STUDY ON BACTERIAL PIGMENTS

[1]Shikha Chatterjee, [2]Riddhima Khatri
[1]PG research scholar,[2]Guide (Assistant professor)
[1]Microbiology
[1] Dolat-Usha Institute of Applied Science &[2] Dhiru-Sarla Institute of Management and Commerce, Valsad, Gujrat, India.

Abstract **:**
In recent years, the Utilization of natural pigments in food product, dyes, cosmetics, & pharmaceutical manufacturing processes has increased exotically. In order to fulfill the increasing demands for natural sources pigment producing microorganisms has been exploited at the industrial level. Natural sources like plants and microorganisms are used for the pigment production process. Pigment extraction from the plant will be somehow difficult and expensive as compared to the pigment extraction from the microorganisms. Microorganisms uses various cheap sources for growth and it make it easy to grow them on controlled condition and production of pigments in larger amount. Pigments are produced by growing microbes on solid nutrient agar medium and then it is purified by using centrifugation and filtration form the microorganisms and then isolation of pigments using various solvents. In our study various water, fruit, soil samples are collected from different areas of valsad Gujrat. From which we had isolated various pigment producing microorganisms of This paper will describe about various bacterial pigments and its application.

Index terms: Pigments, Production, Extraction, Antioxidant.

[I] INTRODUCTION

Colors have a strong impact on every creation of life, including the clothes we wear and the food we eat. The success of any pigment produced by fermentation depends on its acceptability in the market, regulatory approval, and the size of the capital investment required to bring the product to market. Colors plays a special role in the food we eat for example, when confronted with an unattractive color the consumers assumes that the food is poor or spoiled on the other hand products with atypical color-For example, green cheese or blue drink-in most cases, are rejected by the consumers. Typically one associates colors with food items such as cherry with red, lemon with yellow, or carrot with orange. Therefore, colors can serve as the primary identification of foods and also a protection measures. Natural colors are pigments, which are produced by living organisms such as Fungi, Yeast and Bacteria. Synthetic colors have been proved to be toxic and dangerous to mankind. The main source of natural pigments are plant and microorganisms. The most common plant pigments are carotenoids, chlorophylls and betalains. The usage of plant pigment have many limitations due to its non availability throughout the year, its stability and solubility and the large scale pigment production which may lead to loss of the species.

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Natural dyes produce very uncommon, soothing and soft shades as compared to synthetic dyes. Although, synthetic colors are widely available at economical price in wider range of colors but these dyes produce skin allergies, less stable and also produce highly toxic wastes that pose a threat. The color stability under extreme temperature, variable pH, and processing conditions is the pre requisite for industrial application. Therefore, microbial diversity has been a great source for exploration for application of bio pigments. The Fungal and Bacterial pigments are becoming an alternative source of naturally derived pigments. Pigments have been extensively used in food production, fish industries, textile industries, paper production, agricultural practices and other technology and also having biological activities as antioxidants, antimicrobial agents and anticancer agents.

There are many limitations of synthetic pigments. The precursors, used in the production process of synthetic pigment have many carcinogenic hazardous effects on the workers. The wastes of the production process are also harmful. They are itself non-environment friendly and non-biodegradable.

1.1. Classification of pigments:

Pigments are classified as either organic/inorganic or natural/synthetic. Plants, animals and microbes are the major sources of natural pigments. Naturally derived pigments are represented by carotenoids, flavonoids (anthocyanin's), and some tetra pyrroles (chlorophylls and phycobiliproteins). Synthetic pigments are synthesized in the laboratories through chemical manufacturing. Pigments from natural sources have been obtained since long time ago, and their interest has increased due to the toxicity problems caused by those of synthetic origin. In this way the pigments from microbial sources are a good alternative. Some of more important natural pigment are the carotenoids, flavonoids (anthocyanin's) and some tetrapirroles (chlorophylls, phycobiliproteins). Other less important groups are the betalains and Quinone's. The specific color of the pigment is characteristic for each microbe. Some microbes produce pigments as part of their normal metabolism. A large number of microorganisms (bacteria, molds and yeasts) produce pigments.

1.2. Advantages of microbial pigments over synthetic pigments

Microbial pigments offer following advantages:

- Easy propagation and wide strain selection.
- Highly versatile and productive over plants and synthetic sources.
- Fermentation is inherently faster and more productive compared to any other chemical process.
- Simple and fast culturing techniques allowing continuous bioreactor operation.
- Structural complexity suits for industrial needs.
- Bacterial pigments extracted using simple liquid–liquid extraction technique minimizing operation cost. Cheap substrates (maltose, glucose, galactose, peanut oil, sesame oil etc.) used for bulk production (Shahitha and Poornima, 2012).
- Microorganisms produce a large variety of stable pigments such as carotenoids, flavonoids, quinones, and rubramines, and the fermentation has higher yields in pigments and lower residues compared to the use of plants and animals (Hobson and Wales, 1998).

1.3. Industrial importance of microbial pigments

Pigments are compounds with characteristics of importance to many industries. In the food industry they are used as additives, color intensifiers, antioxidants etc (Tibor, 2007). The industrial production of natural food colorants is already well-established and expanding. However, the range of natural color shades is still limited compared to synthetic dyes. Besides, the use of plant extracts is known to be expensive and uncompetitive to synthetic dyes due to their high production costs. Consequently, microorganisms are becoming a more popular alternative source for natural food grade pigments. Development of microbial food grade pigments are likely to cut down the high production cost of natural colors, thus leading to a cheaper source of natural food colorants among the modern consumers. Pigments like indigoids, anthraquinones and naphthoquinones will hold potential applications in food industry in near future (Jacobson *et al.*, 1997). These pigments are looked upon for their safe use as a natural food colorants and will not only benefit human health but also preserve biodiversity, as harmful chemicals released into the environment while producing synthetic colorants could be stopped (Neeraj *et al.*, 2011).

[II] RESEARCH AND METHODOLOGY

2.1 Isolation of pigment producing microorganisms

1gm of soil sample was dissolved in 10 ml of sterile distilled water blank in test tube and used as a suspension. A loopful of suspension was evenly spread on sterile Nutrient agar plates containing 1% glycerol and the plates were incubated at Room temperature for 48-72 hours. The same procedures were followed for all the samples. The pigment producing colonies were restreaked and purified on Nutrient agar plates and for used for production. (Srimathi R *et al.*, 2017).

2.2 Production and Extraction of the Bacterial pigments

The organism were grown in Sterile Nutrient broth for 72 h with 2% glycerol supplementation. Then the culture medium were centrifuged at 1000 rpm for 15 minutes to separate cells. The harvested cells were washed twice and centrifuged at 1000 rpm The cell pellet then suspended in respective Methanol, Chloroform, Acetone solution

solvents (5:5 v/v) and centrifuged at 1000 rpm for 10 minutes. The coloured supernatant was collected and the process was repeated until the pellet turned white or colourless. λ max were determined using colorimeter from 400-700nm range (Vijay Lakshmi. K *et al.,* 2016).

2.3 Presumptive test of the pigment

I. Pink and Red color pigment

The isolated organism was inoculated in the nutrient broth and incubated for the observation of pigment production. The culture broth was centrifuged at 10,000 rpm for 10 minutes. The supernatant was discarded and the pellet was suspended in 95% methanol to extract the pigment from the cells. The suspended pellet was centrifuged at 10,000 rpm for 10 minutes. Debris was removed and the supernatant was taken in two tubes. The content of the first tube was acidified with a drop of concentrated hydrochloric acid and the second tube was alkalinized with a drop of concentrated ammonia solution. Then the colour change was observed and the results were interpreted.(Kurbanoglu *et al.,* 2013).

II. Yellow color pigment

Yellow color pigmented colonies was streaked onto nutrient agar plate and after incubation the colonies were flooded with a 20% potassium hydroxide solution is. A color change from gold to red-brown was observed and again with the addition of acetic acid it revert back to its initial color the results were noted.(Venil *et al..,* 2015).

III. Green color pigment

Blue- green pigmented isolate grown in nutrient medium after the centrifugation of the broth at 10,000 rpm for 15 mins. The pigments were placed into two different test tubes and the production of this pigment was also confirmed by an alteration in the color of the pigment from deep pink to red upon addition of chloroform and acidified using 0.2 (N) HCl **(**Sudhakar *et al.,* 2014)

2.4 Anti-oxidant activity

The anti-oxidant activity of the pigment were performed by using Potassium ferric reducing antioxidant power (P-FRAP) method with Butylated Hydroxy Toluene (BHT), (Nayan *et al.,* 2020) modified method. According to this method, the aliquots of various concentrations of the standard and test sample extracts (1 ml) in 1.0 ml of deionized water were mixed with 1 ml of (pH 6.6) phosphate buffer and 1 ml of (1%) potassium ferricyanide. The mixture was incubated at 55°C in water bath for 20 min after cooling. Aliquots of 1 ml of (10%) trichloroacetic acid were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution 1 ml was mixed with 1 ml distilled water and a freshly prepared 0.5 ml of (0.1%) ferric chloride solution. The absorbance was measured at 700 nm in UV spectrometer (Systronic double beam-UV-2201). A blank was prepared without adding extract. BHT Butylated Hydroxy Toluene at various concentrations (0.001 to 1 µg/ml) was used as standard. The result indicates that increase in absorbance of the reaction mixture indicates increase in reducing power.

[III] RESULTS AND DISCUSSION

3.1 Isolation of pigment producing bacteria

By performing isolation procedures from (Srimathi R *et al.,* 2017) method. Different shades of pink, red, yellow, green pigment producing bacteria's were isolates on nutrient agar plate as shown in the Figure 3.1.

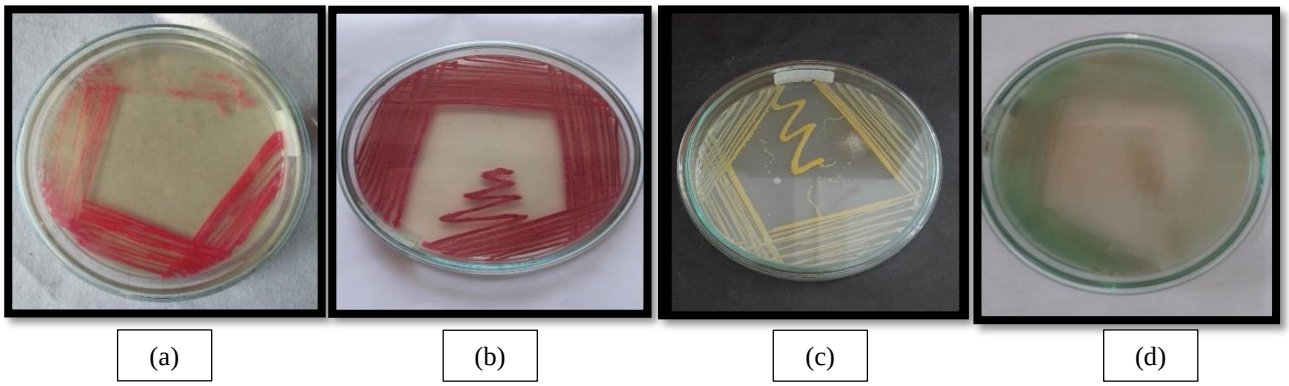


Figure 3.1: Pigment producing bacterial isolates on nutrient agar plate with 1% glycerol were (a)pink, (b)red, (c) yellow and (d) green.

3.2 Pigment Production and Extraction

3.2.1) Production of the pigments

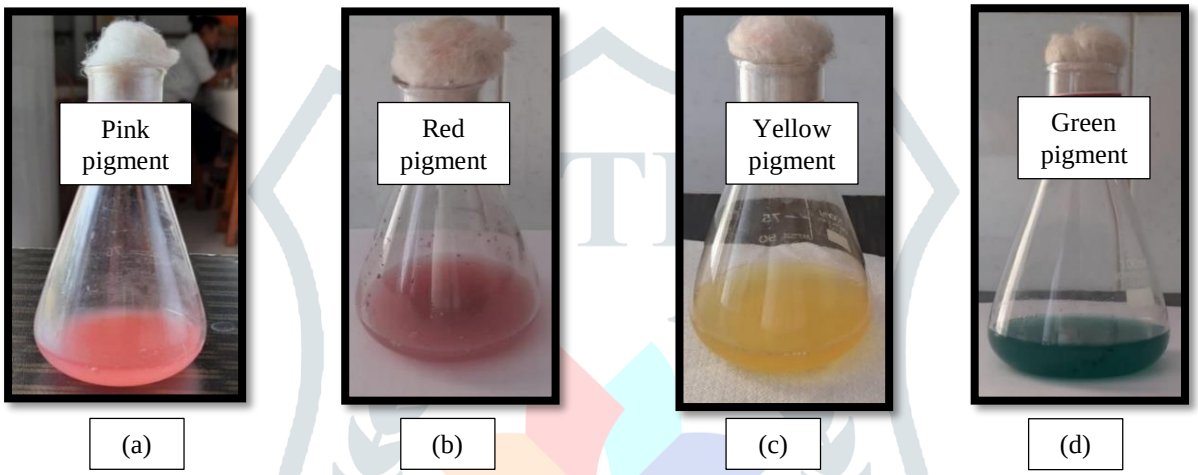


Figure 3.2.1) Pure culture of pigment producing bacterial isolates in nutrient broth supplemented with 1%glycerol were (a)pink, (b)red, (c) yellow and (d) green.

3.2.2) Extraction of the pigment

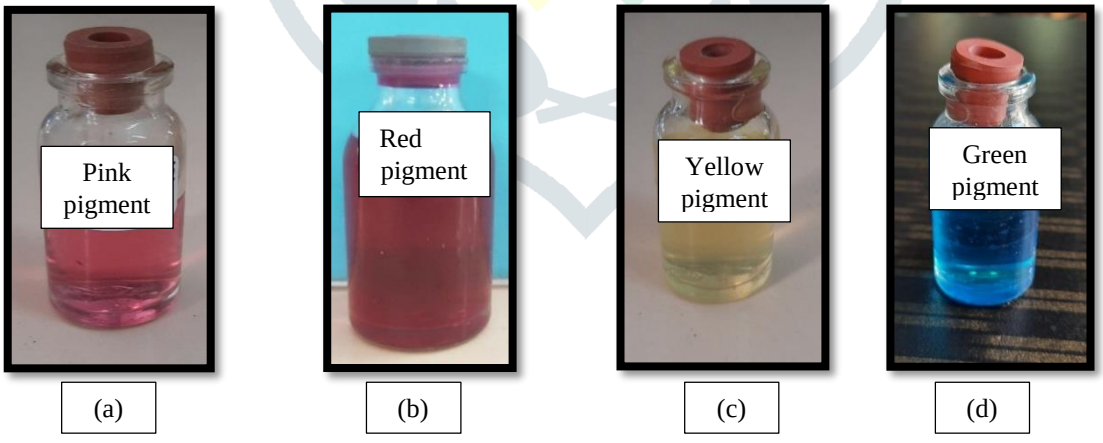


Figure 3.2.1) Extracted Bacterial pigments (a) Pink pigment, (b) Red pigment both were dissolved in Methanol solvent, (c) Yellow pigment dissolved in Acetone and (d) Green pigment dissolved in Chloroform solvent.

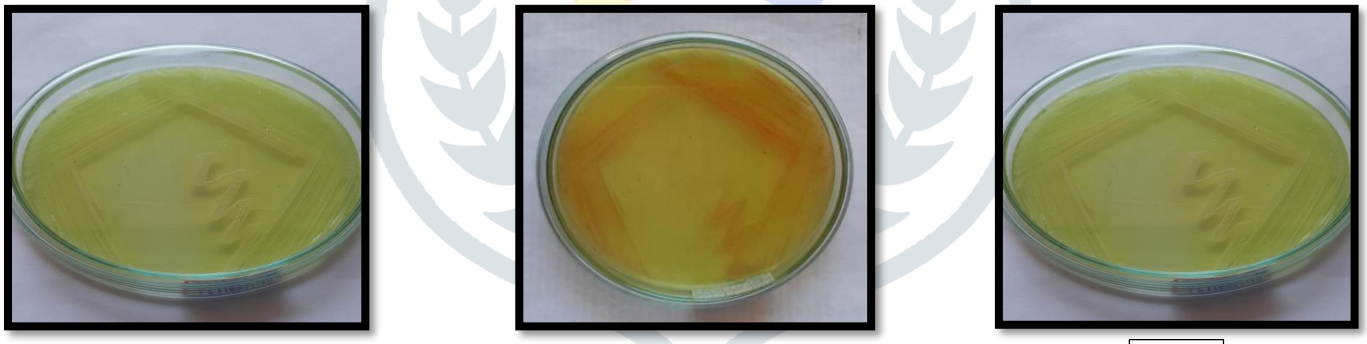
3.3) Presumptive test of the bacterial pigments

(a) (b) (c)

Figure 3.3.1) Presumptive test for the pigments (a) pink, (b) Red and (c) Green

Presumptive test for Pink and Red pigment (from left to right) -Control, red & pink pigment extracted in 95% Methanol solvent, pink and red pigment extract acidified with a drop of con HCL and – red & pink pigment alkalinized with a drop of con Ammonia solution Similar method has been carried out by Hariyali *et al.*,(2018). In their study pigment were extracted using acidified methanol. Fig 3.3.1 (a) and (b) shows the Red/Pink color in acidic condition and Yellow/ Tan color in alkaline condition which indicates in this study there may be the presence of Prodigiosin pigment.

Presumptive test for Bluish green color pigment fig 3.3.1 (c) (from left to right) Control without any solvent, Bluish green pigment extract in chloroform solvent and Bluish green pigment extract in 0.2N HCl Aziz *et al.*, (2012). In their study the pigment were extracted using chloroform as a solvent. The blue layer is separation were observed which may indicates the presence of Pyocyanin pigment , the blue layer is further purified by adding 0.2N HCL in it. Further confirmation of the pigment can be obtain using TLC separation technique.



(a) (b) (c)

Figure 3.3.2) Presumptive test for the Yellow pigment.

In present study, it shows the Rapid detection test for the flexirubin type of pigment which is corresponds to the work reported by Dhiraj Chaudary (2016), exhibited a color shift fig 3.3.2 (a) and (b) shows when yellow bacterial colony containing plate is flooded with 20% KOH which turns into yellow/golden to red/brown color and again with the addition of acetic acid it will revert back to its initial color as shown in fig 3.3.2 (c) which may indicates the presence of Flexirubin type of pigment. Further confirmation of the pigment is obtain by performing TLC separation technique.

3.4) Anti-oxidant activity of the bacterial pigments.

Sr no.	Test Tube's	Optical Density at 700nm
1	Blank	0.0
2	Standard (BHT)	0.3
3	Pink	0.2
4	Yellow	0.18
5	Green	0.07
6	Red	0.24

Table 3.4): Reducing power assay by (Nayan *et al*., 2020) modified method

The anti-oxidant activity of the pigment were performed by using Potassium ferric reducing antioxidant power (P-FRAP) method with Butylated Hydroxy Toluene (BHT) by (Nayan *et al*., 2020) modified method. Reducing power assay method is based on the principle that substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric–ferrous complex that has an absorption maximum at 700 nm.

[IV] CONCLUSION AND FUTURE PROSPECTS

From the above all results it can be stated that the pigment producing bacteria were isolated and the production and extraction of crude pigment is done successfully in a favorable solvents. Hence by substituting synthetic pigment with natural pigments such as bacterial pigments it will be very much beneficial for human health by using various field such as pharmaceutical, textile, cosmetics etc as an antioxidant activity because of its low toxicity.

Studies should be concern especially on finding the easiest method for harvesting bacterial pigments in order to increase their industrials applications. Also there is a need to look on various operational parameters that may cause a variation due to change and develop a new low cost process for the production of bacterial pigments by using agro-waste as substrate in the future. Future investigation on various technologies that would reduce the cost and increase yields for large scale production.

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