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Research Article

ISOLATION AND CHARACTERIZATION OF PIGMENT PRODUCING BACTERIA AND THEIR POSSIBLE USE IN TEXTILE INDUSTRY

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ABSTRACT

The environment where we live is the habitat for various microorganisms, mostly bacteria which are used for various industrial applications like enzyme production, pharmaceutical production, fabric manufacturing and more. Colorants are used in lots of products including cosmetics, food and textiles. These colorants are synthetic and very harmful for humans. It is very harmful to environment because it contain toxic and non-biodegradable compounds. Now a days there is a growing demand for natural dyes which can be biodegradable and less harmful for the humans. Industry have lots of interest in microbial pigments, because they are more stable and soluble than pigments produced from plants and animals sources. It also can grow rapidly which will produce high yield throughout the year. Carotenoid are the very important compounds and are widely used in textile industry. Most of the fruits and vegetables contain carotenoids. These pigments have some limitations which include solubility, sensibility and instability on exposure to light, temperature and pH. The aim of the present study was to isolate pigment producing bacteria from three different soil samples. In this study yellow and orange pigmented bacteria were isolated and developed in to pure culture. Morphological and biochemical characterization was carried out and these isolates were observed for maximun pigment production at diffrent pH, Temperature and NaCl Concentrations. Yellow and Orange colour bacterial extracts of carotenoids pigments were extracted and used as natural colorants for dying of cloth. The present study indicated that pigment production was influenced by physical factors such as pH, Temperature and NaCl. Absorbance of carotenoids pigments were tested by using UV-Visible spectroscopy and these pigments shows a promising result in textile industry as natural colorants.

Keywords: Soil samples, Pigment extraction, Natural colorants, Textile application.

1. INTRODUCTION

Microorganisms are the most important tools in biotechnology to produce variety of molecules like enzymes, antibiotics and pigments. Microorganisms are a promising source for natural colours. The presence of pigments has been reported in entire microbial world including bacteria, fungi, yeast, algae and protozoa. Industrial production of natural pigment by microbial fermentation has several advantages such as cheaper production, easier extraction and higher yields throughout the year. In the food industry they are used as colouring agent and antioxidants. Pigments are present in various colours [1].

The synthetic dyes gained much attraction due to several characteristics such as easy to develop, economical, no undesirable flavours, excellent colouring properties, and needed in a small amount to use. Most of the synthetic dyes used had never been tested for their toxic effects on the environment and our health [2]. Synthetic dyes are made up of chemical compounds composed of benzene, lead, copper, mercury, chromium and sodium chloride that are harmful to human health. Lots of synthetic colorants previously permitted by the Food and Drug Administration (FDA) to use in medicines, food, and cosmetics development were later found to cause cancer. Some of these synthetic dyes previously used are now not used due to apparent hazards such as carbon black, a potential carcinogen and benzidine a causative agent of bowel cancer [3].

Microorganisms produce variety of pigments, so they can be used as natural colorants instead of synthetic colorants. Microorganisms found in different environments produce various pigments. These pigments are carotenoids, flavins, chlorophyll, quinines, prodigiosins. These natural pigments can be used in various commercial fields of pharmaceutical, food, textile and

food industry. B-Carotene, arpink red, riboflavin, lycopene and monascus are food grade pigments which are used in food industries [4].

Pigment are the important organic constituents of bacterial protoplasm. Some of the pigments are melanine, prodigiosin, iodinine, pyocyanin, violacein, phenazine and pulcherrimin are metabolic by-products formed under certain conditions. Natural pigments have lots of demands in the food industry. Ingredients, such as colours, are considered natural when it is derived from biological sources like plants and microorganisms. So lots of industries are now able to produce some microbial pigments for applications in food, cosmetics and textiles. In nature, colour rich and pigment producing microorganisms like fungi, yeasts and bacteria are quite common. The pigments was isolated from species such as: *Serratia plymuthica*, *Serratia rubidaea*, *Hahella chejuensis*, *Vibrio gazogenes*. These pigment have been reported to have antifungal, antibacterial, algicidal, antiprotozoal, antimalarial activities, immunosuppressive and anticancer activities. Carotenoids is a group of bioactive compounds which are responsible for yellow, orange and red pigments. Carotenoids are abundantly present in plants, micro-organisms and animals and are widely distributed in the nature [5].

Natural pigments that are an alternative to synthetic pigments could be obtained from plants and micro-organisms. Natural pigments obtained from microbes are more ideal over plants and animals due to the solubility, and stability of pigments and the easy availability of microbes for culturing [3]. Among these metabolites, pigments are charismatic traits of microorganisms that can be used in several industrial applications. Pigments production mostly occurs within cytoplasm [6].

Carotenoids are fat-soluble, highly unsaturated red, orange and yellow pigments that are naturally present in plants, fungi, bacteria and algae. The intensity of colour is generally related with the number of carotenoids. They are naturally found in abundance in vegetables and fruits. Moreover, certain photosynthetic bacteria and algae are also found to be the good source of carotenoid compounds. The phytochemical carotenoids belong to the isoprenoids. The basic structure is made up of eight isoprene units, having C 40 backbone. There are two types of carotenoids. One is Carotenes-the pure hydrocarbons. Second one is xanthophylls which are derivatives that contain one or more oxygen functions. Carotenoids collaborate with other biomolecules such as proteins and lipids have anti-oxidant activity [7]. Carotenoids gets cleaved into apocarotenoids, responsible for aroma, colour and phytohormone production, which is very helpful in producing signals among the plant cells [8].

Carotenoids are able to scavenge the free oxygen radicals in our body. Free radicals can help in curing certain types of cancer and reduce the formation tumour in cancer patients. Certain carotenoids are found to activate the antioxidant gene expression which helps in decreasing neurological disorder and diabetes [9]. To prove other sources than plants and animals, a carotenoid rich fraction of *D. salina* was taken and tested to prove that carotenoids can attenuate the cardiac dysfunction in obese rats [10].

In nervous system, increase of oxidative stress results into several neurodegenerative diseases such as Alzheimer's, Huntington's and Parkinson's disease. Main reason for several diseases are inability of Ca^{2+} to signal the molecules. But pigments like astaxanthin, β-carotene and lycopene are involved in Ca^{2+} ion transportation in brain. So proper dietary of carotenoids malfunction can be reduced due to improper signalling [11].

Prodigiosin is a red colour pigment primarily reported from *Serratia marcescens*. Prodigiosin was named after its extraction from *Bacillus prodigious* and later given the name of *S. marcescens*. Biosynthesis of prodigiosin is controlled by quorum sensing [2].

Violacin is a violet or purple colour pigments and the maximum UV absorption capacity of violacein is $\lambda = 260$ nm, which suggests its crucial role in the protection of cells from UV radiations [13]. Pigments can act as an antimicrobial agent [14]. Depending upon concentration of pigments, the production could visibly provide coloration to show such as green due to chlorophylls, various yellow shades due to xantophylls, and orange to red due to carotenoids [15]. This indicates that the pigments produced by microorganisms would rule the pigment industries very soon [16]. Natural pigments will replace the synthetic dyes in the textile industry with environmentally friendly dyes. Natural pigments obtained from microorganisms are eco-friendly. So it is considered appropriate for the textile industry [17].

Natural pigments are used in food industries; Violacein obtained from bacterial sources has been used in food industries [18]. Canthaxanthin is used in food items such as beverages, snacks, meat, fish, candy, cheese and fruits. Several pigments obtained from microorganisms are used in food industry [19]. Infectious diseases are the second major reason for global human deaths [20]. Free radicals present inside our body increases the risks of chronic diseases such as cancer, diabetes and autoimmune

disorders [21]. Antioxidant compounds are used which donate electrons to free radicals and neutralize them to protect cellular damage in our body [2]. Microbial pigments also can act as anticancer agents. Novel red pigment extracted from *Athrobacter* sp. G20 exhibited anticancer potential against the oesophageal cancer cell line [22].

The objective of our study was to isolate and characterize pigment producing bacteria from different soil samples. These bacterial isolates were cultured in different temperatures, pH and NaCl concentration to evaluate the maximum pigment production. These pigments are extracted and used as natural colorant for cloth dying.

2. MATERIAL AND METHODS

2.1. Isolation of pigment producing bacteria

Various samples like soil, spoiled fruits and vegetables were collected. Soil samples were collected from differenet area of Tamilnadu such as Kothagiri, Rameshwaram and Coimbatore. All the samples are collected in a clean and dry container. From this, pigment producing bacteria were isolated and used for the present study. One g of each sample was mixed with 9 ml of saline (0.85% NaCl w/v). The mixture was vortexed for uniform suspension. From this, 100 µl was inoculated in nutrient agar plate using spread plate technique. The inoculated plates were incubated at 37°C for 24 hours. From this, yellow and orange color pigment producing bacteria was selected and repeatedly sub cultured for pure culture [23].

2.2. Identification of bacteria

Identification of bacterial isolate was performed by morphological characteristics and biochemical tests.

2.2.1. Morphological Characteristics

Colony characterization of pigment producing bacteria from nutrient agar plate was done by colony size, colour, shape, margin, opacity, consistency, elevation, motility and gram staining [24].

2.2.2. Biochemical test

Biochemical test performed were Indole test, Methyl Red (MR), Voges Proskauer (VP), Simmon's citrates test, Oxidase test, Catalase test, TSI test, Urease test and nitrate reduction tests recommended in the Bergey's Manual of Determinative Bacteriology [25].

2.3. Screening of pigment production

The colour pigment producing bacteria was taken from the nutrient agar for pigment production. A loop full of culture was inoculated in to sterile 100 ml Nutrient broth mixed with 2% glycerol and incubated at 37°C for 2 days in a rotatory shaker [26].

2.4. Extraction of pigments

The pigment producing bacteria was centrifuged at 2000 rpm for 20 mins. The supernatants were discarded and then the pellets were resuspended in acidified ethanol. The mixture was vortexed and the suspension was centrifuged at 2000 rpm for 10 mins and the super-natant was collected and pigment extract were filtered through Whatmann filter paper. The absorbance of the filtrates was measured on UV- visible spectrophotometer in the range of 300- 400nm [26].

2.5. Strategic manipulation of the growth media for maximum pigment production by bacterial isolates [23]

2.5.1. Effect of Temperature on the pigment production

Petriplates containing nutrient agar were prepared in four sets (I, II, III, IV) and inoculated with bacterial isolates and incubated for 24 hours at different temperature. The petriplate belonging set I, II, III, IV were incubated at 25°C , 30°C, 35°C and 40°C respectively, to study the effect of temperature on the pigment production.

2.5.2. Effect of pH on the pigment production

Petriplates containing nutrient agar were prepared in four sets (I, II, III, IV). Prior to autoclaving, the pH of the petriplate belonging set I, II, III, IV was adjusted to 6, 7, 8 and 9 respectively and inoculated with bacterial isolates and incubated for 24 hours at 37°C to study the effect of pH on the pigment production.

2.5.3. Effect of NaCl on the pigment production

Petriplates containing nutrient agar were prepared in four sets (I, II, III, IV). Prior to autoclaving the NaCl concentration of the petriplate belonging set I, II, III, IV were used are 2% , 4%, 6% and 9% respectively and inoculated with bacterial isolates and incubated for 24 hours at 37°C to study the effect salt concentration on the pigment production.

2.6. Pigment Extracts on textile application

The bacterial carotenoid yellow and orange colour pigment extract was mixed with alum potassium aluminium sulphate (6%). The cotton fabric was kept soaked in the solution for 5 minutes and kept for drying [27].

2.7. Washing performance

Dried cotton fabric was soaked in detergent solution for 20 minutes and washed using tap water. The fabric was dried for 30 minutes.

3. RESULTS AND DISCUSSION

3.1. Isolation of Pigment producing bacteria

Various samples like soil, spoiled fruits and vegetables were taken for isolation of bacteria. Among the colonies of bacteria formed on the nutrient agar, yellow and orange colour colonies are produced. From these, yellow and orange colour colonies are repeatedly sub cultured in nutrient agar to produce pure cultures (Fig. 1).

3.2. Identification of bacteria

Yellow and orange colour bacterial isolates were grown on nutrient agar. Identification of bacteria were done by performing morphological and biochemical tests.

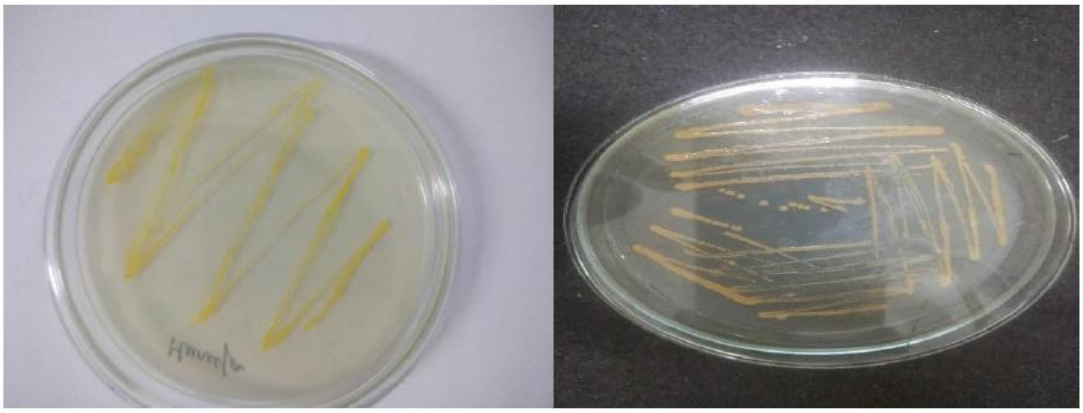

Fig. 1: Nutrient agar containing Yellow and Orange colour colonies

3.2.1. Morphological Charaterization

Characterization of Yellow pigment producing bacteria from nutrient agar plate was done by colony shape, colour, margin, opacity, consistency, elevation and gram staining are mentioned in Table 1.

Table 1: Morphological characterization of isolates

CHARACTERS	OBSERVATION (Yellow)	OBSERVATION (Orange)
Color	Yellow	Orange
Shape	Rod	Rod
Margin	Entire	Entire
Elevation	Convex	Convex
Opacity	Non-Opaque	Non-Opaque
Consistency	Non-sticky	Non-sticky
Gram Staining	Gram Positive	Gram Positive

3.2.2. Biochemical Characteristics

The results of biochemical characterization carried out are presented in Table 2. Catalase test was the first biochemical test done according to the key for identification of Gram positive bacteria (Bergey's manual). Catalase is an enzyme that decomposes hydrogen peroxide into oxygen and water molecules. Microorganism that produces catalase is introduced into hydrogen peroxide, rapid elaboration of bubbles of oxygen, the gaseous product is produced.

Table 2: Biochemical characterization of isolates

TEST	OBSERVATION (YELLOW)	OBSERVATION (ORANGE)
Indole test	Negative	Negative
Voges-proskauer test	Negative	Positive
Methyl red test	Negaive	Positive
Citrate utilization test	Negative	Negative
Oxidase test	Negative	Positive
Catalae test	Negative	Positive
TSI	Positive	Positive
Urease test	Negative	Negative

From the above observation the yellow pigment isolates was found to be *Lactobacillus* sp. and orange pigment isolates was found to be *Exiguobacterium* sp.

3.3. Screening and Extraction of pigments from pigment producing bacteria

Isolated pigment were grown in nutrient agar broth for 2 days at 37°C for screening (Fig 2a). Yellow and Orange colour pigment was extracted from nutrient broth using different technique like centrifugation and filteration and addition of acidified ethanol so the cells

get lysed (Fig 2b). The absorbance was measured at UV-Visible spectrophotometer and yellow and orange pigments was found to be 5.35 µg/ml and 2.59 µg/ml. The extracted pigments were used for further experiments.

Orange Pigment **Yellow Pigment**

Fig. 2a: Visual Observation of cultural of isolated pigment producing oranism

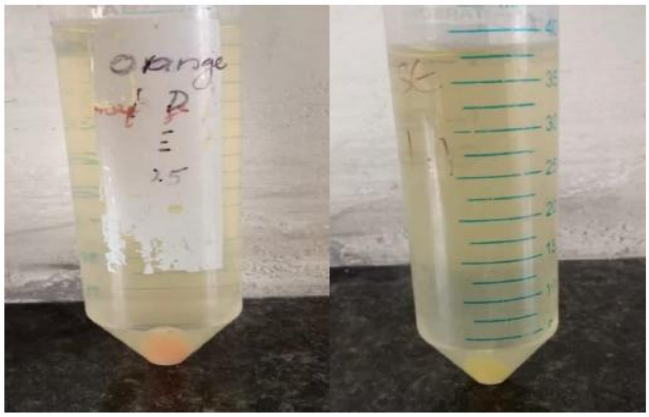

Fig. 2b: Extracted pigments of orange and yellow

3.4. Strategic manipulation of the growth media for maximum pigment production by bacterial isolates

3.4.1. Effect of Temperature on the pigment production

Results in Table 3 and Fig. 3 show that the yellow pigment production was found to be least at 25°C compared to 30°C, 35°C and 40°C. So increase in temperature showed an increased in growth of bacteria and its pigment production capacity. But the results in Table 2 and Fig. 2 shows that the orange colour pigment production was found to be maximum at 25°C, 35°C and 40°C respectively. The production of microbial pigments is greatly affected by the temperature of incubation depending upon the type of inoculated microorganism. Among the different range of temperatures on the pigment production by the selected bacteria, it was seen that there was a gradual increase in growth and pigment production with increase in temperature up to 40°C.

3.4.2. Effect of pH on the pigment production

The results for the effect of pH on pigment production are presented in Table 4 and Fig. 4. The pigment production increased with the increase in pH. Maximum pigment production was observed at pH 9. At pH 6, yellow pigment production was found to be minimum but Orange pigment production was not found. The results indicated that increase in pH favours pigment production. They studied the effect of pH on pigment production by *Micrococcus flavus* with different pH ranges (6, 7and 7.5). It was observed that pH 7.5 was found to be optimum for maximum pigment production.

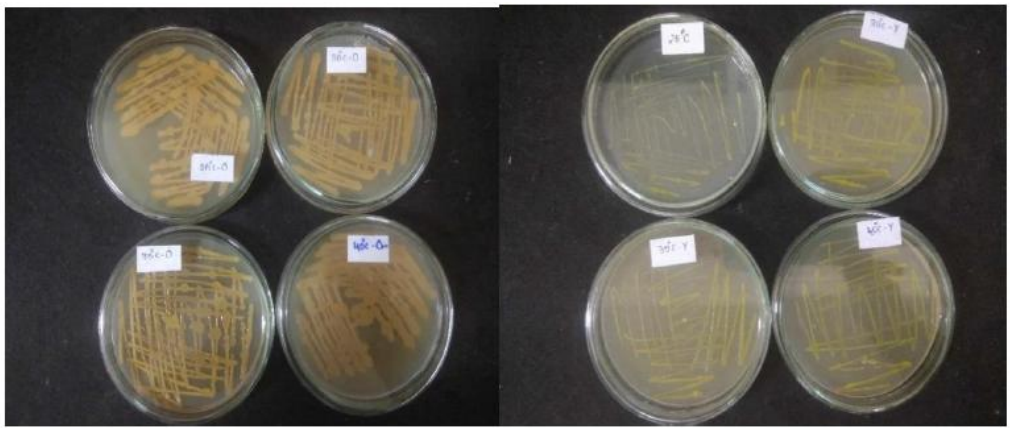

Fig. 3: Effect of Temperature on the pigment production on nutrient agar

3.4.3. Effect of NaCl on the pigment production

The results for the effect of various concentrations of NaCl are presented in Table 5 and Fig. 5. There was a gradual decrease in pigment production with increase in salt concentration from 4% to 8%, however, maximum pigment production was observed at 2% salt concentration. Orange colour pigment production was found to be present in 2%, 4% and 6% NaCl but absent in 8% Nacl concentration. In yellow colour pigment production was found to be present only in 2%, minimum in 4% and 6% and absent in 8% NaCl concentration.

3.5. Application of pigment extracts to cotton fabric

The isolated bacterial pigments were mixed with alum potassium aluminium sulphate to dye cotton fabric and kept for drying. The fabrics retained the yellow and orange colour after washing (Fig. 6). These pigments can be utilized in textile industry instead of synthetic dyes because it is more eco-friendly.

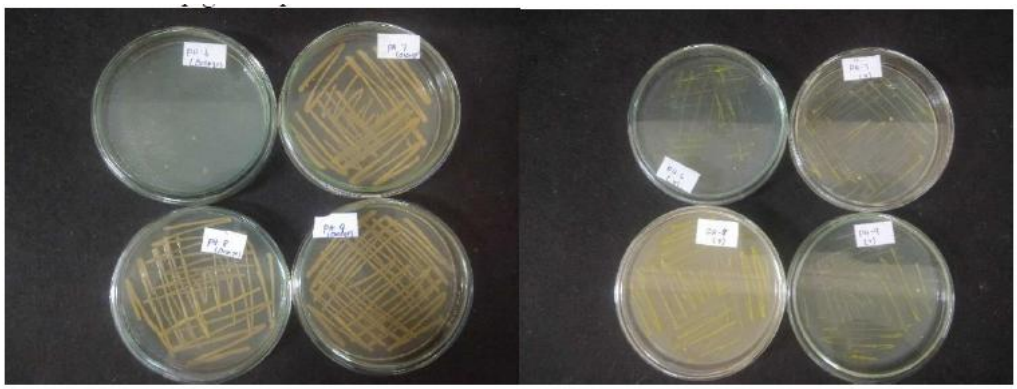

Fig. 4: Effect of pH on the pigment production on Nutrient agar

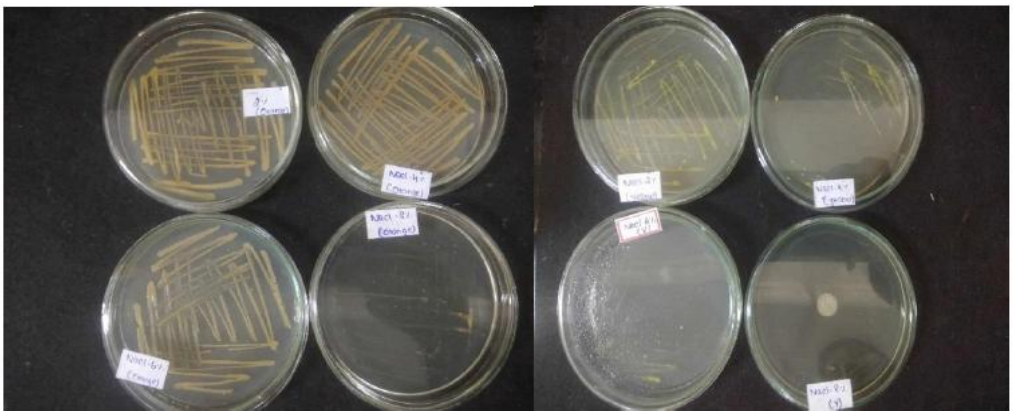

Fig. 5: Effect of NaCl Concentration on the pigment production on Nutrient Agar

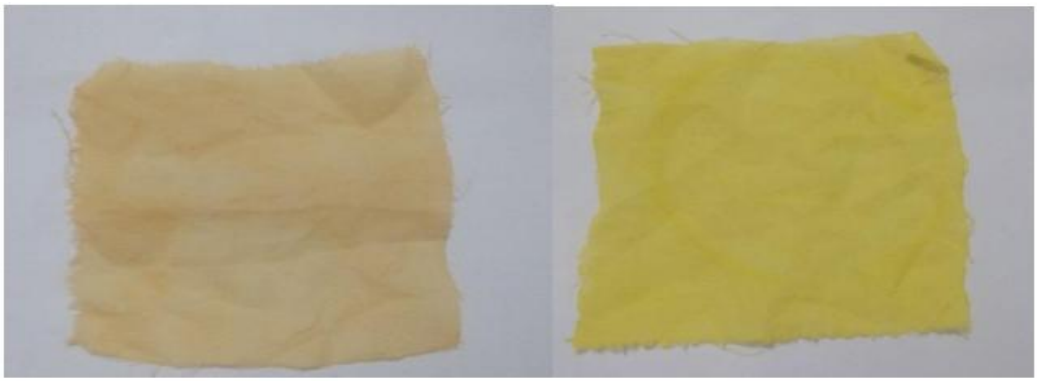

Fig. 6: Colourization done in cotton cloth using yellow and orange pigments

Table 3: Effect of Temperature on the pigment production

Temperature	Yellow	Orange
25˚C	++	+++
30˚C	+++	+++
35˚C	+++	+++
40˚C	+++	+++

Where; +++ (excellent); ++ (good); + (Present) - (nil)

Table 4: Effect of pH on the pigment production

pH	YELLOW	ORANGE
6	+	-
7	++	+++
8	+++	+++
9	+++	+++

Where; +++ (excellent); ++ (good); + (Present) - (nil)

Table 5: Effect of NaCl Concentration on the pigment production

NaCl concentration	Yellow	Orange
2%	++	+++
4%	+	+++
6%	-	++
8%	-	+

Where; +++ (excellent); ++ (good); + (Present) - (nil)

4. CONCLUSION

The present study indicated that pigment production was influenced by physical factors such as temperature, pH and NaCl concentration of the isolated culture medium. There may be many other factors affecting the pigmentation of the microorganism. An understanding of these factors can help to develop a controlled bioprocess for the enhanced production of the desired pigment. The extracted pigment were used for dying cotton cloth. So this will be a opening to the new avenues for further research in this field.

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Conflict of interest

The authors declared that they have no conflict of interest.

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