PLANT GROWTH PROMOTION BY RHIZOBACTERIA
for Sustaianble Agriculture

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Accessing Actinomycetes from Herbal Vermicomposts and their Evaluation for PGP Traits

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Abstract

Role of actinomycetes in plant growth promotion and biocontrol are getting new insights as better alternative for sustainable agriculture production over agro-chemicals. Composts prepared using widely available herbs can be rich in actinomycetes with agriculturally beneficial traits such as siderophore production, chitinase production, and suppression of disease causing fungi. Selection of novel, active actinomycetes can be done by examining microbial rich natural sources like composts and organic amended agriculture soils. Twenty nine different herbal plants foliage (with properties to help manage insect-pests) was used to prepare vermicompost in 12" d plastic pots and were used as source to isolate actinomycetes. Some of these isolates were present in large numbers (10^5 per g compost). Actinomycetes (136) were isolated, purified and further studied for plant growth promoting traits on different media and anti fungal activity against plant pathogenic fungi. The results indicated that 73%, 37%, and 53% were siderophore, chitinase and protease producers respectively whereas none of the 136 isolates were able to solubilize rock phosphate. Bio control studies indicated that 79%, 53% isolates inhibited *Macrophomina phaseolina* and *Fusarium oxysporum f. sp. Ciceri* respectively. Regular and liberal use of composts prepared from these botanicals would highly likely enhance soil health.

Introduction

Sustainable agriculture is an integration of traditional techniques with modern advances and its progress depends on development of new methodologies to understand the fundamental processes involved in soil fertility (Hameeda *et al.,* 2009). Application of organic matter, low cost biological inputs and biofertilizers have positive effects on nutrient levels, soil biota and crop production (Rupela *et al.,* 2006).

Application of beneficial microorganisms from soil, microbial rich composts could supply macro and micro nutrients for plant health and also can decrease associated problems with chemical fertilizers (Abdelaziz *et al.*, 2007). Plant growth promoting microorganisms (PGPM) includes bacteria, fungi and actinomycetes, a group of symbiotic or free-living microbes that colonize rhizosphere and benefit plant growth (Magdoff and van Es 2000). PGPM directly stimulate growth by nitrogen (N) fixation, solubilization of nutrients like Phosphorus (P), Potassium (K), Zn etc., (Han *et al.* 2006), production of growth hormones, 1-amino-cyclopropane-1-carboxylate (ACC) deaminase (Correa *et al.*, 2004), and indirectly by antagonizing pathogenic fungi by production of siderophores, chitinase, β-1, 3-glucanase, antibiotics, fluorescent pigments, and cyanide (Pal *et al.*, 2001). Role of actinomycetes in plant growth promotion and biocontrol are getting new insights as a better alternative for sustainable agriculture production. Novel actinomycetes can be accessed from microbial rich natural sources like composts and organic amended agriculture soils. This paper reports accessing agriculturally beneficial actinomycetes from different herbal vermicomposts.

Materials and Methods

Isolation and screening of actinomycetes for plant growth promoting traits

After 45-50 days of inoculation of herbal plants foliage with earthworms in 12- inch diameter pots, 10 g of sample was collected and plated on Starch Casein Agar (SCA) with antibiotics to isolate actinomycetes. Purified actinomycetes were screened for different plant growth promoting traits on phosphate solubilization (Gyaneshwar *et al.*, 1998, Pikovskaya et al, 1948), indole acetic acid (IAA) (Vivas *et al.*, 2006), siderophore production (Bernhard and Neilands, 1987), hydrolysis of chitin (Reid and Ogrydziak, 1981), cellulose degradation, lipase producers and protease producers were determined on specific media. After inoculation, plates were incubated at 30±°C for 24 to 144 h as required based on the trait under study. Rating of isolates positive for plant growth promoting trait was recorded based on growth and/or zone size accordingly. Screening was done thrice with two replications each.

Screening of actinomycetes for in vitro antagonism

All the 136 isolates were also screened for in-vitro antagonism against plant pathogenic fungi *Macrophomina phaseolina, Fusarium solani, and Fusarium oxysporum* f.sp. *ciceri, Sclerotium rolfsii* by dual culture method. Growth of pathogen was recorded and percent inhibition of fungus was calculated by using the formula:

$$I = (C - T)^{*}\ 100/C$$

Where I is the percent inhibition of mycelial growth, C is the radial growth of fungus in the control plate (mm), and T is the radial growth of fungus on the plate inoculated with bacterium (mm).

Evaluation of actinomycetes on plant growth of sorghum in glasshouse conditions

Based on plant growth-promoting traits, inhibition % against *M. phaseolina,* top ten actinomycetes isolates were selected and evaluated for growth on Sorghum (cultivar RS-16). Unsterilized glasshouse mixture of red soil, black soil, FYM in the ratio of 3:2:1 was used for plant growth studies. Seeds coated with *Azotobacter chroococcum* HT-54 were used as positive control (Alka *et al.,* 2001) and only seeds treated with broth served as control. Plants were irrigated once every 2 days with 100 ml 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 to 35°C (Average 26°C).

Data Analysis

All glasshouse experiments were arranged in completely randomized block design with three replications in each treatment and repeated twice. The data were subjected to analysis of variance using Genstat 8.1 statistical package (Lawes Agricultural trust, Rothamsted, UK). Mean values in each treatment were compared using least significant differences at 5% probability (P=0.05).

Results

Microbiology of Compost and isolation of actinomycetes

Actinomycetes population ranged from log 7.74 CFU/g in Anona compost to log 2.00 CFU/g in Tobacco compost. Diversity of actinomycetes was recorded high in Chrisanthimum (12), lowest in Onion (1) and Jatropha seed compost (1). Population of bacteria was ranged from log 9.31 CFU/g in Anona compost to log 7.40 CFU/g in Chrisanthimum compost. Diversity of bacteria was noted maximum in Neem compost (17) and minimum in Vitex compost (6). Fungal population ranged from log 7.33 CFU/g in Pongamia foliage to log 6.27 CFU/g in Ipomia compost. Diversity of fungi was recorded high in Pongamia foliage (9) and low in Yellow oleander compost (3). pH of the compost samples ranged from 9.07 in Turmeric compost to 7.07 in Rice straw compost. A total of 136 actinomycetes isolates were isolated from twenty-nine different composts and highest 12 isolates were isolated from Masalamatti compost.

Screening of actinomycetes for plant growth promoting traits and in vitro antagonism

Among all 136 actinomycetes, siderophore producing population was 73%, chitinase producers were 35%, protease producers were 53% and Phosphate solubilizers were recorded as 7%. None of the isolates were positive for rock phosphate solubilization, cellulose degradation and lipid degradation. Among 15 isolates studied for Indole acetic acid production, 10 isolates were noted as positive with highest production of CAI 17 (10.24 μg/ml) followed by MMA 32 (8.17 μg/ml). Isolates with antagonism against *M. phaseolina* were recorded as 58% and antagonism against *F. oxysporum* f.sp. *ciceri* were 24%. None of the isolates inhibited *F. udum, S. rolfsii* in dual culture conditions. Isolates with three and above plant growth promoting traits were recorded as 43% (Table 1).

Table1. Screening of actinomycetes for plant growth promoting and biocontrol traits.

Trait	Total number of screened isolates	% Positive isolates
Siderophore	136	73
Indole Acetic Acid	15	67
Rock Phosphate Solubilization	136	0
Phosphate Solubilization	136	7
Cellulase (Cellulose degradation)	136	0
Chitinase (Chitin degradation)	136	35
Lipase (Lipid degradation)	136	0
Protease (Protein degradation)	136	53
Macrophomina phaseolina	100	79
Fusarium oxysporum f sp. Ciceri	62	53
Fusarium udum	136	0
Sclerotium rolfsii	136	0

Evaluation of actinomycetes for plant growth of sorghum in glasshouse conditions

Ten isolates with highest inhibition % against *M. phaseolina* were selected to screen in glasshouse for their plant growth promotion ability with sorghum in the presence of *M. phaseolina*. Plant shoot dry weight was increased by 22% with isolate MAM 32 followed by CAI 17 (20%) over uninoculated control. Root dry weight was increased by 27% with CAI 26 followed MMA 32 (25%) and CAI 68 (25%) over uninoculated control. Shoot, root dry matter ratio was observed highest with isolate CAI 17 (5.25) and 20% increase over control (Figures 1, 2, 3, 4).

This study demonstrated that the selected actinomycetes strains have multifunctional PGP properties as other soil borne micro-organisms such as *Pseudomonas spp. Bacillus* spp. (Pandey *et al.*, 2006), *Acinetobacter* sp. PSGB03 and *Serratia* sp. PRGB11 (Indiragandhi *et al.*, 2008) that were able to produce IAA, chitinase, *b*-1,3-glucanase, siderophores and antifungal substances to improve plant growth. The release of beneficial substances by these actinomycetes is expected to precisely match the plant needs, avoiding pollution in the environment. This work is thus expected to contribute to the development of a sustainable agriculture by using natural resources.

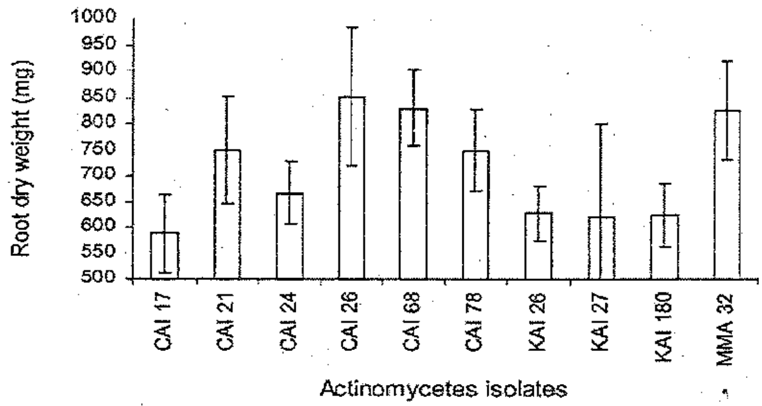

Fig 1. Effect of actinomycetes on Sorghum root dry weight in glasshouse.

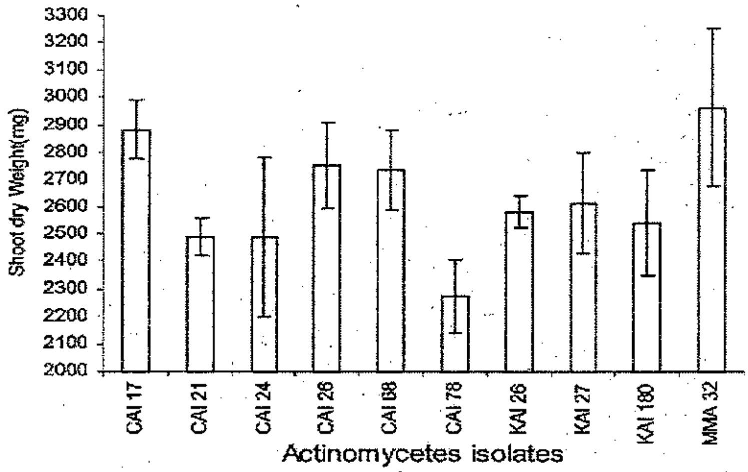

Fig 2. Effect of actinomycetes on Sorghum shoot dry weight in glasshouse.

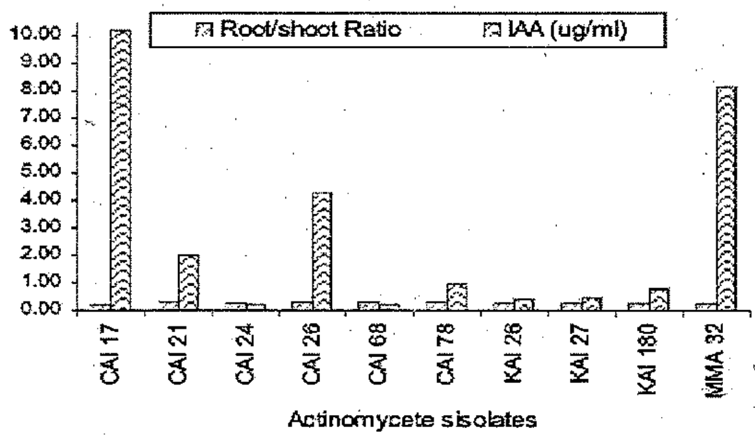

Fig. 3. Effect of actinomycetes on Sorghum shoot/root ratio glasshouse and IAA production.

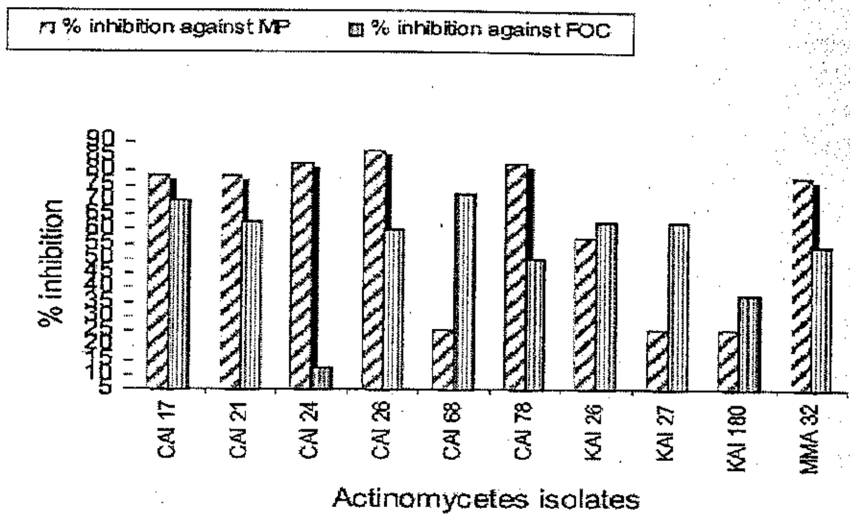

Fig. 4. Antifungal activity of actinomycetes against *M. phaseolina, F. oxysporum f.sp. ciceri* in dual culture conditions.

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