Journal of Global Biosciences
Peer Reviewed, Refereed, Open-Access Journal
ISSN 2320-1355
Volume 11, Number 6, 2022, pp. 9315-9326
Website: www.mutagens.co.in
URL: www.mutagens.co.in/jgb/vol.11/110602.pdf

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Research Paper

SCREENING AND ISOLATION OF PIGMENT PRODUCING BACTERIA

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Abstract

Bacterial pigments have various applications in current scenario. Pigmented bacteria otherwise called as chromo bacteria, mostly aerobic produce the pigments. Our present study is aimed at isolating the bacterial pigments from different soil samples (garden area, lake & agricultural areas), Flower samples were collected such as, Hibiscus (shoeblack plant), Gokarna (Clitoria Ternatea), Rose , Marigold (Tagetes erecta), etc. & Vegetable samples such as Spinach , Lufa from kitchen waste . Six different bacteria were selected for pigment production. These isolates were identified on the basis of morphological and biochemical test. Most of the isolated pigment producers were gram negative and cocci in shape.

Key words: *pigment production, bacteria, biochemical test*.

INTRODUCTION

Pigments are the chemical substances that absorb the light of visible region. The produced color is because of the chromophore, a molecule specific structure which captures the sun energy and causes an excitation of electron from external orbital to higher orbital, where the non-absorbed energy is refracted or reflected to be captured by eye. The modern meaning related to the word pigment has its origin in the twentieth century, meaning a substance constituted of small particles which is practically insoluble in the applied medium and is used due to its colorant, protective or other properties. Pigments are compounds with uniqueness of importance to many industries. In the food industry they are used as additives, antioxidants, color intensifiers, etc. Pigments come in a wide selection of colors, some of which are water-soluble.

They have widely been used in foodstuff, dyestuff, cosmetic and pharmaceutical manufacturing processes; encompass various hazardous effects. All synthetic food components suffered severe criticism, including synthetic additives and predominantly food pigments. Today, all food color additives are cautiously regulated by federal authorities to ensure that foods are safe to eat and accurately labeled. Pigments produced from natural sources are of worldwide interest and is gaining significance. These are looked upon for their safe use as a natural food dye in substitute of synthetic ones in spite of having of undesirable market. It is therefore, essential to explore various natural sources of food grade pigments and their potentials. The utilization of natural pigments in foodstuff, dyestuff, cosmetic and pharmaceutical manufacturing processes has been mounting in recent years . Natural colorants or dyes derived from flora and fauna are believed to be secure because of non-toxic, non-carcinogenic and biodegradable in nature. Natural pigments are attained from ores, insects, plants and microbes[26].

Among all, microbial pigments are dominant sources. The microbial production of carotenoids, pigments from vegetables or chemical synthesis, have problems of seasonal and geographic variability in the production and marketing .The microbial production of carotenoids, They are of great interest owing to the stability of the pigments produced and the accessibility of cultivation technology . The advantages of pigment production from microorganisms comprise easy and fast growth in the cheap culture medium, independence from weather conditions and colors of different shades [26].

The accessible authorized natural pigments from animals and plants have numerous drawbacks such as limited range, volatility against light, heat or adverse pH, low water solubility and are often non-availability throughout the year. Moreover microbial pigments are of great interest owing to the stability of the pigments produced and the availability of cultivation technology. The benefits of pigment production from microorganisms include easy and fast growth in the cheap culture medium, independence from weather conditions and colors of different shades. Hence, microbial pigment production is now one of the promising and emerging fields of research to reveal its potential for various industrial applications.

MATERIALS AND METHOD :

Sample procurement :

Various types of samples were collected from Aurangabad City region in December-January. Soil samples were collected from garden area, lake & agricultural areas. Flower samples were collected such as, Hibiscus (shoeblack plant), Gokarna (Clitoria Ternatea), Rose , Marigold(Tagetes erecta), etc.,& Vegetable samples such as Spinach , Lufa from kitchen waste were collected. Flower & Vegetable samples were dried under sunlight for 2-3 days and converted into fine powder and stored in seal-packed bags.

Screening and isolation of Microorganisms:

Screening of microorganisms was done by Enriching the pigment producing organisms from various collected samples was done in nutrient broth for 48h at 37^{o}C .After enrichment the pigment producing organisms were isolated using spread plate method. The serial dilution of various sample suspension (10^{-6}) was spreaded on nutrient agar plates. The plates were incubated at 37^{o} C for 24 to 48hrs. The plates were checked for isolated colonies. All the pigment-producing, morphologically different bacterial colonies that appeared on the plates were picked, and further isolated on sterile nutrient agar plates to obtain a pure culture. The colonies were further sub-cultured on the sterile Nutrient agar slants and maintained as stock cultures for further studies. Cultures were maintained on Nutrient agar slants and stored at 4°C in the refrigerator [26].

fig:1Enrichment and isolation of pigment producing bacteria.

Characterization of isolated pigment producing bacteria :

The bacterial isolates were studied for their Gram Characters by Gram staining. Morphological colony characters such as size, shape, colour, texture, opacity, elevation and margin were noted. (MeghaWaghela et al., 2018).

Identification of potential pigment-producing bacteria

Identification of the most promising bacterial isolate was done based on the morphological, cultural and biochemical tests.

Biochemical Analysis:

Hydrolytic enzyme activities: Biochemical tests are mainly done to identify bacteria capable of producing various exoenzyme which explore their properties of hydrolyzing waste material. The biochemical test like IMViC, catalase test , amylase production, gelatinase production, and caseinase production was checked. The organism was checked for indole test, methyl red test, vogues proskauer test. Agar plates were prepared containing protein, starch and lipid for testing, amylase, gelatinase, and Caseinase activity, respectively. If the inoculated bacterium secretes the respective exoenzyme, a clear zone of hydrolysis is observed around the inoculums. (Handbook of Media Stains and Reagents by .Deshmukh A M).

RESULT AND DISCUSSION :

Screening Isolation of pigment producing bacteria:

The samples obtained were streaked on Sterile Nutrient agar plates and incubated at 37^{o}C for 48-72 hours. A mixture of pigment producers and non-pigment producers were obtained on Nutrient agar plate after incubation of 48 hours out of which 6 morphologically different pigmented colonies were selected.

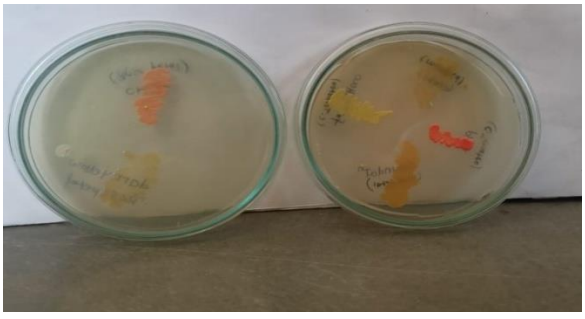

Fig2: isolated pigment producing organisms

Morphological and biochemical characteristics of isolated strains:

Microorganisms produce colonies with characteristics which could be seen by naked eyes that are called as cultural characteristics. The cultural characteristics were

observed on Nutrient Agar Medium plates after incubation. These morphological characteristics were observed in different forms such as colony form, colony elevation, surface of the colony and colony colour. The collected samples of soil, flowers, &vegetables were enumerated for their microbial load of total bacteria. The maximum number of bacterial population was exhibited in dilution 10^{-6} and $10^{-7.}$

The morphological examinations of the isolates were determined by standard procedure of gram staining.Many of the isolates were gram positive Cocci, from bergey's mannaul of systemic bacteriology the bacteria belong to *Staphalococcus spp. & Macrococcus spp.* The confirmation of the bacteria was done with ABIS software.

Table no. 1: Morphological characteristics.

	PP-1	**PP-2**	**PP-3**	**PP-5**	**PP-6**	**PP-7**
Colour	Orange	yellow	Pink	Dark brown	Reddish brown	Brown
Size	3mm±2mm	4mm±2mm	3.5mm±2mm	3mm±2mm	4mm±2mm	3mm±2mm
Shape	Round	Irregular	Round	Round	Oval/circular	Circular
Elevation	Convex	Convex	Convex	Flat	Raised	Raised
Consistancy	Thick	Thick	Thick	Thin	Thick	Thick
Margine	Entire	Irregular	Smooth	Smooth(entire)	Irregular	Cilliate
Opacity	Opaque	Opaque	Opaque	Slightly transperant	Opaque	Opaque
Gram's Nature	Positive	Positive	Positive	Positive	Positive	Positive

IMViC test:
In biochemical identification the IMViC test of the isolates was performed. The results observed are according to following table.

Table 2: IMViC test results

Isolated Pigment	**Citrate Test**	**Indole Test**	**MR Test**	**VP Test**
PP Isolate -1	**+**	**+**	**-**	**-**
PP Isolate -2	**+**	**+**	**+**	**-**
PP Isolate -3	**+**	**+**	**+**	**-**
PP Isolate-5	**+**	**+**	**+**	**-**
PP Isolate-6	**+**	**+**	**+**	**-**
PP Isolate-7	**-**	**+**	**+**	**-**

Sugar Fermentation Test:

Sugar fermentation test of the isolated pigments was performed the results observed are according to the following table.

Table 3: Sugar fermentation test

Isolated Pigment	Sucrose	Fructose	Lactose	Manitol	Glucose
PP isolate -1	-	+	-	+	-
PP isolate-2	-	+	-	-	+
PP isolate-3	+	+	-	+	-
PP isolate-5	-	+	-	-	-
PP isolate-6	-	+	-	-	+
PP isolate-7	-	+	-	+	-

In the Sugar fermentation Test the isolated pigments like in sucrose solution only PP isolate-3 shows positive result and in Fructose solution all the isolated pigments shows postitve results. Similarly, in Lactose solution all the isloted pigment shows negative result & in Manitol solution PP isolte-1,3, &7 shows positive results and in Glucose solution only PP isolate 2 & 6 shows positive results.

Enzyme hydrolysis :

The isolates were checked for exoenzyme production viz. amylase, caseinase, gelatinase and catalase & pectinases. The results were checked after incubation.

Table 4: Enzyme hydrolysis table

	Amylase	Gelatin hydrolysis	Casein	Catalase	Pectinases
PP Isolate-1	-	-	+	+	+
PP Isolate -2	-	+	+	+	-
PPisolate-3	+	+	-	+	+
PP isolate-5	+	+	-	+	-
PP isolate-6	+	+	+	+	+
PP isolate-7	+	+	+	+	+

Fig3: Casein hydrolysis test of isolate PP1, PP2, PP6,PP7.

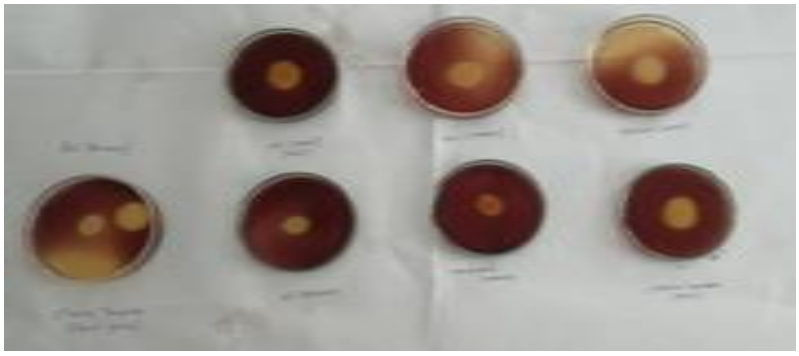

Fig 4:Gelatin hydrolysis test

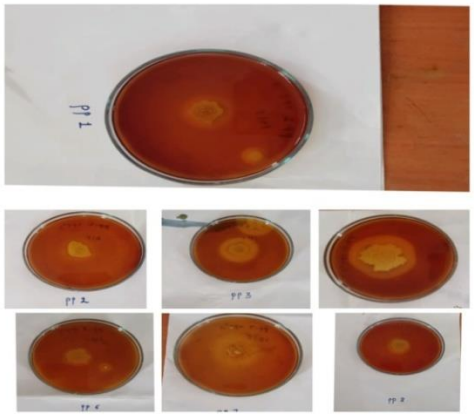

Fig5: Pectin hydrolysis.

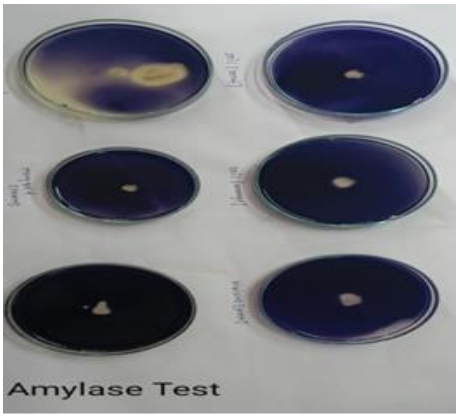

Fig 6: Amylase hydrolysis

DISCUSSION :

The present study reports that the pigment producing organisms were isolated from from different soil samples , kitchen waste , flowers etc. Collected from different region of Aurangabad district. 6 pigment producing bacteria were isolated producing orange, brown, reddish brown, yellow, dark brown and cream colour. In ealier studies a total of 30 morphologically different bacterial strains were isolated from the coffee plant rhizosphere soil sample in Yercaud hills region, Tamil Nadu. Among the 30 bacterial strains, only four bacterial strains producing antibacterial pigments. However, the brown pigment producing colonies were found predominant. Studies done in 15 pigment-producing bacteria were isolated from eight soil samples. and characterized which were red, yellow, cream, and light orange in color[36].

The present study report that the isolates were purified by quadrant streaking on nutrient agar incubated for 48 h at 37°C. After incubation, pure cultures of each isolates were preserved on nutrient agar slants for further use. The potential pigment producer was then characterized by Bergey's Manual of Determinative Bacteriology based on

morphological and biochemical characterization. In earlier reported that the pigment producers were then identified according to Bergey's Manual of Determinative Bacteriology [19] based on Gram staining, biochemical characteristics, and growth pattern on selective and differential media. The strains were found to belong to the genera Aeromonas (20%), Pseudomonas (20%), Chromobacterium (13.3%), Flavobacterium (6.7%), Bacillus (13.3%), Xanthomonas (6.7%), and Escherichia (20%) [29]. A taxonomic identification of the test isolates under study was reported by performing Gram's test, studying cultural characteristics of the isolates on selective and differential media and biochemical tests based on the Bergey's Manual of Determinative Bacteriology. Biochemical tests performed were IMViC, oxidase,gelatinase ,casienase and catalase tests. Samyuktha and Sayali Naphade Mahajan [37] has identified that the pigment-producing bacteria were carried out the three isolates which were found to be Gram-negative coccobacilli.

CONCLUSION :

The pigment producing bacteria were successfully isolated, This study deals with the pigment production and extraction from pigment producing organisms isolated from different soil samples , kitchen waste and garden flower samples. From the morphological and biochemical analysis the isolated pigment producing organisms belong to *Staphylococcus spp. and Micrococcus spp.* The organism were confirmed by ABIS software.

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