INTERNATIONAL JOURNAL OF HIGHER EDUCATION AND RESEARCH
IJHER, Vol., 6(2), 2016, 132-140.
www.ijher.com
IJHER
(ISSN 2277 260X)
CODEN: IJHER ORIGINAL ARTICLE

EXPLORATION OF PIGMENT PRODUCING ACTINOMYCETES, ISOLATED FROM MADHYA PRADESH REGION OF INDIA

Ramendra Singh Parmar*, Charu Singh, Pragya Jadon, Gaurav Bhadauriya and Ajay Kumar
Dept. of Life Sciences, ITM University, Gwalior
*rsparmar.biotech@gmail.com

ABSTRACT

Actinomycetes are biologically important microorganisms and well known for its importance for human welfare. Actinomycete produces many kinds of bioactive compounds and they also have excellent ability to produce natural pigments. In present study the aim was to isolate pigment producing actinomycets and partial purification of natural pigment. The isolation of actinomycetes was done from different regions of Madhya Pradesh. Out of 85 actinomycetes only 5 actinomycetes showed pigment producing ability but one actinomycete AR-ITM02 was selected on the basis of morphological observation such as fast growth and pigment diffusion ability in media. The antimicrobial activity was also tested by agar overlay method on bacteria, fungi and yeast. The extraction of pigment was done by solvent extraction and purification of pigment compound done by chromatographic techniques.

Keywords: *Actinomycetes, Antimicrobial activities, Pigment, Thin layer chromatography*

Parmar, R.S., Singh, C., Jadon, P., Bhadauriya, G. and Kumar, A. (2016). **Exploration of Pigment Producing Actinomycetes, Isolated from Madhya Pradesh region of India.** *International Journal of Higher Education and Research*, 6(2), 132-140. www.ijher.com

1. Introduction

Actinomycetes are biotechnologically important microorganism and they produce many secondary metabolites and natural pigments which are beneficial for various industries. It is observed that many synthetic colours causes many environmental pollution related serious problems and this creates great interest towards natural pigments from microorganisms.

Natural pigments produced by actinomycetes are good alternatives of chemically synthesised colours. The synthetic colours widely used in many industries in foodstuff, dyestuff, cosmetic and pharmaceutical sectors. The negative effect of synthetic colours generated an interest to replace them by naturally extracted colours [1], [2]. Some actinomycetes are capable to produce natural pigments in culture media [3] and actinomycetes produces many antibiotics and natural pigments [4].

The commercial word uses colours widely in many industries like food, cloths, paintings, cosmetics and pharmaceuticals and also plays interesting role in many other industries like bio-plastic and biopolymers [5]. 22500 biologically active compounds have been reported in which 17% isolated from bacteria. Around 38% compounds were isolated from fungi and 45% isolated from actinomycetes [1]. Actinomycetes are reported as an ecological diverse group. India has wide ecological diversity which constitute the large microbial population like actinomycetes in soil which producing active secondary metabolites and also the natural pigment producing ability. This geographic diversity of Indian soil offers the variability of pigment producing and secondary metabolites producing ability of actinomycetes so the exploration of unexplored regions of Chambal territory and some other parts of Madhya Pradesh is necessary to explore some novel actinomycetes. The present study focus on novel actinomycetes, which have diffusible pigment ability in media and antimicrobial abilities against various pathogenic microorganisms.

2. Materials and methods

2.1. Isolation & screening of pigment producing actinomycetes

Rhizospheric soil, were collected from different parts of Madhya Pradesh regions, from 8-10 cm depth of surface in a sterilized polythene bags and stored in 4^{o}C in BOD incubator [6]. One gram soil samples was mixed in sterile distilled water and allowed for shaking in rotator shaker for 10 minutes and serially diluted. Different ISP media like Starch Casein Agar (SCA), Actinomycetes Isolation Agar (AIA), Yeast Extract-Malt Extract Agar (YEMA) ,Inorganic Starch-Salt agar (ISSA), Nutrient agar (NA), Starch Agar (SA) media and Peptone Yeast Iron Agar (PYIA) used and incubated for 6-7 days at 30^{o}C. [7], [8]. Among 85 actinomycetes isolates only five actinomycetes showed pigment producing ability but on the basis of pigment diffusion ability only one actinomycete ARITM02 was selected for further study which also possessed antimicrobial activities. Pigment producing ability of selected isolate ARITM02 was tested on solid as well as in broth media.

2.2. Characterization and antibiogram of actinomycete isolate

ARITM02 showed the spore chain along with substrate and areal mycelium under light microscopic view. A characteristic of the spore bearing hyphae and spore chains were determined by the direct microscopic examination of the culture. Adequate magnification used to establish the presence or absence of spore chains. Different biochemical tests as catalase reduction, nitrate reduction, H_2S production, starch hydrolysis, casein hydrolysis, citrate utilization, indole, Methyle Red test, Voges-Proskauer test, gelatin hydrolysis and utilization of different sugars were performed [9]. The selected actinomycete ARITM02 was also tested for antimicrobial activity against various microorganisms like bacteria and fungus including dermatophytes by agar overlay method and well agar diffusion method [10]. The bacterial cultures as *Escherichia coli* (MTCC 40), *Pseudomonas aeruginosa* (MTCC 8165), *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 1134), *Enterobacter aerogenes* (MTCC 7325), *Bacillus cereus* (MTCC 1307), *Proteus vulgaris* (MTCC 1771) and fungi as *Aspergillus niger* (MTCC 9651), *Aspergillus fumigatus* (MTCC 2551), *Candida albicans* (MTCC3017), *Microsporum canis* (MTCC 2820), *Microsporum fulvum* (MTCC 2837) and *Trichophyton rubrum* (MTCC 296) were tested.

2.3. Extraction and purification of Pigment from Actinomycetes

The selected actinomycete isolate ARITM02 was inoculated into starch casein broth and incubated at 30°C in incubator shaker (180 rpm) for seven days filtered through Whatmannn No.1 filter paper. The filtrate was transferred aseptically into a sterilized conical flask and stored at 4°C for further assay. Culture filtrate, equal volume of various solvents chloroform, acetone and methanol was added separately and centrifuged at 8000 rpm for 10 min at 4°C [11]. The purification of pigment was firstly done by thin layer chromatography using methanol, chloroform and water (40:40:20). After the sample running on TLC plate, the plate was taken out and dried, then ninhydrin was sprayed. The purification of pigment was carried out using gel column chromatography and acetone-methanol mixture was used as an eluting solvent [12],[13].

3. Result

3.1. Isolation & screening of pigment producing actinomycetes

In the field of microbial biotechnology and many industries actinomcetes have shown its wide importance. The isolation and characterization of actinomycetes is an important approach for industrially important natural colours. The actinomycetes, specially *Streptomyces* produces many important economical and beneficial secondary metabolites, hormones, enzymes and vitamins which have economic and clinical importance. In present study, total 85 actinomycetes were isolated from different sites of "Chambal territory" and Gwalior regions of MP (Figure 1).

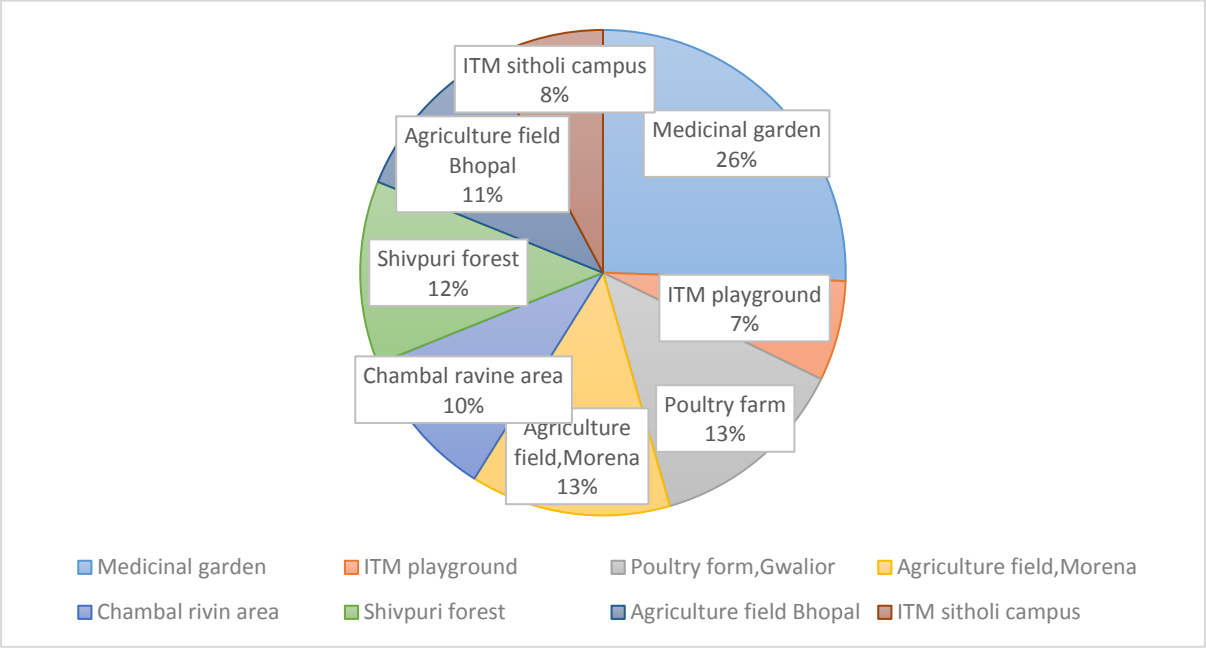

Figure.1. percentage distribution of isolated actinomycetes,from different soil samples at Madhya Pradesh regions.

Media used	Growth	Aerial mycelium	Substrate mycelium	Soluble pigment
Yeast extract-Malt extract	Good	White	Yellow	None
Oatmeal agar (ISP-3)	Good	Grey	Yellow	None
Inorganic Starch-Salt agar	Good	Grey	Yellow	None
Glycerol asparagines agar	Good	Grey	Yellow	None
Peptone yeast extract iron agar	Good	Grey	Yellow	None
Starch agar	Good	Grey	Yellow	None
Starch casein agar	Excellent	White	Red	Yes

Table 1- Cultural characteristics of the selected isolate AR-ITM02 on different ISP media

3.2 Identification and characterization of selected isolate (ARITM02)

The selected isolate ARITM02 showed dark wine red color pigment, produced on starch casein agar which utilized various carbon sources for growth as glucose, arabinose, xylose, mannose and fructose for its growth, while sucrose, rhamnose and rhafinnose and were not utilized. Degradation of starch, casein and citrate were observed but the selected isolate does not degrade gelatin (Table 2 & 3).

Biochemical Test	Reaction
Citrate utilization	+ve
H_2S production	-ve
Gelatine	-ve
Nitrate reduction	-ve
Catalase	-ve
Starch	-ve
Indole	+ve
Casein	-ve
MR	+ve
VP	-ve
Catalase	-ve

+ve = Positive, -ve = Negative

Table –2 Biochemical characteristics of the actinomycete ARITM02

Source	Utilization	Source	Utilization
Glucose	AG	Xylose	G
Fructose	AG	Maltose	AG
Rhamnose	G	Sucrose	AG
Raffinose	G	Ribose	G
Galactose	AG	Maltose	AG

A- Acid production, G- Gas production

Table-3 Utilization of different carbon sources by the selected actinomycete ARITM02

In the light microscopic examination, gram positive dichotomously branched spore chains was seen (Figure 2). Morphological, physiological and biochemical characteristics revealed that ARITM02 was similar to *Streptomyces*, according to the Bergey's Manual of Determinative Bacteriology [14]. The isolate ARITM02 was found aerobic, Gram positive,

non acid fast. The isolate is susceptible to streptomycin (10 µg/mL). Growth was best observed on starch casein agar medium at 30°C temperature.

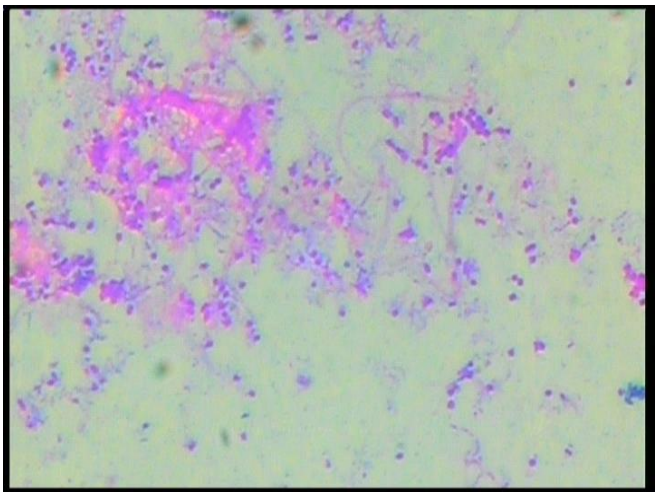

Figure2. Light microscopic view of selected isolate under 100x observation.

3.3. Antibiogram of isolate Streptomyces ARITM02

The antibiogram of the ARITM02 isolate have shown good antibacterial activity against *S. aureus, B. cereus, E. coli, B. subtilis, E. aerogenes, P. aeruginosa* and *P. vulgaris.* The isolate also has good antifungal activity against *C. albicans* and *A. niger* but did not show any activity against skin pathogens. The isolate has broad spectrum activity against test bacterial cultures (Figure 3).

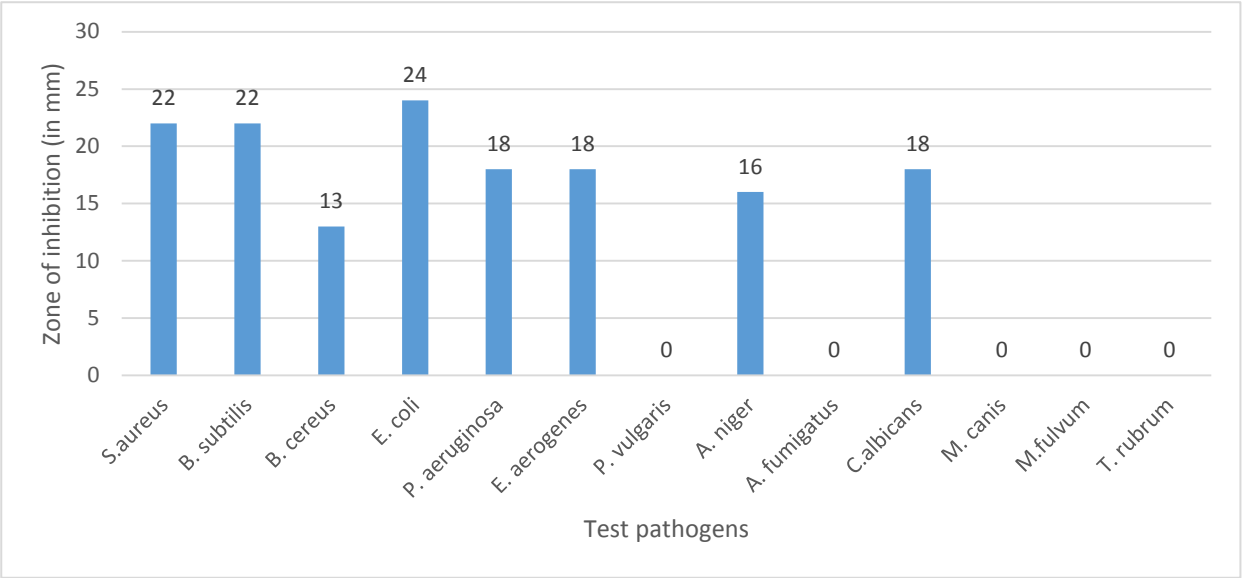

Figure.3.- Antibiogram of selected isolate AR-ITM02 by well agar diffusion method against test pathogens.

3.4. Fermentation, Extraction and Purification

The extraction of pigment was done by solvent extraction method using mixture of solvents. The separation was done by thin layer chromatography and Rf value found 0.73 in which pigment showed antimicrobial activity. The purification process through column chromatography packed with silica gel was eluted with a mixture of methanol and chloroform 95:5 (v/v) (Figure 4).

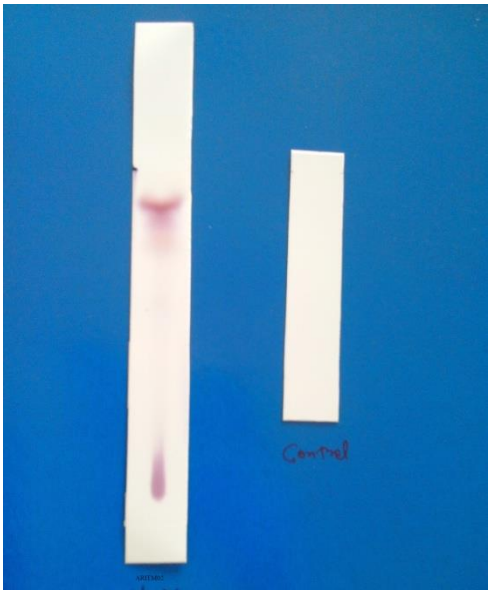

Figure.4. Thin layer chromatography showed single spot of pigment

4. Conclusion

Streptomyces sp. are considered as a robust source for production of pigment which have also able to produce a broad spectrum antimicrobial against Gram-positive, Gram-negative bacteria and fungi. It was successfully cultivated by submerged fermentation in a bioreactor. The purification and identification of antimicrobial compound was carried out using thin layer chromatography and column chromatography. The study concludes that actinomycetes have excellent ability to produce natural pigment and has antimicrobial activity which could be very useful for pharmaceuticals and other industries.

5. Acknowledgment

Authors are thankful to Department of Science and Technology (DST-SERB) for providing financial support (Sanction letter no. SB/FT/LS-220/2012) and Department of Life Sciences, ITM University, Gwalior for providing necessary facilities.

References

[1] Berdy, J., "Bioactive microbial metabolites: Review article," J. Antibiot. **58**: 1-26 (2005).

[2] Unagul, P. Wongsa, P. Kittakoop, P. Intamas, S. Srikiti-Kulchai, P. Tanticharoen, M. "Production of red pigments by the insect pathogenic fungus *Cordyceps unilateralis* BCC 1869", J. Ind. Microbiol. Biotechnol. **32**:135-140 (2005).

[3] Dastager, S.G., Li W.J., Dayanand, A., Tang, S.K., Tian, X.P., Zhi, X.Y., Xu, L.H., Jiang C.L.,"Separation, identification and analysis of pigment (melanin) production in *Streptomyces*". Afr. J. Biotechnol. **5:**1131-1134 (2006).

[4] Miyaura, J., Tatsumi, C., "Studies on the antibiotics from actinomycetes, An antibiotics pigment from *Streptomyces* F-23b", Bull: Univ .Osaka Pref. SerB 129-137 (1960).

[5] Nakato, L., "Melanin and Bio/Nanotechnology", Black herbals at the Source of the Nile, UG Ltd (2006).

[6] Flardh, K., "Growth polarity and cell division in *Streptomyces* .Curr. Opin. Microbiol., **6:** 561–571 (2003).

[7] Mohanshrinivasan, V., Sriramkalyan, P., Ipsita N., Subhadra Devi, C, Selvaranjan E, Sugandthi, V and Jeminah NS. "Fermentative production of extracellular pigment from *Streptomyces coilicolor"*. Res. J. Biotechnol. **8**,1-8 (2013).

[8] Mangamuri, U. K., Muvva, V., Poda, S., Kamma, S., "Isolation, identification and molecular characterization of rare actinomycetes from mangrove ecosystem of Nizampatnam". Malaysian. J. Microbiol. **2**, 83-91(2012).

[9] Krishna, J.G., Basheer, S.M., Beena, P.S., Chandrasekaran, M., "Marine bacteria as source of pigment for application as dye in textile industry", Proc Internatl Conf Biodiv Conserv Mgt.743-4 (2008).

[10] Kumar, V., Bharti, A., Gupta, V.K., Gussain, O.P., Bisht, G.S., "Actinomycetes from solitary wasp mud nest and swallow bird mud nest: isolation and screening for their antibacterial activity", W. J. Microbiol. Biotechnol. **28:**871-880 (2012).

[11] Parthasarathi, S., Sathya, G., Bupesh, R., Durai Samy, M., RamMohan, G., Selva Kumar, M., Manikandan, C.J., Kim, Balakrishnan, K., **"**Isolation and Characterization of Antimicrobial Compound from Marine *Streptomyces hygroscopicus* BDUS 49", W. J. Fish. Mar. Sci., 4, 268-277 (2012).

[12] Dharumaduari, D., Nooruddin, T., Annamalai, P., "An antifungal compound: 4' phenyl-1-napthyl- phenyl acetamide from *Streptomyces* sp." Medi. Biol., 15, 7-12. (2008).

[13] Williams, S.T., Davies, F. L., "Use of antibiotics for selective isolation and enumeration of actinomycetes in soil"., J. Gen. Microbiol., **38,** 251-262 (1965).

[14] Bergy, D.H., Holt, J.G., "Bergy's manual of determinative bacteriology", Baltimore: Williams and Willkins publications (1994).