EFFECTS OF *STREPTOMYCES* SP. ON GROWTH OF RUBBERWOOD SAPSTAIN FUNGUS *LASIODIPLODIA THEOBROMAE*

KL Sajitha* & EJM Florence

Kerala Forest Research Institute, Peechi Thrissur, Kerala, India

Received May 2012

SAJITHA KL & FLORENCE EJM. 2013. Effects of *Streptomyces* sp. on growth of rubberwood sapstain fungus *Lasiodiplodia theobromae*. An actinomycete isolated from the soil, identified as *Streptomyces* sp. SA18, was found to be an effective antagonist against the fungus *Lasiodiplodia theobromae* that caused sapstain on rubberwood. One of the antagonistic factors responsible for this inhibitory effect was identified as chitinase enzyme production, which degraded the chitin of fungal cell wall to N-acetyl D-glucosamine. In the chitin agar plate inoculated with *Streptomyces* sp. SA18, the dye bound with stable chitin and fluoresced under ultra violet light. On the contrary, chitin-free portions appeared dark indicating degradation of chitin by chitinase enzyme produced by *Streptomyces* sp. SA18.

Keywords: Antagonism, chitin, chitinase, calcoflour white, SA18

SAJITHA KL & FLORENCE EJM. 2013. Kesan *Streptomyces* sp. terhadap pertumbuhan kulat cacat warna gubal *Lasiodiplodia theobromae* pada kayu getah. Aktinomiset *Streptomyces* sp. SA18 yang diasingkan daripada tanah didapati antagonis yang berkesan terhadap kulat *Lasiodiplodia theobromae* yang mengakibatkan cacat warna gubal pada kayu getah. Salah satu faktor antagonis yang bertanggungjawab terhadap kesan perencatan ialah penghasilan enzim kitinase yang mendegradasikan kitin pada dinding sel kulat kepada N-asetil D-glukosamin. Bagi plat agar kitin yang diinokulasi dengan *Streptomyces* sp. SA18, pewarna terikat dengan kitin yang stabil dan berpendafluor di bawah cahaya ultra unggu. Sebaliknya, bahagian yang tidak mengandungi kitin kelihatan gelap. Ini menandakan degradasi kitin oleh enzim kitinase yang dihasilkan oleh *Streptomyces* sp. SA18.

INTRODUCTION

Natural forest resources are depleting rapidly. The growing demand for conventional timbers can be met to some extent by utilising rubberwood as an alternative species. Felled rubberwood is always prone to attack by mould, stain and decay fungi. *Lasiodiplodia theobromae* the dominant sapstain fungus in tropical countries plays a major role in reducing the economic value of rubberwood by discolouring the wood. The main wood preservatives used to prevent biodeterioration and biodiscoloration are creosote, chromate copper arsenate, sodium pentachlorophenates, inorganic arsenicals and borax boric acid solutions. However, these chemicals are not very effective against the sapstain caused by *L. theobromae*. Now, biological control agents are seriously being considered in wood protection due to health, environmental concern and high cost. Actinomycetes especially *Streptomyces* spp. as potential biocontrol agents against plant and human pathogenic fungi have been investigated (Gomes et al. 2000, Augustine et al. 2005, Oskay 2009). However, their efficiency in wood biocontrol is little studied (Croan & Highley 1994a, b, Croan 1997).

Actinomycetes are a diverse group of heterotrophic prokaryotes which form hyphae at some stages of their growth. Actinomycetes, in particular the genus *Streptomyces*, are gram positive bacteria. They are ubiquitous in soil and well known as producers of many extracellular enzymes, including chitinases which degrade fungal cell wall (Gupta et al. 1995). The genus *Streptomyces* has many characteristics which make it useful as a biocontrol agent (Crawford et al. 1993).

Chitin, a polymer of N-acetyl D-glucosamine, is the second most abundant carbohydrate in nature and a major structural polysaccharide of arthropods, coelenterates and fungi. Chitinases

* *sajithananda@yahoo.co.in*

are glycosyl hydrolases which catalyse the degradation of chitin. It is an enzyme complex which comprises exochitinase, endochitinase and chitobiase components. The first one liberates soluble low molecular weight dimmers, the second releases multimers of N-acetyl-glucosamine (NAG) and third hydrolyses chitobiose to NAG (Vyas & Deshpande 1989). The combined effect of these enzymes results in a complete degradation of the entire chitin. A comparative study of the three chitinolytic enzymes showed that endochitinase (EC 3.2.1.14) was most effective for antifungal and lytic activities (Lorito et al. 1996).

Various studies on the effect of chitinase have been conducted by Quecine et al. (2008) and Anitha and Rabeeth (2010) on plant pathogenic fungi as well as Mendonsa et al. (1996) on moulting process of insects and mosquito control. Apart from acting as biocontrol agents, chitinase has received renewed interest due to its wide range of biotechnological applications such as generation of fungal protoplasts (Vyas & Deshpande 1989), crustacean chitin waste management (Suresh & Chandrasekaran 1998) and production of single cell protein (Vyas & Deshpande 1991). Almost all soft wood species are susceptible to sapstaining by *L. theobromae* and rubberwood is most severely and frequently affected (Florence & Sharma 1990). This situation can be improved by adopting appropriate control measures. In view of known environmental and health hazards caused by traditional control methods such as chemical preservatives, the possibility of biological control of this fungus was evaluated.

No studies have been conducted on the biological control of rubberwood against the sapstain fungus *L. theobromae* and the influence of antagonistic properties on inhibition of fungal growth. Therefore, the objective of this study was to isolate any actinomycete antagonistic to the sapstain fungus *L. theobromae* and identify its antagonistic properties responsible for growth inhibition of *L. theobromae* on rubberwood.

MATERIALS AND METHODS

Isolation of antagonistic microorganisms and detection of antagonistic activity

Microorganisms were isolated from the soil, compost and rubberwood. Samples were serially diluted and plated on starch casein agar (starch 1%, casein 0.03%, KNO_3 0.2%, NaCl 0.2%, K_2HPO_4 0.2%, $MgSO_4.7H_2O$ 0.05%, $CaCO_3$ 0.002%, $FeSO_4$ 0.001%, agar 1.8%) and incubated at 37 °C for 1 week for microbial growth. All cultures were purified by streak plate method and confirmed by colony morphology. From the isolated actinomycetes, the antagonistic ones were screened using dual culture technique (Johnson & Curl 1972) on potato dextrose agar (potato 20%, dextrose 2%, agar 1.8%) medium. In one method, the selected actinomycete culture was streaked on one side and allowed to grow. Since actinomycetes have a relatively lower growth rate on agar plates than most of the fungi, preinoculation was used to establish the actinomycete colonies on the agar surface. After 1 week, a 5-mm mycelial disc from a fresh culture of *L. theobromae* was inoculated on the side opposite the actinomycete. In the other method, actinomycete culture was grown at three positions on the agar plate and the fungus was placed in the centre. After incubation for 7 days at 28 °C, the radial growth of *L. theobromae* and the zone size (distance between the edge of *L. theobromae* and the actinomycete cultures) were measured in triplicates. Control plates with the test fungus and without the antagonist were also maintained. From among the several actinomycetes tested, the effective one (SA18) was selected for further studies.

Microscopic examination of the fungus and associated mode of action of SA18

The actinomycete SA18 was grown in starch casein broth for 2 weeks at 37 °C. After incubation the culture was centrifuged at 5000 rpm for 10 min. The supernatant fluid was concentrated using an ultra membrane filter, cut-off 5000 Da MW. The concentrated filtrate was filtered through 0.22 µ membrane filter. *Lasiodiplodia theobromae* was inoculated on one side of the plate and the sterile culture filtrate was simultaneously poured into the well bored on the opposite side and incubated for 1 week. Normal mycelium (control plates) and mycelium from the colony edge nearest the inhibition zone were taken and observed under light microscope according to the method of Basha and Ulaganathan (2002). The microscopic examination of the mycelia from the zone of inhibition confirmed the effect of an active ingredient responsible for the growth inhibition of *L. theobromae*.

Test for chitinase production

Preparation of substrate (colloidal chitin)

Crude chitin was treated with concentrated HCl in a ratio 1:10 (w/v) and the mixture was stirred well for 1 hour and cold water added to the mixture. The colloidal chitin mixture was filtered and washed with several changes of water until the pH became 6. The chitin was filtered, dried and used for detecting the chitinase activity of the culture according to the method of Hsu and Lockwood (1975).

Reduction of turbidity in colloidal chitin broth

The actinomycete SA18 was inoculated in the colloidal chitin broth (0.5% colloidal chitin, 0.02% yeast extract, 0.07% KH_2PO_4, 0.03% K_2HPO_4, 0.4% NaCl, 0.05% $MgSO_4$, 0.001% $FeSO_4$, 0.0001% $ZnSO_4$ and 0.0001% $MnSO_4$) with pH 7 and incubated at 37 °C for 2 weeks at 100 rpm.

Plate assay for chitinase production

Actinomycete SA18 was inoculated on colloidal chitin agar (0.5% colloidal chitin, 0.02% yeast extract, 0.07% KH_2PO_4, 0.03% K_2HPO_4, 0.4% NaCl, 0.05% $MgSO_4$, 0.001% $FeSO_4$, 0.0001% $ZnSO_4$, 0.0001% $MnSO_4$ and 2% agar) and incubated at 37 °C for 2 weeks. About 0.01% of the dye calcofluor white (fluorescent brightener 28) was added, the plate incubated at 37 °C for 2 hours and viewed under ultra violet light.

Identification of isolate

Purified isolate of actinomycete SA18 was identified by comparing the morphology of spore-bearing hyphae indicating the structure of spore chain with classification standards (Holt 2000). This was done by inserting a cover slip at an angle of 45° close to the streaked portion in the medium. As the actinomycete grew, its spore-bearing hyphae grew over the cover slip. The hyphae could then be gently removed and viewed under the microscope.

Amplification of the 16 S rDNA of SA18

The genomic DNA of SA18 was isolated according to Ausubel et al. (1994). To characterise the SA18 strain, the small ribosomal DNA unit was amplified by polymerase chain reaction (PCR) using the conserved primers (forward StrepB 5'-ACAAGCCCTGGAAACGGGGT 3' and reverse Strep F 5'- ACGTGTGCAGCCCAAGACA 3') (Rintala et al. 2001, Suutari et al. 2001). The PCR reaction mixture (25 µL) was prepared by mixing the following components: 15–30 ng of template DNA, 0.05 µl of 0.2 µmol L^{-1} of each primers, 200 µM of each dNTPs, 5 µl of 5xHF buffer, 0.02 u/µL of DNA polymerase and 17.4 µL of sterile distilled water. Amplification was carried out. A preliminary denaturation step was done at 98 °C for 5 min, followed by 30 cycles of denaturation (1 min at 95 °C), primer annealing (40 s at 58 °C) and extension (2 min at 70 °C). After 30 cycles, a final extension of 10 min at 72 °C was completed. The PCR product was checked by electrophoresis. The product was sequenced at MWG BIOTECH, Bangalore for species identification. The similarity search for sequences was carried out using the BLAST (N) programme of National Center of Biotechnology Information, USA.

Wood block tests

Twenty-five fresh rubberwood blocks (7 cm × 5 cm ×1 cm), steam sterilised at 100 kPa pressure for 20 min were used for testing in the laboratory. Actinomycete SA18 was grown in yeast glucose broth (yeast extract 0.4%, dextrose 0.2%, NaCl 0.5%, K_2HPO_4 0.25%, KH_2PO_4 0.05%) for 2 weeks. The wood blocks were dipped in the culture for 10 min, drained and kept in sterile Petri dishes with a moist filter paper. An 8-mm diameter disc taken from the edge of an actively growing agar culture of *L. theobromae* was placed aseptically over each wood block. The plates were sealed and incubated. Wood blocks dipped in sterile water and inoculated with the test fungus were kept as control and incubated at room temperature. After the incubation period of 1 month, the wood blocks were observed for fungal growth and staining.

RESULTS

Isolation of antagonistic microorganisms and detection of antagonistic activity

Diverse actinomycetes were isolated: 30 actinomycetes from compost, 14 from wood and 9 from soil. In the dual culture method for screening antagonistic microbes, 11 actinomycetes

showed inhibition to the test fungus *L. theobromae*. Among these, SA18 (Figure 1), which was isolated from the soil, was chosen for further studies.

Microscopic examination of the fungus and associated mode of action of SA18

Actinomycete SA18 and its crude extract showed distinct inhibition of the sapstain fungus *L. theobromae* (Figures 1 and 2). Light microscopic studies of the mycelial portion from the zone of inhibition revealed the presence of abnormal hyphae with large extended vacuoles and clear deformation (Figure 3). Swollen individual cells were common at both hyphal tips and intermediate positions in the fungal mycelium.

Test for chitinase production

Reduction of turbidity in colloidal chitin broth

In the colloidal chitin broth containing 0.5% colloidal chitin, SA18 completely degraded the chitin (Figure 4) within 2 weeks of incubation.

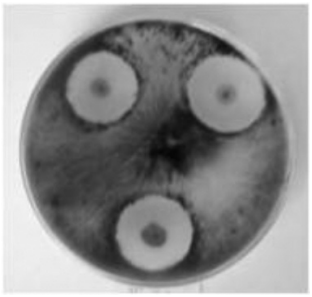

Figure 1 Actinomycete SA18 isolated from the Kerala Forest Research Institute garden soil inhibiting the growth of sapstain fungus *Lasiodiplodia theobromae*

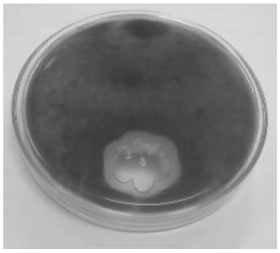

Figure 2 Ultrafiltrate of actinomycete SA18 grown in colloidal chitin broth showing effective inhibition of the growth of sapstain fungus *L. theobromae*

Plate assay for chitinase production

A clear zone was observed around the SA18 culture. When these plates were stained with calcoflour white and viewed under ultra violet (UV) light, the zone around the culture appeared dark while the rest of the portion fluoresced (Figure 5).

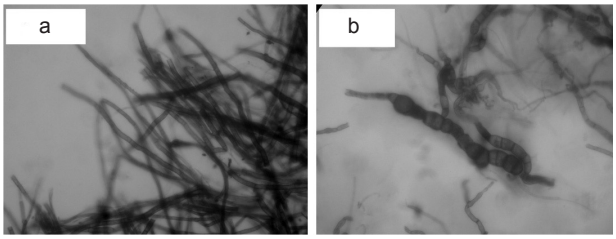

Figure 3 Light microscopic views of (a) normal and (b) malformed mycelia of sapstain fungus *L. theobromae* taken from the edge of the inhibition zone shown in Figure 2

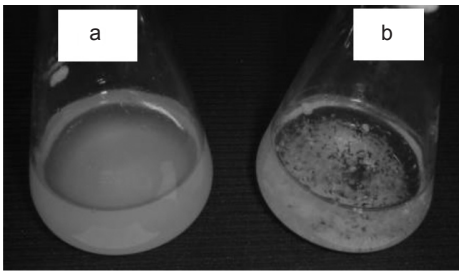

Figure 4 (a) Control flask showing the original colloidal chitin broth (CCB) before inoculation of actinomycete SA18 and (b) flask showing the decolouration of CCB medium after 2 weeks of inoculation of actinomycete SA18

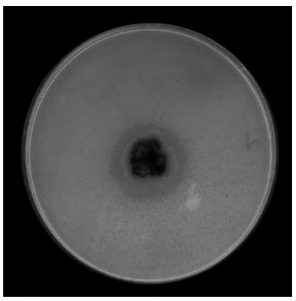

Figure 5 Calcoflour white stained colloidal chitin agar plate inoculated with SA18 in the centre; chitinase production is confirmed by the presence of dark zone around the SA18 culture

Identification of isolate

The microscopic examination of actinomycete SA18 revealed the presence of aerial and branched mycelia. The conidial chain which arose from the branched aerial mycelium was differentiated into rectiflexibles carrying spores.

Amplification of 16 S rDNA of SA18

The 16S rDNA of SA18 genome was amplified using the specific forward and reverse primers for *Streptomyces*. When the partially sequenced 16S rDNA (997bp) was subjected to BLAST sequence similarity search, two *Streptomyces* sp. (Accession No. JN560156 and FJ200398) showed a maximum of 100% identity. Around 100 different *Streptomyces* spp. deposited in the gene bank showed maximum identity of 99% with SA18. *Streptomyces* sp. SA18 (accession number FJ643450) had since been deposited in the gene bank for future reference.

Wood block tests

Streptomyces sp. SA18 grew on the wood surface and inhibited the growth of *L. theobromae* (Figure 6). Actinomycete growing on the surface of the wood was easily removed by brushing.

DISCUSSION

Actinomycete diversity was found higher in compost compared with other substrates. This is an indication that actinomycetes are tolerant to higher temperatures. The in-vitro antagonism assays showed that 11 (20%) of the 53 isolates were antagonistic to *L. theobromae*. Among the 11 actinomycetes, inhibitory effect was found to be greater in *Streptomyces* sp. SA18 and it was chosen as the most suitable antagonist for further studies.

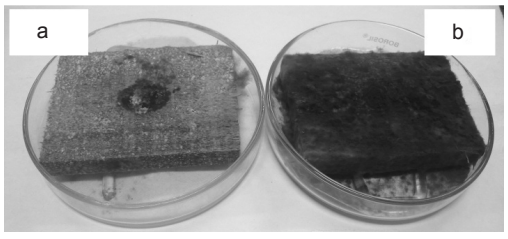

Figure 6 (a) Plate showing *Streptomyces* sp. SA18 treated rubberwood inoculated with *L. theobromae,* SA18 itself grew on the block and inhibited sapstain infection; (b) control plate showing *L. theobromae* infection on rubberwood

Several mechanisms, including antibiosis and parasitism, have been proposed to explain antagonistic phenomenon. In the present experiment of dual culture technique with the actinomycete and fungus, any molecule could act, resulting in growth inhibition. In some cases, inhibition may be caused by hydrolytic enzymes such as chitinases (Yan et al. 2008), though other enzymes such as glucanases or proteases may act against the fungal cell wall and antibiotic production may be involved (Jain & Jain 2007, Oskay 2009). Antibiotics are diffusible compounds which inhibit growth or developmental changes in the pathogen while hydrolytic enzyme results in physical destruction of the fungal cell wall.

Crude culture extracts concentrated through 5000 cut-off membranes have been shown to exclude low molecular weight antifungal antibiotics (Gomes et al. 2000). Hence in this study, the ultrafiltration might have removed the possible antibiotics and the filtrate contained only high molecular weight proteins especially the cell wall hydrolysing enzymes. Thus, the antagonism against *L. theobromae* could be better explained by polymeric substances such as enzymes, which inhibited fungal growth by cell wall lysis. Light microscopic observations of the *L. theobromae* mycelium from the edge of the inhibition zone created by the ultrafiltered crude extract of *Streptomyces* sp. SA18 revealed a number of hyphal morphological changes in the test fungus. As ultrafiltration removed antibiotics from the filtrate, the hyphal damage could be explained by some cell wall degrading enzymes especially chitinase produced by the actinomycete SA18. Fungal cell wall contains chitin as the major component and chitinases are well known for lysing fungal cell walls (Gomes et al. 2001, Quecine et al. 2008). The importance of adhesion of chitinase to its crystalline substrate chitin was studied by Blaak and Schrempf (1995). Vionis et al. (1996) attributed the hole formation on fungal cell walls by the action of chitinase enzyme.

Antagonistic activity of several *Streptomyces* spp. against fungal pathogenic species has been known for a long time (Crawford et al. 1993). *Streptomyces* sp. SA18 possessed good chitinolytic potential, as demonstrated in our work from its

growth and chitin hydrolysis in colloidal chitin broth and colloidal chitin agar. Colloidal chitin was used to test for chitinase production and nearly all chitin was degraded within 2 weeks. The increase in extracellular activity with the disappearance of chitin from the medium was due to the binding of the enzyme to the substrate, which was progressively released (Nawani et al. 2002). Calcofluor white M2R or fluorescent brightener is a specific dye which binds to intact cellulose and chitin and emits light blue fluorescence under UV light. If any portion of chitin is degraded to its individual subunits, the dye will not bind and that portion will appear as a dark band against a fluorescent background under UV light. In this study, an easy and quick method was adopted to analyse the production of fungal cell wall degrading chitinase enzyme by the *Streptomyces* sp. SA18. Here 0.01% of fluorescent brightener was added directly to the chitin agar plate with the grown actinomycete SA18 to detect the chitinase production. When the plate was viewed under UV light, the presence of dark zone around the culture proved the chitin degradation ability of the antagonistic microbe SA18. This method could be used for the evaluation of chitinase production by different microbes by comparing the zone of clearance developed by each microbe on the chitin agar plate. It can detect even negligible enzyme production.

Biological control of wood deteriorating fungi by living cells of actinomycetes is not much studied. It may be due to the slow growing nature of actinomycetes. Even though actinomycetes especially *Streptomyces* are very effective antifungal agents, they cannot grow effectively on the substrate. The synergistic effect of boron on *Streptomyces rimosus* as bioprotectant in preventing spore germination of sapstain and mould fungi on southern yellow pine were studied by Croan and Highley (1992). Boron along with *S. rimosus* metabolites was found to be effective in controlling the sapstain and mould infection. In the present study, *Streptomyces* sp. SA18 alone showed effective inhibition against *L. theobromae* infection on rubberwood. *Streptomyces* sp. SA18 itself was growing on the wood surface, did not cause any stain on the rubberwood and could be easily removed by brushing.

Of all the biocontrol agents used in controlling fungal wood deterioration, actinomycetes particularly *Streptomyces* spp. are the best sources of antifungal enzymes and antibiotics. They have great potential to be exploited as broad spectrum biocontrol agents against wood deteriorating fungi. In the present study, it is proven that *Streptomyces* sp. SA18 along with its ultrafiltrate is a very effective sapstain biocontrol agent.

ACKNOWLEDGEMENT

This study was supported by grants from the Department of Science and Technology, Ministry of Science and Technology, Government of India.

REFERENCES

Anitha A & Rabeeth M. 2010. Degradation of fungal cell walls of phytopathogenic fungi by lytic enzyme of *Streptomyces griseus*. *African Journal of Plant Science* 4: 61–66.

Augustine SK, Bhavsar SP & Kapadnis BP. 2005. Production of a growth dependent metabolite active against dermatophytes by *Streptomyces rochei* AK39. *Indian Journal of Medical Research* 121: 164 –170.

Ausubel MF, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA & Struhl K. 1994. *Current Protocols in Molecular Biology*. Volume 1. John Wiley and Sons Inc, New York.

Basha S & Ulaganathan K. 2002. Antagonism of *Bacillus* species (strain BC121) towards *Curvularia lunata*. *Current Science* 82: 1457–1463.

Blaak H & Schrempf H. 1995. Binding and substrate specificities of a *Streptomyces olivaceoviridis*, its corresponding gene, putative protein domains and relationship to other chitinases. *European Journal of Biochemistry* 229: 132–139.

Crawford DL, Lynch JM, Whipps JM & Ousley MA. 1993. Isolation and characterization of actinomycetes antagonists of a fungal root pathogen. *Applied and Environmental Microbiology* 59: 3899–3905.

Croan SC. 1997. *Environmentally Benign Biological Wood Preservatives by Streptomyces rimosus, SC-36*. Document No. IRG/WP/ 97-10196. International Research Group on Wood Preservation, Stockholm.

Croan SC & Highley TL. 1992. *Synergistic Effect of Boron on Streptomyces rimosus Metabolites in Preventing Conidial Germination of Sapstain and Mold Fungi*. Document No. IRG/WP/1565–92. International Research Group on Wood Preservation, Stockholm.

Croan SC & Highley TL. 1994a. Biological treatment for controlling wood deteriorating fungi. Patent No. 5356624.

Croan SC & Highley TL. 1994b. Biological control of sapwood inhabiting fungi by metabolites from *Streptomyces rimosus*. *Biodeterioration Research* 4: 243–255.

Florence EJM & Sharma JK. 1990. *Botryodiplodia theobromae* associated with blue staining in commercially important timbers of Kerala and its possible biological control. *Material und Organismen* 25: 194–199.

Gomes RC, Semedo LTAS, Soares RMA, Alviano CS, Linhares LF & Coelho RRR. 2000. Chitinolytic activity of actinomycetes from a cerrado soil and their potential in biocontrol. *Letters in Applied Microbiology* 30: 146–150.

Gomes RC, Semedo LTAS, Soares RMA, Alviano CS, Linhares LF, Ulhoa CJ, Alviano CS & Coelho RRR. 2001. Purification of a thermostable endochitinase from *Streptomyces* RC1071 isolated from a cerrado soil and its antagonism against phytopathogenic fungi. *Journal of Applied Microbiology* 90: 653–661.

Gupta R, Saxena RK, Chaturvedi P & Virdi JS. 1995. Chitinase production by *Streptomyces viridificans*: its potential in fungal cell wall lysis. *Journal of Applied Microbiology* 78: 378–383.

Holt. 2000. *Bergey's Manual of Determinative Bacteriology. The Actinomycetales*. Ninth edition. Lippincott Williams and Wilkins, Philadelphia.

Hsu SC & Lockwood JL. 1975. Powdered chitin agar as a selective medium for enumeration of actinomycetes in water and soil. *Applied Microbiology* 29: 422–426.

Jain PK & Jain PC. 2007. Isolation, characterization and antifungal activity of *Streptomyces sampsonii* GS 1322. *Indian Journal of Experimental Biology* 45: 203–206

Johnson LF & Curl EA. 1972. Testing for antibiotic activity. Pp 153–155 in *Methods for Research on the Ecology of Soil-Borne Plant Pathogens*. Burgess Publishing Company, Minneapolis.

Lorito M, Woo SL, D'ambrosio M, Harman GE, Hayes CK, Kubicek CP & Scala F. 1996. Synergistic interaction between cell wall degrading enzymes and membrane affecting compounds. *Molecular Plant–Microbe Interaction* 9: 206–213.

Mendonsa ES, Vartak PH, Rao JU & Deshpande MV. 1996. An enzyme from *Myrothecium verrucaria* that degrades insect cuticles for biocontrol of *Aedes aegypti* mosquito. *Biotechnology Letters* 18: 373–376.

Nawani NN, Kapadnis BP, Das AD, Rao AS & Mahajan SK. 2002. Purification and characterization of a thermophilic and acidophilic chitinase from *Microbispora* sp. V2. *Journal of Applied Microbiology* 93: 965–975.

Oskay M. 2009. Antifungal and antibacterial compounds from *Streptomyces* strains. *African Journal of Biotechnology* 8: 3007–3017.

Quecine MC, Araujo WL, Marcon J, Gai CS, Azevedo JL & Pizzirani-Kleiner AA. 2008. Chitinolytic activity of endophytic *Streptomyces* and potential for biocontrol. *Letters in Applied Microbiology* 47: 486–491.

Rintala H, Nevalainen A, Ronka E & Suutari T. 2001. PCR primers targeting the 16S rRNA and gene for the specific detection of *Streptomycetes*. *Molecular and Cellular Probes* 15: 337–347.

Suresh PV & Chandrasekaran M. 1998. Utilization of prawn waste for chitinase production by marine fungi *Beauveria bassiana* by solid state fermentation. *World Journal of Microbiology and Biotechnology* 14: 655–660.

Suutari T, Rintala H, Ronka E & Nevalainen A. 2001. Oligonucleotide primers for detection of *Streptomyces* species. Patent No. F12001 1004.

Vionis AP, Niemeyer F, Karagouni AD & Schrempf H. 1996. Production and processing of a 59-kilodalton exochitinase during growth of *Streptomyces lividans* carrying pCHIO12 in soil microcosms amended with crab or fungal chitin. *Applied Environmental Microbiology* 62: 1774–1780.

Vyas P & Deshpande MV. 1989. Chitinase production by *Myrothecium verrucaria* and its significance for fungal mycelia degradation. *Journal of General and Applied Microbiology* 35: 343–350.

Vyas P & Deshpande MV. 1991. Enzymatic hydrolysis of chitin by *Myrothecium verrucaria* chitinase complex and its utilization to produce SCP. *Journal of General and Applied Microbiology* 37: 267–275.

Yan R, Hou J, Ding D, Guan W, Wang C, Wu Z & Li M. 2008. In vitro antifungal activity and mechanism of action of chitinase against four plant pathogenic fungi. *Journal of Basic Microbiology* 48: 293–301.

