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Antifungal potential of actinomycete isolate *Streptomyces exfoliatus* MT9 against wood-rotting fungi

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Abstract

An actinomycete isolate, *Streptomyces exfoliatus* MT9 was assessed for *in vitro* antagonism against wood-rotting fungi. Strain MT9 showed strong antagonistic activity (ZOI ≥ 25 mm) towards various tested wood-rotting fungi. Extracellular production of antifungal metabolite(s) including primary and secondary was monitored up to 10 days of submerged fermentation. Antagonist *S. exfoliatus* MT9 produces fungal cell-wall lytic enzymes, namely chitinase (3.098 U ml^{-1}), β-1,3 glucanase (2.4 U ml^{-1}) and protease (144.0 U ml^{-1}) and also showed antifungal activity towards tested *P. chrysosporium* MTCC 787 (12.0 mm) and *P. placenta* MTCC 144 (16.0 mm). Extracellular culture filtrate (ECF) of *S. exfoliatus* MT9 also exhibited strong antifungal activity (ZOI ≥ 25 mm) towards tested wood-rotting fungi and *n*-butanol was found to be the suitable solvent for complete extraction of antifungal metabolite(s) from ECF. Reduced antifungal activity of *n*-butanol extract against *P. chrysosporium* MTCC 787 (11.00 mm) and *P. placenta* MTCC 144 (10.00 mm) on ergosterol agar plate, no activity against bacteria and characteristic UV spectra at 224 nm revealed the polyene nature of antifungal metabolite(s) present in the *n*-butanol extract. A novel actinomycete isolate, *S. exfoliatus* MT9 is producing antifungal metabolite(s) that makes it suitable for biotechnological processes and has the potential to be used as a bioactive agent for controlling wood-rotting fungi.

Key words

Cell-wall lytic enzymes, Polyene, *Streptomyces exfoliatus*, Wood-rotting fungi

Introduction

Wood degradation due to fungal infection is the major problem with wood and timber industries that causes huge economic loss. Among all microorganisms, fungal pathogens mainly basidiomycetes are the major cause for damaging wood products (Kumar and Gupta, 2006). Broad-spectrum chemical fungicides such as copper chrome arsenate, sodium pentachlorophenates, copper oxychloride and inorganic arsenicals are being used for the preservation of wood and its products, unfortunately these synthetic fungicides causes serious environment pollution and are also hazardous to human health (Weiss *et al*., 1992; Hingston *et al*., 2001; Nagpure *et al*., 2014a). The ever-increasing public concern and the new environmental regulations on the use of chemicals have created the need for the development of alternative methods for wood protection (Susi *et al*., 2011). The development of environmentally friendly bioactive wood preservatives would offer an alternative to synthetic chemical fungicides.

Biological control system is one of the best alternatives to protect the environment from chemical fungicides and wood decaying microorganisms (Ara *et al*., 2012; Tomar *et al*., 2014). Biological wood protection by antagonistic microbes alone or in combination with chemicals, is one of the most promising ways for the environmentally sound wood protection (Kundu *et al*., 2008). Actinomycetes, especially *Streptomyces* is a Gram positive, free living saprophytic bacteria, well known for the

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production of antibacterial, antifungal, antimural, antiparasitic, antiviral compounds along with the production of mycolytic enzymes (Morakchi *et al*., 2009; Nagpure *et al*., 2014 a, c). The genus *Streptomyces* has many species that exhibits antifungal activity e.g. *S. chrestomycetius, S. rimosus* and *S. violaceusniger* (Trejo-Estrada *et al*., 1998; Nagpure *et al*., 2014 a). The objective of present study is to examine the antagonistic potential of *Streptomyces exfoliatus* MT9 against wood-rotting fungi and its ability to produce extracellular antifungal metabolite(s).

Materials and Methods

Microorganisms and culture conditions : Antagonist *Streptomyces exfoliatus* MT9 was isolated from soil of Loktak lake, Manipur, India and identified by morphological, cultural characteristics, FAME and phylogenetic analysis (Choudhary *et al*., 2014). Antagonist strain MT9 was grown on M93 agar plates containing (g l^{-1}) glucose 4.0, yeast extract 4.0, malt extract 10.0, calcium carbonate 2.0, agar 15.0, pH 7.0±0.2) at 30ºC for 5 d and then stored at 4ºC until use. The following wood-rotting fungi were used: Brown-rot such as *Gloeophyllum trabeum* MTCC 355*, Postia placenta* MTCC 144*, Polyporus agaricans* ITCC 761, *Polyporus friabilis* ITCC 335 and white-rot fungi included *Phanerochaete chrysosporium* MTCC 787*,Coriolus versicolor* MTCC 138*, Polystictus versicolor* ITCC 13 and *Schizophyllum commune* ITCC 3751. MTCC and ITCC fungal cultures were procured from Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology, Chandigarh and Indian Type Culture Collection (ITCC) of Indian Agricultural Research Institute (IARI), New Delhi, India, respectively. All the test fungi were grown on PDA (Potato Dextrose Agar) plates and incubated at 30ºC for 7 day and stored at 4ºC.

***In vitro* antagonism assay :** Antifungal potential of *S. exfoliatus* MT9 was checked against various wood-rotting fungi by using "cross plug" assay (Crawford *et al*., 1993; Nagpure *et al*., 2014 b). Briefly, a loopful of *S. exfoliatus* MT9 culture was smeared onto the centre of M93 agar plates and incubated at 30ºC for 3 d to establish the culture on the agar surface. Then, seven days old fungal agar plugs (8.00 mm) were transferred onto the periphery of each agar plate at both sides which were previously smeared with the antagonist. The fungal culture grown on M93 agar plate without any biocontrol agent served as control. Plates were incubated at 30 °C, and examined for fungal growth inhibition after 14 day of paired incubation. Two replicates were examined for each experiment and Hi-media zone reader scale was used to measure the zone of inhibition (ZOI) around *S. exfoliatus* MT9 smear.

Extracellular fungal cell-wall lytic enzymes production : Seed culture was prepared in 20 ml of M93 broth medium containing (g l^{-1}) glucose 4.0, yeast extract 4.0, malt extract 10.0, pH 7.0±0.2. A loopful of vegetative cells of *S. exfoliatus* MT9 was used as inoculum to inoculate the seed medium and then incubated in shaker (180 rpm) at 30°C for 24 hr. For the production of fungal cell-wall lytic enzymes, 10% of seed culture (5.95×10^5 CFU ml^{-1}) was transferred to 200 ml production medium containing (g l^{-1}) K_2HPO_4 0.7, KH_2PO_4 0.3, $MgSO_4$ 0.5, $FeSO_4$ 0.01, $ZnSO_4$ 0.001, $MnSO_4$ 0.001, $(NH_4)_2SO_4$ 0.25 and yeast extract 1.0 supplemented with 0.5% (v/v) colloidal chitin and incubated in an orbital shaker (180 rpm) at 30ºC. Chitinase production and growth of *S. exfoliatus* MT9 was monitored up to 10 d at 24 hr interval. To collect the cell-free culture filtrates (CCF) for enzymatic activity, centrifugation was done at 10,000 rpm for 20 min at 4ºC. Viable cells present in the production medium were determined by serial dilution followed by plate count method.

Preparation of colloidal chitin : Five grams of commercial chitin (Sigma#C7170) was added to 50 ml of 85% phosphoric acid and kept in a refrigerator (4ºC) for 24 hr. Thereafter, phosphoric acid treated chitin was added to 1 l of ice chilled tap water under continuous stirring and afterwards the gelatinous white material formed was separated by filtration through filter paper. The retained cake was washed with tap water until the filtrate had pH 7.0. The colloidal chitin obtained had a soft, pasty consistency (Rojas Avelizapa *et al*., 1999).

Quantitative determination of cell-wall lytic enzymes : Enzyme assays for the production of cell-wall lytic enzymes were carried out using CCF as the source of chitinase, β-1,3 glucanase and protease. Chitinase activity was measured by colorimetric method of Miller, 1959. The reaction mixture (2.0 ml) contained 1 ml of diluted crude enzyme (CCF) with 1 ml of 1% (w/v) suspension of chitin (C9752, Sigma-Aldrich Co., USA) in 50 mM sodium phosphate buffer, pH 7.0. The reaction mixture was incubated at 37°C for 60 min. After that blank and sample tubes were centrifuged at 8,000 rpm for 10 min and then 1.5 ml of supernatant from each tube was collected and assayed for *N*-acetylglucosamine (GlcNAc). One unit of enzyme activity was defined as the amount of enzyme that catalyzed the release of 1 μmol of *N*-acetyl glucosamine per ml in 60 min.

The β-1,3 glucanase activity was determined by diluting crude enzyme in 50 mM sodium phosphate buffer, pH 7.0 and then measuring the reducing sugars (Miller, 1959) produced from laminarin (L9634, Sigma-Aldrich Co., USA) after 30 min of incubation at 37°C (Singh *et al*., 1999). One unit of enzyme activity was defined as the amount of enzyme that catalyzed the release of 1 μmol of glucose per ml in 60 min.

Protease activity in CCF was measured spectrophotometrically by the azocasein hydrolysis method (Secades and Guijarro, 1999). Briefly, 0.120 ml of crude enzyme was added to 0.480 ml of 1% (w/v) azocasein (A2765, Sigma-Aldrich Co., USA) in reaction buffer (phosphate buffer, pH 7.0) and the mixture was incubated at 37°C for 30 min. The reaction was terminated by adding 0.600 ml of 10% (w/v) trichloroacetic acid (TCA), and kept on ice for 30 min. Followed by separation of un-reacted azocasein precipitate by centrifugation at 15,000 rpm, 4°C for 10 min. Then 0.800 ml of the supernatant was mixed and neutralized by adding 0.200 ml of 1.8 N NaOH. Absorbance was read at 420 nm (A_{420}) using a spectrophotometer (U-2800, Hitachi High Technologies, Tokyo, Japan). One unit of enzyme activity was defined as the amount which yielded an increase in A_{420} of 0.01 in 30 min at 37°C. All the assays were performed in duplicates and control without enzyme was used as a blank.

***In vitro* antifungal activity of cell-free culture filtrate (CCF) :** The antifungal activity of CCF was estimated by "agar well diffusion" assay performed on PDA plates. Seven days old fungal agar plugs (8.0 mm) of *S. commune* ITCC 3751 and *P. placenta* MTCC 144 were placed at 1·5 cm from the edge of the petri dish and then 100 µl filter sterilized CCF was pipetted into the well at the centre of the plate. The inoculated plates were placed in an incubator at 30°C for 7 day. Simultaneously, CCF treated with proteinase K (0.1 mg ml^{-1}) (0706, Amresco, Solon, Ohio, USA) or boiled for 45 min was also tested. Zone of inhibition (mm) was measured on 7th day of incubation by using a zone reader scale (Hi-Media, Mumbai, India).

Production of extracellular antifungal secondary metabolite(s) : Cultivation of *S. exfoliatus* MT9 was carried out in M93 medium for monitoring the production of antifungal secondary metabolite(s). Seed culture was prepared by inoculating a loopful active culture of strain MT9 into M93 fermentation medium and incubated at 30ºC, 180 rpm for 24 hr. After incubation, 10% of seed culture (1.62×10^6 CFU ml^{-1}) was transferred into 125 ml production medium (same composition as seed medium) and incubated in an incubator shaker at 30ºC, 180 rpm and fermentation was carried out for 10 d. Antifungal metabolite(s) production and growth of strain MT9 was monitored at 1 day interval.

Extracellular culture filtrate (ECF) was collected by centrifugation at 8,000 rpm for 10 min at 4°C and filtered aseptically through a sterile membrane with 0.22-µm pore size and stored at 4°C. The antagonistic activity of ECF was checked by "cross plug" assay on PDA plates against *P. chrysosporium* MTCC 787 and *P. placenta* MTCC 144, as described earlier. Each day ECF (100µl) and heat treated samples of ECF were tested for their antifungal activity. Plates were incubated at 30ºC for 7 d. Zone of inhibition (mm) was observed by using a zone reader scale (Hi-Media, Mumbai, India).

Organic solvent extraction of antifungal metabolites from ECF : As maximum antifungal metabolite(s) production was observed on 3rd day of fermentation, so the fermentation was terminated on the 3rd day and ECF was collected by centrifugation at 10,000 rpm for 20 min at 4ºC. ECF was sequentially extracted using different organic solvents (*n*-hexane, diethyl ether, chloroform, ethyl acetate and *n*-butanol) in 1:1 proportion, with increasing order of polarity. All organic phases were collected and concentrated to dryness. All crude extracts were dissolved in methanol and assayed for antifungal activity against *P. chrysosporium* MTCC 787, *G. trabeum* MTCC 355, *P. placenta* MTCC 144 using methanol as control by "agar well diffusion" method.

Screening of polyene or non-polyene antifungal metabolite(s) : Crude antifungal metabolite(s) i.e. *n*-butanol extract was screened for the detection of polyene or non-polyene metabolite(s) via ergosterol inhibition assay, antibacterial activity and UV-Vis spectra (Nagpure *et al*., 2014 b).

Ergosterol inhibition assay : PDA plates were prepared with and without ergosterol (0.5%) and then used to perform the antifungal assay of *n*-butanol extract (100 µl) against *P. chrysosporium* MTCC 787 and *P. placenta* MTCC 144.

Antibacterial activity : Antibacterial activity of *n*-butanol extract was performed against *E. coli* MTCC 119 and *B. subtilis* MTCC 121, plates were incubated at 37°C for 24 hr and then zone of inhibition was observed.

UV-Vis spectra : UV absorption spectrum of *n*-butanol extract was recorded in the UV region (200-400 nm) by using a UV-Vis spectrophotometer.

Results and Discussion

Biological control of pathogenic fungi is generally attributed to the secretion of antifungal metabolites by antagonistic microorganisms. Extracellular production of antifungal metabolites is a characteristic feature in *Streptomyces* ecology and these metabolites can be from cell-wall lytic enzymes, siderophores and antibiotics (Yuan and Crawford, 1995; Macagnan *et al*., 2008; Prapagdee *et al*., 2008; Nagpure *et al*., 2014 b). To achieve our goal, a broad spectrum fungal antagonism of *S. exfoliatus* MT9 was evaluated against both white-rot and brown-rot fungi using "cross plug" assay. The actinomycete, *S. exfoliatus* MT9 showed strong antagonism (≥ 25 mm) (Fig. 1) against three wood-rot fungi namely, *G. trabeum* MTCC 355, *P. placenta* MTCC 144 and *P. agaricans* ITCC 761, while moderate

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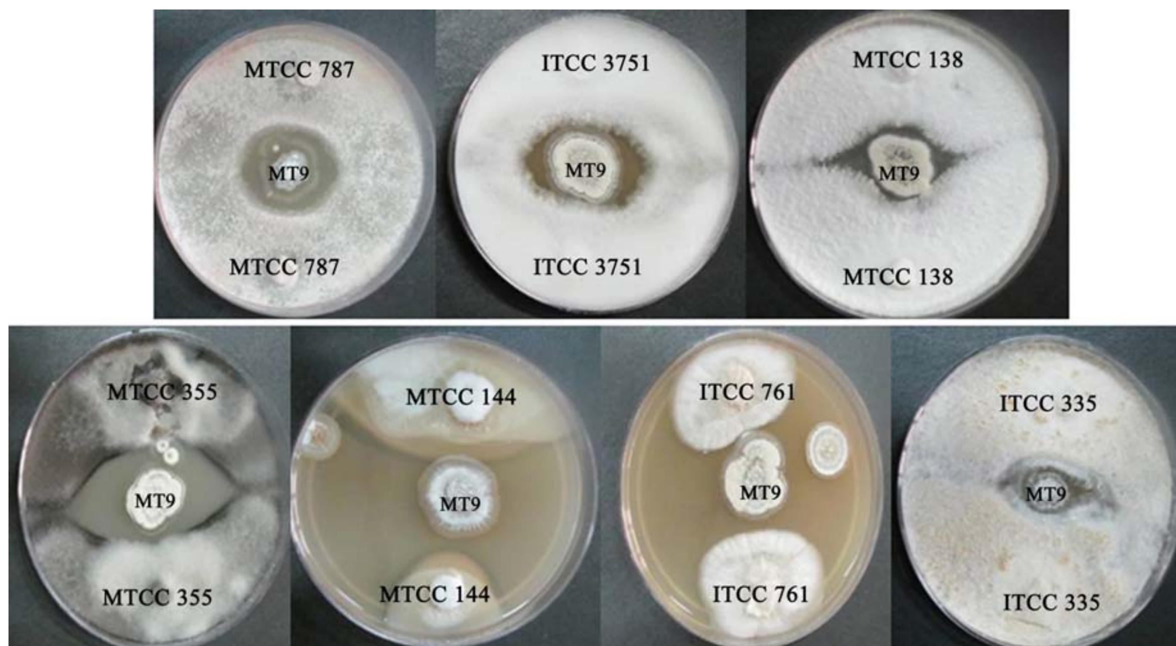

Fig. 1 : *In vitro* antagonism of *S. exfoliatus* MT9 against wood-rotting fungi. *P. chrysosporium* MTCC 787, *S. commune* ITCC 3751, *C. versicolor* MTCC 138, *G. trabeum* MTCC 355, *P. placenta* MTCC 144, *P. agaricans* ITCC 761, *P. friabilis* ITCC 335. Fungal mycelial growth inhibitory activity was observed on 14 days of paired incubation of "cross plug assay" plates which indicated that there was no direct contact between *S. exfoliatus* MT9 and inhibited fungi. This suggests that *S. exfoliatus* MT9 excreted diffusible antifungal metabolites in agar medium.

inhibition (≥15 mm) was observed towards *P. chrysosporium* MTCC 787, *S. commune* ITCC 3751, *C. versicolor* MTCC 138 and *P. friabilis* ITCC 335 after 14 day of paired incubation at 30°C (Table 1). Several previous reports of *Streptomyces* also suggest the release of antifungal metabolite(s) during paired incubation of antagonist and fungi (Thakur *et al*., 2007; Nagpure *et al*., 2014 b).

Microbial antagonism is often attributed to a combination of mechanisms by fungal cell-wall lytic enzymes, siderophores and antifungal secondary metabolites produced by antifungal actinomycetes (Macagnan *et al*., 2008). Fungal cell-wall is primarily composed of chitin, glucan and proteins, therefore the fungal cell-wall lytic enzymes such as chitinase, β-1,3 glucanase and protease may play a significant role in fungal antagonism by hydrolyzing the chitin, glucan and proteins present in the fungal cell-wall (Aktuganov *et al*., 2008; Choudhary *et al*., 2014). In general, higher chitinase activity can be correlated with higher fungal inhibition. Due to this reason, chitinolytic *Streptomyces* strains are a likely choice as potential biological control agents (Quecine *et al*., 2008). The production of extracellular cell-wall lytic enzyme i.e. chitinase was determined at different growth phases of antagonist *S. exfoliatus* MT9 in colloidal chitin medium. Chitinase activity was significantly detected in the cell free culture filtrate (CCF) after 1 d of incubation and progressively increased till the 6th day, after which it started decreasing. The level of chitinase was at peak (3.098 U ml^{-1}) when the cells entered the stationary phase and declined thereafter (Fig. 2). Co-production of other mycolytic enzymes such as β-1,3 glucanase (2.4 U ml^{-1}) and protease (144.0 U ml^{-1}) was also measured. *In vitro* antagonism of CCF was also performed against wood-rotting fungi namely *P. chrysosporium* MTCC 787 (12.0 mm) and *P. placenta* MTCC 144 (16.0 mm) (Table 2), whereas heat and proteinase K treated CCF did not exhibit any antagonism. The results of this study confirmed that the antifungal potential of CCF is due to heat labile proteins such as cell-wall lytic enzymes, namely chitinase, β-1,3 glucanase and protease. All these results were in accordance with the earlier studies (Bar-Shimon *et al*., 2004; Nagpure *et al*., 2014 c).

Filamentous actinomycetes bacteria especially *Streptomyces* are known producers of 75% of all known antibiotics (microbial secondary metabolites). Microbial secondary metabolites are low molecular mass products of secondary metabolism, usually produced during the late growth phase (idiophase) of microorganisms (Demain and Fang, 2000; Ruiz *et al*., 2010; Demain, 2014). Antifungal potential of antagonistic microorganisms is generally due to

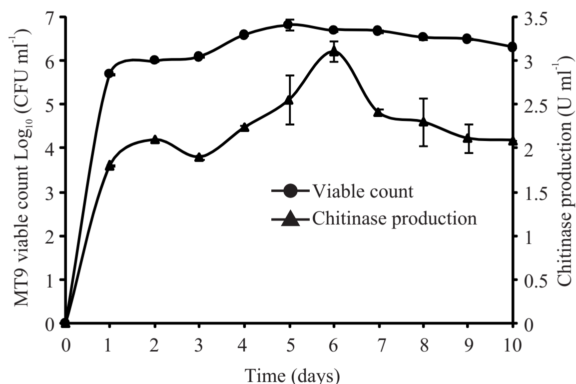

Fig. 2 : Time-course experiments related to growth of *S. exfoliatus* MT9 (●) and chitinase production (▲) in chitin enriched medium. At indicated times, whole cells culture fluid was collected for growth determination by serial dilution followed by plate count method (CFU ml^{-1}) at 1 day intervals along with chitinase production by DNS assay (Miller, 1959). Values presented are the mean ±SD of two independent experiments. One-way analysis of variance (ANOVA) was performed by Duncan's multiple range test using SigmaPlot 11.0 software (Sigma Plot Software; San Jose, California, USA) at $p < 0.05$. Mean values ($p < 0.05$) were considered statistically significant

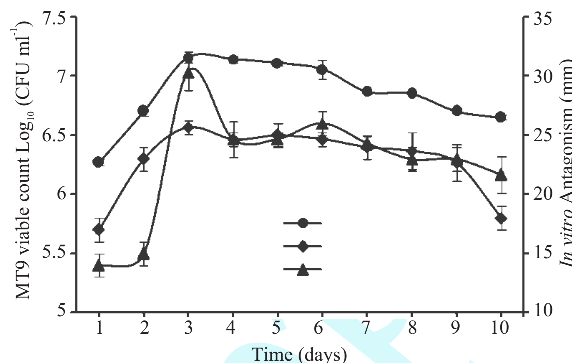

Fig. 3 : Time-course experiments related to growth (●) of *S. exfoliatus* MT9 in M93 medium and antifungal activity of extracellular culture filtrate (ECF) towards *P. chrysosporium* MTCC 787 (♦) and *P. placenta* MTCC 144 (▲). At indicated time, *S. exfoliatus* MT9 growth was monitored by measuring colony forming units (CFU ml^{-1}) at 1 day intervals up to 10 days of incubation along with antifungal activity of extracellular culture filtrate. Values presented are the mean ± SD of two independent experiments; One-way analysis of variance (ANOVA) was performed by Duncan's multiple range test using SigmaPlot 11.0 software (SigmaPlot Software; San Jose, California, USA) at $p < 0.05$. Mean values ($p < 0.05$) were considered statistically significant

Table 1 : Antagonistic activity of *Streptomyces exfoliatus* MT9 against wood-rotting fungi

Wood-rotting fungi	Zone of inhibition (mm)[a]
P. chrysosporium MTCC 787	23.5±0.7
S. commune ITCC 3751	23.5±0.7
C. versicolor MTCC 138	17.0±1.4
G. trabeum MTCC 355	26.0±2.8
P. placenta MTCC 144	26.0±2.8
P. agaricans ITCC 761	25.5±2.1
P. friabilis ITCC 335	16.5±0.7

In vitro antagonism was performed using "Cross plug assay" against wood-rotting fungi. The results are means of three replicates for each fungus; [a]Rating: Strong inhibition = ≥ 25 mm; Moderate inhibition = ≥ 15 mm; Weak inhibition = < 10 mm; One-way analysis of variance (ANOVA) was performed by Duncan's multiple range test using SigmaPlot 11.0 software (Sigma Plot Software; San Jose, California, USA) at $p < 0.05$. Mean values ($p < 0.05$) were considered statistically significant

Table 2 : Antifungal activity of CCF, ECF and *n*-butanol extract against wood-rotting fungi

Wood-rotting fungi	Zone of Inhibition (mm)		
	CCF	ECF	*n*-Butanol ex.
P. chrysosporium MTCC 787	12±0.0	26±1.0	33±1.0
P. placenta MTCC 144	16±0.0	30±1.0	28±1.0

CCF = Cell-free culture filtrate; ECF = Extracellular culture filtrate; Antifungal activity of CCF, ECF and *n*-butanol extract was tested against wood-rotting fungi using "agar well diffusion" method; The results are means of two replicates for each fungus; Rating: Strong inhibition = ≥ 25mm; Moderate inhibition = ≥ 15mm; Weak inhibition = < 10mm; One-way analysis of variance (ANOVA) was performed by Duncan's multiple range test using Sigma Plot 11.0 software (Sigma Plot Software; San Jose, California, USA) at $p < 0.05$. Mean values were not significantly different

the production of secondary metabolite(s) (Shobha and Onkarappa, 2011; Nagpure *et al*., 2014 b). In order to investigate the production of antifungal secondary metabolite(s), *S. exfoliatus* MT9 was cultivated in M93 broth medium for 10 day at 30°C. Extracellular antifungal secondary metabolite(s) production was determined at different growth phases. Time-course antagonistic activity of 10 day sample of extracellular culture filtrate (ECF) of *S. exfoliatus* MT9 was observed against *P. chrysosporium* MTCC 787 and *P. placenta* MTCC 144. The maximum antifungal metabolite(s) production was achieved in the early stationary phase (3rd day) (Fig. 3). *In vitro* antifungal activity of metabolite(s) present in ECF suggested that *S. exfoliatus* MT9 produces diffusible and extracellular antifungal metabolite(s) (Table 2). Moderate antagonism was also shown by heat treated sample (used as control) depicting the heat-stable nature of metabolite (s).

Organic solvents with different polarities were used to extract the antifungal metabolite(s) from ECF of strain MT9. All the organic extracts were tested against wood-rotting fungi, among them *n*-hexane, diethyl ether, chloroform and ethyl acetate were inappropriate solvents for

extracting antifungal metabolite(s) as they did not exhibit antifungal activity towards any of the tested fungi whereas *n*-butanol extract showed strong and broad spectrum inhibitory effect against *P. chrysosporium* MTCC 787 (33±1.0) and *P. placenta* MTCC 144 (28±1.0) (Table 2). Hence, *n*-butanol can be used as an efficient solvent system for complete extraction of antifungal secondary metabolite(s) from the ECF because no antifungal activity was exhibited by other organic solvent extracts and aqueous layer. Solvent extraction is usually employed for the extraction of antibiotics from the culture filtrates and it was found that most of antibiotics produced by microorganisms are extracellular (Augustine *et al*., 2005; Hacene *et al*., 2000), which might also be the case in the present study.

Ergosterol plate assay, antibacterial assay and UV spectra analysis revealed that *n*-butanol extract of ECF contains polyene antifungal metabolite(s). Polyene antibiotic like amphotericin B, binds ergosterol present in the fungal cell-wall and opens channels in the cell membrane that causes leakage of cellular components with subsequent fungal cell death. *n*-Butanol extract (100 µl) showed an inhibition zone of 22.0 mm against *P. chrysosporium* MTCC 787 and 18.0 mm against *P. placenta* MTCC 144 on PDA plate without ergosterol whereas the same amount of *n*-butanol extract showed reduced zone of inhibition towards tested fungi i.e. *P. chrysosporium* MTCC 787 (11.0 mm), and *P. placenta* MTCC 144 (10.0 mm) on PDA plates containing the reversal agent i.e. ergosterol (0.5%). In addition, *n*-butanol extract did not show antibacterial activity towards bacterial strains, *E. coli* MTCC 119 and *B. subtilis* MTCC 121 (cell-membrane without sterols). UV spectral analysis of *n*-butanol extract resulted in characteristic maximum absorbance peak at 224 nm, confirming the secretion of polyene group of antifungal metabolite(s) by *S. exfoliatus* MT9. Polyene nature of antifungal secondary metabolite (s) was confirmed by reduced inhibition zone on ergosterol plate, no antibacterial activity and characteristic UV spectra between 215 and 270 nm (Ilic *et al*., 2005; Thakur *et al*., 2007; Nagpure *et al*., 2014 b).

The soil isolate, antagonist *S. exfoliatus* MT9 showed broad spectrum inhibitory effect on mycelial growth of various wood-rotting fungi. It secretes antifungal metabolites i.e. hydrolytic enzymes and polyene group of antibiotic(s). Therefore, *S. exfoliatus* MT9 and its metabolites could be well suited for biological control of wood-rotting fungi. Furthermore, feasibility can be checked by performing field tests.

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