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Original Research Article

Characterization of Bioactive compound isolated from *Myrothecium spp.* with UV, FTIR and HPLC Analysis

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ABSTRACT

Development of new drugs, especially in area of infectious diseases, represents today one of the most important research. Fungal isolates are receiving increasing attention by natural product chemists due to their diverse and structurally unprecedented compounds making them interesting candidates for drug discovery. In fact, need for novel, safe and more efficient antibiotics is a key challenge to the pharmaceutical industry today, moreover, increase in opportunistic infections in the immune compromised host has influenced this demand. Nowadays, evaluating morphological and biochemical differences as well as studying fungal genetic diversity via molecular indicators seem to be the most common method for screening this genus. In this research we evaluate the potential of bioactive compound, production and characterize the UV and FTIR spectroscopy and HPLC (High performance liquid chromatography) analysis pattern of isolated *Myrothecium* spp. MRP001. Process development for high level production of bioactive compound was applied using OFAT method. Then, following the extraction of secondary metabolite, the UV and FTIR spectroscopy analysis was carried out for characterization of the various extracts. Considering the coordinate analysis of UV and FTIR spectroscopy pattern, the isolate MRP001 with substantial antimicrobial activity exhibited absorption at 3411 cm-1 which is indicator of hydroxyl groups, absorption at 2856 and 2915 cm-1 indicating hydrocarbon chassis, and absorption at 1649 cm-1 indicating a double bond of polygenic compound. These results highlight the importance of *Myrothecium* isolates in antibiotic production. HPLC confirmed the production when compared with standards.

Introduction

Natural products play a major role in the discovery of leads for the development of drugs in the treatment of human diseases. Natural products are an unsurpassed source of bioactive compounds and constitute a relevant economic resource for the pharmaceutical, cosmetic and food industry. The problems associated with hospital infections caused by drug resistant bacteria become increasingly evident and Staphylococcus aureus is the most common pathogen associated with serious gram positive bacterial infections[1]. The prevalence of methicillin resistant Staphylococcus aureus (MRSA) has increased over the past several decades in most countries[2-5]. Natural products still remain the most important resource for discovery of new and potential drug molecules[6]. Plant endophytic fungi are well known as sources of bioactive secondary metabolites. The fungal species of the genus Pestalotiopsis have been demonstrated to be rich sources of bioactive secondary metabolites with diverse structural features[7].

Recently many of studies on the isolation, characterization and genotyping of soil streptomycetes have been conducted [8-9]. Further studies showed various streptomycetes isolates inhibiting the growth of several multi-resistant Gram-positive pathogens. Experiments on the nature of the inhibitory metabolite produced by S. violaceusniger showed a maximum absorption in the UV region at 210-260 nm[10]. In a different study, the various extract of Streptomyces isolates exhibit inhibitory effects against Candida albicans, therefore the properties of the extract determined by UV-spectra absorbance

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peaks. Similarly, a study by Ilić et al. on 20 different Streptomyces isolates from the soils of Southeastern Serbia indicated that the UV spectra of the culture extracts for the active isolates showed absorbance peaks ranging between 221 and 240 nm[11]. The UV spectra of the active compounds in methanol showed peaks at 217 and 221 nm.

Therefore this study was undertaken to demonstrate the potential of *Myrothecium* isolated from Akola region of Maharashtra, India. The further evaluation of the promising isolates was carried out using the UV & FTIR spectroscopy.

Materials and Methods

Isolation of Microbial strain

Collected soil samples, from 10-15 cm depth, were kept in 4 °C until incubation, while noting sampling region's explicit features such as pH and altitude (Ishii et al. 1983). A soil samples were collected from local area at Akola, Maharashtra, India. Strain MRP001 was isolated from the sandy soil sample on starch–casein agar18 adjusted to pH 10.5 (1 M NaOH) and was preserved as a mixture of spores and mycelium fragments in glycerol (20% v/v) at 28 °C. The strain is deposited in Microbial culture collection, Department of Biotechnology at Amravati University, Maharashtra, India. It was examined for chemotaxonomic and morphological properties known to be of value in *myrothecium* systematic and by partial 16S rRNA gene sequence analysis in our previous study[12-13].

Target organisms

A series of indicative bacteria have been used in this experiment including: *Escherichia coli* (MTCC 443), *Staphylococcus aureus* (MTCC 96), *Bacillus cereus* (MTCC 430), *Candida albigans* (MTCC 227), *Klebsiella pneumonia* (MTCC109) and *Salmonella typhimurium* (MTCC98).

UV-Spectra of *Myrothecium* extracts

Myrothecium isolates were cultured in 250 ml Erlenmeyer flask containing 50 ml liquid medium (containing, beef extract 3.0 g; peptone 5.0 g; glucose 2.0 g; pH 6.5). Flasks were inoculated with 1 ml of *Myrothecium* spores suspension (10^7 CFU/ml) and incubated at 28 °C for 120 h on rotary shaking at 120 rpm. Control flasks were not inoculated with the *Myrothecium* spores and were maintained as above. After reaching the fungal bomass to the special concentration, the content of each flask was centrifuged at 2000 g for 10 min. Approximately 20 ml of each of the centrifuged fermented broth was extracted with 15 ml of n-Butanol after which absorption spectra in UV region (200-450 nm) were determined using a UV-visible spectrophotometer (Lab-India 3000+). The organic solvent for extraction of the culture broth and UV spectrophotometer used in different studies are mentioned in Table 1.

Ultraviolet (UV) and Fourier transform infrared (FTIR) spectral analysis

UV-spectra of various *Myrothecium* isolates obtained from this study were subjected to comparison of general pattern, maximum absorbance peaks and range of wave length. Each active extract was determined in the UV region (200-400nm) by using a Perkin-Elmer Lambda 30 UV/VIS spectrophotometer (AH and Aysel, 2003). Then FTIR spectrum of each active extract was detected using Shimadzu IR-470 plus. The spectra were also scanned in the 400 to 4000 cm-1 range and plotted as intensity versus wave number [14-15].

High performance liquid chromatography (HPLC) chromatography

Qualitative analysis was performed by silica gel thin-layer chromatography with a solvent mixture of petroleum ether: acetone (19:1, v/v) as mobile phase and the development was observed under ultraviolet lamp. Separation of carotenoids was also carried out by HPLC on a C18, 3µm column with acetonitrile: methanol: propanol (40:50:10). The flow rate was 0.8 ml/min[5,16].

Results

This study demonstrates the potential of *Myrothecium* in production of antibiotic and further evaluates the antimicrobial activity of the various isolates through the UV & FTIR spectroscopy. Various soil samples of Akola region of Maharashtra, India were isolated and identified to be the disparate colonies. Among them isolate MRP001 shows distinguished antibacterial activity against *Escherichia coli* (MTCC 443), *Staphylococcus aureus* (MTCC 96), *Bacillus cereus* (MTCC 430), *Candida albigans* (MTCC 227), *Klebsiella pneumonia* (MTCC109) and *Salmonella typhimurium* (MTCC98). Surprisingly, some of the *Myrothecium* isolates including MRP001, MRP011, MRP012, MRP013, MRP014 and MRP015 revealed a significant antibacterial activity against indicator microorganisms. The following percentage of *Myrothecium* isolates exhibited inhibitory effect against the indicator bacteria including: *Salmonella typhimurium* (MTCC98) (58%), *Klebsiella pneumonia* (MTCC109) (37%), *Bacillus cereus* (MTCC 430) (24%), *Staphylococcus aureus* (MTCC 96) (42%), and *Escherichia coli* (MTCC 443) (33%), *Candida albigans* (MTCC 227) (17%) mentioned in Table 1.

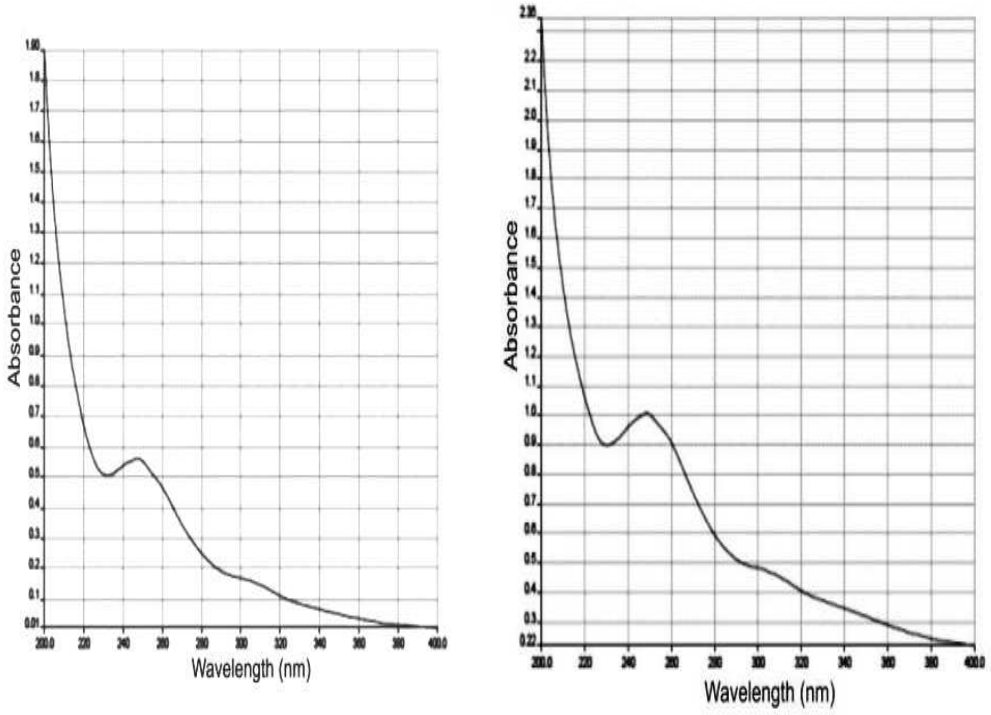
According to the result (Fig. 1A,1 B and Table 1) absorbance peak ranges (215-270nm), as well as the characteristics of absorption peaks signifies a highly polygene nature of the extract. The bioactive compound exhibited a maximum UV absorption at 217–221 nm in ethyl acetate extract. Therefore these strains produced a broad-spectrum of anti-microbial compound or several compounds with different activities. Maximum absorbance peaks observed at 240 nm and again the characteristics of absorption peaks showed a high polygene nature.

Accordingly, the FTIR spectrum of ethyl acetate extracts of MRP001 exhibited absorption at 3411 cm-1, which indicates hydroxyl groups, the absorption at 2856 and 2915 cm^{-1} indicating hydrocarbon chassis and the absorption at 1649 cm^{-1} indicating a double bond of polygenic compound (Fig.2). More or less similar trend was observed by Augustine *et al.* (2005), when they tested the FTIR spectrum of ethyl acetate extract of *S. albidoflavus* PU23 that exhibited absorption

bands at 3296 and 1031.8 cm-1, which indicated hydroxyl groups and absorption at 1639 cm^{-1} indicating double bonding. Despite the fact that, HPLC results of antimicrobial agents at conditions of pressure 71.96% with a flow rate of 1.5 where the mobile phase 0.1% phosphoric acid and pH 4, gave a peak at 7.567 retention time (Fig.3)[15-17].

Table-1: Maximum absorbance peak for the putative isolates in the UV spectroscopy

Stain	λ max (nm)
MRP001	251
MRP011	249
MRP012	243
MRP013	247
MRP014	250
MRP015	245



(A)MRP001 (B) MRP014

Figure-1: Result of UV spectroscopy for ethyl acetate extracts of MRP001 (A) and MRP014 (B)

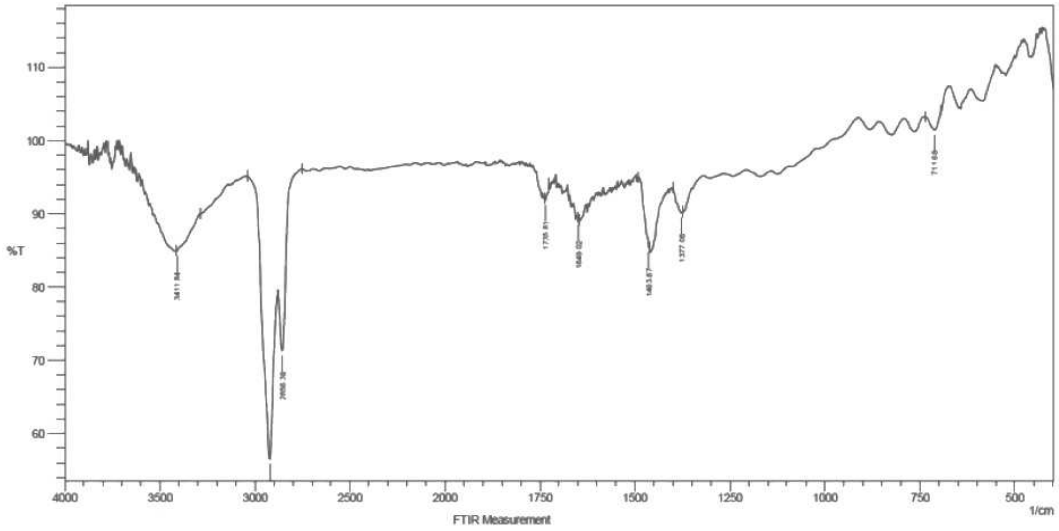


Figure-2: FTIR spectrum of the secondary metabolite isolated from MRP001

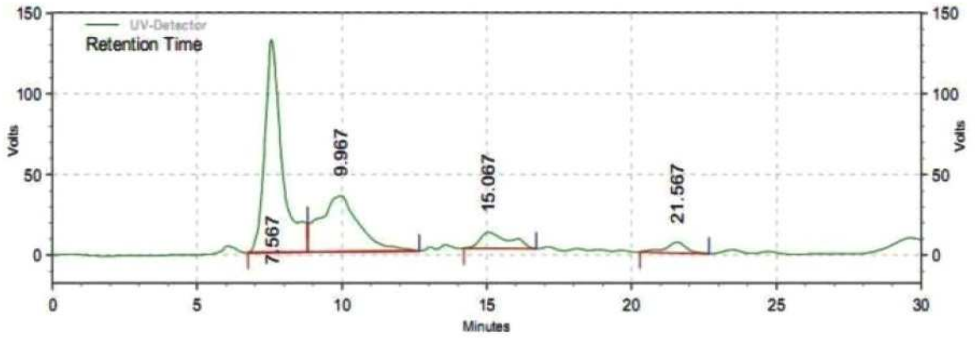


Figure-3: HPLC chromatography of ethyl acetate extraction of MRP001

Discussion

Considering UV & FTIR spectroscopy and according to the result (Figs.1, 2 and Table 2) of absorbance peak ranges (215-270nm), as well as the characteristics of absorption peaks which signifies a highly polygene nature of the extract it is besides similarities in the general UV spectra and maximum absorbance peaks. The result presented in this investigation could explain the ability of the *Myrothecium* sp. to produce antibiotics.

Accordingly there is a demanding need for new and more effective antibacterial for use in more economical uses through industries. Considering the results on antibiotic production potential of MRP001 isolate it might be cited that *Myrothecium* potential in antibacterial production could meet this demand.

HPLC results confirmed the production of colorless carotene and phytoene. The results of the present finding correlate with previous findings of light-induced carotenogenesis in Streptomyces coelicolor.

Conclusion

Fungal isolates are receiving increasing attention by natural product chemists due to their diverse and structurally unprecedented compounds making them interesting candidates for drug discovery. In fact, need for novel, safe and more efficient antibiotics is a key challenge to the pharmaceutical industry today, moreover, increase in opportunistic infections in the immune compromised host has influenced this demand. Since production of novel and more efficient antibiotics needs detection of high yielding microorganisms, in the current study, we evaluated soil sample (collected randomly from different zones of Akola region of India) towards their antibiotic production potential using UV and FTIR spectroscopy and HPLC methods. Based upon UV, FTIR and

HPLC analyses, the isolate MRP001 and MRP014 displayed promising results, inhibiting some important pathogens.

Conflict of interest statement
We declare that we have no conflict of interest.

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References

1. Corey GR. Staphylococcus aureus bloodstream infections: definitions and treatment. Clinical Infectious Diseases 2009;48: S254–S259.
2. Cornaglia G, Rossolini GM. Forthcoming therapeutic perspectives for infections due to multidrug resistant Gram-positive pathogens. Clinical Microbiology and infection 2009;15:218–223.
3. Kumar Ashutosh, Patil Deepak, Rajamohanan P.R, Ahmad Absar Isolation, purification and characterization of Vinblastine and vincristine from an endophytic fungi of *Catharanthus roseus*. PLOS ONE, 2013;8(9):e71805
4. Kumar Ashutosh, Ahmad Absar Biotransformation of vinblastine to vincristine by endophytic fungus *Fusarium oxysporum* isolated from *Catharanthus roseus*. 2013, Biocatalysis and Biotransformation journal 2013;13: 1-5
5. Kumar Ashutosh, Abnave Prasad, Ahmad Absar Isolation and identification of endophytic strain *Fusarium solani* producing Vinca alkaloids from *Catharanthus roseus* , IJBR 2013; 3(2):1-12
6. Strobel GA, Daisy B. 2003 – Bioprospecting for microbial endophytes and their natural products. Microbiology and Molecular Biology Reviews 2003;7:491–502.
7. Harper JK, Arif AM, Ford EJ, Strobel GA, Porco JA, Tomer DP, Oneill KL, Heider EM, Grant DM. Pestacin: a 1,3-dihydro isobenzofuran from Pestalotiopsis microspora possessing antioxidant and antimycotic activities. Tetrahedron 2003;59:2471–2476.
8. Saadoun I, Al-Momani F, Malkawi HI and Mohammad MJ. Isolation, Identification and Analysis of Antibacterial Activity of Soil Streptomycetes Isolates From North Jordan. Microbios, 1999a;100(395):41.
9. Saadoun I, Schrader KK and Blevins WT. Identification of 2-Methylisoborneol (MIB) and Geosmin As Volatile Metabolites of Streptomyces Violaceusniger. Actinomycetes, 1997;8: 37-41.
10. Saadoun I, Hameed KM and Moussauui A. Characterization and Analysis of Antibiotic Activity of Some Aquatic Actinomycetes. Microbios,1999b; 99(394):173.
11. Ili SB, Konstantinovi SS and Todorovi ZB. UV/VIS Analysis and Antimicrobial Activity of Streptomyces Isolates. Facta universitatis-series: Medicine and Biology, 2005;12(1):44-46.
12. Ishii K, Kondo S and Nishimura Y. Decilorubicin, a New Anthracycline Antibiotic. Journal of Antibiotics, 1983;36(4):451-453.
13. Rizwan M. P., Musaddiq M., Thakare P. V. *In-Vitro* Antimicrobial Studies of Isolated Myrothecium Spp Mrp001 against Human Pathogens, International Journal of Basic and Applied Medical Sciences. 2012;2 (3): 228-236.
14. AH N and Aysel U. Investigation of the Antimicrobial Activity of Some Streptomyces Isolates. Turk J Biol, 2003;27:79-84.
15. Augustine SK, Bhavsar SP and Kapadnis BP. A Non-Polyene Antifungal Antibiotic From Streptomyces Albidoflavus PU 23. Journal of Biosciences, 2005;30(2):201-211.
16. Dharmaraj S, Ashokkumar B and Dhevendaran K. Fermentative production of Carotenoids from Marine Actinomycetes. Iranian Journal of Microbiology, 2009;1(4).
17. Slavica BI, Sandra SK and Zoran BT. UV/VIS Analysis and Antimicrobial Activity of Streptomyces Isolates. Medicine and Biology, 2005;12:44-46.

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