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IMPACT OF DIFFERENT MEDIA FOR GROWTH AND PRODUCTION OF DIFFERENT SOLUBLE PIGMENTS IN ACTINOMYCETES ISOLATED FROM SOILS OF HADHRAMOUT, YEMEN

Ali Mohammed Abdullah Bawazir[1], Gunashree B. Shivanna[2] and Manjula Shantaram*[3]

[1,2]Department of Studies and Research in Microbiology, Post Graduate Centre, Mangalore University, Jnana Kaveri, Chikka Aluvara, Kodagu- 571232, Karnataka, India.
*[3]Department of Studies and Research in Biochemistry, Post Graduate Centre, Mangalore University, Jnana Kaveri, Chikka Aluvara, Kodagu- 571232, Karnataka, India.

***Corresponding Author: Prof. Manjula Shantaram**
Department of Studies and Research in Biochemistry, Post Graduate Centre, Mangalore University, Jnana Kaveri, Chikka Aluvara, Kodagu- 571232, Karnataka, India.

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ABSTRACT
In this study, twelve isolates of actinomycetes were segregated from different habitats like dams, mountains and caves of Hadhramout, Yemen on different media, such as starch casein agar, glycerol asparagine agar, tyrosine agar, yeast extract- malt extract agar, glycerol yeast extract agar and starch yeast extract agar for exploring their ability to produce diffusible pigments. Most of the isolates produced pigments, which means that these actinomycetes could have commercial values and open doors for food colors and beverages, in the pharmaceutical industries, and also in cosmetic industries. The same isolates of actinomycetes, when grown on different media also gave different colors. This indicates that actinomycetes have the ability to produce different pigments when culture media are changed.

KEYWORDS: Actinomycetes; Hadhramout; Soluble pigments; Media.

INTRODUCTION
Several microscopic organisms will just produce their pigments under certain environmental conditions. Different species produce pigments as the colonies age or when a particular nutrient is available or lacking in the culture media.[1] Such pigments can help recognize the microscopic organisms. For instance, a few microscopic organisms create water-soluble pigments which spread through the medium in which they develop. Others produce pigments that are soluble in fat. To decide this, one can expel some of the pigmented colonies and shake it in oil. In the event that the oil becomes pigmented, then the pigment is fat soluble.

Natural pigments are a superior substitute to chemical dyes utilized in the industries and research centers. Out of numerous strains of Streptomyces found universally in the soil, *S. coelicolor* and *S. violaceoruber* are shown to produce an important red-blue anti-toxin actinorhodin and associated compounds like α-, β-, ε- actinorhodin collectively known as Actinorhodin- related "Blue Pigments". These pigments have an extensive application in the scientific, medical and industrial sector.[2]

Microorganisms can create distinctive colors, for example, carotenoids, melanins, flavins, quinones, prodigiusens, monacens, violasin or indigo.[3] The temperature of the culture is the primary factor that relies upon the kind of microorganisms. Carotenoids are yellow-to-orange red pigments found in varieties of plants, bacteria and fungi.[4] Recently, carotenoids are utilized commercially for nutraceuticals, beauty care products and pharmaceutical purposes.[5] Carotenoids can repress different types of cancers and can enhance immunity response.[6] It can likewise prevent life-related diseases due to its propitamin action and higher antioxidant capacity.[7] In future, through genetic engineering enzymatic pathways of microorganisms could be manipulated to produce high measures of carotenoids. Since people started to experience the ill effects of different infections they started to look for anti-infection agents from microorganisms. Numerous infectious bacteria have become highly resistant to different antibiotics causing a major healthcare problem.[8] So, there is a critical need to research into antibiotics against multidrug resistance.

Several different combinations of media have been suggested from earlier reports for isolation of actinomycetes, such as starch casein agar[9], yeast malt extract agar[10], glycerol asparagine agar[11], tyrosine agar[12], glycerol yeast extract agar[13] and starch yeast

extract agar.[13] Growth media composition is important for the basis of pigment formation. The formation of deep brown to black pigments on organic media containing proteins and protein derivatives, notably the amino acid tyrosine, is an important characteristic feature.[14] In the present study, actinomycetes were isolated from different locations in Hadhramout, Yemen. The large number of colonies were identified by preliminary grouping on the basis of their pigmentation characteristics.

MATERIALS AND METHODS

Sample collection

The soil samples were collected from different places of Governorate of Hadhramout, Yemen like mountain, caves and dams using sterile containers and were aseptically brought to laboratory.

Isolation of actinomycetes

The soil samples from each of the locations were serially diluted using sterile distilled water and plated on starch casein agar medium using 10^{-1}, 10^{-2} and 10^{-3} dilutions by spread plate and pour plate techniques. They were incubated for 5-7 days at 30℃. The pure colonies were isolated and again plated on starch casein agar medium by streak plate method.[8]

Growth of actinomycetes on different media and their pigment production

Growth characters of actinomycetes were studied for 14 days at 28°C using 9 different culture media at pH 7. The compositions of the culture media used are as follows,

1. **Starch casein agar (SCA)** (g/l): Starch, 10.0; Casein, 1.0; $CaCo_3$, 0.02; $FeSO_4$, 0.01; KNO_3, 2.0; 1000 ml of distilled water and Agar, 20. 0.[6]
2. **Glycerol asparagine agar (GAA):** (g/l): Glycerol, 10.0; L- asparagine (anhydrous), 1.0; K_2HPO_4, 1.0; Trace salt solution, 1ml; $FeSO_4.7H_2O$, 0.1; $ZnSO_4.7H_2O$, 0.1; $MnCl_2.4H_2O$, 0.1 and 1000 ml of distilled water and Agar, 20.0.[8]
3. **Yeast extract-malt extract agar (YMA):** (g/l): Yeast extract, 4.0; Malt extract, 10.0; Dextrose, 4.0, 1000 ml of distilled water and Agar, 20.0.[7]
4. **Starch yeast extract agar (SYA):** (g/l): Starch, 10.0; Yeast extract, 4.0; K_2HPO_4, 1.0; Trace salt solution, 1ml; 1000 ml of distilled water and Agar 20.0.[10]
5. **Tyrosine agar (TA):** (g/l): Glycerol, 15.0; L-tyrosine, 0.5; L- asparagine, 1.0; KH_2PO_4, 0.5; $MgSO_4.7H_2O$, 0.5; NaCl, 0.5; $FeSO_4.7H_2O$, 0.01; Trace salt solution, 1ml; 1000 ml of distilled water and agar, 20.0.[9]
6. **Glycerol yeast extract agar (GYA):** (g/l): Glycerol, 10.0; Yeast extract, 3.0; K_2HPO_4 1.0; Trace salt solution, 1ml; 1000 ml of distilled water and agar 20. 0.[10]

RESULTS AND DISCUSSION

Several different media have been suggested for isolation of actinomycetes from the soil, for example SCA, YMA, GAA, TA, GYA and SYA were selected to see their efficacy for growth and production of different soluble pigments. Twelve strains of actinomycetes were isolated from soil samples of different habitats like caves, mountain and dams of Hadhramout, Yemen grown on different media as mentioned in Fig. 1.

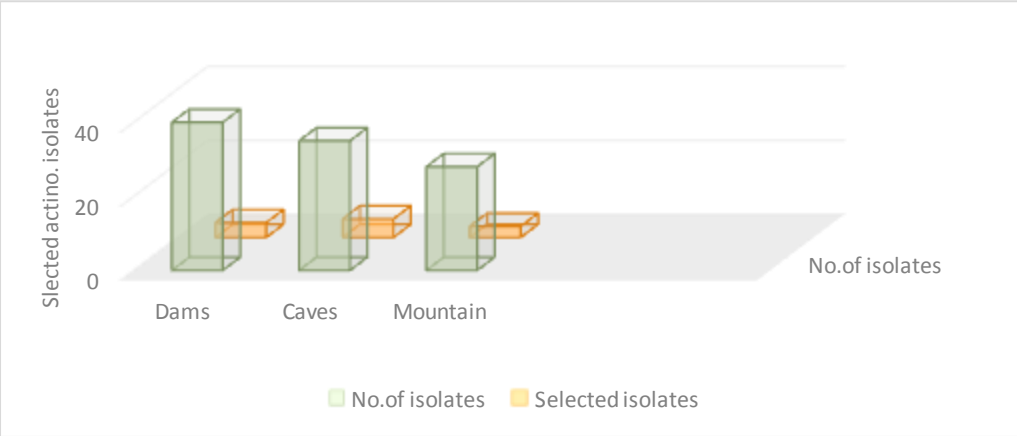

Fig. 1: Selection of actinomycete from different soils.

Growth was best observed on SCA, YMA, GAA, TA, GYA, and SYA media at 37°C. The Dnj-2 and C.S-3 produced brownish pigment on yeast malt extract agar. On SCA and other media, C.S-3, C.S-6, C.B-1, C.B-2 D.S-1, D.S-2, Dsh-1 produced different diffusion pigments (Table 1 & 2; Fig.2).

Determination of color or pigment production by various isolates: For color determination, the media which had been prepared for morphological characterization was used. RAL color chart was used and color determination was done for mature sporulating aerial surface growth, the color as seen from the reverse side of the plate. The color of the aerial surface growth was determined as white, gray, yellow, red, blue, green

and violet. The presence of soluble colors other than melanin pigmentation was determined. Soluble colors other than brown or black were produced on any medium or brown was distinctively modified with red, yellow, green, blue or violet.

Actinomycetes are a group of productive source of secondary metabolites and by far most of these compounds are obtained from the single family Streptomyces. Actinomycetes have demonstrated their significance both biotechnologically as well as industrially. The isolation and characterization of actinomycetes are an important approach to industrially important natural colors.[15] The isolation of actinomycetes from soil has been described by Selvameenal *et al.*,[16] and their potential for pigment-producing ability along with antimicrobial activities have also been shown. Many industries are using the natural color producing actinomycetes.[17]

Fig. 2: Diffusion of pigments in SCA, GAA, TA, GYA, SYA and YMA.

Table 1: Growth and cultural characteristics of the isolates on different media.

Culture medium	Dnj-2	Dnj-3	Dj-1	C.S-1	C.S-2	C.S-3
Starch casein agar						
Growth	Good	Good	Good	Good	Good	Good
Arial mycelium	White	White	Grey white	Grey white	Grey white	Grey white
Substrate mycelium	Red orange	Melon yellow	Brown red	Red orange	Brown yellow	Orange brown
Soluble pigment	Non	Non	Maize yellow	Non	Non	Dark ivory
Yeast- malt extract agar						
Growth	Moderate	Good	Good	Moderate	Good	Good
Arial mycelium	None	White	Grey	None	Grey	Grey
Substrate mycelium	Brownish	Brown yellow	Green brown	Yellow	Ivory	Orange brown
Soluble pigment	Pale yellow	None	Brownish	None	Ivory	Brown
Glycerol- asparagine agar						
Growth	Moderate	Poor	Moderate	Moderate	Moderate	Moderate
Arial mycelium	White	Yellow	Grey	None	White	Cream
Substrate mycelium	Brown	Beige	Black red	Light brown	Beige	Cream
Soluble pigment	None	None	None	Yellow	None	None
Tyrosine agar						
Growth	Moderate	Moderate	Good	Good	Good	Good
Arial mycelium	White	None	Grey	None	Grey	Grey
Substrate mycelium	Brown	Yellow orange	Brown	Ochre brown	Beige	Cream
Soluble pigment	None	None	Beige	Honey yellow	None	None
Glycerol yeast extract agar						
Growth	Good	Moderate	Good	Moderate	Good	Good
Arial mycelium	White	White	Grey white	None	Grey	Blake grey
Substrate mycelium	Brown yellow	Cream	Zinc yellow	Yellow	Golden brown	Golden brown
Soluble pigment	None	None	Traffic yellow	None	None	None
Starch yeast extract agar						
Growth	Good	Good	Moderate	Good	Good	Good
Arial mycelium	White	White	None	Yellow orange	Grey	Grey
Substrate mycelium	Yellow	Golden yellow	Beige	Deep orange	Golden brown	Ochre yellow
Soluble pigment	None	None	None	Sun yellow	None	None

Table 2: Growth and cultural characteristics of the isolates on different media.

Culture medium	C.S-6	C.B-1	C.B-2	Ds-1	D.W-3	C.S-5
Starch casein agar						
Growth	Good	Good	Good	Moderate	Good	Good
Arial mycelium	White	White	White	None	White	White
Substrate mycelium	Beige	Yellow	Yellow	Red orange	Beige	Red orange
Soluble pigment	None	Yellow	Yellow	None	None	Beige
Yeast- malt extract agar						
Growth	Moderate	Moderate	Moderate	None	Good	Moderate
Arial mycelium	White	Grey to white	Yellow	None	White	White
Substrate mycelium	Broom yellow	Yellow to grey	Yellow	None	Traffic yellow	Orange brown
Soluble pigment	None	None	Yellow	None	Light ivory	Sun yellow
Glycerol- asparagine agar						
Growth	Moderate	Good	Good	Good	Moderate	Good
Arial mycelium	None	White	White	None	Light ivory	None
Substrate mycelium	Beige	Beige	Yellow	Cream white	Pearl white	Brown
Soluble pigment	None	None	Yellow	None	None	Honey yellow
Tyrosine agar						
Growth	Moderate	Moderate	Moderate	Good	Good	Good
Arial mycelium	None	Cream brown	None	Blue to white	White	White
Substrate mycelium	Cream	Cream brown	Cream	Brown red	Light ivory	Brown
Soluble pigment	None	None	None	Brown red	Ivory	Brown
Glycerol yeast extract agar						
Growth	Good	Poor	Good	Poor	Good	Good
Arial mycelium	Beige red	None	None	Blue to white	White	White
Substrate mycelium	Yellow Orange	orange	Yellow	Light red orange	Saffron Yellow	Brown
Soluble pigment	Cream beige	Yellow orange	Yellow	None	None	Brown
Starch yeast extract agar						
Growth	Good	Good	Moderate	Poor	Good	Good
Arial mycelium	Beige red	Grey to white	Yellow	None	White	White
Substrate mycelium	Coral red	Cream brown	Yellow	Beige	Saffron Yellow	Brown red
Soluble pigment	None	None	Yellow	None	None	Yellow Orange

CONCLUSION

It is concluded in the present study that the actinomycetes isolated from various habitats of Hadhramout, Yemen have the ability for diffusible pigment- production on different culture media. The present findings also need further characterization of the pigments for their diverse applications such as in food and beverage, pharmaceutical, cosmetic and textile industries.

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