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Mycelial and Pigmentation studies of Dibenzothiophene desulfurizing *Streptomyces* species isolated from oil contaminated sites

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Abstract: *The present work is the part of study on Dibenzothiophene (DBT) desulfurizing actinobacteria viz., Streptomyces sp. VUR PPR 101 and Streptomyces sp. VUR PPR 102 isolated from oil contaminated sites of mechanical workshops in Karimnagar area of Andhra Pradesh. Both the species were grown on ten different media including ISP and other media to observe the growth, colour of aerial and substrate mycelia, and of the soluble pigment. The time taken for the onset of sporulation on each medium was also recorded. After incubation, both the species had shown some colour variations in aerial mycelia, substrate mycelia and soluble pigments. Extent of growth was also varied. The time taken for the onset of sporulation slightly varied among different media but not between the bacteria with respect to a particular medium.*

Key words: *DBT, ISP media, mycelium, pigment colour, Streptomyces.*

I. Introduction

The actinomycetes are the important oil degrading bacteria and species like *Streptomyces rochei, Streptomyces plicatus, Streptomyces diastaticus, Rhodococcus fascians*, *Nocardia* sp. and *Frankia* sp. commonly occur in oil contaminated sites. In some oil contaminated sites, actinomycetes represent the dominant group [1]. Streptomycetes are Gram-positive and have genomes with high GC content [2]. The spores of actinomycetes will germinate and produce substrate and aerial mycelia under favourable conditions [3]. On the solid medium, actinomycetes appear as tough powdery and pigmented colonies. The pigment production is very prominently seen in actinomycetes group than any other. Production of pigments by actinomycetes is used as a key cultural characteristic feature to describe the organisms [4]. *Streptomyces* is the largest genus of Actinobacteria produces a variety of pigments like bluish grey, whitish grey, yellow, greyish yellow and many more pigments. The toxic hazards caused by synthetic pigments to the environment have initiated interest towards natural pigments. Microbial pigments serve as best alternatives to synthetic pigments. Natural pigments can also be obtained from plants but they have many drawbacks like unstable to light, heat or changes in pH, mild solubility in water and even they are not available throughout the year. The benefits of microbial pigment production are - microbes can be cultivated in cheap cultural medium in the laboratory, free from environmental fluctuations and they produce variety of colours of different shades [5]. In this present research work, two Dibenzothiophene (DBT) desulfurizing bacteria were isolated from oil contaminated sites of different automobile workshops located in Karimnagar Town (India) and were identified as *Streptomyces* sp. VUR PPR 101 and *Streptomyces* sp. VUR PPR 102. The present paper deals with cultivation of these two *Streptomyces* species on various solid media to study the time taken for the onset of sporulation, colour of aerial and substrate mycelia, soluble pigment colours and nature of growth on each media.

II. Materials And Methods

Ten different media were selected for pigmentation and growth pattern studies of the DBT desulfurizing *Streptomyces* sp. VUR PPR 101 and *Streptomyces* sp. VUR PPR 102. The two species were grown on ISP medium1 (Tryptone-Yeast extract broth), ISP medium 3 (Oat meal agar), ISP medium 4 (Inorganic salts-starch agar), ISP medium 5 (Glycerol-Aspargine agar), ISP medium 6 (Peptone -Yeast extract iron agar), ISP medium 7 (Tyrosine agar), Bennet's agar medium, Kuster's agar medium, Starch Casein agar and Nutrient agar medium, and recorded the observations [6,7,8,9]. The aerial mycelium (front side), substrate mycelium (reverse side), soluble pigment colours and nature of growth of each bacterium on every medium were observed as per ISP. Time taken for the onset of sporulation was also recorded. During observation of the colour of the pigment produced by the *Streptomyces* species on a particular medium, the same uninoculated medium was used as control.

III. Results And Discussion

The two *Streptomyces* species grew on all the media but abundantly on ISP3, ISP5, ISP7 and Bennet's media when compared to the others and shown various colours on different media. The growth pattern of the *Streptomyces* species varied showing poor, moderate and abundant growth on the different media. The colour of aerial and substrate mycelia of the *Streptomyces* sp. VUR PPR 101 and *Streptomyces* sp. VUR PPR 102 on ten different media are given in Table-1. The aerial mycelium colour (front) of *Streptomyces* sp. VUR PPR 101 varied from white to grey and pale grey and that of *Streptomyces* sp. VUR PPR 102 from white to greyish white, pale grey, grey and dark grey on different media. The substrate mycelium colour (reverse) of *Streptomyces* sp. VUR PPR 101 had shown brown and grey colours at different intensities and the substrate mycelium of the *Streptomyces* sp. VUR PPR 102 had shown the brown, grey and orange colours at different intensities on different media.

Both the *Streptomyces* species had produced the soluble pigments only in five different media viz., ISP3, ISP4, ISP5, ISP7 and Bennet's agar (Table-2). *Streptomyces* sp. VUR PPR 101 had produced soluble greenish dark brown, yellowish brown and yellow pigments and *Streptomyces* sp. VUR PPR 102 produced dark grey-dark brown and yellow pigments on different media. Interestingly these species had produced soluble pigments on all the media in which they had grown abundantly. While characterizing *Streptomyces* speices pigment production by them is widely studied. It was evident from the present study that when pigments produced by the *Streptomyces* species were compared, the nature and colour of the pigments differed depending on the type of carbon and nitrogen sources present in the media [10]. The substrate mycelium colour is important in grouping the *Streptomyces* species [11]. Pigmentation pattern on ISP media is one of the important features for the identification and classification of *Strepotmyces* species. The findings of the present study are in concurrence with some earlier reports [12,3,11] which recorded variation in the extent of growth, color of aerial as well as substrate mycelia and pigmentation of different *Streptomyces* strains on different media.

Time taken for the onset of sporulation on various media by the two *Streptomyces* species was almost similar except on ISP5 medium (Table-3). Both the species showed minimum duration of 3 days for the onset of sporulation on ISP3, ISP6, ISP7, Bennet's agar and Starch Casein agar media. However, the maximum period of 5 days for onset of sporulation was observed on Nutrient agar medium in case of both species. Media on which organisms had produced spores earlier had shown abundant growth on the same media except *Streptomyces* sp. VUR PPR 102 on ISP5 medium. Some what delayed sporulation was observed on the media where the species exhibited poor and moderate growth except *Streptomyces* sp. VUR PPR 102 on ISP5 medium.

IV. Tables

Table-1: Colour of Aerial and Substrate mycelia of two *Streptomyces* species isolated from oil contaminated sites on different media

S.No.	Medium	Colour of Aerial Mycelium		Colour of Substrate Mycelium	
		Streptomyces sp. VUR PPR 101	*Streptomyces* sp. VUR PPR 102	*Streptomyces* sp. VUR PPR 101	*Streptomyces* sp. VUR PPR 102
1.	ISP1	White	White	Brown	Light orange
2.	ISP3	Light grey	Dark grey	Dark brown	Dark grey
3.	ISP4	Grey	Grey	Light brown	Dark brown
4.	ISP5	White	Greyish white	Grey	Brown
5.	ISP6	White	Light grey	Light grey	Dark grey
6.	ISP7	White	Pale grey	Dark grey	Brown
7.	Bennet's agar	Pale grey	White	Light grey	Grey
8.	Kuster's agar	Pale brown	Light grey	Grey	Dark grey
9.	Starch Casein agar	Pale grey	Light yellow	Brown	Light brown
10.	Nutreint agar	White	White	Light brown	Light brown

Table 2: Soluble pigment colour and Growth of two *Streptomyces* species isolated from oil contaminated sites on different media

S.No.	Medium	Colour of Soluble Pigment		Growth	
		Streptomyces sp. VUR PPR 101	*Streptomyces* sp. VUR PPR 102	*Streptomyces* sp. VUR PPR 101	*Streptomyces* sp. VUR PPR 102
1.	ISP1	Absent	Absent	Poor	Poor
2.	ISP3	Brown to Greenish dark brown	Dark Grey to dark brown	Abundant	Abundant
3.	ISP4	Light yellow	Yellow	Poor	Poor
4.	ISP5	Yellow	Dark yellow	Abundant	Abundant
5.	ISP6	Absent	Absent	Poor	Poor
6.	ISP7	Yellow	Yellow	Abundant	Abundant
7.	Bennet's agar	Brownish yellow	Dark yellow	Abundant	Abundant
8.	Kuster's agar	Absent	Absent	Poor	Poor
9.	Starch Casein agar	Absent	Absent	Moderate	Moderate
10.	Nutreint agar	Absent	Absent	Poor	Poor

Table-3: Time taken for the onset of sporulation of two *Streptomyces* species isolated from oil contaminated sites on different media

S.No.	**Medium**	**Onset of Sporulation (days after)**	
		Streptomyces sp. VUR PPR 101	*Streptomyces* sp. VUR PPR 102
1.	ISP 1	4	4
2.	ISP 3	3	3
3.	ISP 4	4	4
4.	ISP 5	3	4
5.	ISP 6	3	3
6.	ISP 7	3	3
7.	Bennet's agar	3	3
8.	Kuster's agar	4	4
9.	Starch Casein agar	3	3
10.	Nutrient agar	5	5

V. Conclusion

This present work has revealed the varied nature of the two DBT desulfurizing actinobacteria viz., *Streptomyces* sp. VUR PPR 101 and *Streptomyces* sp. VUR PPR 102 isolated from oil contaminated sites with regard to mycelial colour and pigment colour when grown on ten different media. All the ten media used favoured the sporulation in both species. This result reflects the ability of the *Streptomyces* species in secreting different pigments basing on the composition of different media.

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