainy season, in June or July. In each year of the first 6 years of the experiment, different crops were grown, as seen in Footnote to Table 35.1, but they were always the same across all four treatments. The experiment is providing an excellent field site for testing the overall hypothesis that treatments receiving high biomass as a source of nutrients — and that consequently exhibit high soil biodiversity and support higher levels of biological activity (both intervening variables being tested in our experiment) — will produce good agronomic results.

Rather than conduct the experiment on a large number of small replicated plots, the design was to use larger plots, 0.2 ha for each treatment, with a total area of 1.02 ha including noncropped area. This design has permitted observation of the effects of using biopesticides (bacteria in particular) for insect–pest management on fields of normal size and under conditions matching those of farmers' fields. We have monitored *Helicoverpa* pod borer, the major pest in the area, and also two of its natural enemies as well. This approach to evaluation of field-scale treatments is not new (Guthery, 1987; Guldin and Heath, 2001). It seems acceptable and appropriate for our purposes of evaluation since small replicated plots could not control for and assess so well the effects of above- and belowground biotic relationships.

Each of the treatments, T1 to T4, has 30 plots, each 9 × 7.5 m, laid out in six strips with five plots. Observations for yield and some other parameters have been made and analyzed for all plots. For those observations that are more costly, such as soil properties, samples are drawn from all the plots and are pooled strip-wise (and depth-wise where relevant) before analysis. There are thus 30 data points (internal replications) for parameters such as yield in our evaluation, with six data points (based on internal replications) for the different soil properties.

The concepts of sustainable agriculture expressed in Figure 35.1 apply to the first two of the four treatments, T1 and T2, in this ongoing experiment. They receive plant biomass as their major source of crop nutrients and depend on herbal extracts and agriculturally

TABLE 35.1

Treatments Used in a Continuing Long-Term Experiment at ICRISAT, Patancheru, India, June 1999 to December 2004[a]

Treatments	T1	T2	T3	T4
Inputs	Low-cost system I, based on rice straw	Low-cost system II, based on farm waste	Conventional agriculture	Conventional agriculture + T2 biomass
Land preparation and intercultivation	None	None	Conventional (bullock plow)	Conventional (bullock plow)
Sowing	Bullock-drawn drill	Bullock-drawn drill	Bullock-drawn drill	Bullock-drawn drill
Microbial inoculants	Added	Added	None	None
Biomass (first 3 years only)	$10\ t\ ha^{-1}\ yr^{-1}$ with rice straw as surface mulch	$10\ t\ ha^{-1}\ yr^{-1}$ with farm waste, stubble and hedgerow foliage as surface mulch	None	$10\ t\ ha^{-1}\ yr^{-1}$ with farm waste, stubble and hedgerow foliage incorporated
Compost	$1.5–1.7\ t\ ha^{-1}\ yr^{-1}$	$1.5–1.7\ t\ ha^{-1}\ yr^{-1}$	$1.8\ t\ ha^{-1}$ in years 2, 4, 6	$1.8\ t\ ha^{-1}$ in year 2, 4, 6
Fertilizer (N)	None	None	$80\ kg\ N\ ha^{-1}$ in 2 split doses yr^{-1}	$80\ kg\ N\ ha^{-1}$ in 2 split doses yr^{-1}
Fertilizer (P)	$20\ kg\ ha^{-1}$ as rock phosphate	$20\ kg\ ha^{-1}$ as rock phosphate	$20\ kg\ ha^{-1}$ as single super phosphate (SSP)	$20\ kg\ ha^{-1}$ as single super phosphate (SSP)
Plant protection	Biopesticides	Biopesticides	Chemical pesticides	Chemical pesticides
Weeding	Manual, weeds retained	Manual, weeds retained	Manual, weeds discarded	Manual, weeds discarded

[a] Same crops were grown in all plots each year. Crop rotations for all four treatments were: Year 1. Pigeon pea–chick pea sequential (June 1999 to May 2000); Year 2. Sorghum/pigeon pea intercrop (June 2000 to May 2001); Year 3. Cowpea/cotton intercrop (June 2001 to May 2002); Year 4. Maize/pigeon pea intercrop (June 2002 to May 2003); Year 5. Cow pea/cotton intercrop (June 2003 to May 2004); Year 6. Maize/pigeon pea intercrop (June 2004 to May 2005), pigeon pea not yet harvested.

beneficial microorganisms as soil inoculants and biopesticides. Both are cultivated with minimum tillage, where only the sowing is done with bullock-drawn implements. For the first 3 years, T1 received 10 t ha^{-1} of rice straw and T2 was given the same quantity of farm waste (crop stubble, leftovers after cattle have eaten, and tree leaves). Both treatments received these applications as surface mulch soon after sowing.

The conventional agriculture treatment, T3, received: 80 kg N and 20 kg P ha^{-1} yr^{-1}; regular tillage (land preparation, sowing, and intercultivation to remove weeds with a bullock-drawn tropicultor); chemical pesticides for managing pests; manual weeding; and 1.8 t ha^{-1} compost in alternate years. The T4 plots had the same inputs used for conventional agriculture, but in addition, they received 10 t ha^{-1} yr^{-1} of biomass (for the first 3 years only) similar to the T2 plots. This biomass has been incorporated into the T4 plots rather than left as surface mulch. From year 4, no further biomass from external sources has been added to any of the four treatments, except compost at rates shown in Table 35.1. The uneconomic parts of plants, e.g., leaves and stem stover, have all been retained on plots in treatments T1, T2, and T4. From year 5, loppings of *Gliricidia* grown on the plot bunds have been added during the crop growth period in equal quantities two to three times a year to all four treatment plots.

As depicted in Figure 35.1, the foliage of *Gliricidia sepium* and neem (*Azidrachta indica*) has been composted in separate tanks, and the wash from this (50 l ha^{-1} at least five times per season) has been sprayed on plants in T1 and T2 to protect crops from insect pests. The wash from neem, a known biopesticide, and from *Gliricidia* has been found to contain siderophore-producing bacteria (O.P. Rupela, unpublished study). These microbes have also been reported as promoting plant growth (Kloepper et al., 1980).

Certain bacterial preparations, e.g., EB35 and CDB35, which degrade cellulose, solubilize phosphorus, promote plant growth, and suppress disease-causing fungi (H. Bee, unpublished studies), have been applied as sand-coat inoculants and sown along with seeds in T1 and T2. A certain bacterium (*Bacillus subtilis* strain BCB 19) and also a selected fungus (*Metarrhizium anisoplliae*), both ICRISAT research products, have shown the ability under laboratory conditions to kill young larvae of *Helicoverpa armigera*, a major pest of cotton and legumes in the region. These preparations have been used as biopesticides in T1 and T2 only, along with other low-cost materials of traditional knowledge. Earthworms plus cattle dung (applied as 1% dung slurry in water to soak into the biomass as a food for earthworms) are important ingredients for composting in the tank shown in Figure 35.1.

The experiment completed its first 5 years in May 2004, so we are able to report and discuss here all the variables, including yield, with particular attention to soil biological factors. The work is ongoing, so there are also some data from the sixth year. More details on the crop yields are published in Rupela et al. (2005).

35.3   Crop Growth and Yield

The high variability in precipitation that farmers in this region have to cope with can be seen from the annual rainfall totals (in mm) for the different years: 580 (year 1), 1473 (year 2), 688 (year 3), 628 (year 4), 926 (year 5), and 610 (year 6). The different crops grown in the last 6 years (soybean, pigeon pea, maize, sorghum, cow pea, and cotton) all emerged well, including those in T1 and T2, which had to emerge through about 10 cm of biomass applied as surface mulch. The incidence of collar rot, caused by Sclerotium, was expected to increase on T1 and T2 in the presence of biomass, but this problem has been virtually nonexistent (<5% mortality of seedlings), at par with or even marginally lower than in T3.

Except in year 1, when T1 and T2 yields were 35 to 62% lower as the transition was made to biological production methods, as discussed further below, the yields of the different crops in T1 and T2 over the first 5 years, produced with lower cash cost, have been on a par with T3 or at most 14% lower. The reasonably high yields of pigeon pea in year 2 and of cotton in year 3 for both T1 and T2 were associated with the effective management of *Helicoverpa* by using biopesticides. Conversely, the low yields in T1 and T2 from pigeon pea in 2002 (year 4) and cotton in 2003 (year 5) were associated with poor success in managing insect pests mostly other than *Helicoverpa*. Detailed information and data on crop yields in the different years are given in Rupela et al. (2005). Annual productivity of T1 and T2 — the combined yield of legumes + nonlegumes, e.g., the mass of cow pea grains and seed cotton (lint + seeds) in year 5 — was high in all 5 years except year 1 (Figure 35.2).

Most significant for farmers, the net income from crops in each year except year 1, which was essentially a year of learning, has been higher — even much higher — in T1 and T2 than T3. The differential has ranged between 1.3 and 4.6 times (Figure 35.2), showing that in economic terms, the low-input strategy is proving to be much more profitable. In this calculation, each input was costed (except the cost of biomass and labor). Biomass was assumed to be available with little or no opportunity cost, having been saved from burning and being handled by family labor. Labor is not a free resource, of course, but it is the one most available to poor households, who are primarily constrained in terms of their land area and cash. Thus, labor was not considered to be the resource from which economic returns had to be maximized.

It should be noted that in year 3, there was a substantial loss (US$156 ha^{-1}) from growing cotton in T3 in contrast to a substantial net income gained from the cotton crop on the sustainable agriculture plots — US$210 ha^{-1} from T1 and US$140 ha^{-1} from T2

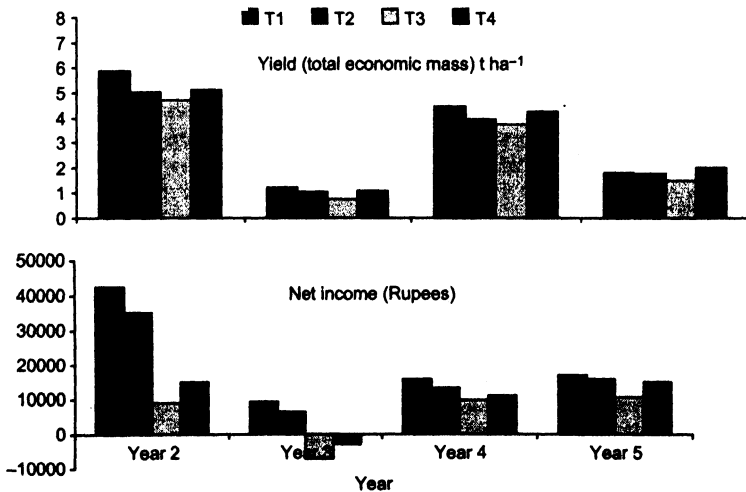
FIGURE 35.2
Yield and net income (in rupees) over years 2 through 5 from the four different systems of crop production (T1 to T4) in long-term experiment at ICRISAT, Patancheru, India. Income was calculated by putting a common price across all treatments for each item (both inputs and outputs). Per-day labor was priced @ Rs. 75 per day for both farmers and family members. (1 US$ = ~Rs. 45)

(Figure 35.2). The low-input strategy of T1 and T2 has, therefore, in some years performed much better agronomically than the more costly conventional cropping system. This makes the economic advantages even greater.

35.4 Soil Properties and Nutrient Balances

Every year in April/May, for all treatments, soil samples from three depths (0 to 15, 15 to 30, and 30 to 60) were collected from each plot before sowing crops, using a 40 mm diameter soil core. The samples from a set of five plots were pooled as indicated previously and were analyzed for total and available nitrogen, total and available phosphorus, total potassium, and organic carbon. Methods of analysis for the different parameters were the same as described by Okalebo et al. (1993). Soil bulk density measured in April 2002 (at the end of year 3) was similar across the treatments and ranged from 1.19 to 1.36 at the different depths. Electrical conductivity and pH were measured. Data for total nitrogen, total and available phosphorus for the first 4 years are given in Table 35.2 as means from the three depths for which measurements were made.

It was important to note that at the same time that T1 and T2 produced yields comparable to T3 — without receiving any chemical fertilizer amendments — they actually showed increases (rather than decreases) in their concentrations of soil nutrients compared with T3. In years 3 and 4, there were increases of 11 to 34% in total nitrogen and 11 to 16% in total phosphorus in T1 and T2, relatively more than in T3. However, it was noted that the mean nitrogen and phosphorus in all four treatments, after improving up to year 3, was reduced in year 4 (Table 35.2). The reasons for this reduction are still being considered.

Soil biological properties, presented in Table 35.3, were assessed only once, close to the time of crop harvest in year 5, using soil depths of 0 to 10 and 10 to 20 cm. The methods used were the same as those in Jenkinson (1988) for microbial biomass and microbial biomass nitrogen; in Anderson and Domsch (1978) for microbial biomass carbon; in Casida et al. (1964) for soil dehydrogenase activity; and in Eivazi and Tabatabai (1977) for acid and alkaline phosphatases.

Of the different parameters measured to assess the biological activity in soil samples from the four different systems of crop husbandry, more activity was noted in T1, T2, and T4 compared with T3. Soil respiration was more by 17 to 27% than in T3; microbial biomass carbon was 28 to 29% higher; microbial biomass nitrogen was 23 to 28% more; and acid and alkaline phosphatases were 5 to 13% higher. While these different parameters are reported as point-in-time measurements of microbial activity under laboratory conditions, they depict treatment differences.

In this experiment, 79 to 109 kg N ha^{-1} were noted to be associated with microbial biomass in the top 20 cm profile, which is more than usually reported for such soils, and this needs further examination. Wani et al. (2003) reported 42 kg N ha^{-1} in the top 60 cm profile of plots using traditional methods of cropping, compared with 86 kg N ha^{-1} in plots using an improved system of cropping. The microbially bound nitrogen is likely to be mineralized for use by plants when microorganisms die naturally or due to unfavorable factors, such as soil drying or application of chemical pesticides to soils.

The overall results on the different soil biological parameters strongly suggest that the soils from plots T1 and T2 were consistently more active microbiologically than those of T3 (Table 35.3). While the total bacterial populations were not that different across all four treatments, 5.3 to 5.7 ($\log_{10} g^{-1}$ soil), the population of Pseudomonas spp. was about 10

TABLE 35.2

Total Nitrogen (mg kg^{-1} soil), Total and Available Phosphorus (mg kg^{-1} soil) in Top 60 cm profile (Mean of Three Depths: 0 to 15, 15 to 30 and 30 to 60 cm), Field BW3, ICRISAT, Patancheru, AP, India

Treatment	Total N					Total P					Available P				
	Year 1	Year 2	Year 3	Year 4	Mean	Year 1	Year 2	Year 3	Year 4	Mean	Year 1	Year 2	Year 3	Year 4	Mean
T1	462 (18.0)	569 (21.1)	690 (30.1)	492 (17.5)	553	175 (6.3)	231 (7.0)	253 (15.7)	194 (9.0)	213	1.2 (0/08)	1.7 (0.34)	2.1 (0.31)	0.7 (0.24)	1.4
T2	488 (12.6)	643 (16.2)	681 (30.9)	489 (32.4)	575	189 (7.2)	257 (10.5)	263 (10.9)	213 (11.3)	230	0.7 (0.02)	1.3 (0.26)	1.7 (0.33)	0.6 (0.24)	1.1
T3	506 (22.1)	651 (73.4)	514 (12.3)	440 (17.9)	528	204 (3.9)	263 (49.2)	227 (3.3)	175 (8.1)	222	1.00 (0.13)	1.4 (0.34)	2.0 (0.29)	0.4 (0.11)	1.2
T4	500 (10.5)	588 (49.3)	586 (61.9)	429 (13.4)	526	244 (23.7)	213 (21.1)	232 (3.9)	177 (1.8)	218	0.5 (0.09)	1.6 (0.34)	2.4 (0.47)	0.3 (0.10)	1.2
Mean	489	613	618	462		203	247	244	189		0.8	1.5	2.0	0.5	

Data in parentheses are ± SE.

TABLE 35.3

Biological Properties of Soils with Different Cropping System Treatments Assessed in top 20 cm
Profile, Field BW3, ICRISAT, Patancheru, Close to Harvest in Year 5

Properties	T1	T2	T3	T4	Mean
Soil respiration (kg C ha^{-1}10 d^{-1})	330 (19.5)	360 (18.6)	283 (14.3)	436 (25.9)	352
Microbial biomass C (kg C ha^{-1})	1550 (110.3)	1535 (120.1)	1202 (66.8)	1510 (104.1)	1449
Microbial biomass N (kg N ha^{-1})	97 (6.7)	109 (8.9)	79 (4.0)	98 (7.5)	96
Organic carbon (t C ha^{-1})	23 (1.5)	20 (1.1)	17 (0.9)	22 (1.1)	20
Acid phosphatase (μg p-NP g^{-1} h^{-1})[a]	310 (38.8)	332 (32.5)	294 (36.0)	357 (39.8)	323
Alkaline phosphatase (μg p-NP g^{-1} h^{-1})[a]	937 (103.2)	1008 (111.3)	890 (114.8)	1011 (113.1)	962
Dehydrogenase (μg TPFg^{-1} 24h^{-1})[b]	133 (28.0)	137 (29.2)	130 (23.8)	142 (27.7)	136
Bacterial population (log$_{10}$ g^{-1} soil)	5.6	5.6	5.3	5.7	5.6
Pseudomonas spp populations (log$_{10}$ g^{-1} soil)	4.1	4.6	3.3	3.2	3.8

Numbers in brackets are ± SE

[a] p-NP = para nitro phenol

[b] TPF = triphenylformazan

times more in T1 and T2 than in T3 and T4 (4.1 to 4.6 vs. 3.2 to 3.3 log$_{10}$ g^{-1} soil). Several
soil isolates of this species are suppressive to disease-causing fungi and nematodes, and
this trait can therefore be regarded as an indicator of soil health. The measured differences
are likely to be due to the inoculant bacteria that were added at sowing of the T1 and T2
crops each year.

It should be noted that less than 10% of microorganisms that live in the soil can be
cultured in laboratory media (Ward et al., 1990). Some researchers think that this number
is less than 5 or even 1%. One cannot say the exact number since the denominator is
unknown, which is indicative of how little we know yet about the earth's microbiota.
This fact suggests, in any case, that soil respiration and microbial biomass carbon and
nitrogen are going to be more reliable parameters of soil biological activity, reflecting the
total microbial community, than are counts of microbial population using laboratory
media.

A balance sheet of nitrogen and phosphorus, the two macronutrients considered critical
for crop production, was prepared for all four treatments. For this purpose, all the
materials added to the different treatments plots, e.g., crop residues, compost, and those
removed (e.g., grain), were fully accounted for. Figure 35.3 shows the amounts of total
nitrogen and phosphorus added and removed, and the balance for the first 5 years across
the four different crop husbandry systems. T1 and T2, which received plant biomass,
compost, and microorganisms as their major sources of crop nutrients, ended up receiving
substantially more nitrogen (27 to 52%) and phosphorus (50 to 58%) than was added to T3
(604 kg N ha^{-1} and 111 kg P ha^{-1}, largely as chemical fertilizers). Of course, T4, having
both sources, received the largest quantities of nitrogen (1232 kg ha^{-1}) and phosphorus
(193 kg ha^{-1}). It is therefore not surprising that T1, T2, and T4 resulted in having a much
larger balance of nitrogen (2.5 to 10 times) and phosphorus (12 to 13 times) than was
measured for T3 (55 kg N ha^{-1} and 5 kg P ha^{-1}).

This does not mean, however, that the crops in the low-cost systems, T1 and T2, had
access to more nitrogen and phosphorus than those in T3, the conventional system.
Nutrients when added as biomass are not in a readily available form for crops and need to
be mineralized by microbial activity. Also, since the biomass was added as surface mulch,
microbial activity at the soil surface might not be sufficient for its decomposition. It is
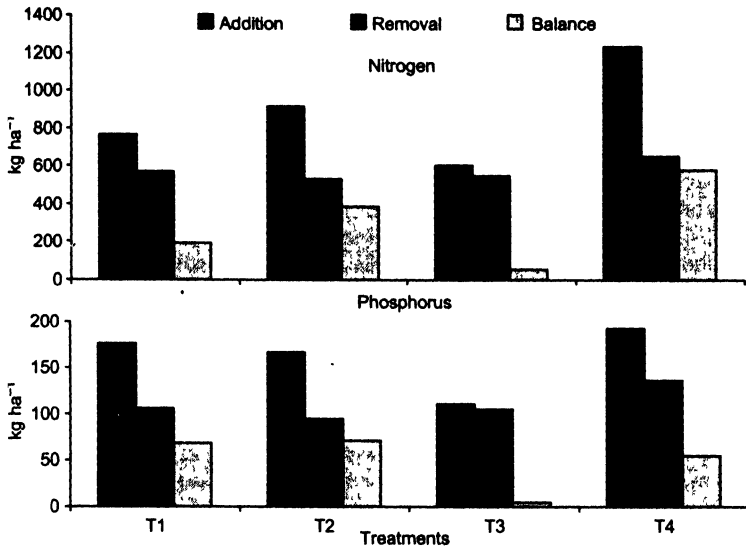
FIGURE 35.3
Nutrient (N and P) balance of the four different systems of crop production (T1 to T4) after five years, in long-term experiments, ICRISAT, Patancheru, India.

widely accepted that only a proportion of the nitrogen applied as biomass to the soil through soil incorporation is recovered by the crop (Schomberg et al., 1994; Thönnissen et al., 2000). According to T. J. Rego, ICRISAT (unpublished data), under Patancheru conditions this proportion would be less than 10% in year 1.

This helps to explain the lower yield obtained in year 1 in T1 and T2 (lower by 35 to 62%) than that produced by T3, which received chemical fertilizer. The longitudinal yield data suggest, however, that in subsequent years, microorganisms, whether in the soil or applied externally, were able to decompose the biomass sufficiently so that the released nutrients could readily meet crop demand, when T1 and T2 yields were on a par with or very close to those from T3.

If T1 and T2 received substantially more nitrogen and phosphorus and their removal was similar to that in T3 (Figure 35.3), then the soil systems of T1 and T2 should have substantially higher amounts of nitrogen and phosphorus. This was observed, at least in the measurements up to the end of year 4 (data for subsequent years are yet to be analyzed). The top 15 cm soil profile for T1 and T2 had 30 to 41% more nitrogen (an additional 355 to 483 kg ha^{-1}) and 0.2 to 17% more phosphorus (an additional 2 to 129 kg ha^{-1}) compared with the level of nitrogen and phosphorus for T3 (1192 kg N ha^{-1} and 746 kg P ha^{-1}). The amounts of nitrogen and phosphorus in the biomass still remaining as surface mulch on T1 and T2 from recent additions are not accounted for in this analysis. Much of the biomass applied at sowing had largely, except for thick plant stems, disintegrated by the end of the rainy season each year, suggesting that all the leafy materials added at sowing time were decomposed during the rains, particularly in a normal to good rainfall year.

35.5 Discussion

From the data collected during the first 5 years of the long-term experiment presented here, it is apparent that the two crop husbandry systems, T1 and T2, which received locally available, low-cost and eco-friendly materials such as biomass and compost, along with agriculturally-beneficial microorganisms, were able to produce yields that match those from the T3 system that relies on purchased inputs, e.g., chemical fertilizers and pesticides, and that also continued conventional tillage practices. Labor was the major input in T1 and T2. While this has opportunity costs for small and marginal farmers, these producers have relatively more access to labor than to cash, so their binding constraint is land and capital rather than labor.

Inputs of the agriculturally-beneficial microorganisms used in this study are not yet widely available, although efforts are beginning, in India, not just to produce them in large commercial operations but also at village level by villagers, as discussed in Chapter 45 (see also Bhattacharyya and Dwivedi, 2004).

In the second year, 20 mm of rain was received in the first week of January 2001, about 10 days before pigeon pea was to be harvested. For a conventional system (T3), this rain meant less strenuous tillage effort for the bullocks after harvest. For the no-till systems (T1 and T2), it was an opportunity to harvest more. Pigeon pea, particularly the non-determinate cultivars, has a tendency to regrow after harvest if soil moisture is conducive. Since such regrowth was noticed, it was decided to harvest by picking pods rather than by the normal method of cutting plants close to the ground. This resulted in 0.69 to 0.77 t ha^{-1} additional pigeon pea harvest, about 25% of total yield. The no-till system gives farmers more flexibility for using opportunities given by nature.

Sowing crops when there is surface mulch is a potential hindrance to adoption of the concept of sustainable agriculture represented in Figure 35.1. Sowing in the long-term experiment described here was done using a bullock-drawn implement. Manual sowing is an option, but both have high labor requirements. Before using the bullock-drawn implement for sowing, we had to rake off the biomass (largely crop stems) from the soil surface and spread it again soon after sowing. A machine punch-planter, which is able to sow crops through surface mulch, has recently become available in India and will be used and evaluated in the future. This machinery will reduce labor requirements substantially.

Earthworms are widely accepted as having a beneficial influence on soil structure and chemistry that promotes plant growth. We have recorded the presence of large numbers of siderophore-producing bacteria (1.2×10^4 to 4.5×10^6 ml^{-1}) in the wash of compost that was made from neem and *Gliricidia* foliage using earthworms (O.P. Rupela, unpublished study). It is likely that other agriculturally beneficial microorganisms, such as ones able to suppress disease-causing fungi, are present in certain compost used by organic farmers (Rupela et al., 2003). If locally available earthworms that feed aggressively on biomass placed on the soil surface can be identified and introduced in large numbers in the future, this will obviate the need to spray compost wash on the crop, reducing further the labor requirement for such biological management of the crop and soil systems.

It was apparent that plant biomass was the engine of crop productivity in T1 and T2, mediated by biological processes that enhance soil fertility. It is generally argued that biomass is required to feed cattle in South Asia, and therefore is not available for application to the soil to enhance crop production as has been done in T1 and T2. Being able to apply the levels of biomass used in T1 and T2 over time will require special efforts from any farmers who want to utilize this biologically-based cropping system. However, there are many ways in which biomass supply can be augmented for a system such as this.

In the long-term experiment, 4.5 t of biomass (containing 103 kg N and 6.7 kg P ha^{-1}) was available annually from year 5 on from the fast-growing Gliricidia grown on bunds (190 m long × 1.5 m wide, separating the four treatments) and on the boundary (218 m long) around the 1.02 ha field. Some crops, such as pigeon pea, which drop their leaves, can contribute biomass and nutrients directly to the soil system. In this experiment, 22 kg N and 2 kg P in year 2 were assessed to be added through the 3.1 t ha^{-1} of fallen leaves of pigeon pea when this was grown as the economic crop.

Fallen leaves and loppings of tree branches on-farm are another source of biomass, and many nonarable areas within the farming community could produce more biomass cheaply from fast-growing shrubs and trees introduced on wasteland, not displacing any agricultural production, provided that there is sufficient rainfall. It is important to note that deep-rooted shrubs and trees are an important biological tool that can acquire nutrients for crops, extracting them from lower layers of the soil and providing them on the surface layer in the form of fallen leaves, thus improving soil fertility; alternatively, these can be used as surface mulch or applied after composting.

A number of leguminous species offer opportunities to enhance biomass availability as cover crops or green manures, as discussed in Chapter 30. Farmers practicing alternative agriculture need to appreciate the value of biomass and to develop multiple practices and technologies that can harness this source of nutrients for crop production. Producing yields on a par with or higher than their neighbors without incurring the cash costs of chemical fertilizers and pesticides offers farmers a significant incentive for change.

A recent study by Delate and Cambardella (2004) has reported yields and differentials similar to those we report here, for the production of corn and soybeans in Iowa, U.S.A., using organic (nonchemical) vs. conventional farming practices over a 3-year period converting from conventional to organic production. The study reported here from India likewise suggests that biological approaches to crop production can sustain soil systems profitably for farmers, provided they have sufficient labor and its opportunity costs are not too high.

Making alternative agriculture systems more productive than conventional agriculture will be essential for their spread, although we must remember not to consider yield alone, a physical measure of success that ignores economic considerations. Costs of production per unit of output need to be assessed, including water-use efficiency. This was not considered in our trials because water provision was beyond our control in a purely rainfed system. However, rainwater harvesting was better in the low-cost systems (T1 and T2) than from the conventional system (T3), as seen from the reduced runoff (Rupela et al., 2005).

The scientific underpinnings for more biologically-based systems have been built up by researchers and practitioners over the past 50 years while Green Revolution technologies were receiving all the public attention and most of the public financial support. Many more studies are needed to be certain of the net value of alternative production systems, for different cropping patterns, on different soil types, and in different climatic regimes. Moreover, one cannot expect to evaluate the effects of biologically-based systems in a single year or two. Longitudinal evaluations are necessary to track the dynamic changes, positive and/or negative, in the many factors that operate in soil systems. This is why this particular long-term experiment was undertaken.

Overall, the biological approaches reported here — use of plant biomass as surface mulch, agriculturally beneficial microorganisms, and other practices — have enhanced soil biological and chemical properties of a rainfed Vertisol in the semiarid tropical environment in southern India. Yields were comparable to the conventional system of crop production that used standard agrochemical inputs. In the ·crop husbandry systems receiving biological inputs only, depending on the crops grown that year, stover yield ranging from 6.6 to 11.6 t ha^{-1} and grain yield ranging from 4 to 5.9 t ha^{-1} was harvested

annually when there was ≥ 628 mm of rainfall. There is, however, the need to evaluate such systems in other locations for soil and climatic differences, so that we can better understand the many interfaces between biotic and abiotic subsystems as they respond to anthropogenic interventions in pursuit of human livelihoods and sustenance.

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