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Isolation and characterization of pigment producing bacteria isolated from waste

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Abstract
The natural colour, easy production and non-toxic nature of the pigments obtained from bacteria have led them to gain worldwide significance and provided to be an alternative over its chemical counterparts. This study involves the isolation of pigment-producing bacteria from different sources like rotten vegetables, soil and air flora. Ten bacterial isolates were selected and named PR 1 to 10. The morphological characteristics and Grams nature of these isolates were studied. The isolates were identified using VITEK 2, Nucleus Diagnostic Laboratory, Kalyan. The effect of various Physical (Temperature, pH, Incubation period) and Chemical (Concentration of NaCl, Tryptone) parameters were checked for pigment production. The pigments produced were extracted with different solvents like ethanol, methanol, chloroform and acetone. The isolated pigments were characterized using Uv-Vis Spectrophotometer and TLC and were found to be Carotenoids in nature.

Keywords: Bacterial pigment, pigment production, carotenoids, pigment extraction

1. Introduction
Pigments are molecules that have colour. Examples of pigments obtained from natural sources are ores, insects, plants, and microbes. Bacteria that produce pigment are called as Chromogenic bacteria. Some bacteria produce pigment as part of their normal metabolism including black, white, brown, golden, silver, fluorescent green, yellow or blue. The specific colour of the pigment is characteristic of a bacterium; pigmented bacteria will form cultures that exhibited some colour. Production of pigments can be extracellular or intracellular depending on the micro-organisms. Pigment production is very useful for bacteria. Pigment production in bacteria is associated with morphological characteristics, cellular activities, pathogenesis, protection, U.V. radiation and, survival. Pigments of extremophiles are very colourful and are required for respiratory and photosynthetic functions (Rokade and Pethe, 2016) [1]. Micro-organisms produce a variety of pigments including carotenoids, prodigiosin, melanins, quinones, flavins, monascins, violacein, etc some of these are water-soluble (Sinha *et al*., 2017) [2]. Examples of micro-organisms that produce pigments in high yields include species of *Monascus, Paecilomyces, Serratia, Cordyceps, Streptomyces, Penicillium herquei, Penicillium atrovenetum, Sarcina, Cryptococcus, Monascus purpureus, Phaffia rhodozyma, Bacillus sp., Achromobacter, Yarrowia,* etc. (Malik *et al*., 2012) [3].
Natural starting materials are favourable for today's production lines according to green technology. Due to the carcinogenicity of some synthetic dyes and the effect of the disposal of their industrial wastes into the environment, its manufacturing is prohibited (Dufosse`, 2009) [4]. The newfound awareness in environmental safety and conservation has kindled fresh enthusiasm for exploring natural sources of colours. Colourants derived from natural sources are believed to be safe because they are non-carcinogenic, non-toxic and biodegradable (Pattnaik *et al.,* 1997) [5]. Recent studies have focused on employing microbial pigments as the source of natural colourants instead of the commonly used plant pigments (Ahmad *et al.,* 2012) [6]. Microbial pigments offer rapid and unlimited productivity throughout the year irrespective of the season (Gunasekaran and Poorniammal, 2008) [7]. Pigment production from micro-organisms is easy, lesser requirement of expensive culture medium and it is independent of weather conditions (Bhat *et al.,* 2013) [8]. The present study was carried out with an aim to isolate pigment-producing bacteria from different sources, optimizing the physicochemical parameters required to scale-up the production, extraction, and characterization of the pigment.

2. Materials and methods

2.1 Test chemicals

The media components (Nutrient agar and Nutrient broth) required for this study were procured from Himedia, India. All the chemicals were made in distilled water. Solvents like Ethanol, Methanol, Chloroform and Acetone were obtained from SRL.LTD. India.

2.2 Isolation of pigmented bacteria Pigmented bacteria were isolated from three different sources- Rotten fruits and vegetables, Soil, and Air flora.

2.3 Sample collection:

Rotten fruits and vegetables were collected from Kalyan area and were used for the isolation of pigment-producing bacteria. From the collected samples, a mixture was made by mashing the samples with distilled water in a mortar and pestle. A loopful of the mixture was streaked on sterile Nutrient agar plates and the plates were incubated at 37°C for 48 hours (Barth *et al*., 2009) [9]. The soil sample was collected from the garden area of B.K. Birla College, Kalyan at a depth of 10 to 15 cm from the surface. Soil suspensions were prepared using sterile distilled water and a loopful was streaked on sterile Nutrient agar plates and kept for incubation at 37^0C for 48 hours. The plates were observed for growth after 48 hours of incubation (Samyuktha and Naphade-Mahajan, 2016) [10]. The sampling of air flora was carried out by keeping the Nutrient agar plates exposed to air for 30 minutes. The plates were incubated at 37^0C for 48 hours (Bharmal *et al*., 2012) [11].

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 (Shaikh, 2016) [12].

2.4 Characterization of isolated pigmented 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 (Sinha *et al*., 2017) [2].

2.5 Identification of potential pigment-producing bacteria

Identification of the most promising bacterial isolate was done based on the morphological, cultural and biochemical tests using VITEK 2, Nucleus Diagnostic Centre, Kalyan.

2.6 Optimization of various growth parameters for maximum pigment production (pH, Temperature, Sodium chloride and Tryptone)

The effect of various physiological parameters such as temperature, pH trptone and salt concentration was studied on the growth of the selected bacterial isolates to determine which gave maximum pigment production.

2.6.1 Effect of temperature

Bacterial isolates were streaked on sterile Nutrient agar slants (pH 7.4) and incubated at a varying range of temperature viz. 10^0C, RT and 37^0C for 24-48 hours.

2.6.2 Effect of trptone concentration

The effect of tryptone (as a Carbon Source) was determined by streaking the cultures on sterile Nutrient agar slants with 1%, 2%, 3% tryptone concentrations and were incubated at 37ºC for about 24- 48 hours, further assayed for pigment production.

2.6.3 Effect of pH

Sterile Nutrient agar slants with different pH (5, 7, 9, and 12) were prepared. The isolated bacteria were streaked and kept for incubation at 37°C for 24-48 hours.

2.6.4 Effect of salt concentration

The effect of Sodium chloride was determined by streaking the bacterial isolates on sterile Nutrient agar slants with sodium chloride concentrations of 2.5%, 4.5% 6.5%, and 8.5% and incubated at 37°C for 24-48 hours.

For all the physiological parameters, the positive result in the form of pigmented growth was checked after 48 hours of incubation using negative control (sterile plain Nutrient agar) and positive control (sterile plain Nutrient agar streaked with bacterial isolates).

2.7 Production of pigment

For the production of the pigment, the isolated bacterial cultures were suspended in two flasks-one containing plain sterile Nutrient broth (Priya *et al*., 2017) [13] (Fig. 1) and the other with sterile Nutrient broth containing 2% glycerol (Sinha *et al*., 2017) [2] (Fig. 2). Both the flasks were incubated at 37^0C for 7 days for observation of maximum pigment production.

Fig 1: Inoculated Nutrient Broths

Fig 2: Inoculated Nutrient Broths (2% Glycerol)

2.8 Extraction of pigment

Different organic solvents like Ethanol, Methanol, Chloroform, and Acetone were screened for maximum

extraction of pigment. After incubation, the culture broth was centrifuged at 3000 rpm for 10 minutes. The colourless supernatant was discarded. The pellet was mixed with the different solvents via vortex mixing repeatedly until the pellet turned colourless (Priya *et al*., 2017) [13]. The supernatant was separated and filtered through Whatman No.1 filter paper and kept in a hot air oven for solvent evaporation. The dry pigment residues left after evaporation were suspended in the solvent and then re-evaporated, this step was repeated 2-3 times so that pure pigments were obtained.

2.9 Characterization of pigment:

2.9.1 Spectrophotometric analysis

Pigment expression can be monitored spectrophotometrically as most pigments absorb light at some defined wavelength. The extract was analyzed in the range of 400nm to 700nm by an UV-visible spectrophotometer to find out the maximum absorption spectra. Methanol was used as a blank (Priya *et al*., 2017) [13].

2.9.2 Chromatography analysis

Thin-Layer Chromatography

Qualitative analysis of pigments was carried out by Thin Layer Chromatography (TLC). The samples were spotted on the baseline of the TLC plates with the help of a capillary tube and then allowed to dry at room temperature; this step was repeated three to four times. The TLC plates were then placed in a pre-saturated TLC chamber containing the mobile phase (chloroform: methanol in the ratio of 9:1) (Priya *et al*., 2017) [13]. After 45 minutes, the TLC plates were carefully removed and the Retention factor *(*R_f*)* value was calculated according to the following equation from the chromatogram.

$R_f = d / D$

Where,
d= Distance travelled by solute front
D= Distance travelled by solvent front.

2.10 Stability study/ effect of time and incubation period

The bacterial isolates were continuously sub-cultured over a period of seven days to determine their growth and pigment production.

3. Result and Discussion

3.1 Isolation of pigment-producing bacteria

The samples obtained were streaked on Sterile Nutrient agar plates and incubated at 37^0C for 48 hours. A mixture of pigment producers and non-pigment producers were obtained on Nutrient agar plate (Fig. 3) after incubation. 48 hours of incubation out of which 10 morphologically different pigmented colonies were selected and named PR 1 to PR 10 (Fig. 4).

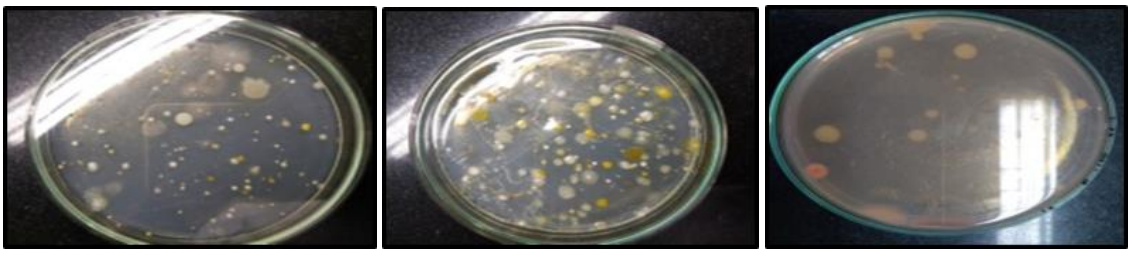

Fig 3: Pigment producers and non-pigment bacterial producers obtained on Nutrient agar plate

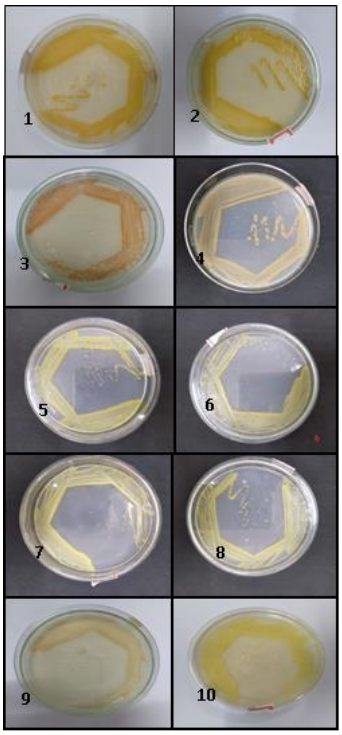

Fig 4: Bacterial isolates PR 1-10(1-10)

3.2 Characterization of isolated pigmented bacteria

The well-isolated bacterial colonies obtained were selected from the plate and its characteristics were noted down. Gram staining was performed to know the Gram nature of the isolates. Table.1 illustrates the results of colony characteristics study.

Table 1: Colony characteristics of selected pigment producing isolates

	Size	Shape	Colour	Margin	Opacity	Consistency	Elevation	Gram Nature
PR1	2mm	Circular	Orange	Entire	Opaque	Butyrous	Concave	Gram Negative
PR2	1mm	Circular	Orange	Entire	Translucent	Butyrous	Flat	Gram Negative
PR3	2mm	Circular	Dark Orange	Entire	Opaque	Butyrous	Flat	Gram Negative
PR4	3mm	Circular	Orange	Entire	Opaque	Sticky	Concave	Gram Positive
PR5	1mm	Circular	Yellow	Entire	Opaque	Butyrous	Concave	Gram Positive
PR6	2mm	Circular	Yellow	Entire	Opaque	Butyrous	Concave	Gram Positive
PR7	1mm	Circular	Yellow	Entire	Opaque	Butyrous	Concave	Gram Positive
PR8	2mm	Circular	Yellow	Entire	Opaque	Butyrous	Concave	Gram Positive
PR9	1mm	Circular	Light Orange	Entire	Translucent	Butyrous	Concave	Gram Positive
PR10	1mm	Circular	Yellow	Entire	Opaque	Butyrous	Concave	Gram Positive

3.3 Identification of the bacterial isolates

The cultures of PR3 and PR6 were identified as *Kocuria rosea* (Fig. 5) and *Micrococcus luteus* (Fig. 6) respectively, by cultural, morphological and biochemical analysis using VITEK 2, at the Nucleus Diagnostic Laboratory, Kalyan.

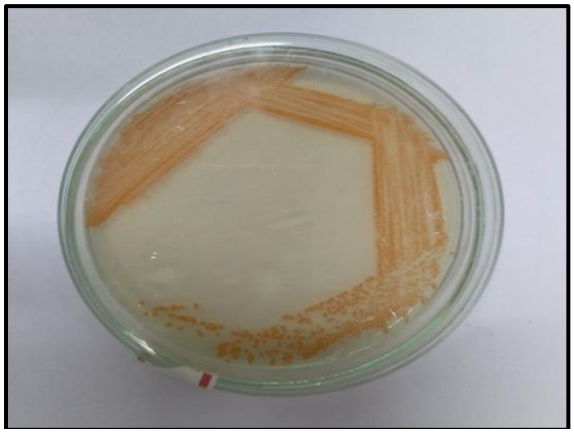

Fig 5: PR 3 identified as *Kocuria rosea*

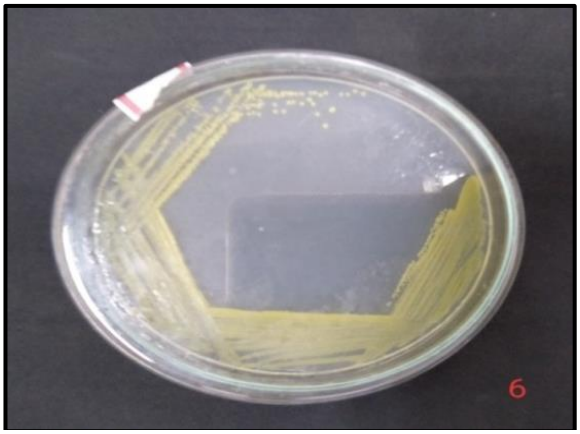

Fig 6: PR 6 identified as *Micrococcus luteus*

3.4 Optimization of various growth parameters for maximum pigment production (pH, Temperature, Sodium chloride, Tryptone source)

The pigment production is affected when the cells are exposed to stressful conditions which may include elevated temperatures, osmotic pressures, metabolic inhibition, etc. The yield of pigment from fermentation is increased as bacterial growth is restrained due to adaption to conditions of stress (Venil *et al*., 2014) [14]. Effect of various physiological parameters such as temperature, pH, tryptone and salt concentration on the growth of the 10 bacterial isolates was studied.

3.4.1 Effect of temperature

The bacterial isolates were streaked on sterile Nutrient agar slants and incubated at various temperatures, 10^0C, RT and 37^0C for 24-48 hours. Maximum growth and pigment production of all the bacterial isolates was visually observed at RT and 37^0C after 48 hours of incubation, whereas no growth was observed for all the isolates at 10^0C (Table.2, Fig.7). Although bacteria grow over a broad range of temperatures, reduction in pigment production at elevated temperatures is well documented (Shaikh, 2016) [12].

Table 2: Effect of Temperature on pigment growth

Temperature	10^0C		RT		37^0C	
	24hr	48hr	24hr	48hr	24hr	48hr
PR1	-	+	+	++	+	++
PR2	-	-	+	++	+	++
PR3	-	-	+	++	+	++
PR4	-	-	+	++	+	++
PR5	-	-	+	++	+	++
PR6	-	-	+	++	+	++
PR7	-	-	+	++	+	++
PR8	-	-	+	++	+	++
PR9	-	-	+	++	+	++
PR10	-	-	+	++	+	++

Key: + Normal growth, ++ Good growth and – No growth.

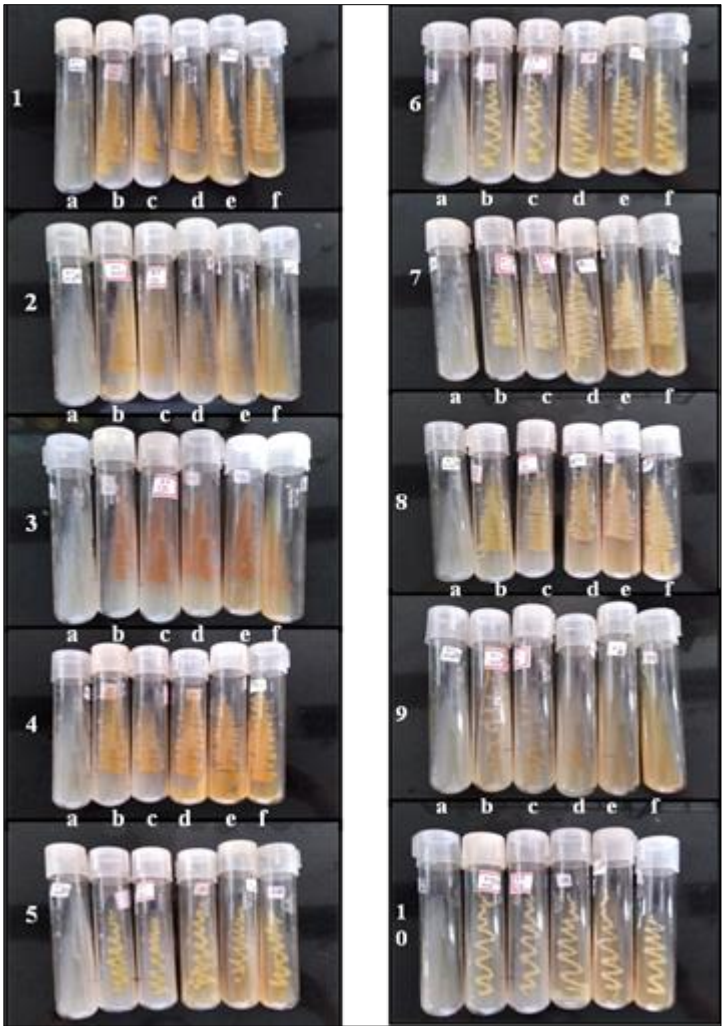

Fig 7: Effect of temperature (a =10^{0}C, b= RT, c= 37^{0}C) and tryptone concentrations (d= 1%, e= 2%, f= 3%) on bacterial isolate PR 1 to 10.

3.4.4 Effect of tryptone

The bacterial isolates were streaked on sterile Nutrient agar slants with different tryptone (carbon source) concentrations of 1%, 2%, and 3% and incubated at 37^{0}C for 24-48 hours. All the bacterial isolates showed growth and pigment production at all the concentrations of tryptone except for PR 2 and PR 9 (Table.3, Fig. 7). The addition of maltose or glucose enhanced pigment production since the nutrient broth is devoid of carbon source. A two-fold increase in yield was observed when maltose and glucose were added in nutrient broth over only nutrient broth or peptone glycerol broth. Slightly enhanced pigment production was observed in peptone glycerol broth at 30°C over nutrient broth at 28°C and this could be attributed to glycerol which was the carbon source. This justifies the fact that carbon does support cell growth and thereby pigment production (Venil and Lakshmanaperumalsamy, 2009) [15].

Table 3: Effect of Tryptone on pigment growth

Tryptone	1%		2%		3%	
	24hr	**48hr**	**24hr**	**48hr**	**24hr**	**48hr**
PR1	+	+	+	+	+	+
PR2	+	+	+	+	-	-
PR3	+	+	+	+	+	+
PR4	+	+	+	+	+	+
PR5	+	+	+	+	+	+
PR6	+	+	+	+	+	+
PR7	+	+	+	+	+	+
PR8	+	+	+	+	+	+
PR9	+	+	-	-	-	-
PR10	+	+	+	+	+	+
Key: + Normal growth, ++ Good growth and – No growth.						

3.4.3 Effect of pH

The bacterial isolates were streaked on sterile Nutrient agar slants of various pH (5.0, 7.0, 9.0 and 11.0) and incubated at 37^{0}C for 24-48 hours. It was found that no growth was observed at pH 5 for all the bacterial isolates. PR 2, PR 3 and PR 9 did not show pigment production at pH 12. Maximum growth and pigment production for all the bacterial isolates was seen at pH 7 after 48 hours of

incubation (Table. 4, Fig. 8). The bacterial isolate exhibited carotenoids production and growth in the range of pH 7 to 10, however, maximum carotenoids production observed to be 482.82 µg/g at pH 7 (Shatila, 2013) [16].

Table 4: Effect of pH on pigment growth

pH	pH5		pH7		pH9		pH12	
	24h	48h	24h	48h	24h	48h	24h	48h
PR1	-	-	+	++	+	+	+	+
PR2	-	-	+	++	+	+	-	-
PR3	-	-	+	++	+	+	-	-
PR4	-	-	+	++	+	+	+	+
PR5	-	-	+	++	+	+	+	+
PR6	-	-	+	++	+	+	+	+
PR7	-	-	+	++	+	++	+	++
PR8	-	-	+	++	+	++	+	++
PR9	-	-	+	++	+	+	-	-
PR 10	-	-	+	++	+	++	+	++
Key: + Normal growth, ++ Good growth and – No growth.								

3.4.4 Effect of salt concentration

The bacterial isolates were streaked on sterile Nutrient agar slants with different salt (Sodium chloride) concentration 2.5%, 4.5%, 6.5% and 8.5% and incubated at 37^0C for 24-48 hours. Maximum pigment production for most of the bacterial isolates was observed at 2.5%, 4.5% and 8.5 % after 48 hrs of incubation (Table.5, Fig. 8). The bacterial isolate PR 1 showed pigment production at all the salt concentrations. No pigment production was seen in both PR2 and PR3 for all salt concentrations. For most isolates, with the increasing salt concentration (8.5%) there was no pigment production.

Table 5: Effect of Sodium Chloride on pigment grow

NaCl	2.5%		4.5%		6.5%		8.5%	
	24h	48h	24h	48h	24h	48h	24h	48h
PR1	+	++	+	++	+	++	+	++
PR2	-	-	-	-	-	-	-	-
PR3	-	-	-	-	-	-	-	-
PR4	-	+	-	-	-	-	-	-
PR5	-	+	-	-	-	-	-	-
PR6	+	++	+	++	+	++	+	+
PR7	+	+	+	+	+	+	-	-
PR8	+	++	+	++	+	++	+	+
PR9	-	+	-	-	-	-	-	-
PR 10	++	++	++	++	++	++	-	-
Key: + Normal growth, ++ Good growth and – No growth.								

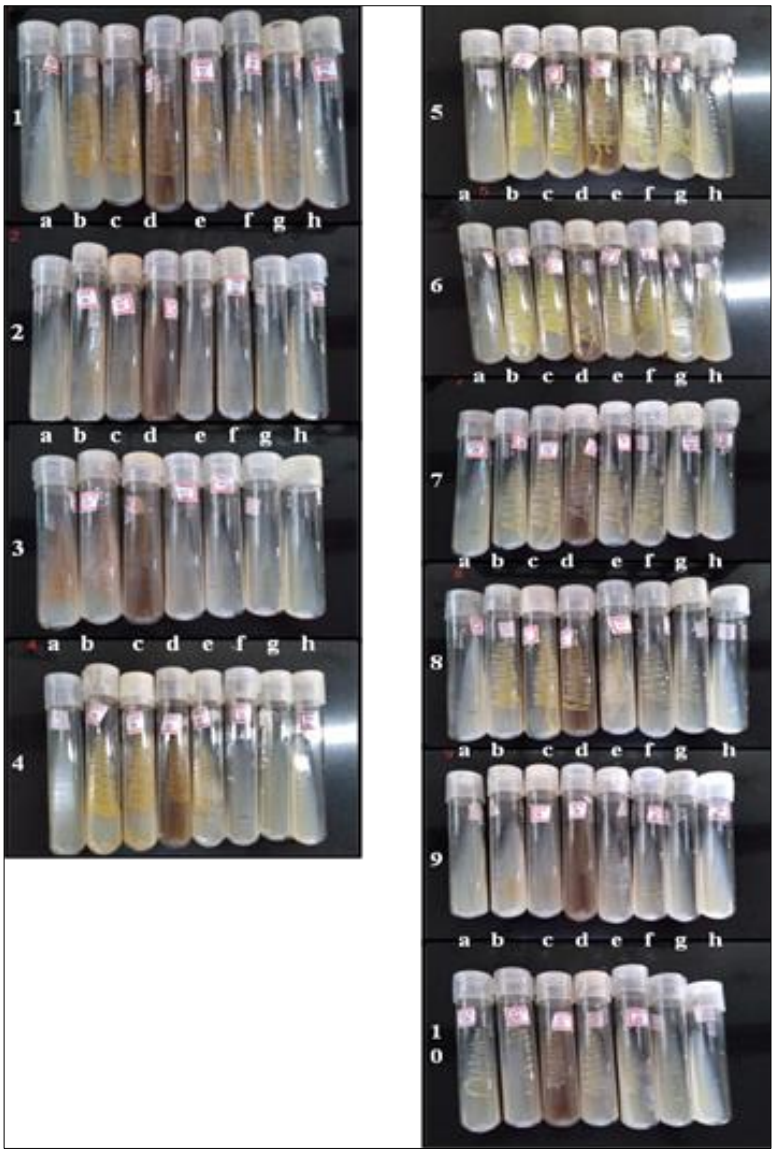

Fig 8: Effect of pH (a =pH5, b= pH7, c= pH9, d= pH12) and salt concentrations (e= 2.5%, f= 4.5%, g= 6.5%, h=8.5%) on bacterial isolate PR 1 to 10.

3.5 Production of pigment

The production of pigments was carried out by growing the cultures in plain nutrient broth and 2% glycerol in nutrient broth for seven days. Both of the enlisted methods gave good results with visible pigments settled at the bottom of the flask as shown in Fig.9 and Fig.10 respectively. Nutrient broth contains peptone and meat extract which act as an organic carbon and nitrogen source respectively, providing the organism with the essential growth factors and vitamins that are necessary for their propagation (John and Aruna, 2019) [17]. Besides, the addition of glycerol also being a carbon source helps further in their growth.

Fig 9: Pigment production in nutrient broth

Fig 10: Pigment production in nutrient broth with 2% glycerol

3.6 Extraction of pigment

For the extraction of pigment-producing bacteria, methods like centrifugation, filtration were used with ethanol as an organic solvent (Fig. 11 and 12). The chemical composition of the extracted pigment influences the choice of organic solvent and yield directly. Carotenoids are lipophilic in nature and are soluble in organic solvents, like chloroform, acetone, methanol, ethanol etc. (Priya *et al*., 2017) [13]. Different solvents like chloroform, ethanol, petroleum ether, etc were screened for extraction of pigments from bacterial species, in various studies (John and Aruna, 2019) [17]. Acetone and methanol are the solvents, can extract the pigment from the cell. But the highest extraction of pigment was shown in methanol (Vora *et al*., 2014) [18]. It was reported in a study that the effective use of chemicals such as ethanol and acetic acid as an inducer and precursor of carotenoid (astaxanthin) synthesis in *Phaffia rhodozyma* strain. In their study, the addition of 10g/L ethanol or 5g/L acetic acid resulted in a relatively high concentration of astaxanthin i.e., 45.62 mg/L and 43.87 mg/L respectively (Geun *et al*., 2003) [19].

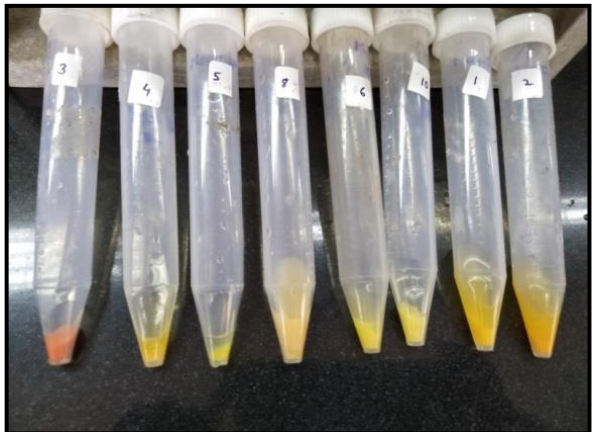

Fig 11: Bacterial pellets

Fig 12: Pigment extracts

3.7 Characterization of pigment

3.7.1 Spectrophotometric analysis

All the isolated pigments were found to be carotenoids as they showed maximum absorption in the range of 420 nm which is the indication for carotenoids. Most of the carotenoids show peak between 400-500nm ranges. A similar result was observed for standard beta carotenoids showing a peak at 450nm (Priya *et al*., 2017) [13]. In plants, flowers, fruits and vegetables the orangish-yellow colour is due to the presence of carotenoids.

3.7.2 Chromatography analysis

Thin-Layer Chromatography

Carotenoids are a group of bioactive compounds that are widely distributed in nature, which are responsible for yellow, orange, and red pigments in various plants, micro-organisms, and animals. The TLC profile of PR 3 showed R_f value of 0.73 (Fig. 13) and PR 6 with two bands showed R_f values of 0.78 and 0.9. The orange-yellow coloured pigments were confirmed to be carotenoids because the R_f value of carotenoids lies within the range of 0.92 to 0.34 (Reddy *et al.*, 2003) [20] which matches with the results.

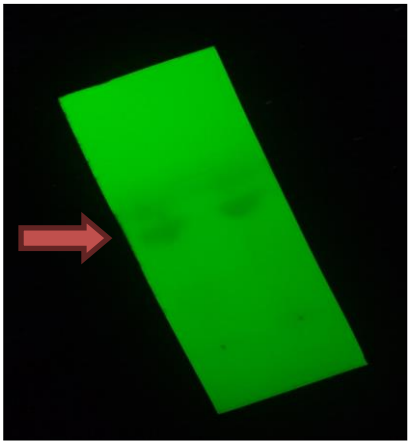

Fig 13: The TLC plate showing the bands of PR 3

3.8 Stability study/effect of time and incubation period determine

The bacterial isolate was sub-cultured over days to its growth and pigment production. It was observed that after 6 days of continuous sub-culturing there was no growth and pigment production (Fig.14). The isolate can be genetically manipulated to produce more pigment in large scale production of pigment.

Fig 14: Isolate PR3 growth from day1 to day 6

4. Conclusion

Colourants are used in majority of industries. Most of the synthetic colourants are harmful to the environment and are difficult to biodegrade. The study involved isolation of pigment-producing bacteria from different sources like rotten vegetables, soil and air flora. The pigment production was influenced by physical factors like temperature, pH and trptone concentration of the culture medium. The effect of time and incubation on the bacterial isolates showed that after 6 days of continuous sub-culturing no pigmentation was observed. The study was intended to isolate and characterize pigment-producing micro-organisms from different sources. Colours acquired from natural origins have many uses in numerous sectors as colouring agents and hence could provide as an alternative for synthetic chemicals which have serious ill effects.

5. References

1. Rokade MT, Pethe AS. Isolation and identification of chromogenic bacteria from various sources. European Journal of Pharmaceutical and Medical Research. 2016; 3(5):295-299.
2. Sinha S, Choubey S, Kumar A, Bhosale P. Identification, characterization of pigment producing bacteria from soil and water and testing of antimicrobial activity of bacterial pigments. Int. J Pharm. Sci. Rev. Res. 2017; 42(2):119-124.
3. Malik K, Tokkas J, Goyal S. Microbial Pigments: A review. International Journal of Microbial Resource Technology. 2012; 1(4):2278-3822.
4. Dufosse` L. Pigments, microbial. Encyclopedia Microbiology. 2009; 4:457-71.
5. Pattnaik P, Roy U, Jain P. Biocolours: new generation additives for food. Indian Food Ind. 1997; 16(5):21-32.
6. Ahmad WA, Ahmad WYW, Zakaria ZA, Yusof NZ. Application of bacterial pigments as colourant: the Malaysian perspective. Springer Briefs in Molecular Science, 2012, 57-74.
7. Gunasekaran S, Poorniammal R. Optimization of fermentation conditions for red pigment production from *Penicillium* sp. under submerged cultivation. Afr. J Biotechnology. 2008; 7(12):1894-1898.
8. Bhat SK, Khan SS, Amin T. Isolation and characterization of pigment producing bacteria from various foods for their possible use as biocolours. International Journal of Recent Scientific Research. 2013; 4(10):1605-1609.
9. Barth M, Hankinson T, Zhuang H, Sperber F, Doyle MP. Microbiological spoilage of fruits and vegetables food microbiology and food safety, 2009. DOI 10.1007/978-1-4419-0826-1_6.
10. Samyuktha S, Naphade-Mahajan S. Isolation and identification of pigment producing bacteria and characterization of extracted pigments. International Journal of Applied Research. 2016; 2(7):657-664.
11. Bharmal MH, Jahagirdar N, Aruna K. Study on optimization of prodigiosin production by *Serratia marcescens* msk1 isolated from air. International Journal of Advanced Research in Biological Sciences. 2012; 2(4):671-680.
12. Shaikh Z. Biosynthesis of prodigiosin and its applications. IOSR Journal of Pharmacy and Biological Sciences. 2016; 11(6):01-28.
13. Priya M, Selvi CS, Kousalya M. Screening for potential carotogenic producing bacteria from rhizospheric soil and it act as an antibacterial activity. World Journal of Pharmacy and Pharmaceutical Sciences. 2017; 6(12):1033-1043.
14. Venil CK, Aruldass CA, Dufosse` L, Zakaria ZA, Ahmad WA. Current perspective on bacterial pigments: emerging sustainable compounds with coloring and biological properties for the industry-an incisive evaluation. RSC Adv. 2014; 4:39523-39529.
15. Venil CK, Lakshmanaperumalsamy P. An insightful overview on microbial pigment, prodigiosin. Electronic Journal of Biology. 2009; 5(3):49-61.
16. Shatila F, Yusef H, Holail H. Pigment production by *Exiguobacterium aurantiacum* F H, a novel Lebanese strain. International Journal of Current Microbiology and Applied Science. 2013; 2:176-191.
17. John M, Aruna K. Optimization of pigment production by *Kocuria flava* majod isolated from garden soil. Journal of Global Biosciences. 2019; 8(2):5946-5965.
18. Vora JU, Jain NK, Modi HA. Extraction, characterization and application studies of red pigment of halophile *Serratia marcescens* KH1R KM035849 isolated from Kharaghoda soil. International Journal of Pure and Applied Bioscience. 2014; 2(6):160-168.

19. Geun Joong, Kim KSJ, Park DH, Ryu YW. High level production of astaxanthin by fed-batch culture of mutant strain *Phaffia rhodozyma* AJ-6-1. Journal of Microbiology and Biotechnology. 2003; 13(2):175-181.
20. Reddy SN, Prakash SS, Vadivel P, Matsumoto G, Stackebrandt E, Shivaji S. *Kocuria polaris sp*. nov., an orange pigmented psychrophilic bacterium isolated from an Antarctic cyanobacterial mat sample. International Journal of Systematic and Evolutionary Microbiology. 2003; 53:183-187.