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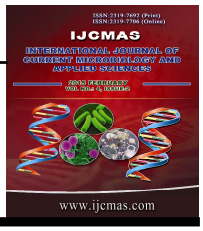

Original Research Article

Studies on the Bioactive Actinomycetes of Estuarine Soils of South West Coast of India

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A B S T R A C T

The present study was designed to isolate chitinolytic actinomycetes from the estuarine soil sediments of South west coast of Tamilnadu. A total of 20 chitinase producing actinomycetes were isolated from the estuaries of Thengapattanam, Manakudy and Rajakkamangalam. All the 20 isolates were subjected to antifungal activity against 6 phytopathogenic fungi *Aspergillus niger, Fusarium oxysporum, Fusarium solani, Phytophthora capsici, Rhizoctonia solani* and *Penicillium* sp. by disc diffusion method. Starch casein agar media was used for the isolation of actinomycetes. Out of the 20 antifungal isolates screened, the isolate from Manakudy estuary ESM9 showed good inhibitory activity against the test fungal pathogens. A maximum zone of inhibition of 32 mm was expressed against *Fusarium oxysporum* and *Phytophthora capsici*. The isolates EST4, ESR2 and ESR8 were also found to be potential inhibitors of pathogenic fungi

Keywords

Estuaries, Chitinolytic actinomycetes, Antifungal activity

Introduction

Actinomycetes comprise an extensive and diverse group of gram positive, aerobic bacteria frequently filamentous and sporulating with DNA rich in G+C nucleotide content (75%). They play an important ecological role in soil cycle (Holt *et al*., 1989). Actinomycetes have a distinctive and diverse macroscopic and microscopic morphology and they are traditionally considered to be transitional forms between bacteria and fungi. Like fungi, they form a mycelial network of branching filaments, but like bacteria they are thin, possess cell walls containing muramic acid. Actinomycetes are well known as a good source of microbial secondary metabolite producer in drug discovery programs. Many species especially those belonging to the genus *Streptomycetes* have been studied as potential producers of metabolites with diverse structures and biological activities (Berdy, 2005). They are widely distributed in soil, water and natural environments where their population and types in an ecosystem are determined by numerous

physical, chemical and biological factors. Identification of novel ecological systems is therefore crucial for the discovery of novel actinomycetes (Wang *et al.*, 1999). In recent years there has been a growing awareness of the potential value of fresh water habitat as source of actinomycetes that produce useful metabolic products. In fact some investigators emphasized that freshwater habitats are fruitful as those isolated organisms are from terrestrial habitats (Cross, 1981). The genus *Streptomyces* is found worldwide and they play an important role in soil and plant ecology. These organisms have been widely investigated as agents of biological control of several plant diseases.

Chitinases are a group of enzymes which hydrolyse the β -1, 4 – linkages and cleaving a bond between the c1- c4 of two consecutive N-acetyl glucosamines of chitin to low – molecular weights products and have been shown to be produced by a number of microorganisms. Generally chitinase producing strains will use chitin or colloidal chitin a carbon source. Chitinases are widely distributed in bacteria such as *Serratia, Chromobacterium, Klebsiella, Pseudomonas, Clostridium, Vibrio, Arthrobacter, Aeromonas* and *Streptomyces*. They are also found in fungi *Trichoderma, Penicillium, Neurospora, Mucor, Beauveria, Aspergillus, Myrothecium, Metarrhizium* and *Agaricus*. Chitinase is known to be produced by a wide range of actinomycetes (Hsu and Lockwood, 1975). *Streptomyces* are also explored for chitinase production and they are thought to be one of the major chitinivorous microbial groups in the soil due to their ability to degrade chitin. This has long been regarded as a characteristics feature of soil *Streptomycetes* (Blaak *et al*., 1993). Several chitinolytic enzymes have been identified in several *Streptomyces* sp. including *S. antibioticus, S. griseus, S. plicatus, S. lividans, S. aureofaciens* and *S. halstedi* (Taechowisan *et al*., 2003). *Streptomyces viridificans* was found to be a good chitinase producer and its crude and purified enzyme has potential cell wall lysis of many fungal pathogens (Gupta *et al.*, 1995).

Lysis of the host structure by secretion of extracellular lytic enzymes is one of the important mechanisms that are involved in the antagonistic activity of biocontrol agents (Kim and Ji, 2001). Among these, chitinase (EC 3.2.1.14) plays a vital role in the biological control of many plant diseases by degrading the chitin polymer in the cell walls of fungal pathogens. It affects fungal growth through the lysis of cell walls. The main mechanisms involved in the antagonism of biocontrol agents are mycoparasitism, competition for space and nutrients, stimulation of the plant's defensive capacity, and secretion of bioactive compounds such as antibiotics and cell wall degrading enzymes. As the skeleton of the fungal cell wall mainly contains chitin, glucan and proteins, mycoparasitism and enzymes that hydrolyze these components are one of the main mechanisms accounting for showing antagonistic important in the hyper-parasitic mechanism. The distribution of chitinases in nature is very common and actinomycetes are found to display strong fungicidal properties. It is related to the production of many types of various fungicidal compounds, including antibiotics and extracellular enzymes such as chitinase and β-1,3-glucanases. Several chitinolytic enzymes have been identified in various *Streptomyces* sp, including, *Streptomyces plicatus*, *S. lividans*, *S. virdificans* and *S. halstedii*. *S. cavourensis* SY224, and *S. halstedii* produce highly active antifungal chitinase which implies the possibility of using them as agents for biological

protection of crops. However studies have shown that the combination of two bacteria *Streptomyces* sp. 385 and *Paenibacillus* sp.300 are found to be more effective against *Fusarium oxysporum* causing cucumber wilt than individual strains or other combinations. Chitinolytic bacteria such as *Aeromonas hydrophila*, *Aeromonas caviae*, *Pseudomonas maltophila*, *Bacillus licheniformis*, *Bacillus circulans*, *Vibrio furnissii*, *Xanthomonas* sp., and *Serratia marcescens* play an important role in biological control of plant pathogenic fungi (Gohel *et al.,* 2005). Interest in biological control of plant pathogens has increased considerably over the past years, partly as a response to public concern about the use of hazardous chemical pesticides, but also because it may provide control of diseases that cannot, or only partially, be managed by other control strategies. The present study explains the broad distribution of chitinolytic bacteria in estuarine habitats which can be used as biocontrol agents against phytopathogenic fungi.

Materials and Methods

Study area

Soil sediments from three different estuaries Manakudy, the second largest estuary in the District has a total area of 145 hectares. It is located at the North West of Cape Comorin falling within the latitude of 8° 4¹ and 8° 21¹N and longitude 77°26¹ and 77°30¹E Thengapattanam is located in the South west coast of India. This minor estuary of Kanyakumari district is formed by the confluence of Tamiraparani River with Arabian Sea in between Thengapattanam and Eraiummanthurai (7°53¹ N Latitude and 77°07¹E longitude) and Rajakkamangalam estuary is situated along the Southwest coast of India. This is the place where the Pantrivaikkal discharge freshwaters into the Arabian Sea after transversing about 85km were selected for this study. Soil samples were collected from the top 5–10 cm sediments from four different stations of the selected estuaries during three seasonal periods (post monsoon, summer and monsoon). The four stations were selected in the following pattern. Station I is the shallow coastline region, Station II is the mid estuarine region, Station III is the fresh water zone and the Station IV is the mouth of the estuary. The collected samples were transferred to a sterile polythene bag and transported immediately. The sediment samples thus collected were then used for the isolation of actinomycetes.

Isolation of Actinomycetes

The sediment samples were stored at 4 °C and analyzed by plating their serial dilution on to the selective agar media Starch Casein Agar (Kuster and Williams, 1964). To minimize the growth of fungi and undesirable bacteria, nystatin (20 µg/ml) and cyclohexamide (100 µg/ml) were added to the isolation media. The plates were incubated at 28 °C for 2–3 weeks. The plates were observed for the presence of actinomycetes colonies. However, colonies started appearing from sixth day onwards (Zhao *et al.,* 2004).

Plates were examined for the appearance of actinomycetes colonies from sixth day onwards up to 20 days. Total number of colonies in each set of plates is recorded as CFU/gm dry soil (Ghanem *et al*., 2000).

Many colonies with different morphological and cultural characteristics generally appeared with a tough leathery or chalky texture; dry or folded appearance and branching filamentous with or without aerial mycelia are picked (Mincer *et al.*, 2002) from the isolation plates and streaked for

purification. The pure cultures were transferred to PDA slopes and incubated for seven to ten days. When sufficient growth had occurred, the tubes were stored at 4 °C in a refrigerator and constituted the stock cultures. The stock cultures were maintained and transferred to fresh PDA slant once in two months and stored at 4 °C. The isolated colonies were enumerated and subjected to purification.

Primary screening of chitinolytic Actinomycetes

The selected colonies were subjected to primary screening for chitinase enzyme activity. The production of chitinase was performed by plate assay method using colloidal chitin (Hsu and Lockwood, 1975). Chitinase activity of the strains were screened in a Yeast Nitrogen Base medium containing 0.2 % (W/V) chitin and 0.5 % yeast extract and 1.5% agar (Watanabe *et al.,* 1990). After incubation at 28 °C for up to 8 days 0.1 % Congo red solution was added.

Finally potential isolates showing chitinolytic activity were selected for studying their activity against phytopathogenic fungi.

Antifungal activity

Antifungal activity of the chitinolytic actinomycete isolates were tested against the fungal pathogens *Aspergillus niger, Fusarium oxysporum, Fusarium solani, Rhizoctonia solani, Phytophthora capsici and Penicillium* sp. The antifungal activity was determined using disc diffusion method. The Fungal lawn of each organism was prepared on Potato dextrose agar plates. One drop of the culture filtrate of the selected actinomycete grown in colloidal chitin broth were loaded onto the filter paper discs (size: 5 mm) laid on to the swabbed plates. 50 µl of the enzyme filtrate was added to the paper discs. The plates were then incubated at 30 °C for 2 to 4 days. The radial diameter of the zone formation was measured (Gomes *et al*., 2001)

Result and Discussion

The soil samples from estuaries are reported as rich habitat for microbial diversity. The investigators from all over the world have isolated actinomycetes for various applications and they have found immense potential and diversity. These filamentous bacteria have been able to colonize geographically distinct locations like plain lands, agricultural soils, compost soil, river waters, estuaries, oceans, mountains, snow covered Arctic regions etc. The total actinomycetes population of the bottom sediments were enumerated using spread plate technique. About 60 isolates were selected from all the estuarine sediments. A number of reports have been reported on the occurrence and distribution of actinomycetes in estuaries & marine environments (Walker and Colwell, 1975). Early reports have shown that actinomycetes are widely spread in various water bodies where they play a great part in the carbon cycle due to their ability to grow at low concentrations of carbonaceous substances and to degrade recalcitrant organic matter (Kuznetsov, 1970).

Chitinases are normally produced by a large amount of organisms. Of all the isolates from Mankudy, Thengapattanam and Rajakkamangalam sediment soils 20 actinomycete isolates with chitinolytic activity which exhibited a clear zone on Colloidal chitin Agar media were chosen to test their anti fungal activity against phytopathogenic fungi. The major producers of chitinase in soils are *Streptomyces*

(Tanabe *et al*., 2000) and this capability makes them a valuable organism in waste management strategies. Chitinase can degrade chitinous waste and also the cell wall of many fungi. This property makes them the most important organism in the fields of biological research for the control of phytopathogenic fungi (Felse and Panda 2000). The antifungal activity of the selected isolates were tested against 6 phytopathogenic fungi like *Rhizoctonia solani, Aspergillus niger, Fusarium oxysporum, Fusarium solani, Phytophthora capsica* and *Penicillium* sp. by disc diffusion method. Also extracellular secretion of chitinase had been demonstrated previously for 13 strains of actinobacteria (Kawase *et al*., 2004).

Out of the 20 strains selected, 6 of them ESM2, ESM7, ESM9, ESM10, ESM14 and ESM15 from Manakudy estuary proved as chitinase producers. Of these 6 isolates the strain ESM9 was found to be the potential actinomycete with maximum antifungal activity. It showed a inhibition zone of about 32 mm against *Phytophthora capsici* and *Rhizoctonia solani*. Among the 6 pathogenic fungi ESM9 showed a lowest clearance zone of 25 mm against *Penicillium* sp. The strain ESM7 and ESM14 expressed a clearance zone of 24 mm against *Rhizoctonia solani.* No antifungal activity was expressed by the strain ESM14 against *Aspergillus niger*. Table.1. Several workers have worked on the antifungal activity of chitinolytic actinomycetes. *Streptomyces* chitinase have been implicated against a variety of plant pathogenic fungi (Gupta *et al*., 1995, Taechowisan *et al*., 2003). In the earlier studies chitinase from *Streptomyces* showed activity against fungi such as *Aspergillus sp, Phycomyces* sp and *Trichoderma reesei* (Williams *et al*., 1983). Hoster *et al.* (2005) reported chitinase activity against *A. nidulans* and phytopathogens such as *Botrytis cinerea*, *Fusarium culmorum* and *Sclerotia sclerotium*. Chitinolytic bacteria *Paenibacillus sp*. and *Streptomyces* sp. were reported to suppress Fusarium wilt of cucumber.

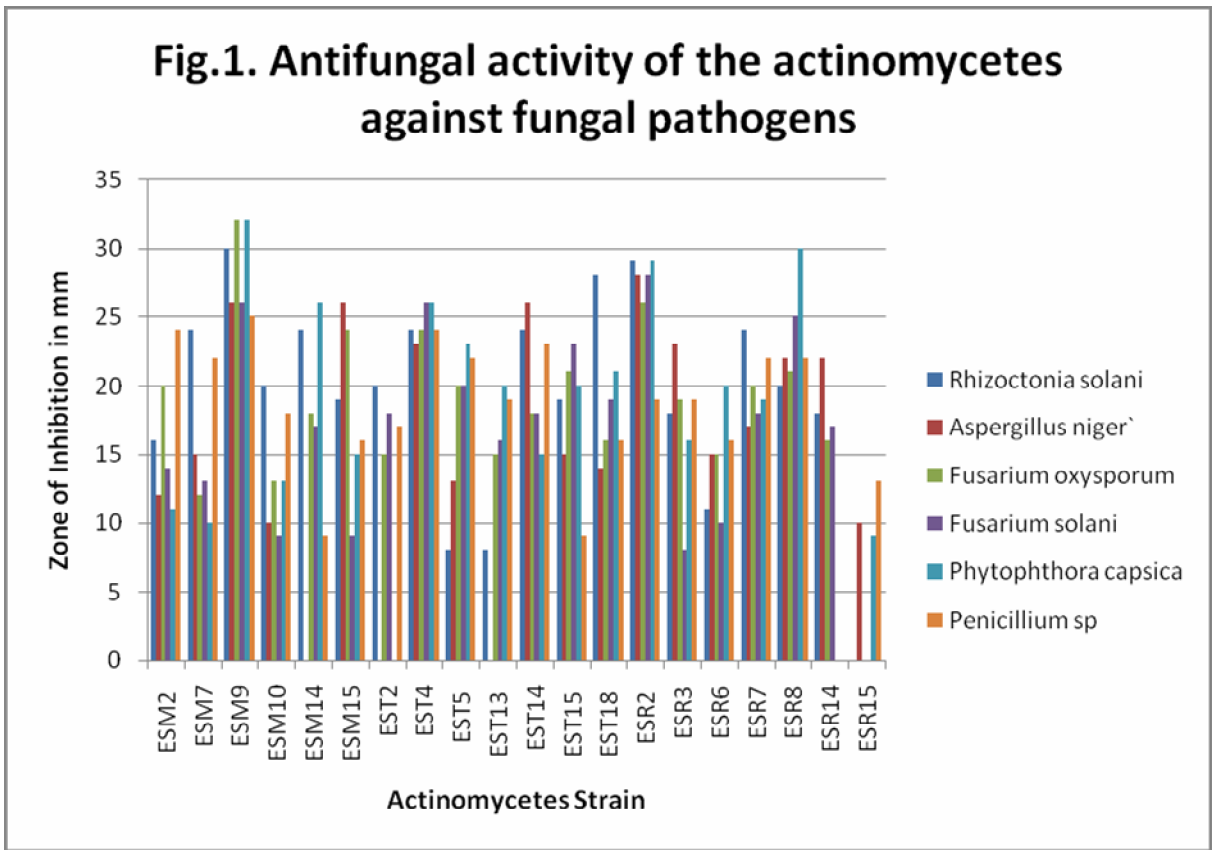
Seven actinomycetes, EST2, EST4, EST5, EST13, EST14, EST15 and EST18 with chitinolytic property were isolated from Thengapattanam estuary. Out of these 7 actinomycetes, the isolate EST4 was observed to exhibit a clearance zone of inhibition against all the test fungi. A maximum zone of inhibition 26 mm was shown against *Fusarium solani* and *Phytophthora capsici*. No activity was shown against *Aspergillus niger* and *Phytophthora capsici* by the strain EST2. Table.1 Anti fugal activity of the crude chitinase of *Streptomyces griseus* showed a clearance zone of 10 mm against *F.oxysporum*. Similarly antifungal activity of purified chitinase enzyme against fungal pathogens was proved previously (El-Tarabily *et al.*, 2000).

The role of chitinase activity against the fungal cell wall is evident (Thara and Gunanamanickam, 1994). Seven chitinolytic actiomycetes were isolated from Rajakkamangalam estuary and they were numbered as ESR2, ESR3, ESR6, ESR7, ESR8, ESR14 and ESR15. Out of these isolates the actinomycete strain ESR2 showed a clearance zone of inhibition of 29 mm against *Rhizoctonia solani* and *Phytophthora capsici.* The isolate ESR8 showed a maximum zone of inhibition of 30 mm against *Phytophthora capsici* and a lowest activity of 20 mm against *Rhizoctonia solani*. The isolate ESR15 was observed to exhibit no activity against *Rhizoctonia solani, Fusarium oxysporum and Fusarium solani.* Similarly antifungal activity of *Streptomyces lydicus* WYEC108 against a wide range of pathogenic fungal strains was reported previously (Yuan and Crawford, 1995).

The present investigation evaluated the potential of chitinolytic actinomycetes isolated from the sediment soils of estuaries of South west coast of Tamilnadu. This study dealt with the application of chitinase enzyme to effectively inhibit the phytopathogenic fungi which bring damage to crops. Therefore the production of this enzyme can be carried out to use as a potential biocontrol agent against phytopathogenic fungi.

Table.1 Antifungal activity of chitinolytic actinomycetes against selected pathogenic fungi

Sl.No	Actinomycetes Strain	Antifungal effect (diameter of inhibition zone in mm)					
		Fungal Strains					
		Rhizoctonia solani	*Aspergillus niger`*	*Fusarium oxysporum*	*Fusarium solani*	*Phytophthora capsica*	*Penicillium sp*
1	ESM2	16	12	20	14	11	24
2	ESM7	24	15	12	13	10	22
3	ESM9	30	26	32	26	32	25
4	ESM10	20	10	13	9	13	18
5	ESM14	24	0	18	17	26	9
6	ESM15	19	26	24	9	15	16
7	EST2	20	0	15	18	0	17
8	EST4	24	23	24	26	26	24
9	EST5	8	13	20	20	23	22
10	EST13	8	0	15	16	20	19
11	EST14	24	26	18	18	15	23
12	EST15	19	15	21	23	20	9
13	EST18	28	14	16	19	21	16
14	ESR2	29	28	26	28	29	19
15	ESR3	18	23	19	8	16	19
16	ESR6	11	15	15	10	20	16
17	ESR7	24	17	20	18	19	22
18	ESR8	20	22	21	25	30	22
19	ESR14	18	22	16	17	0	0
20	ESR15	0	10	0	0	9	13



References

Berdy, J. 2005 Bioactive microbial metabolites. Review article. *J. Antibiot.,* 58: 1–26.

Blaak, H., Schnellmann, J., Walter, S., Henrissat, B., Schrempf, H., 1993. Characteristics of an exochitinase from *Streptomyces olivaceoviribis*, its corresponding genes, putative domains and relationship to other chitinase. *J. Eur. Biochem.,* 214: 659–669.

Cross, T., 1981. Aquatic actinomycetes: A critical survey of the occurrence growth and role of actinomycetes in aquatic habitats. *J. Appl. Bacteriol.,* 50: 397–424

El- Tarabily, K.A., Soliman, M.H., Nassar, A.H., Al-Hassani, H.A., Sivasithamparam, K., McKenna F., Hardy, G.E. 2000. Biological control of *Sclerotinia minor* using chitinoloytic bacterium and actinomyces. *Plant Pathol.,* 49: 573–583.

Felse, P.A., Panda, T. 2000 Production of microbial chitinases A revisit. *Bioprocess Eng.,* 23: 127–134

Ghanem, N.B., Sabry, S.A., El-Sherif, Z.M., Abu, El-Ela, G.A. 2000. Isolation and enumeration of marine actinomycetes from seawater and sediments in Alexandria. *J. Gen. Appl. Microbiol.,* 46: 105–111.

Gohel, V., Vyas, P., Chhatpar, H.S. 2005. Activity staining method of chitinase on chitin agar plate through polyacrylamide gel electrophoresis. *J. Afri. Biotechnol.,* 4: 87–90.

Gomes, R.C., Semedo, L.T.A., Soares, R.M.A., Linhares, L.F., Ulhoa, C.J., Alviano, C.S., Coelho, R.R. 2001. Purification of a thermostable endochitinase from *Streptomyces* RC1071 isolated from a cerrado soil and antagonism against phytopatogenic funga. *J. Appl. Microbial.,* 90: 653–661

Gupta, R., Saxena, R.K., Chadurvedi, P., Virdi, J.S. 1995. Chitinase Production by *Streptomyces viridificans* and it's

potential in fungal cell wall lysis. *J. Appl. Bacteriol.,* 78: 378–383

Holt J.G., Williams, S.T., Sharpe, M.E. 1989. Bergey's manual of systematic bacteriology, Vol. 4. Williams and Wilkins,Baltimore, Maryland, USA. Pp. 2362–2371.

Hoster, F., Schmitz, J.E., Daniel, R. 2005. Enrichment of chitinolytic microorganisms, isolation and characteisation of a chitinase exhibiting antifungal activity against phytopathogenic fungi from a novel*Streptomyces* strain. *Appl. Microbiol. Biotechnol.,* 66: 434–442.

Hsu, S.C., Lockwood, J.L. 1975. Powdered Chitin agar as a selective medium for Actinomycetes in water & soil. *Appl.Microbial.,* 29: 422–426.

Kawase, T., Saito, A., Sato, T., Kanai, R., Fuji, T., Nikadou, N., Miyashita, K., Watanabe, T. 2004. Distribution and phylogenetic analysis of family 19 chitinases in *Actinobacteria*. *Appl. Environ. Microbiol.,* 70: 1135–1144

Kim, K., Ji, H.S. 2001. Effect of chitinsources on production of chtinase and chitosanase by *Streptomyces griseus* HUT 6037, *Biotechnol, Bioprocess Eng.,* 6: 18–24

Kuster, E., Williams, S.T. 1964. Selection of media for isolation of *Streptomycetes*. *Nature,* 202: 928–929.

Kuznetsov, S.1970. Microflora of lakes and its geo chemical activity Leningrad. Nauka 1–9.

Mincer, T.J., Jensen, P.R., Kauffman, C.A., Fenical, W. 2002. Widespread and persistent populations of a major new marine actinomycete taxon in ocean sediments. *Appl. Environ. Microbiol.,* 68: 5005–5011

Taechowisan, T., Peberdy, J.F., Lumyong S. 2003. Chitinase production by endophytic *Streptomyces aureofaciens* CMU Ac 130 and its antagonism against phytopathogenic fungi. *Annal. Microbiol.,* 53: 447–461.

Tanabe, T., Kawase, T., Watanabe, T., Uchida, Y., Mitsutomi, M. 2000. Purification and characterisation of 49–kDa chitinase from *Sreptomyces griseus* HUT 6037. *J. Bios. Bioeng.,* 1: 27–32.

Thara, K.V., Gunanamanickam, S.S. 1994. Biological control of rice sheath blight in India: Lack of correlation between chitinase production by bacterial antagonists and sheath blight suppression. *Plant Soil.,* 160: 277–280

Walker, J.D., Colwell, R.R. 1975. Factors affecting enumeration and isolation of actinomycetes from Chesapeake Bay and South Eastern Atlantic Ocean Sediments. *Biology,* 30: 193–201

Wang, Y., Zhang, Z.S., Ruan, J.S., Wang, Y.M, Ali, S.M. 1999. Investigation of actinomycetes diversity in the tropical rainforests of Singapore. *J. Ind. Microbiol. Biotechnol.,* 23: 178–187.

Watanabe, T., Oyanag,i W., Suzuki, K., Tanaka., H. 1990. Chitinase system of *Bacillus circulans* WL –12 and importance of Chitinase A1 in Chitin Degradation. *J. Bacteriol.,* 172: 4017–4022.

Williams, S.T., Goodfellow, M., Alderson, G., Wilington, E.M., Sneath, P.H., Sneath, M.J. 1983. Numerical classification of *Streptomyces* and related genera. *J. Gen. Microbiol.,* 129: 1747–1 813.

Yuan, W.M., Crawford, D.I. 1995. Characterisation of *Streptomyces lydicus* WYEC 108 as a potential biocontrol agent against fungal root and seed rots. *Appl. Environ. Microbiol.,* 61(8): 3119–3128.

Zhao, H., Kassama,Y., Young, M., Kell, D.B., Goodacre, R. 2004. Differentiation of *Micromonospora* isolates from a costal sediments inwales on the basis of Fourier transform infrared spectroscopy. 16s rRNA sequence analysis, and the amplified fragment length polymorphism technique. *Appl. Environ. Microbiol.,* 70: 6619–6627.