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Short Communication

Recent trend of aminoglycoside resistance among *Staphylococcus aureus* isolates in tertiary care hospital

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Aminoglycosides still play an important role in antistaphylococcal therapies, although emerging resistance amongst staphylococci is widespread. The objective of the present study was to know the percentage of aminoglycoside resistance among *Staphylococcus aureus* isolates. A total of 250 isolates of *S. aureus* were studied from different clinical specimens like blood, pus, wound swabs, sputum, ear swabs and body fluids. All the isolates were tested for their susceptibility to four aminoglycosides namely gentamicin, amikacin, tobramycin and netilmicin by Kirby Bauer disc diffusion method using criteria of standard zone of inhibition. Methicillin resistant *S. aureus* (MRSA) detection was done by cefoxitin disc diffusion method. Out of the 250 *S. aureus* isolates, 66 (26.4%) isolates demonstrated resistance to at least one of the four aminoglycosides tested. Four isolates were resistant to all the four aminoglycosides tested. All these four were MRSA. The most active antimicrobial agent against *S. aureus* was found to be netilmicin followed by amikacin. Continued surveillance at both the genotypic and phenotypic levels as well as adherence to well-designed antibiotic and infection control policies are necessary to understand and limit further rise of resistant isolates.

Key words: Aminoglycosides, *Staphylococcus aureus*.

INTRODUCTION

Staphylococcus aureus is a major cause of hospital and community-acquired infections, and can result in serious consequences. The importance of *S. aureus* as a human pathogen, apart from its ability to cause a diverse range of life-threatening infections, is its extraordinary potential to develop antimicrobial resistance (Lowy, 2003).

One of the classes of antibiotics playing an important role in the therapy of serious staphylococcal infections is aminoglycosides. Although aminoglycosides are predominantly used for the treatment of Gram-negative infections, they are also known to have antistaphylococcal activity (Jana and Deb, 2006). This becomes an important consideration when patients are treated empirically for suspected sepsis with an aminoglycoside and

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Table 1. Phenotypic resistance patterns of *S. aureus* (MRSA and MSSA) for four aminoglycosides.

Type	Resistance pattern				Number (%)		
	GEN	TOB	AMK	NET	MRSA	MSSA	Total
1	R	R	R	R	04 (3.7%)	-	04 (1.6%)
2	R	R	R	S	21 (19.6%)	01 (0.7%)	22 (8.8%)
3	R	R	S	S	35 (32.7%)	01 (0.7%)	36 (14.4%)
4	R	S	S	S	01 (0.9%)	03 (2.1%)	04 (1.6%)
5	S	R	R	S	-	-	-
6	S	S	S	S	46 (43.0%)	138 (96.5%)	184 (73.6%)
Total					107	143	250

GEN- Gentamicin; TOB- Tobramycin; AMK- Amikacin; NET- Netilmicin; S- sensitive; R- resistant; MRSA- methicillin resistant *S. aureus*; * MSSA- methicillin sensitive *S. aureus.*

ureidopenicillin, other penicillins, or cephalosporins susceptible to staphylococcal beta-lactamases. In addition, the emergence of methicillin-resistant *S. aureus* is increasing the clinical importance of the antistaphylococcal activity of aminoglycosides (Hammerberg et al., 1986).

Increased resistance to these drugs have been reported from many countries (Hauschild et al., 2008) and little data is available on their resistance pattern in staphylococci from this part of India. So this study was undertaken to gain some insight into the susceptibility pattern of aminoglycosides among *S. aureus* in our tertiary care hospital.

MATERIALS AND METHODS

This study was performed between November 2010 and April 2012 in the Department of Microbiology at our tertiary care hospital, India. A total of 250 isolates of *S. aureus* were isolated from different clinical specimens like blood, pus, wound swabs, sputum, ear swabs and body fluids. Only one isolate per patient was included in the study. All the isolates were tested for their susceptibility to gentamicin (10 µg), amikacin (30 µg), tobramycin (10 µg), netilmicin (30 µg) by Kirby Bauer disc diffusion method using criteria of standard zone of inhibition (CLSI, 2010). *S. aureus* ATCC 29213 was used as quality control strain for *in vitro* susceptibility testing. MRSA detection was done by cefoxitin disc diffusion method.

RESULTS

Of the 250 *S. aureus* isolates included in this study, 66 (26.4%) were resistant to at least one of the four aminoglycosides tested (Table 1). 04 (1.6%) *S. aureus* isolates were resistant to all the four aminoglycosides tested. All these four were MRSA. The most active antimicrobial agent against *S. aureus* was netilmicin. 21 (19.6%) MRSA isolates were resistant to gentamicin, tobramycin and amikacin and showed susceptibility only to netilmicin. 35 (32.7%) MRSA isolates showed resistance to gentamicin and tobramycin. Of the 107 MRSA isolates, 61 (57.0%) were resistant to at least one of the four aminoglycosides tested.

DISCUSSION

Aminoglycosides are potent bactericidal agents, inhibiting protein synthesis by binding to the 30S ribosomal subunit. Gentamicin and tobramycin are the most active against staphylococci and are often used in combination with either a β-lactam or a glycopeptide, especially in the treatment of staphylococcal endocarditis, as these drugs act synergically (Schmitz et al., 1999). Bacterial resistance to aminoglycosides is widely recognized as a serious health threat. The major mechanism of aminoglycoside resistance in staphylococci is drug inactivation by cellular aminoglycoside-modifying enzymes such as aminoglycoside acetyltransferases (AAC), aminoglycoside adenyltransferases (ANT) or aminoglycoside phosphotransferases (APH) activity (Jana and Deb, 2006).

Since the first report on gentamicin resistance among staphylococci, strains resistant to both methicillin and gentamicin have been the cause of serious infections and extensive outbreaks. Many of these organisms are often resistant to a number of other antibiotics (Anupurba et al., 2003). In 1969, the first clinical gentamicin-resistant MRSA (GR-MRSA) strain was isolated. By 1980s GR-MRSA had become epidemic in Australia, the United States and Europe (Cafferkey et al., 1983). In fact many strains of MRSA exhibit resistance to both β-lactams and aminoglycosides (Anupurba et al., 2003; Tiwari et al., 2008).

In the present study, 66 (26.4%) strains of *S. aureus* and 61 (57.0%) MRSA were resistant to gentamicin which correlates with the study from North India (Tiwari et al., 2008) which reported 55.8% gentamicin resistance among MRSA. Aminoglycoside resistance reported in *S. aureus* isolates from different countries, and especially gentamicin resistance, is of clinical importance because it can compromise the therapeutic effectiveness of these

antibacterial agents (Hauschild et al., 2008).

In this study, 62 (24.8%) *S .aureus* isolates were resistant to tobramycin which correlates well with the report from Europe (Schmitz et al., 1999) which reported 29.0 % resistance among *S. aureus* to tobramycin. A study from South Maharashtra region of India reported that more than 90% *S. aureus* isolates were resistant to gentamicin and tobramycin (Kandle et al., 2003).

In this study, 04 (1.6%) and 26(10.4%) *S. aureus* isolates were resistant to netilmicin and amikacin respecttively. Another study from Poland reported 24.4% *S. aureus* strains were resistant to amikacin and that no strain of *S. aureus* was found resistant to netilmicin (Hauschild et al., 2008). Of the 250 *S. aureus* isolates, 66 (26.4%) were resistant to at least one of the four aminoglycosides tested which is somewhat lower than the finding of the authors (Hauschild et al., 2008) who reported that 38.1% of their isolates of *S. aureus* were resistant to one of the aminoglycosides tested.

In this study, 60 (56.1%) MRSA isolates were resistant to tobramycin, 25 (23.4%) to amikacin and 04 (3.7%) to netilmicin. Tiwari et al. (2008) reported 41.5% amikacin resistance among MRSA. In this study, netilmicin sensitivity is found to be higher than other aminoglycosides. A study from India and Korea reported 5.1% and less than 20% netilmicin resistance in their study, respectively (Rajaduraipandi et al., 2006; Kim et al., 2004).

In our study, members of aminoglycoside group were tested separately because *in vitro* testing of one member, however, may not predict *in vitro* result for other members in the group. Further genotypic studies are needed for the isolates demonstrating phenotypic resistance to any of the member of this class. It will help in the identification of the known or any new gene encoding aminoglycoside modifying enzyme in staphylococcal isolates which could account for the phenotype.

In summary, among the aminoglycosides tested, maximum susceptibility was found for netilmicin (98.4%) followed by amikacin (89.6%). Highest resistance was observed for gentamicin (26.4%). Continued surveillance at both the genotypic and phenotypic levels as well as adherence to well-designed antibiotic and infection control policies are necessary to understand and limit further rise of resistant isolates.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Control of Fusarium wilt using biological agent *Streptomyces* sp.CPP-53 isolated from compost with plant growth promoting effect on tomato under greenhouse condition

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In the present investigation, four different composts were obtained from Supa Biotech (P) Ltd., Muketshwar and LRC, GBPUAT, Pantnagar, India. The highest actinomycetes community was obtained from CPP compost (67.18%), LRC (15.62%), CM (12.5 %) and VE (4.6%). A total of 48 actinomycetes isolated and examined, exhibited ability to degrade starch, solubilize phosphate, produce catalase and siderophores. Isolated actinomycetes strains were checked for their antagonistic potential against seed and soil borne plant pathogens: *Fusarium oxysporum* (Wilt), *Colletotrichum truncatum* (Anthracnose in soyabean), *Colletotrichum capsici* (Anthracnose in chilli) and *Helminthosporium oryzae* (Brown spot in rice). It restricted mycelium growth of all four pathogens: *H. oryzae* (61.53%), *F. oxysporum* (57.5%), *C. truncatum* (54.05%) and *C. capsici* (50%) under *in vitro* condition. A greenhouse study was performed to evaluate efficiency of CPP-53 for controlling disease incidence by *F. oxysporum* in tomato plants. Out of the four treatments in this experiment, significantly lowest disease severity and higher plant vigour was recorded when CPP-53 was inoculated as compared to the control plants. Observation proved the potential of strain CPP-53 under *in vitro* condition and as an amendment in soil leading to suppression of pathogenic effect and efficient biocontrol agent. 23S rDNA region of actinomycetes strains were sequenced and the most potent one, CPP-53 has 98% similarity with *Streptomyces flavofuscus*.

Key words: Siderophore production, wilt, disease severity, *Streptomyces flavofuscus,* 23S rDNA, biocontrol.

INTRODUCTION

The compost may act as growth medium or as a source of beneficial organisms. A rich microbial flora commonly present in compost has proven to have suppressive effects on several plant pathogens. Compost directly or indirectly influences the soil by improving soil health and provides plant protection. A compost may give a

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contribution to biological control and hence suppression of disease caused by pathogens.

Several microorganisms isolated from different composts have turned out to be strongly antagonistic against certain plant pathogenic fungi. Numerous microorganisms antagonistic towards soil borne plant pathogens have been isolated from disease suppressive composts (Kuter et al.,1983; Kwok et al., 1987) and studies has also showed evidence that compost can benefit siderophore producing bacterial populations, which means that compost generally have the potential to create a suitable environment for proliferating rhizosphere bacteria.

Numerous compost of various origins have been investigated over the years regarding the effects on soil borne plant pathogens as well as foliar. Biocontrol organisms in compost inhibit pathogens through several mechanisms commonly found among antagonists, such as competition, antibiosis, hyperparasitism and induced systemic resistance (ISR). A variety of composts microorganisms have proved activity in controlling various plant diseases. Biological control has potential for the management of various seed and soil borne plant pathogens.

Seed and soil borne plant pathogens can significantly reduce yield and quality of vegetable crops. Anthracnose is the most common disease reported in many plant species such as chilli, soybean, tomato, banana, cotton caused by *Colletotrichum* spp., it affects almost all the parts of plant, that is leaves, flowers, pods, roots, stems, fruits. In addition to yield reduction, *Colletotrichum truncatum* may affect seed quality (França Neto and West, 1989).

Fusarium wilt is a common destructive disease in many economically important crops including, tomato (*Lycopersicon esculentum*) cause by soil borne pathogen (*Fusarium oxysporum*) resulting in severe losses in many crop variety of plants. Ever since, two distinct forms of the pathogen can cause either a vascular wilt (*Fusarium oxysporum* f. sp. *Lycopersici* W. C. Snyder & H. N. Hans.) or a crown and root rot (*F. oxysporum* f. sp. radicis-*lycopersici* W. R. Jarvis & Shoemaker) in tomato. Both pathogens occur mostly in tomato growing areas and sometimes destroy whole crop grown in the field. This pathogen is spread through-out almost all crops and developing new races is a continuing trouble. Many researchers have reported and developed resistant variety of tomato but they provide some degree of control over pathogen.

It is well known that actinomycetes are one of the major and important sources of many biologically active substances such as vitamins, antibiotics, extracellular metabolites, alkaloids, plant growth factors, enzymes, etc. Several properties associated with actinomycetes reported in many areas might explain the ability of several of them to act as biocontrol tool, ability to colonize plant surface, antibiosis against plant pathogens, synthesis of extracellular enzymes, and the degradation of phytotoxins.

Weller (1988) reported that the microorganism that colonizes roots is ideal for use as a biocontrol agent against soil-borne diseases. *Streptomyces griseoviridis* is a good example for colonization of plant rhizosphere by actinomycetes. *S. griseoviridis* is an antagonistic microorganism dominant in biocontrol of plant diseases such as the fusarium wilt of carnation (Tahvonen, 1988).

This study, which deals with seed and soil borne plant pathogens and compost, will be a source for isolating biocontrol actinomycetes. The aim of this study was to isolate potential antagonists from different composts and examine their activity against seed and soil borne fungal pathogens with special emphasis on controlling fusarium wilt of tomato. In addition, primary screening of potent isolate was performed under *in vitro* condition; further secondary screening under green house conditions was also performed for the control of fusarium wilt.

MATERIALS AND METHODS

Compost collection

Four composts were collected; three were from Supa Biotech (P) Ltd, Muketshwar and one as from LRC, GBPUAT, Pantnagar, India at different temperature condition. At the time of collection of samples, the nature of compost was wet, semi-wet and dry. The compost was air-dried at room temperature (30 ± 2°C). About 10 g of the sample was taken and the rest stored at 4°C in a refrigerator for further studies.

Isolation and characterization of Actinomycetes

Actinomycetes isolation was done followed by serial dilution on their respective isolation medium. Isolates were purified on actinomycetes isolation agar and ken knight agar medium using streak and counter streak method. Purified actinomycetes isolates were stored at 4°C for further examination. Primary screening was done by morphological behaviour of bacterial colonies such as colony color, size, shape and growth on medium.

Biochemical assay

Starch hydrolysis

The ability of isolates to excrete hydrolytic enzymes capable of degrading starch was checked on starch agar medium. The isolates were inoculated onto starch agar plate and incubated for 4-6 days at 28 ± 2°C. Starch in the presence of iodine produce blue colorization on the plate and yellow zone around the colonies showed amylolytic activity considered as positive result.

Siderophore production

Siderophore production test was conducted with selected isolates; CAS (Chrome Azurole S) agar test method was adopted as reported (Schwyn and Neilands, 1987). Actinomycetes isolates were inoculated on CAS agar plate by spot inoculation using autoclaved tooth pick, incubated at 28 ± 2°C for 4-6 days. After incubation, yellow-orange hollow zone around the bacterial colony was considered positive.

Quantitative indole acetic acid (IAA) estimation

IAA production by actinomycetes was estimated according to Gordon and Weber (1951) method by inoculating in 5 ml Luria Bertanni (LB) broth supplemented with 0.01% tryptophan and incubated for 3 days at 28°C. The bacterial culture were centrifuged at 3,000 rpm for 5-7 min, supernatant were collected in separate tubes. Appearance of pink colour (addition of 4 ml of Salkowski's reagent to 2 ml of supernatant) confirmed the production of IAA. Quantitative measurement of IAA was determined by recording absorbance at 535 nm.

Molecular identification of actinomycetes

Seven day old pure culture grown in ken knight broth was pelleted in 50 ml centrifuge tube by centrifugation at 10,000 g for 10 min at room temperature. Cells pellet was collected and resuspended in lysis buffer (TE buffer, 10% SDS and Proteinase K) followed by C-TAB method (Rogers et al., 1994). The PCR amplification was done from total genomic DNA using actinomycetes 23S rDNA primers Actino 23 F- CCGANAGGCGTAGBCGATGG, Actino 23 R - CCWGWGTYGGTTTVSGGTA, these primers amplify approximately 361 base pairs (bp). The (50 µl) PCR mixture, 25 pmol of primer, 2.5 mM of each dNTP (dATP, dGTP, dTTP, dCTP (GeNei), 10 X Reaction mixture buffer (100 mM Tris/HCl, 500 mM KCl, 15 mM $MgCl_2$) (GeNei), 0.5 unit of Taq DNApolymerase (Larova) and 10–50 ng of genomic DNA. The PCR program was performed on a thermal cycler (Biometra) system. The DNA and ddH_2O were subjected first to a denaturation step of 94°C for 4 min followed by addition of the rest of the PCR mix and 30 cycles of 97°C for 30 s, 55°C for 30 s, 72°C for 1 min; a final re-annealing at 55°C and extension at 72°C for 15 min (Gao and Gupta, 2005). Following thermal cycling, the PCR products were visualized by agarose gel electrophoresis and captured (Bio-red) Gel Doc. Sizes of the amplicons were assessed with 100 bp ladder (GeNei) run in the agarose electrophoresis.

Primary screening of actinomycetes strains for antagonistic activity

In vitro assay (laboratory condition)

A total of four actinomycetes were used in this study to check their antagonistic effect on seed and soil borne plant pathogens, that is, *Fusarium oxysporum* (wilt in tomato), *Rhizoctonia solani* (sheath blight in maize), *Colletotrichum tranctum* (Anthracnose in soyabean), *Colletotrichum capsici* (Anthracnose in chilli) and *Helminthosporium oryzae* (brown spot in rice) by *in vitro* dual-culture assay using the modified method of Cherif and Benhamou (1990).

The mycelium disc (5 mm diameter) was aseptically punched from the fully grown culture plates with a cork borer, placed onto freshly prepared potato dextrose agar and actinomycetes isolation agar.

Actinomycetes isolates were checked for their activity against the same pathogen. Three replicates were maintained for each test organism. Un-inoculated plate with pathogen served as control and incubated for 72-96 h at 28°C. The percentage growth inhibition was determined by calculating the radial growth of fungal mycelium in control and in dual culture plate suggested by Skidmore and Dickinson (1976).

$$\mathbf{PI} = \frac{\mathbf{R1} - \mathbf{R2}}{\mathbf{R1}} \times \mathbf{100}$$

R1 = Radial growth on control plate; R2 = Radial growth on cultured plate

Plant materials

Tomato (*Lycopercisi esculantum*) seeds cv. Pant T-3 were obtained from SPC, Pantnagar. The seeds were surface sterilized with 2% sodium hypochlorite for 10 min (Guo et al., 2004), rinsed three times with autoclaved distilled water. Seeds were sown in sterilized soil and sand (1:1) mixture in a tray under maintained glasshouse condition, supplementary light, 40 µE m-2s-1, with a 16/8 h day/night cycle at 22-28°C and 50% humidity.

Pathogen evaluation for greenhouse studies

After four leaves stages of tomato plant pathogenicity test was carried out to determine the pathogen responsible for wilt host specific to tomato plant. Mycelium disk of desired pathogen were grown in Armstrong medium broth for seven days in orbital shaker incubator at 28°C. Mycelium mat was collected by centrifugation at 3,000 rpm for 10 min. Mycelium was crushed with autoclaved distilled water in sterilized mortar and pestle under laminar condition to avoid microbial contamination. Different concentration of conidial (10^7 spore ml^{-1}) suspension of pathogen (*F. oxysporum*) was taken as 2 ml/100 g, 4 ml/100 g, 8 ml/100 g and 12 ml/100 g substrate. Plant were treated and kept under greenhouse condition until the disease incidence activity was seen, control plants were treated with distilled water alone. Finally, 4 ml (10^7/ml spores) conidial suspension per 400 g substrate concentration was standardized for disease incidence experiment in tomato plant (Figure 2). The experiment was conducted in 500 g pot filled with sterilized substrate (equal ratio of soil and sand mixture).

Screening of *Streptomyces* strain CPP-53 for antagonistic activity

Greenhouse experiment

A greenhouse experiment was conducted for screening of actinomycetes strains under greenhouse condition for their antagonistic activity. Tomato nursery was grown, Four leaf stage tomato seedlings were then transplanted into new pot containing pathogen inoculum (10^{-7} ml^{-1} spores) cell suspension. After a week of pathogen inoculation, actinomycetes strain CPP-53 was inoculated via rootlet system at a concentration of 8 x 10^7 cfu ml^{-1} (seven days old culture). There were three treatments and one control, each treatment had three replicates. The experiment was repeated twice with the same actinomycetes strain. Plants were then allowed to grow under controlled greenhouse condition. Observation was made after 25 days of inoculation.

Disease severity evaluation

Disease severity index was evaluated following the method of Grattidge and O'Brien (1982).

Growth promotion activity by Streptomyces strain CPP-53

Strain CPP-53 grown in 100 ml ken knight broth to evaluate growth promotion activity on tomato (*L. esculantum*) under greenhouse condition. Seven days old culture of actinomycetes with a concentration of 8 x 10^{-7} cfu ml^{-1} inoculated on rootlets one week later of pathogen inoculation, with one control (without actinomycetes). The experiment repeated twice and the end of 25^{th} day plants were uprooted and biometric observation was done, that is, plant height, root length and total plant biomass.

Table 1. Characteristics of compost collected.

Compost name	Temperature (°C) condition	Nature of compost sample	pH
CPP supa biotech (P) Ltd, Muketshwar	26±2	Semi-dry	7.4
Vermi supa biotech (P) Ltd, Muketshwar	26±2	Semi-dry	8.5
Compost supa biotech (P) Ltd, Muketshwar	26±2	Semi-dry	7.7
LRC vermi Pantnagar	30±2	Wet	7.5

Table 2. Number of actinomycetes colony isolated on Actinomycetes isolation agar (AIA) and ken knight agar medium.

Name of collected compost	Decoding	Number of isolated actinomycetes
CPP	CPP	27 (14-58)
Vermi	VE	8 (59-66)
Compost	CM	10 (67-76)
LRC vermi	LRC	3 (77-79)

Table 3. Antifungal activity of *S. flavofuscus* strains CPP-53 by dual culture assay, radial growth of fungus on control and test plate and percentage inhibition (PI).

Plant Pathogen	Growth of fungus (mm)	Suppression of fungus by *S. flavofuscus* CPP-53 (mm)	Percentage of inhibition over control
Helminthosporium oryzae	39	15	61.53
Fusarium oxysporum	40	17	57.5
Colletotrichum truncatum	37	17	54.05
Colletotrichum capsici	40	20	50
SEM	13	-	-
CD @5%	24	-	-

RESULTS AND DISCUSSION

Isolation and characterization of actinomycetes strains

Total 79 actinomycetes were isolated from four different compost samples collected from different place as designated in Tables 1 and 2. Actinomycetes strain were isolated and purified onto ken knight agar medium. Strains were characterized and identified following Bergey's Manual of Determinative Bacteriology (1957) and Nonomura (1974). Strains were differed from their spore chain formation, colony size, shape, color of aerial mycelium, pigment production onto growth medium (Table 3). The potent actinomycetes strains were selected by their morphological and biochemical test such as siderophore production, starch hydrolysis and catalase activity etc.

Molecular identification of actinomycetes

Actinomycetes strains were identified by 23S rDNA sequencing, DNA was amplified using polymerase chain reaction (PCR) with actinomycetes specific primers, directly sequenced and analysed with online available alignment and similarity search tool (NCBI-BLAST). A most potential antagonist strain CPP-53 identified as *Streptomyces flavofuscus* with a maximum hit with genus *Streptomyces* and species *flavofuscus* with 98% similarity. The nucleotide sequence (201 bp) of isolate *Streptomyces flavofuscus* CPP-53 deposited to gene bank database (NCBI) under the accession number KJ465918.

Antagonistic activity

Actinomycetes strains were primary screened *in vitro* (lab

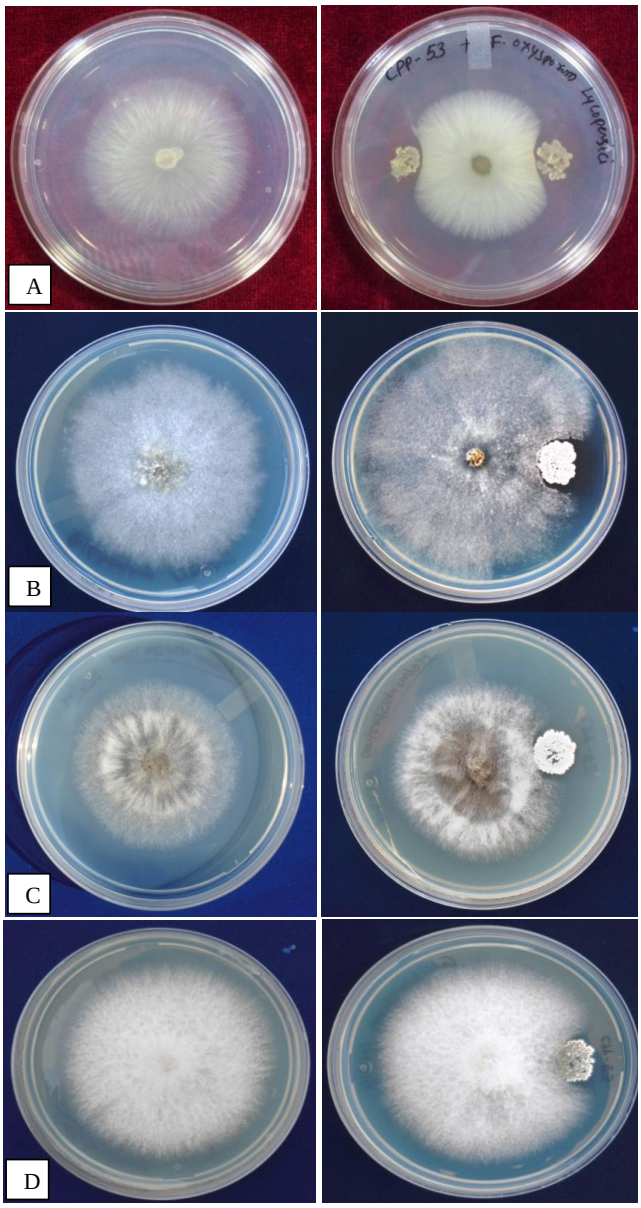

Figure 1. Antagonistic activity of *S. flavofuscus* strain CPP-53 against plant pathogen fungi; A) *Fusarium oxysporum* control, B) *Colletotrichum tranctum*, C) *Helminthosporium oryzae* and D) *Colletorichum capsici*.

condition) by dual-culture assay to evaluate their antagonistic effect against four important crop (Tomato, Soyabean, Chilli and Rice) damaging plant pathogenic fungi, that is, *F. oxysporum, C. tranctum, H. oryzae* and *C. capsici* respectively. A single potent actinomycete strain CPP-53 is found to have antagonistic effect over all four pathogens. The observation was recorded on 3rd and 5th day after dual culture inoculation, *S. flavofuscus* CPP-53 showed maximum (61.53%) inhibition over *H. oryzae* followed by *F. oxysporum* (57.05%), *C. tranctum* (54.05%) and *C. capsici* (50%), and restricts growth of aerial mycelium (Figure 1).

A greenhouse trial, in which *F. oxysporum* served as model pathogens, was set up in order to monitor growth and disease development in tomato plants. *S. flavofuscus* strain CPP-53 was studied against *F. oxysporum* to reduce the effect of pathogen in soil medium. Disease severity was recorded following Grattidge and O'Brien (1982) using 0-4 scale system, four treatments were analysed with three replication of each treatment. Control (T^1) without pathogen was placed 0 in scale followed by T^2 (*F.oxysporum*) counted as dead plant (100%), T^3 (*S. flavofuscus* Strain CPP-53 + *F.oxysporum*) recorded as 1 (0-24%) and T^4 (CPP-53) recorded with no disease incidence with a plant growth promotion effect (Figure 3). When *S. flavofuscus* CPP-53 was inoculated alone, a significant increase in plant height and root volume was also achieved (Figure 4). The ability of Streptomycetes has been shown to be promising biocontrol agents capable of inhibiting fungal pathogens of several plant diseases.

Talc-based formulation of *S. griseus* on tomato seeds and seedlings showed a significant reduction in disease severity caused by *F. oxysporum* f. sp. *lycopersici* (Anitha and Rabeeth, 2009). *S. rochei* ACTA1551 strongly suppressed the growth of *F. oxysporum* f.sp. *lycopersici* in *in vitro* condition. The strain was able to protect tomato seeds from *F. oxysporum* infection *in vivo* (Kanini et al., 2013). A significant reduction in the disease incidence of Fusarium wilt in tomato plant was recorded when the tomato seeds were treated with *S. miharaensis* strain KPE62302H when compared with untreated controls (Kim, 2012). The inhibitory effects of *S. violaceusniger* strain G10 against *F. oxysporum* f.sp. cubense, the causal pathogen of wilt disease of banana was reported by Getha and Vikineswary (2002)

Quantitative estimation of IAA

Production of IAA by actinomycetes strains grown in LB broth with and without tryptophan was estimated. All strains differed in their capacity to produce IAA. It was found that the IAA was produced by *Streptomyces* sp. strain CPP-53 (16.83 µg/ml), when culture medium was supplemented with a concentration ($mg^{-1}ml^{-1}$) of L-Tryptophan at 28°C incubation.

S. atrovirens ASU14 produced high amount of IAA 22 µg/ml isolated from wheat rhizosphere soil (Abd-Alla et al., 2013). Many studies reported the production of IAA by *Streptomyces scabies* (Manulis et al., 1994; Meenakshi et al., 2010).

Conclusion

From the results, the benefit of actinomycetes strains was observed by restricting the mycelium growth of plant pathogenic fungi and controlling fusarium wilt of tomato under greenhouse condition. Biological control is a cost-effective and eco-friendly approach for any disease

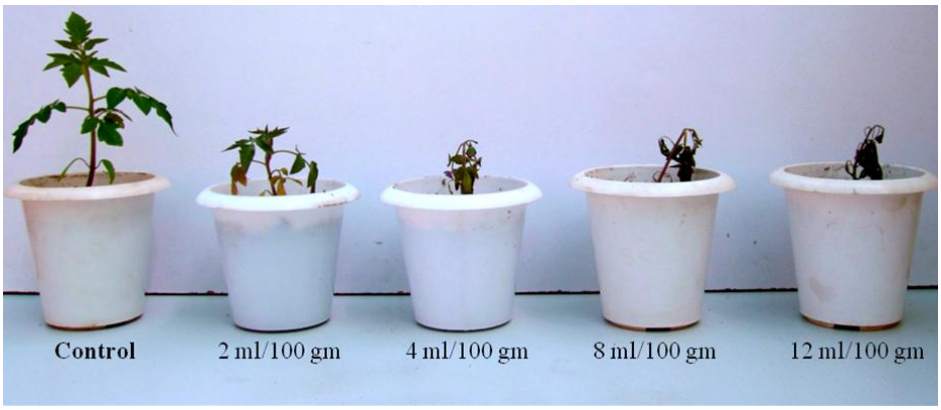

Figure 2. Pathogen evaluation under greenhouse condition: control plant with distilled water and other plants treated with a different concentration (10^{-7} spore ml^{-1}) of pathogen inoculum.

Figure 3. Effect of actinomycetes and *Fusarium oxysporum* inoculation on tomato plant under greenhouse condition. (T1) Treated with distilled water (control), (T2) Treated with *F. oxysporum*, (T3) *F. oxysporum* and actinomycetes strain CPP-53 and (T4) Actinomycetes strain CPP 53 alone.

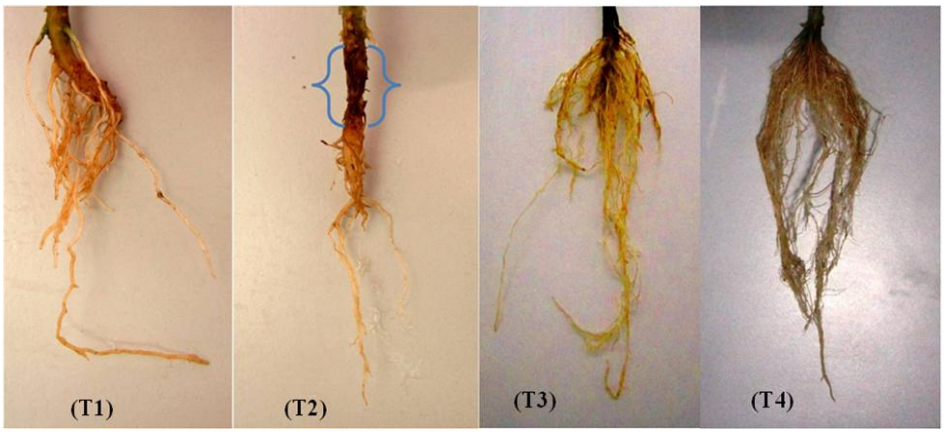

Figure 4. Effect of actinomycetes and *F. oxysporum* on tomato roots: (T1) Treated with distilled water (control), (T2) Treated with *F. oxysporum*, (T3) *F. oxysporum* and actinomycetes strain CPP-53 and (T4) actinomycetes strain CPP 53 alone.

management application. The consequence on seed and soil borne pathogens revealed that the antagonists significantly reduced the growth of all four pathogenic fungi either by suppression or exhibiting inhibition zones.

The outcome of the experiment in laboratory and in greenhouse shows the potential of *Streptomyces* sp. CPP-53 strain control over *F. oxysporum* causing agent of wilt in tomato. The bio-formulation of actinomycetes will accomplish the prerequisite criteria for the development of an eco-friendly disease management strategy to overcome the disease (fusarium wilt) of tomato in the field.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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