Original Research Article

In vitro Screening of Streptomyces spp., against Necrotrophic Pathogen Pythium aphanidermatum Causing Damping-off in Tomato and Chilli

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Abstract

Damping-off in tomato and chilli caused by Pythium aphanidermatum is an opportunistic pathogen more prevalent on young or weak plants causing extensive damage in nurseries and mainfield. Control of this disease by biological method is gaining a momentum because of its high efficiency and environmental friendliness. In the present study, an attempt has been made to explore the bioactive rhizosphere actinomycetes for suppressing the pathogen. Rhizosphere soil samples were collected from various locations in The Nilgiris and Coimbatore districts and twenty seven different actinomycetes were isolated. All the isolated microbes were characterized morphologically by colour series and growth pattern on artificial media which showed that they were belong to Streptomyces spp. All the 27 isolates were screened under in vitro condition which revealed that the isolate ACM 14 showed a maximum inhibition of 26.6 percent over the control, hence the antagonistic actinomycetes may probably be used against the damping-off pathogen.

Keywords

Pythium aphanidermatum, Streptomyces spp., Antagonistic activity, Biocontrol disease

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Introduction

Tomato (Lycopersicon esculentum Mill.,) and Chilli (Capsicum annuum L.,) are the two important versatile vegetable crops with wide usage in Indian culinary tradition. Besides their cultivation and usage worldwide, the productivity is slowed down due to various pests and diseases. Damping-off is one among the diseases and it causes 30% seedlings mortality (Muriungi et al., 2014). Pre-emergence damping-off caused decaying or shrivelling of seeds, whereas post emergence damping-off caused death and toppling of the seedlings. It gets aggravated due to high soil temperature, moisture, poor soil aeration, lack of drainage and thick stand of seedlings. Control of soil borne diseases are tiresome
due to their wide host range, prolonged survival of spores and other resting structures in soil and lack of resistant cultivars (Kilanyet et al., 2015). *Pythium* saprophytic oomycete fungal like organism also called as water mould, is the largest genus causing severe damages in many crop plants. It forms resting structure called sporangia releasing numerous zoospores which later develops into oospores by surviving in soil and greenhouses (Loliam et al., 2013). *Pythium aphanidermatum* causes damage to the economically important crops and is the most pathogenic (Muthukumar et al., 2016). Management using fungicides like metalaxyl, strobilurin results in phytotoxicity, environmental pollution, development of fungicide resistance in plants, detrimental to non-targeted and beneficial microorganisms (Bharathi et al., 2004).

Exploring the beneficial rhizosphere microbiome will be an alternate strategy for combating damping-off disease. The antagonistic microbes act directly by attacking the resting spores or mycelium by interfering with germination, infection process or indirectly by inducing host resistance (Termorshuizen and Jeger, 2008). Actinomycetes are Gram positive saprophytic soil inhabitants, widely distributed microorganisms with antibiotic producing capacity and growth promoting activity used for controlling soil-borne pathogens (El-Tarabily et al., 2008; Palaniyandi et al., 2011). Actinomycetes present in soil mostly belong to *Streptomyces* and 60% of the bioactive molecules obtained from them are used for agricultural purposes (Ilic et al., 2007). *Streptomyces* present in plant rhizosphere protect roots by inhibiting the pathogen growth through production of antifungal compounds and enzymes that degrade fungal cell wall (El-Tarabily et al., 2008) besides, it also enhances plant growth through production of plant growth promoters like auxin and gibberellin (El-Tarabily 2008). It plays a dual role by acting as a plant growth promoter and as a suppressor of plant disease through mechanisms like increasing the supply of nutrients namely phosphorus, sulphur, iron, copper, production of IAA, cytokinin and siderophage (Gowdar et al., 2018). Microbial antagonists like *Streptomyces* spp., *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Trichoderma* spp. have been used for managing damping-off diseases. *Streptomyces* spp., like *S. griseoviridis* (Mycostop) and *S. lydicus* WYEC108 (Actino-Iron) are the potent producer of hydrolytic enzymes that degrades the cellwall of the fungi like *Pythium*, *Phytophthora* and *Fusarium*. Cellulolytic *S. rubrolavendulae* S4 cause lysing of hyphal tips and abnormal swelling of mycelium of *Pythium aphanidermatum* (Loliam et al., 2013), *S. rochei* from tomato rhizosphere produce IAA which aids in increasing seed germination, root elongation thereby increasing the plant growth (El-Tarabily 2008).

The intention of this study is to isolate the novel *Streptomyces* spp., perform preliminary characterization and identify the effective antagonistic for managing *Pythium* under *in vitro* condition.

**Materials and Methods**

**Isolation and phenotypic characterization of rhizospheric actinomycetes**

The soil samples were collected from rhizosphere region of different plants from different locations in The Nilgiris (Muthhorai 11°24'36" N, 76°41'59.99" E, Lovedale 11°22'54" N, 76°42'6" E, Nanjanad 11°36'68" N, 76°64'56" E) and Coimbatore districts (Eastern farm, TNAU 11°07'3.36" N, 76°59'39.91" E). Sampling was done in 40 days old crops to a depth of 10cm by
removing the top soil for about approximately 3cm and mixture of rhizosphere soils collected randomly from three plants in each location and stored in sterile polythene bags. Isolation was done by serial dilution technique using Kenknights agar medium amended with ampicillin (5 µg/ml) and cycloheximide (20 mg/l) to reduce bacterial and fungal contamination, respectively (Trabelsi et al., 2016) and the plates were incubated at 28±2⁰C for 3-5 days. After incubation small white pinhead size powdery colonies appear which are purified and maintained by streaking on starch caesin agar medium (Kumar et al., 2010). The morphological characters like aerial spore mass colour, substrate mycelium colour, colony texture and pigment production were recorded and compared with the observations made in International Streptomyces Project (ISP) medium containing data of 450 species of Streptomyces and Streptoverticillum (Shirling and Gottlieb, 1966).

**Source of pathogen**

*Pythium aphanidermatum* Udumalpet strain (NCBI accession no. MK817574) isolated from the tomato was obtained from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore.

**In vitro screening of antagonistic actinomycetes against *P. aphanidermatum***

The antifungal activity of the isolates against *P. aphanidermatum* was performed using the dual culture technique as described by (Dennis and Webster 1971). A quantity of 20 ml of PDA medium was poured into Petriplates after solidification, the actinomycetes isolates were streaked at one end of the plate and incubated for 3 days at 28±2⁰C. Later, mycelial disc of pathogen (9 mm dia) was placed opposite to actinomycetes and incubated at 28±2⁰C for 2 days. Control was maintained by placing pathogen alone. Efficacy of the isolates was determined by measuring the mycelial growth of pathogen over control. Percent inhibition over control was calculated using the formula.

\[
P_I = \frac{C - T}{C} \times 100
\]

C - growth of pathogen (mm) alone in control plate
T - growth of pathogen (mm) in presence of antagonist isolate.

**Statistical analysis**

All the experiments were analysed independently and the treatment means were compared using the Duncan’s Multiple Range Test (DMRT). SPSS version 16.0 developed by IBM Corporation was used for analysing the experiments.

**Results and Discussion**

**Isolation of actinomycetes**

A significant loss in the yield of many crops are mainly due to soilborne diseases. *Pythium aphanidermatum* (Edson) Fitz causing damping-off of chilli and other solanaceous vegetable crops is a severe threat to vegetable production and various methods like chemical and biological control measures are available for managing the disease (Ghosh, 2002). Actinomycetes have been extracted from many unexplored environments and extreme habitats for the past few years. Many of them could be considered as a unique or novel species with the potential of producing metabolites and enzymes with antagonistic activity (Martinez, 2012). *Streptomyces* occupies nearly 10% of soil microflora having the ability to colonize plant root surfaces under varied environmental conditions and soil types and the antibiotics produced are of
biodegradable used for making pathogen-specific fungicides with less side-effects to ecosystem. Using Kenknightsagar medium actinomycetes were isolated by serial dilutions of rhizosphere soil from $10^{-2}$ to $10^{-6}$. After incubation for 5 days, small pinhead size white powdery colonies started to appear which were further streaked and maintained on starch casein agar medium. Consideration of using antibiotics as a precautionary measure has been suggested by many authors while isolating *Streptomyces* (Kitouni *et al.*, 2005; Errakhi *et al.*, 2009). Hence, for inhibiting the bacterial and fungal contamination ampicillin (5 µg/ml), either cycloheximide (50 µg/ml) or nystatin (50 µg/ml) were used (Fguira *et al.*, 2012). These revealed the importance of constituents added during isolation. Based on morphology, totally 27 different actinomycetes isolates were obtained from the samples collected and labelled from 1 to 27.

**Table 1** Phenotypic characterization of isolated actinomycetes

| Isolate | Substrate Colony | Reverse Colony | Colony texture |
|---------|------------------|----------------|----------------|
| AOR1    | Grey             | Light orange   | Powdery        |
| AOR2    | White            | Light yellow   | Powdery        |
| AOR3    | White            | Ash            | Powdery        |
| AOW4    | White            | Cream          | Powdery        |
| AOW5    | White            | White          | Powdery        |
| AOW6    | White            | White          | Powdery        |
| AOT7    | Grey             | Cream          | Powdery        |
| AOT8    | Cream            | Cream          | Powdery        |
| AOT9    | Grey             | Light orange   | Powdery        |
| AOP10   | White            | White          | Powdery        |
| AOP11   | Light orange     | Light orange   | Powdery        |
| ACM12   | White            | Yellow-Orange  | Powdery        |
| ACM13   | Grey             | Light orange   | Powdery        |
| ACM14   | Grey             | Yellow-orange  | Cottony        |
| ACS15   | White-grey       | Cream          | Cottony        |
| ACS16   | White            | Orange         | Powdery        |
| ACS17   | White            | Pink           | Powdery        |
| ACS18   | Dark brown       | Orange         | Powdery        |
| ACS19   | Grey             | Light orange   | Powdery        |
| ACS20   | Grey             | Brown-yellow   | Powdery        |
| ACS21   | Grey             | Yellow         | Cottony        |
| ACS22   | White            | White          | Powdery        |
| ACD23   | Brown            | White          | Powdery        |
| ACD24   | Grey             | Light brown    | Powdery        |
| ACSO25  | White            | Light yellow   | Powdery        |
| ACPM26  | Cream            | Light yellow   | Powdery        |
| ACPM27  | White            | Cream          | Powdery        |
**Table 2** *In vitro* screening of *Streptomyces* spp. against *P. aphanidermatum*

| Antagonists | Mean mycelial growth (mm) * | Percent inhibition over control ** |
|-------------|----------------------------|-----------------------------------|
| AOR1        | 70                         | 22.20° (28.11)                    |
| AOR2        | 70                         | 22.20° (28.11)                    |
| AOR3        | 70                         | 22.20° (28.11)                    |
| AOW4        | 90                         | 0.00° (1.62)                      |
| AOW5        | 86                         | 4.44° (12.16)                     |
| AOW6        | 88                         | 2.22° (8.57)                      |
| AOT7        | 68                         | 24.40° (29.60)                    |
| AOT8        | 90                         | 0.00° (1.62)                      |
| AOT9        | 90                         | 0.00° (1.62)                      |
| AOP10       | 84                         | 6.60° (14.89)                     |
| AOP11       | 86                         | 4.44° (12.16)                     |
| ACM12       | 70                         | 22.2° (28.11)                     |
| ACM13       | 70                         | 22.20° (28.11)                    |
| ACM14       | 66                         | 26.60° (31.05)                    |
| ACS15       | 70                         | 22.20° (28.11)                    |
| ACS16       | 70                         | 22.20° (28.11)                    |
| ACS17       | 68                         | 24.40° (29.60)                    |
| ACS18       | 70                         | 22.20° (28.11)                    |
| ACS19       | 70                         | 22.20° (28.11)                    |
| ACS20       | 70                         | 22.20° (28.11)                    |
| ACS21       | 68                         | 24.40° (29.60)                    |
| ACS22       | 87                         | 3.3° (10.47)                      |
| ACD23       | 87                         | 3.3° (10.47)                      |
| ACD24       | 70                         | 22.20° (28.11)                    |
| ACSO25      | 90                         | 0.00° (1.62)                      |
| ACPM26      | 90                         | 0.00° (1.62)                      |
| ACPM27      | 90                         | 0.00° (1.62)                      |
| Control     | 90                         | 0.00° (1.62)                      |

*Values are means of three replications. In a column, means followed by a common letter are not significantly different at the 5% level by DMRT; **Values in parentheses are arcsine transformed values.
Phenotypic characterization of actinomycetes

Phenotypic characterization of the isolates was done by observing the colour of matured aerial mycelium, colour of substrate mycelium and powdery or cottony textured colony characters. The isolates showed typical morphology of *Streptomyces* by growing on agar medium with earthy odour. Most of the isolates exhibited the colour series as white, brown, grey, cream and yellow with powdery growth colonies (Table 1). Phenotypic characterization notably the aerial mycelial colour as white, brown, grey and cream with substrate mycelial colours like light orange, yellow, cream, pink with powdery and cottony textured colonies indicated that they were confederated to novel *Streptomyces* genus which are considered as preliminary identification (Taddei et al., 2006). Several authors viz., Nanjwade et al., (2010), Kumar et al., (2010) and Sharma et al., (2014) have also followed the same phenotyping method for their isolates. The difference in colour series of the isolates may be due to the diversity of isolates in the sites chosen.

In vitro screening of actinomycetes

Actinomycetes which are abundant in rhizosphere colonize plant roots and play a role in plant growth promotion. It is well known that most of the *Streptomyces* spp., exhibit antimicrobial activity. Interaction of *Streptomyces* with fungal pathogens leads to the production of cell wall-degrading enzymes such as cellulases, hemicellulases, chitinases, amylases, and glucanases. In order to find the effective one, all the 27 isolates were screened against *P. aphanidermatum* by dual plate method. Isolate ACM 14 showed the maximum percent inhibition of about 26.6% over control (Table 2; Fig. 1) followed by ACS 17 and 21 which showed 24.40 per cent inhibition over the control while, few isolates namely AOW4, AOT8, ACSO25, ACPM26 and ACPM27 had not inhibited the mycelial growth of the pathogen. *Streptomyces* spp., from rhizosphere regions showed antimicrobial activity against *Pythium* similarly *Streptomyces* sp. CA-2 against tomato damping-off with improved seedling vigour as reported by (Goudjal et al., 2014), *S. griseoviridis* against cucumber damping-off and *S. rochei* ERY1 against damping-off of cabbage as reported by (Suwitchaynon et al., 2018) which showed the potentiality of using them as a biocontrol agent for damping-off disease.

Chemical fungicides are unsuitable for damping-off management because of residual effect. Hence biological control might be a better alternative. Thus, it was concluded that...
Streptomyces sp., which possess growth promoting activities directly or indirectly benefit the plant growth and are rhizosphere competent, utilizing all plant sugars available in rhizosphere. Streptomyces sp., with its unique antifungal activity isolated from this study may be exploited to combat damping-off disease of tomato and chilli after field experiments.

Abbreviation

IAA – Indole Acetic Acid, ISP – International Streptomyces Project

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Conflict of Interest: None declared

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