Advanced Journal of Microbiology Research Volume 2014
Available online at http://advancedresearchjournals.org/AJMR

Full Length Research Paper

Antimicrobial activity of the new endophytic *Monodictys castaneae* SVJM139 pigment and its optimization

S. Visalakchi* and J. Muthumary

Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai - 600 025, Tamil Nadu, India.

Accepted 12 August, 2013

The present research work was aimed to screen the endophytic fungal pigment against human pathogenic bacteria and optimize the most suitable medium with specific pH, temperature and elicitors to improve the growth and antimicrobial pigment production. Based on the morphological characters, the isolated strain SVJM139 was identified as *Monodictys castaneae* SVJM139. Among 13 media tested (Table 1), Czapek yeast extract agar/Czapek yeast extract broth (CYA/CYB) was the best suitable medium. Optimized CYA/CYB with specific pH (5), temperature (24°C) and silver nitrate (15 mg/l) strongly influenced the highest fresh weight (23.7 ± 0.4^{a} g/l), dry weight (3.7 ± 0.0^{a} g/l), radial growth (8.9 ± 0.1^{a} cm) and pigment production (193 ± 2.2^{a} µg/g) on 18th day when compared to normal CYA/CYB. Antimicrobial activity of the *M. castaneae* SVJM139 pigments (75 µg/ml) significantly inhibited the growth of human pathogenic bacteria viz., *Staphylococcus aureus* (21.4 ± 0.34^{a} mm), *Klebsiella pneumoniae* (20.0 ± 0.32^{a} mm), *Salmonella typhi* (20.3 ±0.32^{a} mm) and *Vibrio cholerae* (20.7 ± 0.33^{a} mm). The present findings concluded that *M. castaneae* SVJM139 pigment was clearly more active than the commercial antibiotic, streptomycin. Our literature survey suggests that this is the first report of the new endophytic *M. castaneae* SVJM139 pigment with anti-microbial activity against human pathogens.

Key words: Antimicrobial activity, fungal pigment, *Monodictys castaneae,* optimization.

INTRODUCTION

Endophytic microorganisms colonize living, internal tissues of the plants without causing any immediate, overt negative effects (Bacon and White, 2000; Wasser, 2002). Endophytes have proved to be the promising sources of biologically active products which are of interest for specific medicinal applications (Strobel, 2002). Recent investigations have been intensified by the potentialities of endophytic fungal strains in the production of pigments, bioactive metabolites, immune-suppressants, anticancer compounds and biocontrol agents (Wang et al., 2002; Stinson et al., 2003; Strobel and Bryn, 2003; Selvin et al., 2004; Strobel et al., 2004; Gangadevi and Muthumary, 2007) . Presently, our research groups have identified more than hundreds of endophytic isolates from South Indian medicinal plants that showed promising activity against anti-tumour and antimicrobial agents (Gangadevi and Muthumary, 2007, 2009).

*Corresponding author. E-mail: svisalakchi@gmail.com. Tel: +91-9841585715 (O). Fax: +91-44-22352393.

The development of drug resistance in human pathogenic bacteria has prompted a search for more and better antibiotics, especially as diseases caused by pathogenic microorganisms now represent a clear and growing threat to world health (Raviglione et al., 1995; Pablosmendez et al., 1997). The increase of microbial resistance to antibiotics threatens public health on a global scale as it reduces the effectiveness of treatments and increases morbidity, mortality and health care costs (Coast et al., 1996). Evolution of highly resistant bacterial strains has compromised the use of newer generations of antibiotics (Levy, 2002; Levy and Marshall, 2004). Although the active constituents may occur in lower concentrations, endophytic fungal pigments may be a better source of antimicrobial compounds than synthetic drugs. Therefore, the investigations of the antimicrobial activity of natural products have opened new ways for drug development in the control of antibiotic resistant pathogens. The researchers are currently paying more attention to the drug development from the endophytic fungi isolated from medicinal plants (Tan and Zou, 2001) in Asia.

Several microorganisms such as *Monascus*, *Peacilomyces,*

Serratia, Cordyceps, Streptomyces and *Penicillium* have the ability to produced pigments in high yield (Hajjaj et al., 2000; Sameer et al., 2006) which have been developed as a drug and used to treat the wound infections and skin diseases caused by the pathogens. Many of the endophytic fungal strains have attracted special attention because they have the capability of producing different coloured pigments with high chemical stability. Recently, the industrial and biotechnological approaches are mainly focused on fungal growth and their pigments with higher production are essential features. Some parameters like pH, temperature, incubation period, carbon and nitrogen sources and amino acid plays a major role on production of bioactive compounds and antimicrobial agents (Lethimaki et al., 1997; Cho et al., 2002; Adinarayana et al., 2003; Vahidi et al., 2004, Gunasekaran et al., 2008). Therefore, the present investigation was aimed to study the *Monodictys castaneae* SVJM139 pigment against human pathogenic bacteria under *in vitro* conditions and the antimicrobial pigment optimized in CYA/CYB with specific pH, temperature and elicitors.

MATERIALS AND METHODS

Isolation and identification of endophytic fungi

Endophytic fungal strain was isolated from *Cedrus* sp. (medicinal plant), collected from Kodaikanal hills, Tamil Nadu, India. The leaf was sterilized according to the method of Dobranic et al. (1995) and the segments were evenly placed in Petri dishes containing potato dextrose agar (PDA) medium amended with streptomycin (40 µg/l). Endophytic fungal growth was monitored every day and sub-cultured onto PDA and brought into pure culture for further study. The isolated endophytic fungus was identified using standard monographs and deposited with the access code number SVJM139 in Madras University Botany Laboratory (MUBL), Centre for Advanced Studies (CAS) in Botany, University of Madras, Chennai – 600025, India.

Microorganism and their growth conditions

M. castaneae SVJM139 fungal plugs (5 mm in diameter) were removed from the PDA plates with the help of sterilized metal cork borer and transferred to fresh Petri dishes containing 13 different agar media (Table 1) and were incubated at 28 ± 2°C. These agar plates were used to measure the diameter of radial mycelial growth in two directions at 90°C angles. Results of the final radial growth values were expressed as centimeter (cm) per day. Five replications for each medium were maintained in this present study.

Estimation of *M. castaneae* SVJM139 growth and pigment production

M. castaneae SVJM139 (5 mm diameter size of 5 disks for each flask) culture was inoculated in 13 different liquid growth promoting media and incubated at 28 ± 2°C for 18 days in resting condition. Experiments were carried out with aliquots of 200 ml culture medium in 500 ml Erlenmeyer flasks. The mycelium was harvested on 18 days and it was filtered through pre-weighed Whatman No. 1 paper and dried at 80°C for 48 h in an oven, after which the dry mycelial weight (DMW) was measured and its growth was expressed as gram/l. The quantitative determination of pigment production was studied with standard procedure of Nicolaus et al. (1964) . The experiments were performed twice with five replicates and the results are tabulated in the present study.

Antimicrobial activity of *M. castaneae* SVJM139 pigment

M. castaneae SVJM139 pigment screened against human pathogenic bacteria [both Gram (+)ve & (-)ve] *viz*., *Staphylococcus aureus* (MTCC737), *Klebsiella pneumoniae* (MTCC109), *Salmonella typhi (*MTCC531) and *Vibrio chlorae* (MTCC5108). About 1 ml of the inoculum of the test pathogen was spread in to Müller Hinton Agar plates. A 5 mm well was made in each corner of the plate with equal distance using a sterile cork borer. Filter sterilized (0.25 µm) aqueous extract of *M. castaneae* SVJM139 pigment with different concentrations at 25, 50 and 75 µg/ml compared with standard antibiotic, streptomycin (10 µg/ml) were tested. 50 µl of each concentration of pigment and antibiotic were placed in their respective well and the plates were incubated at 37°C for 48 h. Sterile water was used as a control. Each bacterial strain was tested against *M. castaneae* SVJM139 pigment with five replications in this study. After the incubation, the inhibition zone (minimal inhibitory concentration) around the well was recorded and expressed as millimeter (mm).

Effect of different pH, temperature and specific elicitors on *M. castaneae* SVJM139

According to the results shown in Table 1, the CYA/CYB was selected for the optimization study. *M. castaneae* SVJM139 cultured on CYA/CYB with different pH range from 2.0 to 10.0 (at pH=1 intervals), temperatures at 4, 10, 17, 24 and 37°C and six different elicitors such as ammonium citrate, cobalt chloride, ethylene, phenylalanine, silver nitrate and sucrose with different concentrations at 5, 10, 15 and 20 mg/l for 18 days were studied individually to improve their growth and pigment production.

Estimation of *M. castaneae* SVJM139 growth and pigment production on optimized CYA/CYB

M. castaneae SVJM139 was inoculated in optimized CYA/CYB containing specific pH (5), temperature (24°C) and elicitor (15 mg/l of silver nitrate) and maintained for 18 days for the estimation of their growth and pigment production.

Statistical analysis

All the reported values for growth and pigment production are the means of five replications. One-way and two-way (ANOVA) of Dunkan's test was performed to analyze the growth and pigments. All the values were significant at P=0.05 and standard deviation (±) and standard errors (SE) are presented in all tables, the confidence level up to 98%.

RESULTS AND DISCUSSION

The endophytic fungal strain initially appeared as pale yellow on PDA medium and it's turned to reddish brown. Based on the morphological characteristics of the macroscopic and microscopic observations of the SVJM139 isolate shows that the conidia are oblong, rounded at the ends, pyriform, clavate, sub spherical or irregular, usually

Table 1. Growth characteristics of *M. castaneae* SVJM139 on different fungal growth media.

Media	Fresh weight (g/l)	Dry weight (g/l)	Pigment production (µg/g)	Radial growth (cm)
PDB	18.2 ± 0.29^{b}	1.6 ± 0.02^{b}	123 ± 1.9^{b}	7.1 ± 0.11^{b}
PCB	12.4 ± 0.20^{f}	0.82 ± 0.01^{d}	79 ± 1.3^{f}	6.4 ± 0.10^{e}
SDB	11.9 ± 0.19^{g}	0.8 ± 0.01^{d}	68 ± 1.1^{h}	6.8 ± 0.11^{cd}
CDB	16.4 ± 0.26^{d}	0.88 ± 0.01^{d}	117 ± 1.9^{c}	6.2 ± 0.10^{ef}
OMB	17.2 ± 0.27^{c}	1.4 ± 0.02^{c}	106 ± 1.7^{d}	5.9 ± 0.09^{gh}
MEB	13.4 ± 0.21^{e}	0.78 ± 0.01^{d}	92 ± 1.5^{e}	6.0 ± 0.09^{fg}
MID	10.9 ± 0.17^{h}	0.69 ± 0.01^{e}	54 ± 0.9^{i}	5.7 ± 0.09^{h}
CYB	18.9 ± 0.30^{a}	2.9 ± 0.03^{a}	139 ± 2.2^{a}	7.5 ± 0.12^{a}
M1	8.2 ± 0.13^{i}	0.48 ± 0.01^{f}	56 ± 0.9^{i}	7.0 ± 0.11^{bc}
M4	6.4 ± 0.10^{j}	0.46 ± 0.01^{f}	48 ± 0.8^{j}	6.9 ± 0.11^{bcd}
M8	5.7 ± 0.09^{k}	0.38 ± 0.01^{h}	42 ± 0.7^{k}	6.9 ± 0.11^{bcd}
M9	11.8 ± 0.19^{g}	0.76 ± 0.01^{d}	75 ± 1.2^{g}	6.8 ± 0.11^{cd}
M11	12.1 ± 0.19^{fg}	0.8 ± 0.01^{d}	76 ± 1.2^{fg}	6.7 ± 0.11^{d}

*__PDB__; Potato dextrose broth, __PCB__; Potato carrot broth, __SDB__; Sabouraud dextrose broth, __CDB__; Czapex-dox broth, __OMB__; Oat meal broth; __MEB__; Malt extract broth, __MID__; Modified liquid medium, __CYB__; Czapex yeast extract broth, __M1__; Media- 1, __M4__, Media- 4, __M8__; Media- 8, __M9__; Media- 9, __M11__; Media- 11. The radial growth was measured on their agar medium. All the values of fresh and dry weights, pigment production and radial growth were analysed by one-way Dunkan's test with 5% intervals. ± Standard deviation and error of the values were calculated with five replications.

verrucose, brown, basal cells sometimes paler than the others, 14 - 40 × 10 - 25 µm and the endophytic isolate SVJM139 was identified as *M. castaneae* SVJM139 in the present study.

Statistical analysis of the results clearly indicated that the CYA/CYB significantly increased the *M. castaneae* SVJM139 radial growth, fresh and dry mycelial weights and pigment production. Among the 13 media tested, CYA/CYB exhibited the significant increases of radial growth (7.5 ± 0.12^{a} cm) on agar medium, 18.9 ± 0.30^{a} g/l of fresh mycelial weight, 2.9 ± 0.03^{a} g/l of dry mycelial weight and 139 ± 2.2^{a} µg/g of pigment production. Following CYA/CYB, these parameters were relatively high on PDA/PDB (Table 1), the PDA was then used for the isolation of *M. castaneae* SVJM139. Among the different media studied, CYA/CYB was the best one for further optimization study. Our present findings are strongly agreed with the previous reports (Tuttobello et al., 1969; Kim et al., 1995; Cho et al., 2002; Gunasekaran et al., 2008), they suggested that the uses of different substrate(s) in growth medium might improve the fungal growth and other parameters.

Interestingly, the fungal pigment of *M. castaneae* SVJM139 exerted different levels of inhibitory effects against four human pathogenic bacteria. The *M. castaneae* at 75 µg/ml, the per cent increases of the inhibition zone represents the 42.7% of *S. aureus* (21.4 ± 0.34^{a} mm), 66.7% of *K. pneumonia* (20.0 ± 0.32^{a} mm), 35.3% of *S. typhi* (20.3 ± 0.32^{a} mm) and 59.2% of *V. cholerae* (20.7 ± 0.33^{a} mm), compared to antibiotic streptomycin. Moreover, the pigment was found to be more effective than commercial antibiotic, streptomycin, *S. aureus* (15 mm), *K. pneumoniae* (12 mm), *S. typhi* (18 mm) and *V. cholerae* (13 mm)) when tested at different concentrations (Table 2). These results suggest *M. castaneae* SVJM139 pig-ment might be developed as antibiotic drug. However, further studies are required to determine their potential against a wide range of human pathogens and its mode of action. Many studies on isolation and characterization of endophytic diversity from different medicinal plant species have been well established by Johri (2006). Records on antimicrobial activity of endophytes isolated from different gymnosperms of North East India are available (Bala et al., 1999; Saikia et al., 2005). Valan et al. (2009) reported that the strain of *Streptomyces* spp. ERI3 effec-tively inhibited human as well as phytopathogens under *in vitro* conditions. To our knowledge, this is the first report on the antimicrobial activity of *M. castaneae* SVJM139 pigment and it suggests that the potential therapeutic drug could be developed in future to treat human pathogenic bacteria.

The pH of culture medium is one of the determinant factors for the microbial growth, pigment production and biosynthesis of secondary metabolites. *M. castaneae* SVJM139 was cultivated at different initial pH values (2.0 to 10.0) in static conditions. Among the different pH tested, the initial pH 5.0 was found to be very suitable for the maximum fungal radial growth (8.2 ± 0.13^{a} cm), fresh (19.6 ± 0.29^{a} g/l) and dry weights (3.2 ± 0.05^{a} g/l) and pigment production (140 ± 2.1^{a} µg/g). The pH range (5) had a modulating effect on the highest biomass and pig-ment production of *M. castaneae* SVJM139 (Table 3). The pigment production was vigorous at acidic pH and the acti-

Table 2. Antimicrobial activity of *M. castaneae* SVJM139 pigment against human pathogenic bacteria.

Bacteria	Inhibition zone at different concentration (mm)			Streptomycin
	25 µg/ml (% increase)	50 µg/ml (% increase)	75 µg/ml (% increase)	10 µg/ml (Standard antibiotic)
Staphylococcus aureus	17.0 ± 0.27^{c} (13.3)	19.1 ± 0.30^{b} (27.3)	21.4 ± 0.34^{a} (42.7)	15.0
Kelbsiella pneumoniae	15.3 ± 0.24^{c} (27.5)	18.4 ± 0.29^{b} (53.3)	20.0 ± 0.32^{a} (66.7)	12.0
Salmonella typhi	15.8 ± 0.25^{c} (5.3)	17.6 ± 0.28^{b} (17.3)	20.3 ± 0.32^{a} (35.3)	15.0
Vibrio chlorea	18.2 ± 0.29^{c} (40.0)	19.4 ± 0.31^{b} (49.2)	20.7 ± 0.33^{a} (59.2)	13.0

*All the inhibition values of pathogenic bacteria were analysed by one-way Dunkan's test at 5% intervals.
± Standard deviation and error of the values were calculated with five replications

Table 3. Optimization of different pH on selective CYA/CYB for *M. castaneae* SVJM139.

pH	Fresh weight (g/l)	Dry weight (g/l)	Pigment production (µg/g)	Radial growth (cm)
2	5.3 ± 0.08^{g}	0.4 ± 0.01^{g}	0 ± 0.0^{h}	0 ± 0.00^{g}
3	11.7 ± 0.18^{c}	1.6 ± 0.03^{f}	48 ± 0.8^{f}	4.6 ± 0.07^{e}
4	11.7 ± 0.18^{c}	2.5 ± 0.04^{c}	117 ± 1.9^{c}	7.0 ± 0.11^{c}
5	19.6 ± 0.29^{a}	3.2 ± 0.05^{a}	140 ± 2.1^{a}	8.2 ± 0.13^{a}
6	13.3 ± 0.21^{b}	2.6 ± 0.04^{c}	124 ± 2.0^{b}	7.8 ± 0.12^{b}
7	10.6 ± 0.17^{d}	2.7 ± 0.04^{b}	110 ± 1.7^{d}	6.9 ± 0.11^{c}
8	13.6 ± 0.22^{b}	2.5 ± 0.04^{c}	92 ± 1.5^{e}	5.4 ± 0.09^{d}
9	9.6 ± 0.15^{e}	2.4 ± 0.04^{d}	48 ± 0.8^{f}	4.4 ± 0.07^{ef}
10	6.1 ± 0.10^{f}	2.3 ± 0.04^{e}	45 ± 0.7^{g}	4.2 ± 0.07^{f}

*The values of fresh weight, dry weight, pigment production and radial growth on CYA/CYB with different pH were analysed by one-way Dunkan's test with 5% intervals. ± Standard deviation and error of the values were calculated with five replications.

activity decreased as the pH approached to alkaline range. The present findings clearly indicated that the growth parameters were slightly affected when the growth medium pH changes. Sabu et al. (2005) reported that the enzymes, pigments, antifungal substances and other fungal metabolites are very sensitive to changes of pH and they work better over a very short range, with a definite optimum pH. A shift in the pH of the growth medium might require a corresponding change in temperature to get maximum biomass production.

Similarly, *M. castaneae* SVJM139 was cultivated at different temperatures in CYA/CYB. Consequently, the maximum growth and pigment production were recorded at 24°C compared to other temperature tested. The present results registered 8.4 ± 0.13^{a} cm of radial growth, 21.0 ± 0.30^{a} g/l of fresh and 3.4 ± 0.05^{a} g/l of dry mycelial weights and 153 ± 2.1^{a} µg/g of pigment production on CYA/CYB (Table 4). Present investigation strongly proved that the optimized temperature (24°C) be favorable to growth and pigment production of *M. castaneae* SVJM139 and the high and low temperatures may stop the cell func-tion of the fungus. Similarly, our findings strongly agreed with the previous reports of Huang et al. (2001), who founded high production of the antifungal and anti-tumour agents from endophytic fungi at 25°C and 7 days incubation.

The optimized medium of CYA/CYB at the specific pH (5) and temperature (24 °C) strongly influenced the growth and pigment production of *M. castaneae* SVJM139. Significant differences in the growth parameters and pigment production in liquid and solid media were observed in the present study. The selective pH and temperature greatly increased the biomass production of *M. castaneae* SVJM139 (Tables 3 and 4). Therefore, the specific pH (5) and temperature (24°C) were taken together to design the selective CYA/CYB growth medium. Additionally, the different carbon and nitrogen sources were studied for the improvement of pigments production in *M. castaneae* SVJM139 (data not shown). Besides, the physio-chemical properties of *M. castaneae* SVJM139 pigments and stability test (heat, light and chemical) were studied and cently reported. According to these results, the new methods were developed for identifying natural dyes from fungal pigments and these fungal will be tested for the coloring process for foods, feeds and textiles industries. In addition, *M. castaneae* SVJM139 pigments will be in future characterized through analytical methods (UV, IR, MS and NMR) for further confirmation.

Among different elicitors tested, silver nitrate (15 mg/l)

Table 4. Optimization of different temperatures on CYA/CYB for *M. castaneae* SVJM139.

Temperature (°C)	Fresh weight (g/l)	Dry weight (g/l)	Pigment production (µg/g)	Radial growth (cm)
4	2.6 ± 0.04^{e}	0.4 ± 0.01^{e}	13 ± 0.2^{e}	0.0 ± 0.00^{e}
10	3.8 ± 0.06^{d}	0.7 ± 0.01^{d}	43 ± 0.7^{d}	5.2 ± 0.8^{d}
17	11.4 ± 0.18^{b}	1.8 ± 0.03^{b}	106 ± 1.7^{c}	7.0 ± 0.11^{c}
24	21.0 ± 0.30^{a}	3.4 ± 0.05^{a}	153 ± 2.1^{a}	8.4 ± 0.13^{a}
37	8.9 ± 0.14^{c}	1.3 ± 0.02^{c}	122 ± 1.9^{b}	7.9 ± 0.12^{b}

*All the values of fresh weight, dry weight, pigment production and radial growth were analysed by one-way Dunkan's test with 5% intervals.

± Standard deviation and error of the values were calculated with five replications.

Table 5. Effect of different elicitors on *M. castaneae* SVJM139 growth and pigment production in CYA/CYB.

Fungal elicitors	Fresh weight (g/l)	Dry weight (g/l)	Pigment production (µg/g)	Radial growth (cm)
Ammonium citrate (mg/l)				
5	18.5 ± 0.3^{f}	2.7 ± 0.0^{i}	122 ± 1.9^{k}	6.9 ± 0.1^{cd}
10	18.7 ± 0.3^{f}	2.8 ± 0.0^{g}	127 ± 2.0^{ijk}	7.0 ± 0.1^{c}
15	19.1 ± 0.3^{e}	2.9 ± 0.0^{g}	142 ± 2.3^{de}	7.3 ± 0.1^{c}
20	19.3 ± 0.3^{e}	3.0 ± 0.0^{f}	151 ± 2.4^{ab}	7.4 ± 0.1^{c}
Cobalt chloride (mg/l)				
5	18.0 ± 0.3^{g}	2.4 ± 0.0^{k}	132 ± 2.1^{ghi}	6.5 ± 0.1^{e}
10	18.3 ± 0.3^{f}	2.5 ± 0.0^{j}	135 ± 2.1^{fg}	6.8 ± 0.1^{cd}
15	18.7 ± 0.3^{f}	2.7 ± 0.0^{i}	138 ± 2.2^{ef}	7.0 ± 0.1^{c}
20	19.8 ± 0.3^{d}	3.5 ± 0.0^{b}	146 ± 2.3^{bcd}	7.8 ± 0.1^{b}
Ethylene (mg/l)				
5	19.1 ± 0.3^{e}	2.7 ± 0.0^{i}	138 ± 2.2^{ef}	7.2 ± 0.1^{c}
10	19.4 ± 0.3^{e}	2.9 ± 0.0^{g}	142 ± 2.3^{de}	7.3 ± 0.1^{c}
15	19.7 ± 0.3^{d}	3.1 ± 0.0^{e}	145 ± 2.3^{cd}	7.4 ± 0.1^{c}
20	20.0 ± 0.3^{d}	3.2 ± 0.0^{d}	149 ± 2.4^{bc}	7.5 ± 0.1^{c}
Phenylalanine (mg/l)				
5	17.3 ± 0.3^{h}	2.8 ± 0.0^{h}	129 ± 2.0^{hij}	6.4 ± 0.1^{ef}
10	18.0 ± 0.3^{g}	2.9 ± 0.0^{g}	134 ± 2.1^{fgh}	6.8 ± 0.1^{cd}
15	18.4 ± 0.3^{f}	3.0 ± 0.0^{f}	137 ± 2.7^{efg}	7.0 ± 0.1^{c}
20	19.2 ± 0.3^{e}	3.1 ± 0.0^{e}	138 ± 2.2^{ef}	7.3 ± 0.1^{c}
Silver nitrate (mg/l)				
5	20.2 ± 0.3^{d}	2.9 ± 0.0^{g}	125 ± 2.0^{jk}	8.0 ± 0.1^{b}
10	21.6 ± 0.3^{c}	3.0 ± 0.0^{f}	136 ± 2.2^{fg}	8.1 ± 0.1^{b}
15	23.2 ± 0.4^{a}	3.6 ± 0.1^{a}	166 ± 2.5^{a}	8.8 ± 0.1^{a}
20	22.1 ± 0.5^{b}	3.2 ± 0.0^{d}	145 ± 2.3^{cd}	8.3 ± 0.1^{b}
Sucrose (mg/l)				
5	17.9 ± 0.3^{g}	2.8 ± 0.0^{g}	123 ± 1.9^{k}	6.7 ± 0.1^{cd}
10	18.5 ± 0.3^{f}	3.0 ± 0.0^{f}	134 ± 2.1^{fgh}	7.0 ± 0.1^{c}
15	19.0 ± 0.3^{e}	3.3 ± 0.0^{c}	142 ± 2.3^{de}	7.2 ± 0.1^{c}
20	19.4 ± 0.3^{e}	3.5 ± 0.0^{b}	145 ± 2.3^{cd}	7.4 ± 0.1^{c}

*All the fresh and dry mycelia weights, pigment production and radial growth of *M. castaneae* SVJM139 were analysed by two-way Dunkan's test at 5% intervals. ± Standard deviation and error of the values were calculated with five replications.

Table 6. Effects on specific pH (5), temperature (24°C) and fungal elicitors (silver nitrate 15 mg/l) on the growth and pigment production by *M. castaneae* SVJM139 in CYA/CYB.

Component	Number of days					
	3	6	9	12	15	18 (% increases)
Fresh weight (g/l)	6.2 ± 0.10^{f}	11.8 ± 0.2^{e}	16.4 ± 0.26^{d}	17.8 ± 0.3^{c}	20.9 ± 0.3^{b}	23.7 ± 0.4^{a} (18.9)
Dry weight (g/l)	0.7 ± 0.01^{e}	1.7 ± 0.0^{e}	2.1 ± 0.02^{d}	2.2 ± 0.0^{c}	2.6 ± 0.0^{b}	3.7 ± 0.0^{a} (2.9)
Pigment production (µg/g)	84.0 ± 1.3^{e}	97.0 ± 1.5^{d}	112.0 ± 0.10^{f}	124.0 ± 2.0^{c}	135 ± 2.1^{b}	193 ± 2.2^{a} (139)
Radial growth (cm)	2.3 ± 0.0^{f}	4.7 ± 0.1^{e}	6.8 ± 0.11^{d}	7.6 ± 0.1^{c}	8.3 ± 0.1^{b}	8.9 ± 0.1^{a} (7.5)

*All the values fresh & dry weight, pigment production and radial growth of *M. castaneae* SVJM139 on CYA/CYB at different days intervals were analysed by one-way Dunkan's test at 5% intervals.
± Standard deviation and error of the values were calculated with five replications.

significantly increases in the radial growth (8.8 ± 0.1^{a} cm), and the fresh (23.2 ± 0.4^{a} g/l) and dry mycelial weights (3.6 ± 0.1^{a} g/l) and the maximum pigment production (166 ± 2.5^{a} µg/g) of *M. castaneae* SVJM139 were recorded (Table 5). Further, the present study requires to establish the specific elicitors for the improvement of *M. castaneae* SVJM139 growth and pigment production. The selected pH, temperature and elicitors have been found to play a significant role in the determination of the final morphology of the culture. These factors have always been of great interest to the researchers in the biotechnological industry for the development of low cost media design. With this perspective, a study was initiated with the goal of designing the selective medium along with using the pH, temperature and elicitors which would promote maximum growth and pigment production of *M. castaneae* SVJM139.

M. castaneae SVJM139 growth was analyzed by using 18 days old culture in optimized CYA/CYB medium which is tentatively comparable to normal CYA/CYB. The results represents that the percent increases of growth that is, 18.7% of radial growth, 25.4% of fresh and 27.5% dry weights and 38.8% of pigment production. This observation is significant and suggests that optimized CYA/CYB is to be preferred, since the yield parameters such as radial growth (8.9 ± 0.1^{a} cm), fresh weight (23.7 ± 0.4^{a} g/l) and dry weight (3.7 ± 0.0^{a} g/l) have increased considerably. The present investigation showed the maximum pigment production (193 ± 2.2^{a} µg/g) in optimized CYB medium (Table 6). It should be pointed out that, the specific pH, temperature and the elicitors are very important factors for fungal growth and pigment production. The microbial pigments are considered to provide a wide range of beneficial effects. Besides these types of fungal pigments have been used food and feed colouring agents and drug developments and mainly to treat cancer for hepato protection and due to their antimicrobial activity (Hajjaj et al., 2000; Selvin et al., 2004; Vahidi et al., 2004; Wenjum, 2006). Thus, a comprehensive production of the pigments from *M. castaneae* SVJM139 could help to identify novel compounds providing beneficial effects.

Overall analysis of the present study showed that the significant increases of growth and pigments production were obtained in optimized CYA/CYB when compared to normal CYA/CYB (Tables 1 and 6). Especially, the greater increases of pigments (> 50 µg/g) were observed in the present findings. Moreover, our literature survey suggested that this is the first report of new Indian isolates of endophytic *M. castaneae* SVJM139 which showed remarkable enhancement of pigments and additionally with antimicrobial activity against human pathogens. Furthermore, this present study improves our knowledge to test the *M. castaneae* SVJM139 pigment to treat the wound infection caused by the pathogenic bacteria and fungi in future.

In conclusion, the present findings represents that *M. castaneae* SVJM139 pigment found to be the superior than the commercial antibiotic, streptomycin. Therefore, after standardization of the mode of action against pathogenic microorganisms, the *M. castaneae* SVJM139 pigment could be developed as a drug in future. Besides, the optimal pH (5), temperature (24°C) and elicitors (15 mg/l) in CYA/CYB were standardized for an improved growth and pigments production of *M. castaneae* SVJM139. This method suggests that these types of the optimization study will be helpful to biotechnological approach for strain improvement, food industry and also to increase the knowledge to develop a suitable selective medium for other microorganisms.

ACKNOWLEDGEMENT

We are gratefully acknowledged Prof. R. Rengaswamy, Director, CAS in Botany, Chennai, India for providing the laboratory facilities to conduct the research work.

REFERENCES

Adinarayana K, Ellaiah P, Srinivasulu B, Bhavani Devi R, Adinarayana G (2003). Response surface methodological approach to optimize the nutritional parameters for neomycin production by *Streptomyces ma-*

rinensis under solid state fermentation. Process Biochem. 38: 1565-1572.
Bacon CW, White JF (2000). In: Microbial endophytes. Bacon CW, White, JF (Eds), Marcel Dekker, Inc, New York.
Bala S, Uniyal GC, Chattopadhyay SK, Tripathi V, Sashidhara KV, Kulshrestha M, Sharma RP, Jain SP, Kukreja AK, Kumar S (1999). Analysis of taxol and major taxoids in Himalayan yew, *Taxus wallichiana*. J. Chromatogr. 858: 239-244.
Cho YJ, Park JP, Hwang HJ, Kim SW, Choi JW, Yun JW, (2002). Production of red pigment by submerged culture of *Paecilomyces sinclairii.* Letters in Appl. Microbiol. 35: 195-202.
Coast J, Smith R, Miller M (1996). Superbugs: should antimicrobial resistance be included as a cost in economic evaluation?. Health Econ. 5: 217-226.
Dobranic JK, Johnson JA, Alikhan QR (1995). Isolation of endophytic fungi from eastern larch (*Larix laricina*) leaves from New Brunswick, Canada. Can. J. Microbiol. 41: 194-198.
Gangadevi V, Muthumary J (2007). Preliminary studies on cytotoxic effect of fungal taxol on cancer cell lines. Afr. J. Biotechnol. 6: 1382-1386.
Gunasekaran S, Poorniammal R, (2008). Optimization of fermentation conditions for red pigment production from *Penicillium* sp. under submerged cultivation. Afr. J. Biotechnol. 7: 1894-1898.
Hajjaj H, Blanc P, Groussac E, Uribelarrea JL, Goma G, Loubiere P, (2000). Kinetic analysis of red pigment and citrinin by *Monascus rubber*as a function of organic acid accumulation. Enzyme Microbial Technol. 27: 619-625.
Huang Y, Wang J, Li G, Zheng Z, Su W, (2001). Antitumor and antifungal activities in endophytic fungi isolated from pharmaceutical plants *Taxus mairei, Cephalataxus fortunei* and *Torreya grandis*. FEMS Immunol. and Med. Microbiol. 31:163-167.
Johri BN (2006). Endophytes to the rescue of plants. Curr. Sci. 90: 1315-1316.
Kim JK, Park SM, Lee SJ, (1995). Novel antimutagenic pigment produced by *Bacillus licheniformis* SSA3. J. Microbiol. and Biotechnol. 5:48-50.
Lehtimaki J, Moisander P, Sivonen K, Kononen K (1997). Growth, nitrogen fixation and nodularin production by two Baltic Sea cyanobacteria. Appl. Environ. Microbiol. 63: 1647-1654.
Levy SB, Marshall B (2004). Antibacterial resistance worldwide: causes, challenges and responses. Nature Med. 10:122-129.
Levy SB (2002). The antibiotic paradox: how misuse of antibiotics destroys their curative powers. 2nd (ed), Perseus Books, Boston.
Nicolaus RA, Piattelli M, Fattorusso E (1964). The structure of melanins and melanogenesis-IV on some natural melanins. Tetrahedrans. 20: 1163-1172.
Pablosmendez A, Knirsch CA, Barr RG, Lerner BH, Frieden TR (1997). Nonadherence in tuberculosis treatment: predictors and consequences in New York City. Am. J. Med. 102: 164-170.
Raviglione MC, Snider DE, Kochi A (1995). Global epidemiology of tuberculosis. Morbidity and mortality of a worldwide epidemic. JAMA 273: 220-226.
Sabu A, Kiran G, Shegal PA (2005). Purification and characterization of tannin acyl-hydrolase from *Aspergillus niger* ATCC16620. Food Technol. Biotechnol. 43:133-138.
Saikia D, Khanuja SPS, Shasany AK, Darokar MP, Kukreja AK, Kumar S (2005). Assessment of diversity among *Taxus wallichiana* accessions from northeast India using RAPD analysis. PGR Newslett. FAO-IPGRI 121: 27-31.
Sameer AM, Anne SM, Ulf T (2006). Colorimetric characterization for comparative analysis of fungal pigments and natural food colorants. J. Agric. Food Chem. 54: 7027-7035.
Selvin J, Joseph S, Asha KRT, Manjusha WA, Sangeetha VS, Jayaseema DM, Antony MC, Vinitha AJD (2004). Antibacterial potential of antagonistic *Streptomyces* sp. isolated from marine sponge *Dendrilla nigra*. FEMS Microbiol. Lett. 50: 117-122.
Stinson M, Ezra D, Hess WM, Sears J, Strobel G (2003). An endophytic *Gliocladium* sp. of *Eucryphia cordifolia* producing selective volatile antimicrobial compounds. Plant Sci. 165: 913-922.
Strobel G, Bryn D (2003). Bioprospecting for microbial endophyte and their natural products. Microbiol. Mol. Bio. Rev. 67:491-502.
Strobel G, Daisy B, Castillo U, Harper J (2004). Natural products from endophytic microorganisms. J. Nat Prod. 67:257-268.
Strobel SA (2002). Biochemical identification of A-minor motifs within RNA tertiary structure by interference analysis. Biochem. Soc. Trans. 30:1126-1131.
Tan RX, Zou WX (2001). Endophytes: a rich source of functional metabolites. Nat. Prod. Report 18:448-459.
Tuttobello L, Foppen FH, Carilli A (1969). Growth and pigmentation of *Epicoccum nigrum* in submerged culture. Appl. Microbiol. 17: 847-852.
Vahidi H, Kobarfard F, Namjoyan F (2004). Effect of cultivation conditions on growth and antifungal activity of *Mycena leptocephala*. Afr. J. Biotechnol. 3: 606-609,
Valan AM, Duraipandiyan V, Agastian P, Ignacimuthu S (2009). *In vitro* antimicrobial activity of *Streptomyces* spp. ERI-3 isolated from Western Ghats rock soil (India). J. Med. Mycol. 19: 22-28.
Wang J, Huang Y, Fang M, Zhang Y, Zheng Z, Zhao Y, Su W, Brefeldin A (2002). A cytotoxin produced by *Paecilomyces* sp. and *Aspergillus clavatus* isolated from *Taxus mairei* and *Torreya grandis*. FEMS Immunol. Med. Microbiol. 34:51-57.
Wasser SP (2002). Medicinal mushrooms as a source of anti-tumor and immuno-modulating polysaccharides. Appl. Microbiol. Biotechnol. 60: 258-274.
Wenjum Wang, Longjiang Yu, Pengpeng Zhou (2006). Effects of different fungal elicitors on growth, total carotenoids and astaxanthin formation by *Xanthophyllomyces dendrohous*. Biores. Technol. 97: 26-31.