ISOLATION AND CHARACTERIZATION OF ACTINOMYCETES FOR AGRICULTURALLY BENEFICIAL TRAITS

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ICRISAT

To Whom So Ever It May Concern

It is to certify that Ms. Sandhya Menon who was accepted as Apprentice at ICRISAT, Patancheru, India has satisfactorily completed the assigned experiments as a part of the project titled **"ISOLATION AND CHARACTERIZATION OF ACTINOMYCETES FOR AGRICULTURALLY BENEFICIAL TRAITS"** at ICRISAT. The work was done during June 2005 to October 2006 and was of high quality. I appreciate her performance in the laboratory.

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CANDIDATE'S DECLARATION

I, hereby declare that the dissertation or part there of has not been previously submitted by anyone or me for a degree of any University.

SANDHYA MENON

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SUMMARY

The microbial collection at ICRISAT had over 40 different isolates of actinomycetes, out of which 13 were characterized for beneficial traits. More 65 were isolated from soil samples brought from farmer fields growing rice by SRI (systems of rice intensification) and conventional flood method in Medak and West Godavari districts. Major focus of the study was to screen actinomycetes isolates for their ability to kill *Helicoverpa* larvae. The other traits studied were a) siderophore production, b) P-solubilization c) inhibit disease causing fungi and d) ability to promote growth of pearl millet (for eight selected isolates only).

The new isolates were characterized for their morphological characteristics. The isolates represented different sizes (<1 mm to 3 mm) of colonies on culture plates; shapes (irregular, round, regular); colors (white, brown, grey); margins (regular, smooth, serrated); texture (powdery, smooth, rough); consistency (dry); opacity (opaque, slightly translucent) and elevation (elevated, flat, raised). These isolates were streaked to get isolated colonies. The isolates were named as (BCA 500 - BCA 562).

Seventy-five isolates were screened in six different batches (each of 10-17) for their ability to kill *Helicoverpa* larvae. Mean percent mortality in control (no spray) was 34 to 38% in the different batches while for the different isolates it ranged from 10% in BCA 507 to 84% in BCA 549. None of the 13 isolates in batch I and most of the isolates in batch IV to VI were significantly superior to control showing 40 to 84% mortality. This needs a careful consideration. Two isolates (BCA 519, BCA 541) in batch II and four (BCA 502, BCA 519, BCA 541, BCA 559) in batch III were statistically significantly better than control that showed 30 to 38% mortality. Five isolates that showed above sixty percent mortality were evaluated for their ability to affect growth of pearl millet.

Of the seventy-five isolates screened only four (BCA 500, BCA 501, BCA 507 and BCA 514) showed some antagonistic activity against *M. phaseolina*. BCA 538 was

positive for siderophore production. None of the isolates were positive for P-solubilization on Pikovkyas's medium. Only eight isolates were selected for evaluation of their ability to affect growth of pearl millet grown in paper towels. When measured ten days after sowing, only the strain BCA 559 improved shoot length significantly ($P \leq 1.0$) and five of the eight strains significantly ($P \leq 5.0$) improved root length over uninoculated control. Root and shoot mass did not improve significantly.

Overall it was a rewarding learning experience involving isolation and purification of actinomycetes, their evaluation for siderophore production (an indicator trait for plant growth promoting activity), P-solubilization and antagonism to *Macrophomina phaseolina*, a fungus causing root diseases in crops e.g. charcoal rot of sorghum.

INTRODUCTION

The cotton bollworm or legume pod borer, *Helicoverpa* is an important constraint in the crop production worldwide. *Helicoverpa armigera* is a serious pest in Asia, Australia, Africa and Europe (King 1994), while *Helicoverpa zea* is widely distributed in America. Because of the number of crops that this pest affects, it has many common names: scarce bordered straw worm, corn earworm, African cotton bollworm, American bollworm and tomato worm (Begemann and Schoeman 1999).

It is polyphagous and attacks more than 182 plant species and is a serious pest for economically important plants like cotton, maize, chickpea, pigeonpea etc. It causes an estimated loss of US$ 927 million in chickpea and pigeonpea (Sharma 2001). *Helicoverpa* is polyphagous and attacks more than 182 plant species. Crop production in many countries especially Semi-Arid Tropics (SAT) is severely threatened by the increasing difficulty in controlling insect pests. In India it has been recorded on more than 181 plant species from 45 plant families (Manjunath et al. 1989). It results in an annual loss of over $500 million in the semi-arid tropics (ICRISAT 1992). The key pest status of *H. armigera* is due to a combination of several factors like feeding on reproductive structures and on diverse array of host plants. It is a strong disperser and undergoes rapid diapause (Fitt 1989). All these life history features make *H. armigera* one of the world's worst pest (Pimbert et al. 1989). Techniques, that are employed for minimizing losses due to these pests, include cultural manipulation of crops and its environment, host plant resistance, biological control including microbial pesticides, pheromones for population monitoring and chemical control (Bottrell 1979).

Charcoal rot is a disease seen in crops during the hot and the dry weather and when the plant growth is checked by unfavorable environmental conditions. The disease is caused by *Macrophomina phaseolina* and is of worldwide occurrence. It is a pathogen of over 75 different plant families and about 400 plant species (Dhingra and Sinclair 1977). The disease is seen in wide variety of crops like sorghum, maize, groundnut, cotton etc. Its wide host range suggests that it is highly variable

pathogen in both pathogenicity and mycological characteristics. Some isolates are host specific (Hilderbrand et al. 1945) while some attack a wide range of hosts (Holiday and Punithalingam 1970). The disease is of sufficient economic importance in the postrainy season crops in Maharashtra state, India (Uppal et al. 1936). Improved varieties and hybrids that have revolutionized the sorghum production in India in 1970's have proved to be susceptible to the disease (Rao 1982).

Management strategies for these pests require different control techniques based on relationship between population density and economic loss. The practice of pest management has been described as doing the following (a) determining how the life system needs to be modified to decrease its level, (b) applying biological knowledge and current technique to achieve desired modification and devising procedures for the pest control suited to the current technology and compatible with economic and environmental quality aspects, i.e. economic and social acceptance (Gerier 1996).

Actinomycetes are group of Gram-positive organisms like bacteria. They form long thread like branched filaments. They are found in soil and play an important role in decomposition of organic matter such as cellulose and lignin. They are known to produce many metabolites that can be used as biocontrol agents. They have the ability to produce many important compounds that have pharmaceutically useful properties. Hundreds of naturally occurring antibiotics have been discovered in these terrestrial microbes especially Streptomyces (Ward-Rainey 1997). They are also known to produce metabolites like Spinosad and Avermectin that are capable of managing insect pests. An actinomycetes was isolated from the bark of trees of Dehradun in India and was later identified as *Streptomyces violaceusniger*. This strain exhibits strong antagonism towards various wood rotting fungi as *Phanerochaete chrysos porium, Postia placenta, Coriolus versicolor* and *Gloephyllum trabeum* (Shekhar 2006).

In addition to protecting the plants from these pests they also require nutrients for their growth. Plants require nitrogen, phosphorous, sulphur, carbon and some micronutrients. They are available in the soil but in the insoluble form, which cannot

be absorbed by the plants. Phosphorus is a major plant nutrient. Soil phosphorous exists in the bound or dissolved inorganic or organic form. The application of phosphatic fertilizers is essential to enhance the crop yield. However more than two thirds of the phosphatic fertilizer is rendered unavailable due to fixation, which necessities the addition of the fertilizer every year (Mandal and Khan 1972).

Iron is an important micronutrient that is present in the soil in the inorganic form that the plants cannot absorb. Iron is a major component of cytochrome that is involved in the electron transfer chain and therefore a very important nutrient for plant growth.

Currently, synthetic chemicals are used by most farmers for managing crop nutrient and pests. But inappropriate and excessive use of them has not only disturbed the chemical and biological balance of the soil but also adversely affected microbial and other life forms. These have also increased the cost of production. Some chemical pesticides have already been proven to cause adverse affects on human as well as beneficial organisms, as a result they have been banned in developed countries. Hence, other alternative to cope up with this problem is the use of eco-friendly biological means.

Biopesticides are derived from natural sources such as animal, plant, and bacteria. Insect pests can be controlled biologically by use of fungi, bacteria, viruses, protozoa and nematodes (Dent and Jenkins 2000). Microbial pesticides can control many of pests, although each separate active ingredient is relatively specific for its target pests and are also less toxic when compared to the conventional pesticides. They are effective in very small quantity and often decompose quickly, thereby resulting in lower exposure and largely avoiding the pollution problems caused by conventional pesticides. The particular advantage of using biopesticides derives not only from their capacity to kill, but also their unique capacity to reproduce and compound their killing action with time (Thomas and Waage 1996). When used as a component of integrated pest management (IPM) programs, biopesticides can greatly decrease the use of conventional pesticides, while crop rise is high.

The microbial collection at ICRISAT has over 40 different isolates of actinomycetes that have to be characterized for various traits. Focus of the study was to screen actinomycetes isolates and to identify *Avermectin* and *Spinosad* (reported to be eco-friendly and showing instant kill of *Helicoverpa* larvae) like products that can kill *Helicoverpa* larvae. In addition, the identified strain is also characterized for the other plant growth promoting traits such as a) ability to produce siderophores, b) P-solubilization c) suppress disease causing fungi and d) safe for environment.

REVIEW OF LITERATURE

Insect pests affecting plant health are a major and chronic threat to food production and to the stability of the ecosystem worldwide. The cotton bollworm has developed resistance to almost all the insecticides (Adihisson 1969). As agricultural production has intensified over the past few decades, producers have become more dependants on agrochemicals as a relatively reliable method of crop protection. Unlike the other toxic chemicals, pesticides are designed to kill or injure living systems; they are deliberately introduced into the environment. Pesticide residues are found in the food, soil, water and air (www.ars.usda.gov/research/programs/programs). The enormous success of synthetic organic insecticides such as dichloro diphenyl trichloro ethane (DDT) and bromo hydrogen chloride (BHC) during the world war, began a new era of pest control. The number of chemically active ingredient approved for use in pesticides has increased from some dozens in the late 1960's to some 600 in the 1990's (www.pan.UK.org). The use of pesticides has been increasing every ten years since 1950's. The onset of insecticide resistance, was first experienced with DDT within two years after its wide spread use (Brown and Pal 1971). In 1995 global pesticide sales increased by nearly 9%, with increasing sales in Asia, Latin America and to lesser extent in Africa. By the year 2000 the pesticide sales in the developing countries was found to be round 35%.

Chemical pesticides and fertilizers were used to control pests and for improving the soil fertility and to help in the plant growth. But excessive use of them has generated several environmental problems including the green house effect, ozone layer depletion and acidification of water etc (www.sankey.ws/viewpest). They are expensive and make use of non-renewable energy resources like fossil fuel, which can deplete nature's resources. Pesticides reduce the species diversity and cause ecological damage, as they are known to have direct or indirect effect on their habitat and food chain. Such problems can be overcome by the use of biofertilizers and biopesticides which are natural, beneficial ecological and user friendly.

Use of biopesticides is an effective and safe technique for pest control. The use of biopesticides is particularly advantageous because of their ability to kill the pest and also for their unique capacity to reproduce and compound the killing action with time (Thomas and Wagge 1996). Additionally, many biopesticides show high degree of specificity for controlling that makes them safer than chemical pesticides, many of which has adverse affects on non target fauna and the environment.

Helicoverpa is a serious pest of crops, particularly grains of legumes, summer grains and cotton. Intensification of agriculture on the global level is the root cause for the enhanced damage caused by many pests. *H. armigera* has developed resistance to a wide range of insecticides and therefore, it is difficult to control the pest (Kranthi et al. 2002). A conservative estimate is that over US$ 1 billion is spent on insecticides to control these pests. Recent strategies such as area-wide management (AWM) and integrated pest management (IPM) aim to restrict the build-up of *Helicoverpa* populations to below damaging levels. Management strategies for *Helicoverpa* require different control tactics based on relationship between population densities and economic loss. Successful AWM and IPM strategies (which combine biological, cultural and chemical control options) require a more sophisticated understanding of *Helicoverpa's* lifecycle and biology than relying on insecticides alone.

Lifecycle of *Helicoverpa*

Lifecycle of *H. armigera* takes about 4-6 weeks to develop from egg to adult in summer and 8-12 weeks in spring or autumn. It includes 4 stages: egg, larva, pupae and adult.

The **moth.** Adult moth wingspan is 30-45 mm; the forewings are brownish or reddish-brown (females) or dull greenish to yellow or light brown (males); hindwings are pale with a broad, dark outer margin. It has a pale patch near the centre of this dark region (www.cotton.crc.org.au).

They feed on nectar and live for around ten days during which time females lay 3000 eggs (Shanower and Romeis 1999). Eggs are laid singly, or in clusters, on leaves, flower buds, flowers and developing fruits and sometimes on stems and growing points (www.defra.gov.uk/planth/pestnote/helicov.htm). Moths tend to lay eggs on top of healthy plants and on vigorously growing terminals.

Eggs. Fertile eggs hatch in about three days during warm weather (25 °C average) and 6–10 days in cooler conditions. As they develop, eggs change from white to brown to a black-head stage before producing a hatchling. Not all eggs are fertile. Physical factors can dramatically affect egg survival and larval establishment. Heavy rainfall and wind can force eggs off the leaves. High temperatures can dehydrate and kill eggs and very small larvae. The hatching larva (neonate) eats through the eggshell to make an exit hole and emerges.

Larvae. Neonate larvae are 1–1.5 mm long, with a brown–black head and white or yellowish-white, dark-spotted body. Larvae graze on tender young foliage for 1–2 days and then move to feed on buds, flowers or young pods, bolls or fruits (Fig. 1).

Larvae develop through six growth stages (instars) and become fully grown in 2–3 weeks in summer or 4–6 weeks in spring or autumn. The size of the final instar larvae ranges from 3.5 to 4.5 cm in length (King 1994). Development is more rapid at higher temperatures, up to 38 °C, after which development slows. Larval activity and feeding stops when the temperature falls below 12 °C.

Instar	Larval appearance	Actual larval length (mm)	Size category	Age of larvae in days
First		1–3	very small	1–2
Second		4–7	small	2–4
Third		8–13	small medium	4–8
Fourth		14–23	medium large	8–11
Fifth		24–28	large	11–14
Sixth		29–30+	large	14

Figure 1. Age of the larvae (in days) at different instars.

Ninety per cent of all the feeding done (and therefore damage) by *Helicoverpa* is by larva from the third instar (small medium larva that are 8–13 mm long) onwards. Large *Helicoverpa* larvae (longer than 24 mm) are the most damaging stage, since larvae consume about 50% of their overall diet in the fifth and sixth instars. Seedlings along field borders may be completely destroyed by large larvae moving from maturing or cultivated weeds or other host crops (cotton.crc.org.au/insects.htm). This highlights the importance of controlling *Helicoverpa* larvae while they are still very small (less than 7 mm).

It feeds on leaves, flower buds and flowers, developing pods, fruits and seeds (King 1994). In most crops, the third instar larvae or older (8 mm or longer) graze on leaves, feed on developing pods, bolls, cobs and grains. In some crops, such as mung bean and cotton, hatchling larvae infest reproductive structures (flowers, squares) as soon as they hatch. Once established in these concealed feeding locations, larvae are much more difficult to control with insecticides.

Pupae. Once larvae are fully-grown, they crawl to the base of the plant, tunnel up to 10 cm into the soil and form a chamber in which they pupate. The pupa develops into a moth in two weeks (Dillon and Fitt 1997). The moth emerges, feeds, mates and begins the next cycle of egg laying and larval development. As with all insect development, the duration of pupation is determined by temperature, taking around two weeks in summer and up to six weeks in spring and autumn. However, diapausing pupae take much longer to emerge. Diapause is induced when caterpillars are exposed to the falling temperatures and reduced day lengths of autumn. Diapause is a dormant phase that allows them to survive in a state of suspended development for several months. When soil temperatures increase in spring, normal development is resumed and moths emerge soon afterwards. Moths that emerge from dispausing pupae are highly resistant to insecticides.

Biopesticides based on entamopathogenic bacteria, fungi and insect virus such as *Nucleo polyhederosis virus* (NPV) have the potential to play important role in the management of *H. armigera*. HaNPV has shown to be effective in controlling *H. armigera* on a range of crops, including legumes (Rabindra et al. 1992). However, their use is restricted and they are not widely used on major field crops attacked by *Helicoverpa*. Both bacteria and fungi have been found to be pathogenic against *Helicoverpa* (Lutty et al. 1982). Among bacteria, *B. thuringenesis* was the most effective. In fungi, *B. bassiana* and *M. anisopliae* have been reported as major pathogens of *Helicoverpa* (Jing 1999).

Macrophomina phaseolina

Sorghum is rated as the fifth important crop in the world. In the developing countries of the semi arid tropics that accounts for 80% of the total world area, are sown with sorghum. Charcoal root rot, caused by the fungus *Macrophomina phaseolina* (syn. *Sclerotium bataticola*) affects more than 300 species of plants, including many agricultural crop plants, forest tree seedlings, and native weeds. It is also a major disease causing fungi in sorghum. *M. phaseolina* elaborates a number of

phytotoxins namely *asperlin, isoasperlin, phomolactone, phaseolinic* acid, *phomenon* and *phaseolinone* (Bhattacharya et al. 1992a). Phaseolinone appears to be the most important of these and it induces disease symptoms in plants similar to those produced by the pathogen (Mathur 1968). Because of the wide host range of *M. phaseolina* and the long survival times of the microsclerotia, crop rotation would probably have little benefit in reducing charcoal rot. Under these study conditions it may be a better alternative to suppress charcoal rot by using the no-till cropping system to conserve soil moisture and reduce disease progress.

Charcoal root rot is found worldwide in warm temperate and tropical regions of both hemispheres. In forest tree nurseries, the disease is most frequently found in the Southern and Southwestern United States. Charcoal root rot and black root rot, if left unchecked, cause heavy mortality in the nursery. Significant decrease in survival and growth of seedlings occurs when more than 25 per cent of the root system is infected at the time of out planting. Symptoms of charcoal root rot are evident by midsummer and are characterized by a gradual decay of the root tips, lateral roots and root crown. This gradual destruction of the root system causes the seedlings to become stunted and chlorotic and finally to die. *Macrophomina phaseolina* survives as small, black microsclerotia in the soil and infested plant debris. When a growing root of a susceptible plant contacts a microsclerotium in the soil, this resting state germinates and the fungus grows over the surface of the root and penetrates between epidermal cells into the root cortex. From there, the fungus advances through the cortex and inner bark into and up the taproot. The gradual destruction of the root system causes the seedlings to become stunted and chlorotic; finally they die. In the latter stages of decline and after the seedling has died, the fungus forms small, black microsclerotia in the inner bark of the roots and lower stem. As the stem and root system of the dead seedling decay and break down, these microsclerotia are released into the soil, where they serve as inoculum for next season's crop of seedlings (Toko et al. 1989).

Plant root health depends upon its inherent resistance to microbial invasion and on the biological equilibrium developed between the deleterious microbes in the rhizosphere. Streptomyces have been reported to inhibit plant pathogenic fungi. Some of them have even shown promise in disease control and in replicated field experiments. They have shown to reduce the population of fungi both invitro and in the fields (Baker et al. 1971).

Siderophores

Iron is an essential element for most microorganisms owing to its importance in a variety of biochemical reactions, including respiration, photosynthetic transport, nitrate reduction, chlorophyll synthesis, nitrogen fixation and detoxification of oxygen radicals (Guan et al. 2001).

Siderophores are low molecular mass, high-affinity iron chelators of microbial origin that are produced coordinately with their cognate receptors and transporters in response to iron starvation (Brickman and Armstrong 2002). They are synthesized by a variety of soil microorganisms to ensure that the organism is able to obtain sufficient amount of iron from the surrounding. (www.fao.org). Fe3+ ions have very low solubility at neutral pH and therefore cannot be utilized by the soil microorganisms. Siderophore dissolve these ions as soluble Fe3+ complexes that can be taken up by active transport mechanisms. Many siderophores are non-ribosomal peptide (www.wikipedia.org/wiki/Siderophore). Nonribosomal peptides (NRP) are a class of secondary metabolites, usually produced by microorganisms like bacteria and fungi. They are structurally a very diverse family of natural products with an extremely broad range of biological activities and pharmacological properties. Although, iron is one of the most abundant elements, its pivotal role in the evolution of life on earth depends on the development of effective methods in its assimilation. When grown under iron deficient conditions, many microbes synthesize and excrete siderophores in excess of their own dry weight to sequester and solubilize iron. This extreme focus on the need for iron is reflected by its requirement for the proper function of enzymes that facilitate electron transport, oxygen transport and other life

sustaining processes. One potentially powerful application is to use the iron transport abilities of siderophores to carry drug into cells by preparation of conjugates between siderophores and antimicrobial agents (www.nd.edu/~mmiller1/page2.htm). DFO belong to a large family of siderophores (ferrioxamines) excreted by the actinomycetes (Bracina et al. 1987). Siderophores are produced by saprophytes to overcome the inherent aqueous insolubility of ferric iron, which limits its availability in soil. A total of 94 actinomycetes strains were isolated from the marine sediments. Thirty eight per cent of the actinomycetes strains produced siderophore on chromezurol S (CAS) agar plates (You 2005). This result shows that actinomycetes could be promising source as biocontrol. More than 10 distinct species of *Streptomyces* have been reported to produce characteristic desferrioxamine siderophore such as desferrioxamines G_1, B and E (Crawford et al. 2002). Examples of siderophores produced by various bacteria and fungi are ferrichrome (*Ustilago sphaerogena*), enterobactin (*Escherichia coli*), enterobactin and bacillibactin (*Bacillus subtilis*), ferrioxamine B (*Streptomyces pilosus*), fusarinine C (*Fusarium roseum*), yersiniabactin (*Yersiniapestis*) (www.wikipedia.org/wiki/Siderophore, 13 Oct. 2007.

P-solubilization

Phosphate is the second most limiting plant nutrient after Nitrogen. It has many important functions in plants, the primary one being the storage and transfer of energy through the plant. Adenosine diphosphate (ADP) and adenosinetriphosphate (ATP) are high-energy phosphate compounds that control most processes in plants including photosynthesis, respiration, protein and nucleic acid synthesis, and nutrient transport through the plant's cells.

Until comparatively recent times the growth of plants and animals, and hence the productivity of agriculture, was limited by a lack of phosphorus since only small amounts are released annually from rocks and soil minerals by weathering (www.soil.gsfc.nasa.gov/soilfert/npk). Most of the soil contains insoluble organic phosphate but it is of no use to the crops till it is solubilized. Fertilizers play a key role in the modernization of Indian agriculture and in making the country self

sufficient in food grain production. Much of the soluble phosphates applied to the soil is fused by the soil and is less available to the plants.

Phosphate-solubilizing bacteria (PSM's) are immensely important as they have been reported to increase uptake of phosphorous (P) by converting insoluble to soluble forms. These microbes belong to diversified groups of bacteria, actinomycetes (eg. *Streptomycetes*) and several fungi (eg. *Aspergillus and Penicillium*). The population of phosphate dissolving micro-organisms is more in the rhizosphere as compared to the non rhizosphere and the high population in the rhizosphere is of great relevance to the plant especially in 'P' deficient soils as it helps in the solubilization of phosphorous in these soils.

PSMs are reported to dissolve insoluble phosphates by the production of inorganic or organic acids and by the decrease of the pH (Whitelaw 2000). Most of the previous reports state that calcium phosphates are dissolved by acidification. Therefore, any microorganism that acidifies its external medium will show some level of phosphorus solubilizing activity (Goldstein 1986).

PSMs play a vital role in solubilizing insoluble phosphates present in soil and make it available to plant through the production of organic acids. The solubilization of P by these micro-organisms is attributed to excretion of organic acids like citric, glutamic, succinic, lactic, oxalic, glyoxalic, maleic, fumaric, tartaric and Ketobutyric. These organic acids secreted, either directly dissolve the mineral phosphate or chelate Ca, Fe, Al ions associated with phosphate. Soils may immobilize P by binding with Ca++, Mg++, Fe+++ and adsorption to Fe- and Al-hydrous oxides and Al-silicates or via precipitation reactions (e.g., Al in acidic soils or Ca in alkaline soils). On such soils the efficiency of P-based fertilizers can be low, with only 10 to 30% of the P applied available for plant uptake in the year of application. It is estimated that the accumulated P in agricultural soils is sufficient to sustain maximum crop yields worldwide for about 100 years (Goldstein et al. 1993).

Plant growth promotion

Plant associated bacteria act as agents stimulating plant growth and managing soil and plant health (Glick 1995). The most widely studied group of plant growth promoting bacteria (PGPB) are the plant growth promoting rhizobacteria (PGPR) colonizing the root surfaces and the closely adhering soil interface, the rhizosphere. Some of these microorganisms can also enter the root interior and establish themselves as an endophytic population. The extent of endophytic colonization of the host plant organs and tissues reflects the ability of the bacteria to selectively adapt itself to these ecological niches (Gray and Smith 2005). Despite their different ecological niches, free-living rhizobacteria and the endophytic bacteria use some of the same mechanisms to promote plant growth and control phytopathogens (Dobbelaere et al. 2003). The widely recognized mechanisms of biocontrol mediated by PGPB re competition for a substrate, production of inhibitory allochemicals (Haas et al. 2000) and induction of systemic resistance in the host to a broad spectrum of pathogens and abiotic stress.

Streptomyces lydicus WYEC108 is a root- colonizing Actinomycetes that was originally isolated and studied for its antifungal activity this strain was found to be capable of mycoparasitic colonization of fungal root pathogen and excretion of antifungal metabolites (Yuan and Crawford 1995). Streptomyces spp. have been described as an rhizosphere colonizers (Liljeroth et al. 1990), antifungal biocontrol agents (Rothrock and Gottlieb 1984), invitro producers siderophore and plant growth- promoting hormones (Hamby 2001). Yet the overall importance, physiological activities and symbiotic roles of actinomycetes in situ within plant rhizosphere remains little studied at the biochemical and mechanistic levels.

In the present work actinomycetes isolates were used to study the role played by them in eradicating the pests and in improving the soil fertility. Actinomycetes are a higher form of bacteria, similar to fungi and second in number to bacteria. They are unicellular organisms that mass together to form filaments called hyphae. Colonies of actinomycetes can then form a mass of intertwined hyphae called a *mycelium*.

They are gram-positive bacteria, they have DNA with a high GC-content and some actinomycetes species produce external spores.

Actinomycetes do not respond well to acidic conditions (below pH 5) or high moisture conditions, but operate best at medium temperature areas of the compost. Actinomycetes take over during the final stages of decomposition, often producing antibiotics that inhibit bacterial growth. In 1940 Selman Walksman discovered that the soil bacteria he was studying made actinomycin, a discovery which granted him a Nobel prize. Since then hundreds of naturally occurring antibiotics have been discovered in these terrestrial microorganisms, especially from the genus Streptomyces.The soil-dwelling actinomycetes produce a variety of antibiotics including Streptomycin, Aureomycin, Terramycin and Chloromycetin. They are likely to work on tough organic material and give compost its pleasant, earthy smell. They are especially important in the formation of humus. They liberate carbon (C), nitrate nitrogen (NO_3) and ammonium nitrate (NH_4), making nutrients available to plants (www.rdn.bc.edcms/wpattackments/wp/Oct 2007).

These micro-organisms are known to produce secondary metabolites and extracellular enzymes, such as cellulases, chitinases and lignin peroxidases (Wong et al. 1991) that play an important role in soil biodegradation by recycling the nutrients associated with recalcitrant polymers (McCarthy and Williams 1992), and also show some mycoparasitic activity. The discovery and characterization of soil actinomycete *Saccharopolyspora spinosa* opened an opportunity to develop tools for progressive insect pest management (Sparks et al. 1998). It produces a metabolite during aerobic fermentation called spinosad that possess rapid efficacy competitive with the best synthetic standards and safety profiles similar to the biologicals. Spinosad is a mixture of two most naturally occurring metabolites (spinosyns A and D) (Krist et al. 1992). In the present study the isolated strains of actinomycetes were evaluated for their ability a) to kill *Helicoverpa* larvae, b) to suppress the growth of the fungus *M. phaseolina* causing charcoal root rot in sorghum, c) to solubilise insoluble phosphate and d) to produce siderophore.

MATERIALS AND METHODS

In the present study, actinomycetes were isolated from soil samples from experiments on SRI (system of rice intensification) from the farmer fields in Medak and West Godavari districts. The microbial collection at ICRISAT had over 40 different isolates of actinomycetes of which 13 isolates were included in the study. These showed some early signs of producing metabolites and suppressing disease-causing fungi. Previous experiments with bacteria suggested that some isolates besides having the ability to suppress disease-causing fungi also killed *Helicoverpa* larvae, a major pest of several crops including legumes and cotton. This may also be true in case of actinomycetes.

Enumeration, isolation, purification and preservation of actinomycetes

Enumeration. Thirty-six soil samples were collected from farmer's rice fields from two districts namely Medak and West Godavari districts and were enumerated for actinomycetes population by dilution plate method. Ten grams of soil was suspended in 90 ml of sterile water and kept on a shaker for one hour. Serial dilutions were made up to 10^{-2} dilutions. 0.1 ml of 10^{-2} dilution was plated on actinomycetes isolation agar plates. The plates were incubated at 28±2°C in the incubator for one week.

Isolation and purification. Actinomycetes with different morphological characteristics were selected. These were then streaked onto Actinomycetes Isolation Agar (AIA) plates to get the pure culture.

Preservation. The isolated colonies were picked up and were streaked on AIA slants and were covered with sterile liquid paraffin. High quality liquid paraffin was sterilized by autoclaving at 15 lbs for 20 minutes. A total of four slopes were made for each isolate, two of which were preserved in paraffin oil and maintained at 8°C. The isolates were named from (BCA 500 to BCA 562). Along with these 13 isolates from ICRISAT collection were also revived and preserved.

Rearing of *Helicoverpa armigera* in the laboratory

Third or fourth instar larvae were collected from fields and were supplied with artificial diet (Table 1). These larvae developed into sixth instar stage in 5-6 days after which they start pupation. The process of pupation was completed in 10-15 days giving rise to the adult moths. The moths were placed in oviposition cages. The temperature was set to 25°C and humidity was maintained at 70%. The adults were fed on honey that was kept in these cages. The females laid eggs within 2-3 days on the liners kept within the cages. These liners were collected and placed in different boxes having artificial feed at base. The eggs hatched in 2-3 days and developed into neonates. These neonates were divided into two batches, one was used for the experiment and the other was used for the rearing purpose and to continue the next cycle of development of *Helicoverpa*.

Preparation of the larval diet

All the dry ingredients were weighed and all the wet ingredients (Table 1) were taken in appropriate measuring cylinders. All the ingredients except yeast and agar were added to 450 ml of water and mixed thoroughly in a mixer. In a separate pan 800 ml of water was heated, to which yeast was added and mixed thoroughly. Agar was sprinkled and the mixture was stirred and mixed thoroughly to prevent lump formation. The mixture was added into the bowl containing the remaining ingredients. This was poured into stainless steel trays up to a level of 5 mm depth and left to cool in the Laminar Flow.

Evaluation of actinomycetes against *Helicoverpa armigera*

Actinomycetes cultures were inoculated into Bennett broth prepared in 250 ml conical flask on shaker incubator for seven days at 30°C. A total of 76 isolates were screened for larvicidal activity. These cultures were then screened for their ability to kill *Helicoverpa* larvae. A batch of 12 treatments was taken at a time. Five replications were maintained for each treatment. Spintor a commercial product was used as positive reference. In the control sterile distilled water was sprayed. The cake boxes and small Petri dish were surface sterilized by dipping in 5% chlorax followed by

rinsing with distilled water and UV sterilization overnight. 10-15 ml of sterile ¼ Arnon agar was added into the sterile Petri dishes to retain the turgidity of the chickpea seeds. Chickpea seeds were surface sterilized by dipping in 3% chlorax for five minutes followed by rinsing 6–8 times with sterile water. The chickpea seeds were kept at room temperature and under dark conditions. The chickpea seeds sprouted within two days. These sprouted seeds were used as food for larvae. Five ml of each treatment was taken in separate sterile syringes and sprayed on the sprouted seeds. The seeds so treated were left on sterile tissue paper for 30 minutes and were tucked with radicals of three seeds on the agar base in each Petri dish. The Petri dish was then fixed in the cake boxes with cello tapes at the base and the box labeled. Larvae in the neonate stage (first instar larvae), which have been starved for 24 hours, were selected and 10 larvae were released on the seeds placed inside the Petri dish separately. The top of the container was closed with the lid. The boxes were checked at least once to prevent the escape of any larvae and formation of moisture inside the box as this may lead to the interpretation of wrong results. Any excessive moisture was allowed to escape by keeping the boxes open in the Laminar Flow for 5-10 min. After 96 hours observations were made on the number of dead larvae, number of live larvae, % mortality and % mass change over control.

Screening of actinomycetes for antagonistic activity against *Macrophomina phaseolina*

Macrophomina phaseolina, which was preserved in 2 mm sieved sand, was revived by sprinkling the sand particles onto ¼ PDA plate and incubated at 30°C till the mycelial growth was observed. A total of 62 isolates were screened for their antagonistic activity. A pure culture was obtained by transferring a small portion of mycelial growth onto a fresh ¼ PDA plate that was amended with Streptomycin at the concentration of 500 mg per liter. The purified culture was maintained on non-antibiotic media plate. A medical flat containing 100 mL of ¼ PDB was inoculated with 5 mm discs of 48 hours old culture of *M. phaseolina* taken from the non-antibiotic ¼ PDA plates. Two discs were inoculated for each flask. These were incubated at laboratory temperature for seven days at window side. About 0.1 ml of

M. phaseolina culture was spread on ¼ PDA plates and was allowed for air dry. Using pin inoculator, 24 actinomycetes isolate and one reference strain (BWB 21) were inoculated on to dried ¼ PDA plates having *M. phaseolina* culture and were incubated at 30±1°C. The zone of inhibition was recorded after 3-4 days.

Screening of actinomycetes for P-solubilization

Phosphate solubilizers were screened using rock phosphate buffered medium (Gyaneshwar et al. 1998). Using pin inoculator 24 isolates and one reference strain (*Pseudomonas sp.* BWB 21) were inoculated on a Petri plate and incubated at 30°C for 6-7 days. The isolates were observed for zone of pH reduction around the colony indicated by red to coffee brown color and the results were recorded.

Screening of actinomycetes for siderophore production

Siderophore is a low molecular weight substance that binds very tightly to iron. Siderophore are synthesized by variety of microorganisms to ensure that the organism is able to obtain sufficient amount of iron from the environment. Siderophore producing actinomycetes were screened using CAS (Chromozural Sulphate) medium (Schwyan and Neilands 1987). Using pin inoculator (pin inoculator is 88 mm diameter having 25 wells and can be used to screen 25 isolates simultaneously) 250 micro liters of the inoculum of each of the isolates were added to each well with *Pseudomonas* sp. BWB 21 used as a reference strain. The plates were incubated at 30°C for 6-7 days. The isolates were observed for orange halo formation around the colony due to pH reduction.

Screening of microorganisms for plant growth promotion in pearl millet by paper towel method

This experiment was conducted to study the effect of microorganisms on germination and growth promotion of pearl millet cultivar (ICMV 155). It was designed to screen large number of microorganisms in shorter period than in test tube method. The materials required for the experiment were paper towel, actinomycetes cultures, pearl millet seeds and Bennett's broth.

Healthy seeds were selected and were surface sterilized by soaking in 3% chlorax for five minutes and washed in sterile deionised water for 9-10 times. The actinomycetes cultures were inoculated into conical flasks containing 40 ml of Bennett's broth and were incubated at 30°C for one week. The sterilized seeds were added to the culture broth and soaked for 30 minutes. The sterilized germination paper was soaked in sterile water for 30 seconds. Approximately 50 seeds were arranged on the upper half of the paper at equal distance with the forceps under the Laminar Flow. Four replications were maintained for each treatment. For the control the seeds were soaked in Bennett's broth and placed on the paper. The paper was covered with another paper dipped in sterilized water and rolled taking care to retain the seeds intact. The rolls were kept upright in polythene bags and were placed in assay buckets and kept in the glasshouse and incubated at 25°C. The rolls were watered for every two days and on the fifth day ¼ Arnons solution was added. Observations were made on the root length; shoot length, root dry weight and shoot dry weight after an incubation period of 10 days.

RESULTS

Population of actinomycetes in soil samples

The mean population (mean of 14 fields, 7 each from Medak and West Godavari districts of Andhra Pradesh) was similar and ranged from 4.22 to 4.33 $\log_{10}$ g^{-1} dry soil) in the soil samples from fields growing rice by the two different methods (SRI and flood rice). In West Godavari district, the population in the control plots ranged from 3.30 to 4.20 $\log_{10}$ g^{-1} dry soil and in SRI plots from 3.30 to 4.45 $\log_{10}$ g^{-1} dry soil (Table 2). In Medak district the control plots had 4.23 to 5.22 $\log_{10}$ g^{-1} dry soil and SRI plots had 4.41 to 5.02 $\log_{10}$ g^{-1} dry soil.

Identification of the cultural characteristics of actinomycetes

Sixty-two different strains of actinomycetes were isolated from 36 soil samples to represent all available diversity due to different cultural traits. The isolates were of different sizes (<1 mm - 3 mm); shapes (irregular, round, regular); colors (white, brown, grey); margins (regular, smooth, serrated); texture (powdery, smooth, rough); consistency (dry); opacity (opaque, slightly translucent); elevation (elevated, flat, raised). These isolates were streaked to get isolated colonies. The isolates were named as (BCA 500-562) (Table 3). The size of most isolates was <1 mm. Most isolates showed cream coloured colonies. Isolates (BCA 500, BCA 507, BCA 514, BCA 516, BCA 521, BCA 531, BCA 532, BCA 547, BCA 548, BCA 552, BCA 553) showed white coloured colonies. Most isolates showed smooth margins. BCA 522, BCA 533, BCA 545 showed transparent colonies.

Screening of actinomycetes isolates for their ability to kill neonates of *Helicoverpa*

All the 65 isolates were screened for their ability to kill neonates of *Helicoverpa armigera*. In addition to these, 13 isolates from the microbial collection at ICRISAT were also screened for larvicidal activity. In general the % mortality was low for all the cultures screened particularly when negative control treatment also had high mortality (30 to 36%). In batch I the percentage mortality was found to be between 18 % in BCA 430 to 52% in BCA 423 (Table 4). In batch II the percent mortality

ranged between 10% in BCA 507 to 70% in BCA 500 (Table 5). The percentage of larvae dead was between 0% in BCA 507 to 42% in BCA 500. Percentage of larvae missing ranged from 6% in BCA 509 and BCA 515 to 28% in BCA 500 and BCA 516. In batch III (Table 6) the percent mortality ranged between 42% in BCA 523 to 86% in BCA 502. The percentage of dead larvae actually seen was between 12% in BCA 537 to 38% in BCA 531. The percentage of missing larvae between 24% in BCA 523 to 56% in BCA 502. BCA 500 was again repeated in the III batch and showed percent mortality of 64%. In batch IV the isolates showed a mortality ranging from 50% in BCA 531 to 78% in BCA 546 (Table 7). The percentage of dead larvae between 22% in BCA 531 to 50% in BCA 532, BCA 542 and BCA 546. The percentage of missing larvae was actually seen in the range of 14% in BCA 551 to 30% in BCA 533 and BCA 557. Isolates in batch V exhibited a percent mortality ranging between 56% (BCA 524) to 84% (BCA 549) (Table 8). The percentage of dead larvae actually seen was in the range of 28% in BCA 524 and BCA 528 to 46% in BCA 549. The percentage of missing larvae was between 24% in BCA 543 to 40% in BCA 520. Isolates in batch VI (Table 9) showed a mortality ranging between 40% (BCA 503) to 76% (BCA 527). The percentage of dead larvae was between 22% in BCA 550 to 52% in BCA 527. The percentage of missing larvae was between 14% in BCA 503 to 40% in BCA 532. Percentage of missing larvae indicates the percentage of larvae that was decomposed within four days of observation or may have crawled out of the box due to unfavorable conditions. The positive control (Spinosad) showed 100% mortality in all the batches whereas the negative control showed mortality ranging from 30% to 40%.

Some treatments showed reduction while some showed an increase in the larval mass as compared to the negative control. In batch I, some isolates like BCA 430, BCA 405 and BCA 406 showed a negative value (as shown in the Table 4) indicating a decrease in mass over control. In batch II all the isolates showed an increase in mass over control. BCA 500 that showed 70% mortality showed 22.5% increased larval mass over control and BCA 516 showed an increase by 39.6% mass over control (Table 5). In batch III, all the isolates showed a decrease in mass over control.

BCA 502 that showed a mortality of 86%, showed a decrease in mass by 91.1% when compared to the control. BCA 519 showed a decrease in mass over control by 68.1 % (Table 6). In batch IV, the isolate that showed 74% mortality (BCA 542) (Table 7) showed an increase in mass over control whereas the isolates BCA 546 and BCA 532 showed a decrease in mass over control by 44.4% and 43.3% respectively. In batch V, some isolates like BCA 549 and BCA 520 showed an increase in mass over control whereas others like BCA 524 and BCA 548 showed a decrease in the mass over control (Table 8). In batch VI, the two promising isolates BCA 527 (76% mortality) showed a decrease in mass over control by 8.3% and BCA 532 (72% mortality) showed a decrease in mass over control by 16.7% (Table 9).

Evaluation of actinomycetes for suppression of *M. phaseolina*

Four isolates showed some antagonistic activity against disease causing fungi *M. phaseolina*. These were BCA 500, BCA 501, BCA 507 and BCA 514 and had clear zone of inhibition of 0.4 mm, 0.3 mm, 0.2 mm and 0.3 mm diameter respectively (Table 10).

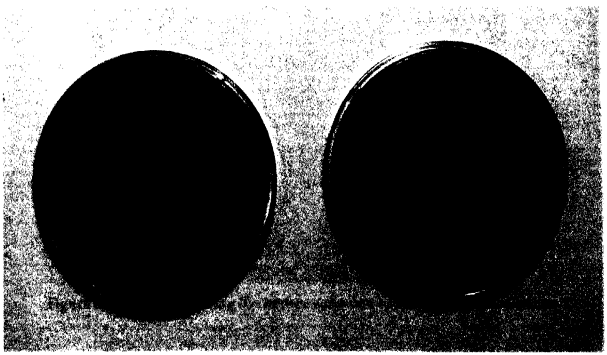

Figure 2. Petri dish showing the zone of inhibition of *M. phaseolina* by actinomycetes.

Evaluation of actinomycetes for P-solubilization

Among the 75 isolates that were screened for P-solubilization using rock phosphate medium, none of the isolates showed positive for phosphate solubilization. No zone of colour change (red halo indicating pH reduction) was seen around the colonies. The reference strain (*Pseudomonas* sp. BWB 21) under the same conditions showed red zone (Table 10).

Evaluation of actinomycetes for siderophore production

Out of the 76 isolates screened, only one isolate (BCA 538) (Fig. 4) produced a zone of 0.3 mm. The zone was indicated by the formation of an orange halo around the colony due to pH reduction as shown by the reference strain (*Pseudomonas* sp. BWB 21) (Table 10).

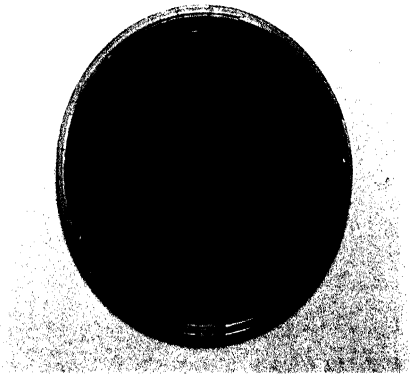

Figure 3. Petri dish showing the orange zone due to siderophore production.

Evaluation of selected isolates for plant growth promotion

Percent increase or decrease in growth parameters of different strains over uninoculated control is shown in Table 11. Increase in shoot length was recorded in BCA 501, BCA 502, BCA 507, BCA 541 and BCA 559. Maximum increase was seen in BCA 559 (27.4%) compared to the control. BCA 559 also recorded the maximum root length (26.0%). All isolates had more root length over control and the increase ranged from 9% in BCA 514 to 26% in BCA 559. Increase in shoot dry mass was recorded in five isolates and ranged from 9% in BCA 541 to 27.2% in BCA 507. Only BCA 502 increased root dry weight (2.1%).

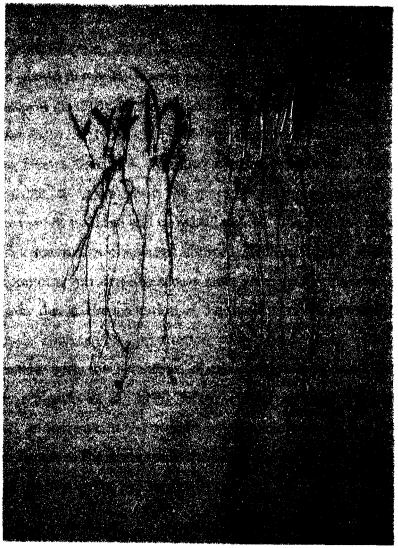

Figure 4. Comparing the length of root and shoot of pearl millet for different treatments.

DISCUSSION

Population of actinomycetes in soil samples

Eight out of fourteen SRI samples had higher population of actinomycetes than those from flood rice plots. The method of SRI is mainly different from the flood rice method due to the fact that there is no standing water all the time. SRI allows wetting and drying cycles such that the soil surface is generally moist for much of the cropping period. It is, therefore, expected that much of the aerobic microorganisms will proliferate (including actinomycetes) in the SRI plot over time. Most farmers were growing SRI rice for the past two years. It is hoped that the differences in the microbial population in the SRI plots and that of flood rice plots will increase over time if treatments are maintained on a given piece of land.

Screening of actinomycetes isolates for their ability to kill neonates of H. armigera

Research on actinomycetes lead to the discovery of soil actinomycetes *Saccharopolyspora spinosa* that opened an opportunity to develop new tools to manage insect pests (Sparks et al. 1998). In the present study the actinomycetes isolates that were screened for their ability to kill *Helicoverpa* larvae showed an average larval mortality ranging from 40% to 86%. Mortality of neonates in control ranged to 30% to 38%, mean was 30.2%. If we adjust the mortality of the promising strains with mean control, the net kill by these will only be 2 to 46%. The positive control Spintor (obtained from *Saccharopolyspora spinosa*) showed 100% mortality. It thus suggests that we have to continue our drive to access more efficient strains from nature. The mode of action may due to the excitation of the insect nervous system that leads to involuntary muscle contractions, prostration with tremors and paralysis. These effects are consistent with the activation of nicotinic acetylcholine receptors and GABA receptors (Salgado et al. 1998). Some of the isolates that showed high mortality showed an increase in the weight while some showed a decrease in the weight of the live larvae's. The decrease in the body weight of the larvae may be due to the cessation of feeding by the exposed insects.

Evaluation of actinomycetes for suppression of *M. phaseolina*

Research on actinomycetes showed that certain actinomycetes belonging to streptomyces species such as *Streptomyces griseoviridis* (Strain K6) and *S. lydicus* act against disease causing fungi. The suppression of Macrophomina phaseolina may be due to the release of certain inhibitory metabolites or may be by depriving them of space and nourishment. Recently *Streptomyces lydicus* WYEC 108 that is a root colonizing actinomycetes was studied for its property as an antifungal biocontrol agent this strain was found to be capable of mycoparasitic colonization of fungal root pathogen and excretion of antifungal metabolites (Gerhardson 2002). In addition the actinomycetes may synthesis an array of biodegradative enzymes like chitinases, glucanases etc. that are involved in mycoparasitic activity (Fridlender et al. 1993). In the present study four isolates (BCA 514, BCA 507, BCA 501 and BCA 500) have shown some activity against *M. phaseolina*. This may be due to the production of antibiotics or due to the synthesis of certain inhibitory enzymes.

Evaluation of actinomycetes for siderophore production

Siderophore are small diffusible molecules excreted by microorganisms that form stable iron complexes. Microorganisms produce different types of siderophore belonging to the catechol phenolic type of siderophores, the hydroxymate type and desferrioximine type. Research shows that more than 10 distinct species of streptomycetes produce desferrioximine siderophores such as desferrioximines G1 (Crawford et al. 2002), desferrioximine B and desferrioximine E (Mincer et al. 2002). In the present study only one of the isolates showed positive for siderophore production. Many genes are involved in the production of siderophores. The inactivation of des D genes that codes for siderophore synthetase that catalysis the key step in desferrioximine synthesis causes abrogation of the production of desferrioximines G1 and B (Schupp et al. 1988) whereas the inactivation of cchH gene leads to the loss of production of hydroximate siderophores. In the present study only one isolate was siderophore positive and its reasons need to be examined.

Evaluation of actinomycetes for P-solubilization

Phosphate solubilizing bacteria are involved in the conversion of insoluble phosphates to soluble forms thus making it available for the plant growth. There are diverse groups of microorganisms like bacteria, fungi and actinomycetes that bring about this conversion. Phosphorus solubilizing microorganisms are reported to dissolve insoluble phosphates by the production of organic acids and by the decrease of the pH (Whitelaw 2000). The solubilization of phosphorous is mainly due to the secretion of organic acids such as citric, succcinic, glutamic acid etc. Most of the previous reports state that calcium phosphates are dissolved by acidification. Therefore, any microorganism that acidifies its external medium will show some level of phosphorus solubilizing activity (Goldstein 1986). In the present study none of the isolates showed positive results this may be attributed to the inability of the actinomycetes isolates to acidify their external medium.

Evaluation of actinomycetes to promote the growth of pearl millet cultivar (ICMV 155)

Most actinomycetes isolates showed an increase in the growth of pearl millet cultivar (ICMV 155). These isolates were initially picked up for their diversity or for plant growth promoting traits. But in the study reported here (disease suppression, P-solubilization in rock phosphate and siderophore production) most isolates failed these tests. But atleast five of the eight isolates had reasonable level of growth promotion of either root or shoot or both. From these results it seems that there are many other factors other than those studied that governs the plant growth. The widely accepted mechanisms of biocontrol mediated by plant growth promoting microorganisms (PGPM) are competition for ecological niche or a substrate, production of inhibitory alleochemicals and induction of systemic resistance in the host plants to a broad spectrum of pathogens (Haas 2002) and abiotic stress. Therefore there is a need to focus on the principles and mechanism of action of PGP and their use as a potential means for the biological control of pests and diseases.

Research on mechanisms of plant growth promotion by microorganisms has provided a good understanding of the multiple facets of disease suppression by the different biocontrol agents. Revelations about the mechanisms of action of PGPB open new doors to design strategies for improving efficacy of biocontrol agents.

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Table 1. Ingredients required for making artificial diet for *Helicoverpa armigera*.

Chickpea flour	300 g
Ascorbic acid	4.7 g
Aureomycin powder	11.5 g
Vitamin stock solution	10 ml
Water	450 ml
Yeast	48 g
Agar	17.3 g
Water	800 ml

Vitamin solution:

Nalidixic acid	1.528 mg
Calcium pentoate	1.528 mg
Riboflavin	0.764 mg
Aneurine	0.382 mg
Pyridoxine	0.382 mg
Folic acid	0.382 mg
Biotin	0.305 mg
Cynacobalamine	0.003 mg
Water	500 ml

Table 2. Actinomycetes population in the soil samples collected from rice fields of SRI and controls plots at vegetative crop growth from the two districts of Andhra Pradesh during 2004-05 post rainy season.

Name of farmer	District name	Actinomycetes population log_{10} cfu/g	
		SRI	Control
Sudhakar Reddy	West Godavari	4.23	4.04
K Bala Ram Raju	West Godavari	3.30	3.90
Sreenivasa Raju	West Godavari	4.04	3.30
Gopal Raju	West Godavari	4.45	4.20
Ramana Murthy	West Godavari	3.60	3.70
DSSN Raju	West Godavari	3.85	3.30
V Krishna Rao	West Godavari	3.48	3.30
Ch Sadasivudu	Medak	4.63	4.23
K Sadasiva Reddy	Medak	4.94	5.18
M Bala Ram Reddy	Medak	4.69	4.74
Pawan Kumar Yadav	Medak	4.81	4.83
T Manohar	Medak	4.87	4.52
N Madhava Rao	Medak	5.02	4.66
G Srinivasa Rao	Medak	4.41	5.22
Mean		4.30	4.22
SE ±		0.100	0.080^{NS}

NS= Statistically not significant

Table 3. Cultural and morphological characteristics of actinomycetes isolated from soil.

Strain No.	Size (mm) in 7 days	Shape	Colour	Margins	Texture	Consistency	Opacity	Elevation
BCA 500	1	R	W	RE	SM	L	O	C
BCA 501	1-2	R	CR	S	SM	L	O	C
BCA 502	1-2	R	CR	S	SM	L	O	C
BCA 503	1-2	R	CR	RE	SM	L	O	F
BCA 504	1-2	R	CR	S	SM	L	O	C
BCA 505	2-3	R	CR	S	SM	L	O	C
BCA 506	2-3	R	CR	S	SM	L	O	C
BCA 507	1-2	R	W	S	SM	L	O	C
BCA 508	1-2	R	CR	S	SM	L	O	C
BCA 509	1-2	R	CR	S	SM	L	O	C
BCA 510	2-3	IR	CR	S	P	D	O	C
BCA 511	<1	R	CR	RE	SM	L	O	C
BCA 512	1-2	IR	CR	S	SM	M	O	C
BCA 513	<1	R	CR	RE	SM	M	O	F
BCA 514	<1	IR	W	RE	P	P	O	C
BCA 515	1-2	IR	CR	S	SM	L	O	C
BCA 516	1-2	IR	W	RE	P	D	O	C
BCA 517	<1	R	CR	RE	SM	L	O	C
BCA 518	2-3	IR	CR	S	SM	L	O	C
BCA 519	2-3	IR	CR	S	SM	L	O	C
BCA 520	1-2	R	CR	S	SM	L	O	C
BCA 521	1-2	IR	W	S	P	D	O	C
BCA 522	<1	IR	CR	S	SM	M	T	C
BCA 523	<1	R	CR	RE	SM	M	O	C
BCA 524	<1	R	CR	RE	SM	L	O	C
BCA 525	1-2	IR	CR	S	SM	L	O	C
BCA 526	<1	R	CR	RE	SM	M	T	F
BCA 527	<1	R	CR	RE	SM	M	O	F
BCA 528	<1	IR	CR	S	SM	M	O	F
BCA 529	<1	IR	W	S	SM	D	O	F
BCA 530	1	IR	CR	S	P	M	O	F
BCA 531	<1	R	W	RE	P	D	O	F
BCA 532	<1	R	W	S	SM	M	T	F
BCA 533	<1	R	CR	RE	SM	M	T	F
BCA 534	<1	IR	W	S	P	D	O	C
BCA 535	<1	R	CR	RE	SM	M	O	F
BCA 536	<1	R	CR	S	SM	D	O	C
BCA 537	<1	IR	CR	S	SM	M	O	F
BCA 538	<1	IR	CR	S	SM	M	O	C
BCA 539	<1	R	CR	RE	SM	M	O	C
BCA 540	1-2	IR	CR	S	SM	D	O	C

Strain No.	Size (mm) in 7 days	Shape	Colour	Margins	Texture	Consistency	Opacity	Elevation
BCA 541	1-2	R	CR	S	SM	D	O	C
BCA 542	<1	IR	CR	S	SM	M	O	F
BCA 543	1-2	IR	CR	S	SM	D	O	C
BCA 544	<1	IR	CR	S	SM	M	O	F
BCA 545	<1	IR	CR	S	SM	M	T	F
BCA 546	<1	R	G	RE	P	D	O	C
BCA 547	<1	R	W	RE	SM	M	O	C
BCA 548	<1	IR	W	S	P	D	O	C
BCA 549	1-2	IR	B	S	P	D	O	C
BCA 550	<1	IR	CR	S	SM			
BCA 551	<1	IR	CR	S	SM			
BCA 552	<1	IR	W	S	P	D	O	C
BCA 553	<1	IR	W	S	P	D	O	C
BCA 554	<1	IR	G	S	P	D	O	C
BCA 555	<1	IR	W	S	P	D	O	C
BCA 556	<1	IR	CR	S	P	D	O	C
BCA 557	<1	R	CR	S	P	D	O	C
BCA 558	<1	R	B	S	P	D	O	C
BCA 559	<1	IR	CR	S	P	D	O	C
BCA 560	<1	IR	CR	S	P	D	O	C
BCA 561	<1	IR	CR	S	P	D	O	F
BCA 562	<1	R	CR	S	P	D	O	C

B = Brown; C = Convex; CR = Cream; D = Dry; F = Flat; G = Grey; R = Irregular; L = Leathery; M = Mucoid; O = Opaque; P = Powdery; R = Round; RE = Regular; S = Serrate; SM = Smooth; T = Transparent; W = White

Table 4. Screening actinomycetes against neonates of *Helicoverpa armigera*-Batch I.

Treatments	nortality	% larval mass change over control
Control	36	NR
Spintor	100	NR
BCA 174b	38	18.3
BCA 177	24	23.5
BCA 253	34	5.2
BCA 253b	28	33.0
BCA 401	40	15.1
BCA 405	46	-22.7
BCA 406	42	-8.4
BCA 408	40	1.5
BCA 416	24	28.4
BCA 417	32	33.7
BCA 422	32	33.7
BCA 423	52	18.5
BCA 430	18	32.8
Mean	32	7.9
SE+	7.5**	18.88 ***

** =statistically significant at P <0.1%

***=statistically significant at P<1%

NR = Not relevant

- = Indicates reduction in mass of the larvae alive on the day of observation compared to mass of the larvae in control treatment. The other values indicate increase in larval growth over control.

Table 5. Screening actinomycetes against neonates of *H. armigera* - Batch II.

Treatments	% of dead larvae	% of missing larvae	% mortality	% mass change over control
Control	18	12	30	NR
Spintor	54	46	100	NR
BCA 500	42	28	70	22.5
BCA 501	6	8	14	149.9
BCA 504	4	8	12	38.8
BCA 507	0	10	10	106.1
BCA 509	8	6	14	106.5
BCA 510	4	20	24	22.2
BCA 511	10	18	28	9.8
BCA 512	2	8	10	134.4
BCA 515	8	6	14	39.8
BCA 516	40	28	68	39.6
BCA 517	28	20	48	65.6
Mean	17	17	35	53.9
SE+	4.6***	5.2***	6.9	23.66

NR = Not relevant

*** = Statistically significant at $P<0.1\%$

Table 6. Screening actinomycetes against neonates of *H. armigera*-batch III.

Treatments	% of dead larvae	% of missing larvae	% mortality	% larval mass change over control
Control	10	28	38	NR
Spintor	52	48	100	NR
BCA 500	22	42	64	-42.5
BCA 502	30	56	86	-91.1
BCA 519	26	48	74	-68.1
BCA 523	18	24	42	-18.0
BCA 530	20	46	66	-29.2
BCA 531	38	36	72	-61.7
BCA 536	26	36	62	-40.3
BCA 537	12	52	64	-49.2
BCA 541	24	40	70	-26.2
BCA 550	16	50	66	-53.8
BCA 553	22	40	62	-33.9
BCA 559	34	42	76	-52.8
BCA 561	16	30	46	-59.4
Mean	24	41	63	-52
SE±	8.8^{NS}	9.0^{NS}	10.1	14.9

NR = Not relevant

NS = Statistically not significant.

- = Indicates reduction in mass of the larvae alive on the day of observation compared to mass of larvae in control treatment.

Table 7. Screening actinomycetes against neonates of *H. armigera* - Batch IV.

Treatments	% of dead larvae	% of missing larvae	% mortality	% mass change over control
Control	24	12	36	NR
Spintor	78	22	100	NR
BCA 525	34	28	62	-21.3
BCA 529	40	28	68	-26.9
BCA 531	22	28	50	-24.0
BCA 532	50	22	72	-43.4
BCA 533	36	30	66	-38.4
BCA 538	34	28	64	-37.9
BCA 539	40	26	66	-42.8
BCA 540	44	16	60	-18.4
BCA 542	50	24	74	103.6
BCA 543	36	24	60	-6.5
BCA 544	42	28	70	-6.0
BCA 546	50	28	78	-44.4
BCA 547	38	24	62	-23.6
BCA 551	40	14	54	-1.9
BCA 556	44	24	68	-22.9
BCA 557	28	30	58	-22.3
BCA562	42	26	68	-25.2
Mean	41	24	65	-21.0
SE±	5.8***	5.4 NS	4.4**	24.71**

= Statistically significant at $P<1\%$, *** = Statistically significant at $P<0.1\%$

NR = Not relevant, NS = Statistically not significant

- = indicates reduction in the mass of the larvae alive on the day of observation compared to the mass of the larvae in the control treatment. The other values indicate increase in the larval growth over control.

Table 8. Screening actinomycetes against neonates of *H. armigera*- Batch V.

Treatments	% of dead larvae	% of missing larvae	% mortality	% mass change over control
Control	30	4	34	NR
Spintor	62	38	100	NR
BCA 502	30	30	60	28.9
BCA 506	30	30	60	27.2
BCA 520	38	40	78	23.6
BCA 524	28	28	56	-47.4
BCA 528	36	28	64	3.0
BCA 537	38	32	70	-25.8
BCA 543	34	24	58	37.6
BCA 548	28	32	60	-9.6
BCA 549	46	38	84	19.2
BCA 558	30	36	66	63.8
Mean	35	31	66	1.9
SE±	4.5***	4.7^{NS}	4.9***	18.74***

* = Statistically significant at $P<0.1\%$

NR= Not relevant, NS= Statistically not significant

- = Indicates the reduction n the mass of the larvae alive on the day observation compared to mass of larvae in control treatment. The other value indicates increase in larval growth over control.

Table 9. Screening actinomycetes against neonates of *H. armigera* - Batch VI.

Treatments	% of dead larvae	% of missing larvae	% mortality	% mass change over control
Control	30	4	34	NR
Spintor	74	26	100	NR
BCA 503	26	14	40	3.9
BCA 505	40	28	68	7.8
BCA 522	36	20	56	73.6
BCA 526	44	24	68	1.7
BCA 527	52	24	76	-8.3
BCA 532	32	40	72	-16.7
BCA 534	40	28	68	-13.3
BCA 535	34	28	62	32.4
BCA 545	30	32	62	30.9
BCA 550	22	28	50	-12.1
BCA 554	36	38	74	61.7
BCA 555	36	24	60	23.7
BCA 557	34	28	62	18.4
Mean	38	26	64	6.9
SE+	5.1***	4.9^{NS}	5.0***	14.14***

*** = Statistically significant at $P<0.1\%$

NR= Not relevant, NS = Statistically not significant.

- = Indicates reduction in mass of the larvae alive on the day of the observation compared to the mass of the larvae in the control treatment. The other values indicate increase in the larval growth over control.

Table 10. Screening actinomycetes for antagonism against *M. phaseolina*, siderophore production and P-solubilization.

Treatments	*M. phaseolina*	Siderophore production	P-solubilization
BWB 21	+	+	+ (Ref.)
BCA 174b	-	-	-
BCA 177	-	-	-
BCA 253	-	-	-
BCA 253b	-	-	-
BCA 401	-	-	-
BCA 405	-	-	-
BCA 406	-	-	-
BCA 408	-	-	-
BCA 416	-	-	-
BCA 417	-	-	-
BCA 422	-	-	-
BCA 423	-	-	-
BCA 430	-	-	-
BCA 500	+	-	-
BCA 501	+	-	-
BCA 502	-	-	-
BCA 503	-	-	-
BCA 504	-	-	-
BCA 505	-	-	-
BCA 506	-	-	-
BCA 507	+	-	-
BCA 508	-	-	-
BCA 509	-	-	-
BCA 510	-	-	-
BCA 511	-	-	-
BCA 512	-	-	-
BCA 513	-	-	-
BCA 514	+	-	-
BCA 515	-	-	-
BCA 516	-	-	-
BCA 517	-	-	-
BCA 518	-	-	-
BCA 519	-	-	-
BCA 520	-	-	-
BCA 521	-	-	-
BCA 522	-	-	-
BCA 523	-	-	-

Treatment	*M. phaseolina*	Siderophore production	P-solubilization
BCA 524	-	-	-
BCA 525	-	-	-
BCA 526	-	-	-
BCA 527	-	-	-
BCA 528	-	-	-
BCA 529	-	-	-
BCA 530	-	-	-
BCA 531	-	-	-
BCA 532	-	-	-
BCA 533	-	-	-
BCA 534	-	-	-
BCA 535	-	-	-
BCA 536	-	-	-
BCA 537	-	-	-
BCA 538	-	+	-
BCA 539	-	-	-
BCA 540	-	-	-
BCA 541	-	-	-
BCA 542	-	-	-
BCA 543	-	-	-
BCA 544	-	-	-
BCA 545	-	-	-
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BCA 551	-	-	-
BCA 552	-	-	-
BCA 553	-	-	-
BCA 554	-	-	-
BCA 555	-	-	-
BCA 556	-	-	-
BCA 557	-	-	-
BCA 558	-	-	-
BCA 559	-	-	-
BCA 560	-	-	-
BCA 561	-	-	-
BCA 562	-	-	-

Table 11. Plant growth-promoting activity of selected isolates of actinomycetes on pearl millet cultivar ICMV 155.

Treatments	Shoot length (cm)	Root length (cm)	Shoot dry weight (mg)	Root dry weight (mg)
A.chroococum HT 54	8.0	28.9	118	118
Control	6.0	20.0	110	140
BCA 501	7.0 (16.6)	24.3 (21.5)	123 (11.8)	135
BCA 502	6.5 (8.33)	23.8 (19.0)	123 (11.8)	143
BCA 507	6.5 (8.33)	25.1 (25.5)	140 (27.2)	135
BCA 514	5.8	21.8 (9.0)	100	133 (2.1)
BCA 516	5.9	22.3(11.5)	110	138
BCA 538	5.5	23.2 (16.0)	103	105
BCA 541	6.8 (13.3)	24.2 (21.0)	120 (9.0)	115
BCA 559	7.7 (28.3)	25.2 (26.0)	125 (13.6)	135
Mean	6.6	24.0	117	130
SE±	0.47**	1.61**	10.9*	9.4*

Values in the parentheses are percent increase over control.

* = Statistically significant at $P<5\%$, ** = Statistically significant at $P<1\%$