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Screening and evaluation of antimicrobial activity of actinomycetes isolated from rhizosphere of *Heracleum candicans*

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ABSTRACT

The present study deals with isolation and screening of industrially and biotechnologically important strains of actinomycetes species for maximum production of antagonistic and proteolytic activities. Total 8 strains were isolated from rhizospheric soil of medicinal plant *Heracleum candicans*. Three strains were more effective against the indicator test bacteria and fungal pathogens present in soil. Maximum production of proteolytic activity was shown by MK-1 (24 mm), MK-5 (25 mm) and MK-8 (28 mm). These hyper-producing strains of actinomycetes can be exploited in industries for commercial product formation.

Keywords: Actinomycetes; *Heracleum candicans*; hyper-production

INTRODUCTION

Microorganisms turned out to be unlimited source for potential drugs, agrochemicals and biocatalysts (Varbrough et al 1999). They are used to produce hundreds of industrially important commercial products valued in tens of billions of dollars worldwide. They provide economic and efficient solution to the rising problems of increasing cost, pollution and worlds renewable resources (Steele and stower 1991). Actinomycetes have potential to produce secondary metabolites that are economical and beneficial to human beings (Vining 1990). Actinomycetes constitute a significant component of microbial population in most soils and are frequently described as bacteria which have the ability to form branching hyphae at some stages of their development (Goodfellow and Williams 1983). They are ubiquitious in both terrestrial and aquatic ecosystem. Many activities of actinomycetes in nature remain to be elucidated. But it is clear that they are able to degrade plant, animal and microbial polymers in soil and litter and some fix nitrogen in a variety of non-

leguminous plants (Goodfellow and Williams 1983). Although they occur in soil rhizosphere there is little knowledge of what role they play in soil processes apart from their possible influence on root pathogens. Streptomyces may protect roots by inhibiting the development of fungal pathogens which they may do readily in vitro by production of antifungal metabolites (Williams 1982). Actinomycetes have established themselves as the most potent group of microorganisms with regard to their capabilities of forming complex organic molecules with diverse biological activities. Notable among these are the large number and variety of antibiotics that actinomycetes produce which today are indispensable for the treatment of variety of infections. They are produced as idiophase metabolites and have no physiological role in growth phase (Srinivasan 1991). They are generally found in soil where they play major role in decomposition of organic material. The aim of this study was to screen and search for hyper-producer strains of actinomycetes for antagonistic and proteolytic secondary metabolite.

MATERIAL and METHODS

Collection of soil samples: Soil samples were collected from the rhizosphere soil of the medicinal plant *Heracleum candicans* from the medicinal plant field of Dr YS Parmar University of Horticulture and forestry, Nauni, Solan, HP and from Rahla farm, Manali, HP.

Enumeration, isolation and identification: Ten gram of rhizosphere soil from the medicinal plant was shaken vigorously in 90 ml of sterile water blank in 250 ml Erlenmeyer flask for fifteen minutes. Serial dilutions were made using 9.9 ml water blanks to yield 100 fold dilution series from 10^{-2} to 10^{-8} for each soil sample. Each diluted sample (0.1 ml) was added to every Petri plate and about 15-20 ml of cool and molten medim was poured to each Petri plate and mixed gently in all directions so as to mix it quickly. Different synthetic and selective media (potato glucose agar, sucrose–nitrate–agar and starch casein-agar) were used for the isolation of actinomycetes species (Waksman 1961). Plates were incubated at 28°C for 7 days.

The most prominent actinomycetes strains isolated from rhizosphere soil of the medicinal plant *H candicans* were tentatively identified on the basis of cultural, morphological and biochemical characteristics as per their genera laid down in the Bergey's manual of systemic bacteriology (Cross 1989). Actinomycetes isolates were maintained on potato glucose agar at 4°C and was sub-cultured periodically on the same media at 28°C.

In vitro screening of isolates for antagonism: Actinomycetes isolates were tested for their antibacterial activity against bacterial pathogens namely *Bacillus subtilis*, *Escherichia coli*, *Klebsiella* sp, *Pseudomonas* sp, *Shigella* sp, *Salmonella typhi*,, *S paratyphi* and *Xanthomonas* sp.

The antibacterial activity was carried out by bit plate assay method. The actinomycetes isolates isolated from the rhizosphere soil of medicinal plants were spotted on the pre-poured nutrient agar plates already having a lawn of indicator test bacteria on it. Plates were incubated at 37°C for 24 h and observed for clear zone formation around the spot/well/bit. Antibacterial activity was expressed in terms of diameter (mm) of clear zone produced around the spot/well/bit at 37°C for 24 h.

Screening for antifungal activity: All the isolated actinomycetes were tested for their antifungal activity against the fungal strains namely *Alternaria sp, Aspergillus s., Fusarium sp, Pencillium sp, Pythium sp, Phytophthora sp* and *Tricotherium sp* The antifungal activity was carried by well/bit plate assay method. seven days old culture of actinomycetes strains was centrifuged at 10000 rpm at 4°C for 30 min and supernatant was collected. Supernatant (100 µl) was added to each well cut on a pre-poured PDA Petri plate with the help of a sterile cork borer already having a bit of indicator test fungi on it. Plates were incubated at 28 ± 2°C for 72 h and observed for the formation of a clear zone around the well. The antifungal activity has been expressed in terms of diameter (mm) of clear zone formed around the well.

Proteolytic activity: All the actinomycetes strains were screened out for the production of proteolytic activities by the method of Flaming et al (1975). Seven day old cell free culture supernatant (100 µl) of each actinomycetes strain was prepared after centrifugation at 10000 rpm for 30 min at 4°C and was added in each well. Wells were cut on skim milk agar plate with the help of sterile cork borer. Plates were incubated at 37°C for 24 h and were observed for the formation of clear zone around the well. Proteolytic activity was expressed in terms of diameter (mm) of clear zone formed around the well after incubation at 37°C for 24 h.

RESULTS and DISCUSSION

In the present study medicinal plant *Heracleum candicans* was employed for isolation and enumeration of actinomycetes sp. The most prominent actinomycetes colonies were found on the potato glucose agar, sucrose nitrate agar and on synthetic media ie starch casein agar (Kuster and Williams 1964) were picked up for further characterization according to Bergey's manual of systemic bacteriology.

Maximum actinomycetes strains isolated from *H candicans* showed high antibacterial effect against *B subtilis* ie by MK4 (50 mm) followed by MK3 and MK8 (40 mm each), MK5 (30 mm). MK1 showed inhibitory effect against maximum number of test bacteria and it was selected for media manipulation studies. Similarly MK8 showed maximum activity against *B subtilis* and also against other test bacteria

Table 1. Screening of actinomycetes species isolated from rhizosphere of *Heracleum candicans* for the production of antibacterial activity at 37°C

Actinomycetes strain #	Antibacterial activity (mm diameter) indicator test bacteria							
	Bacillus subtilis	*Escherichia coli*	*Klebsiella* sp	*Pseudomonas* sp	*Salmonella paratyphi*	*Salmonella typhi*	*Shigella* sp	*Xanthomonas* sp
MK-1	+(20 mm)	-	+(15 mm)	+(30 mm)	-	-	-	+(40 mm)
MK-2	-	+(10 mm)	-	+(10 mm)	-	-	-	-
MK-3	+(40 mm)	-	-	-	-	-	-	+(10 mm)
MK-4	+(50 mm)	-	-	-	-	-	-	+(9 mm)
MK-5	+(30 mm)	-	-	-	-	-	-	-
MK-6	-	+(22 mm)	-	-	-	-	-	-
MK-7	-	-	-	-	+(18 mm)	-	-	-
MK-8	+(40 mm)	-	+(20 mm)	-	-	+(20 mm)	-	-

+ indicates activity, - indicates no activity, Antibacterial activity

Table 2. Screening of actinomycetes sp isolated from rhizosphere of *Heracleum candicans* for the production of antifungal activity at 28 ± 2°C

Actinomycetes strain #	Antifungal activity (mm diameter) indicator test fungal pathogen						
	Alternaria sp	*Aspergillus* sp	*Fusarium* sp	*Pythium* sp	*Phythopthora* sp	*Penicillium* sp	*Trichothecium* sp
MK-1	+(20 mm)	-	-	-	-	-	-
MK-2	-	-	-	-	-	-	-
MK-3	-	-	-	-	+(22 mm)	+(18 mm)	-
MK-4	-	-	-	+(24 mm)	+(14 mm)	-	-
MK-5	+(16 mm)	-	-	-	+(20 mm)	-	-
MK-6	-	-	+(9 mm)	-	+(10 mm)	-	-
MK-7	-	-	+(20 mm)	-	-	-	-
MK-8	+(14 mm)	-	-	+(18 mm)	+(18 mm)	-	-

+ indicates activity, - indicates no activity, Antifungal activity

Table 3. Screening of actinomycetes sp isolated from rhizosphere of *Heracleum candicans* for the production of proteolytic activity at 28 ± 2°C

Actinomycetes strain #	Proteolytic activity (mm dia)
MK-1	+(24 mm)
MK-2	-
MK-3	+(19 mm)
MK-4	+(20 mm)
MK-5	+(25 mm)
MK-6	+(20 mm)
MK-7	+(16 mm)
MK-8	+(28 mm)

+ indicates activity, - indicates no activity, Proteolytic

while MK6 showed inhibitory effect against only one bacteria ie *E coli* (22 mm).

All the isolates of actinomycetes sp isolated from rhizosphere soil of *H candicans* were screened for the production of antifungal activity. The result showed that maximum number of isolates of actinomycetes isolated from the rhizosphere of *H candicans* were inhibitory against *Phytophthora* sp followed by *Alternaria* sp, *Pythium* sp, *Penicillium* sp and *Fusarium* sp. Maximum range of antifungal activity production against *Phytophthora* sp was shown by actinomycetes strains MK3 (22 mm), MK5 (20 mm), MK8 (18 mm), MK4 (14 mm) and MK6 (10 mm). Similarly maximum production of antifungal activity against *Alternaria* sp shown by three actinomycetes strains viz MK1, MK5 and MK8 in the range of 14-20 mm diameter of clear zone while actinomycetes strains MK4 and MK8 showed antifungal effect against *Pythium* sp in the range of 18-24 mm diameter of clear zone. Only one strain MK8 showed inhibitory effect against *Penicillium* sp while two strains MK6 and MK7 showed antifungal effect against *Fusarium* sp respectively.

Arifuzman et al (2010) studied soil samples of Karanjal regions of Sundarbans of Bangladesh and 55 actinomycetes were isolated and screened for antibacterial activity. Out of 55 isolates 20 isolates (36.36%) were active against the test organisms. Dehnad et al 2010 studied the antibacterial activity of *Streptomyces* isolates from soil samples of west of Iran. Sujatha et al (2005) isolated actinomycetes isolates from mangrove sediments of

Pichavaram southeast coast of India which exhibited prominent antibiotic activity against *Candida albicans*.

All the eight actinomycetes strains isolated from the rhizosphere of *H candicans* were screened out for the production of proteolytic activity. All the actinomycetes strains were found to be positive for the production of proteolytic activity except strain MK2 which showed no activity. The four strains viz MK1, MK3, MK5 and MK6 showed maximum production of proteolytic activity in the range of 24-28 mm diameter of clear zone.

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