



# Isolation of Pigments from Bacteria for Textile Industry

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## Abstract

Use of microorganisms for obtaining various natural products has rapidly increased over last few years, as they are main source of many industrially important products. This study was an attempt to find out the extraction of bacterial pigment and its application on cotton clothes. Estimation of bacterial population was done by dilution plate technique. Highest population count was shown in the soil taken from Kanjiram while lowest in Kidangoor. Isolated pigment producing bacteria were *Pseudomonas aeruginosa*, *Serratia marcescens*, *Micrococcus luteus*, *Micrococcus roseus*, *Micrococcus rubrum* and *Staphylococcus aureus*. These bacteria can be used as a source of natural dye for colouring cotton clothes.

**Key words:** Bacterial pigment, *Pseudomonas aeruginosa*, *Serratia marcescens*, Textile dyeing

## Introduction

Pigments produced from natural sources are of worldwide interest. The advantage of pigment production from microorganisms comprises easy and fast growth in the cheap culture medium. Biosynthesis of colourants for textile applications attracted interests in recent years. In plants, pigments not only impart characteristic colour, but also involved in trapping light for physiological processes. Microbial pigment production is one of the emerging fields of research for various applications.

The demand for natural sources of pigments is increasing now because of the consciousness of positive health benefits of natural compound. It is therefore, necessary to explore various natural sources of colorants and their potentials (Delgado Vargas, *et al* .,2000). Though many natural colours are available from ores, insects, plants, and microbes, microbial colorants play a significant role as food coloring agents, because of its production and easy down streaming process (Bechtold and Mussak,2009; Delgado Vargas and O Paredes-Lopez, 2000). Pigments have a well-known pharmacological activity such as anti-cancer and effective against cardiovascular diseases (Cristea and Vilarem, 2006). Pigments produced by organisms as reminiscence of its secondary metabolism are commonly mentioned as bio-pigments which have extensive synthetic and commercial applications.

## Methodology

### Sample selection

Soil samples were collected from four different places of Kottayam District in Kerala-Kanjiram, Thiruvathukkal, Kidangoor and Angadivayal. The obtained soil samples were thoroughly washed with sterile distilled water to separate the dust and debris.

### Isolation of soil microorganisms

The soil suspensions were diluted serially up to  $10^{-5}$ . 1 ml from  $10^{-5}$  concentration was inoculated to the petridish along with nutrient agar medium using pour plate technique. The petriplates were incubated at  $37^{\circ}\text{C}$  for 24 hours. The growth of different microorganisms varies with different media. After 24 hours of incubation at  $37^{\circ}\text{C}$ , the number of colonies of bacteria appearing on dilution plates was counted to obtain the total bacterial population in each of the petriplates. From this, coloured colonies were picked and sub-cultured for further experiments.

### Identification of isolates

The isolates producing higher intensity of pigmentation in nutrient agar plates were selected and identified using Gram-staining. Morphological description of the colony such as elevation and margin were noted. The pure culture of isolates was maintained on Nutrient agar slants for further investigation. Various biochemical tests were conducted to identify the cultured strains. These tests include: Indole test, Methyl red test, Voges-Proskauer test, Citrate utilization test, Urease test, Carbohydrate fermentation test, Nitrate Reduction Test, Catalase Test, Oxidase Test, Mannitol Motility Test, and Triple Sugar Iron Agar Test (TSI).

### Pigment production in broth

Selected isolates were inoculated in to nutrient broth medium, and King's Medium B broth was used for pseudomonas species and incubated in shaking incubator at  $30^{\circ}\text{C}$  for 48-72 hours. Extraction of pigments and their absorption were done by Thin layer chromatography and Spectrophotometry.

### Dyeing of clothes

25ml of the extract was taken in a beaker and the fabrics were immersed in it for one day and dried in the shade.

## Result and Discussion

The soil sample from Kanjiram showed the highest population count while the soil of Kidangoor showed the lowest population. The number of pigment producers was also found high at Kanjiram (Fig.1). Based on the biochemical tests conducted, the isolates were identified as *Pseudomonas aeruginosa*, *Serratia marcescens*, *Micrococcus luteus*, *Micrococcus roseus*, *Micrococcus rubrum* and *Staphylococcus aureus* which were prominent pigment producers (Table:1, Table:2). Among these isolates, the bacterial strains such as *Serratia* species, *Pseudomonas* species and *Micrococcus* species were found good pigment producers in nutrient medium. The colour of the pigment varied with isolates.

From the high optical density value of *Pseudomonas aeruginosa* and *Serratia marcescens*, it was evident that these two species have high pigment producing capacity than other isolates. (Fig.2). From the chromatographic studies it was clear that the pigment contained both lipids and proteins. So it can be concluded that bacteria can be potential source of dyes.

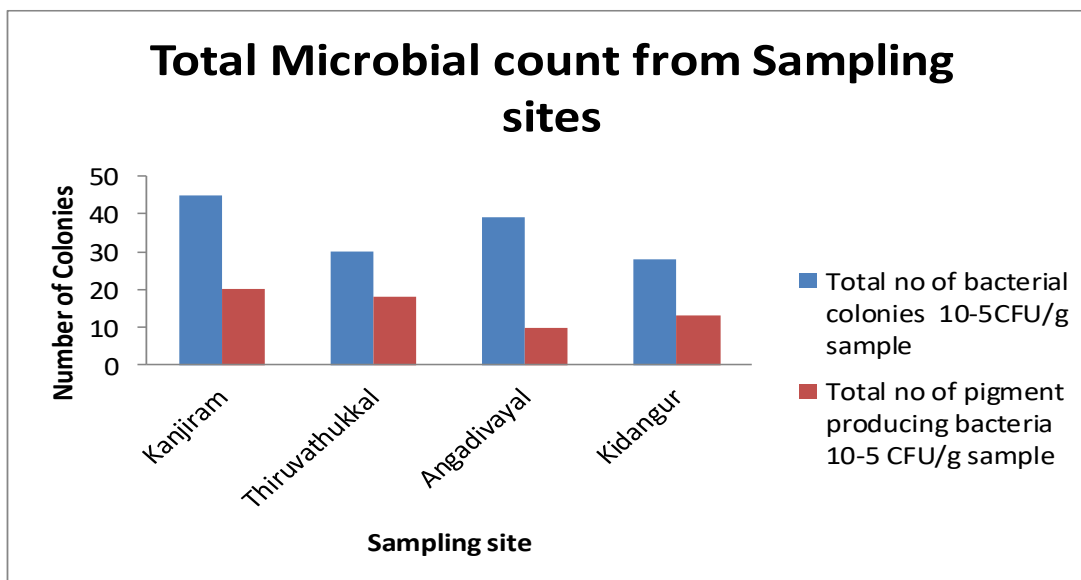
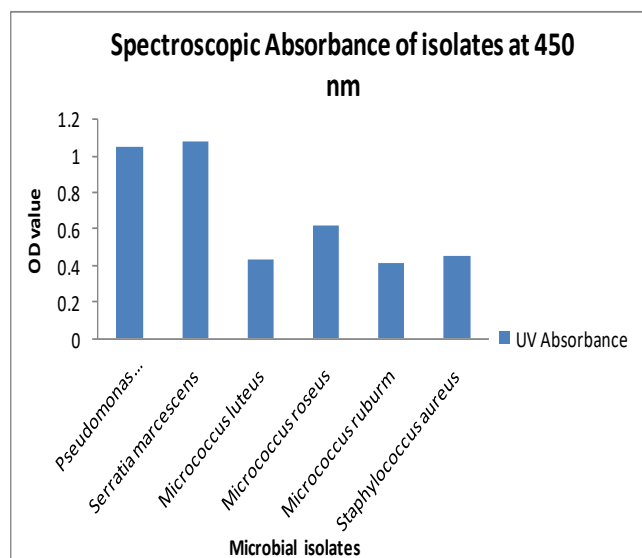


Fig1: Total microbial count from sampling site

ISOLATES	INDOLE	CITRATE	MANITOL	UREASE	TSI	MR	VP	NITRATE	CATALASE	OXIDASE	CARBOHYDRATE TEST			GRAM STAINING
											LACTOSE	GLUCOSE	SUCROSE	
<i>Pseudomonas aeruginosa</i>	-	+	-	-	-	-	-	+	+	+	-	-	-	-/rod
<i>Serratia marcescens</i>	-	+	+	+	-	-	+	+	+	-	+	-	+	-/rod
<i>Micrococcus luteus</i>	-	-	-	+	-	-	-	+	+	-	-	-	-	+/cocci
<i>Micrococcus roseus</i>	+	+	+	+	+	+	+	+	+	+	-	-	-	+
<i>Micrococcus rubrum</i>	+	+	-	+	-	-	-	+	+	+	-	+	+	+
<i>Staphylococcus aureus</i>	-	-	+	-	-	+	+	+	+	-	+	+	+	+/cocci

Table 1: Identification of bacteria by Biochemical Test



Isolates	UV Absorbance
<i>Pseudomonas aeruginosa</i>	1.052
<i>Serratia marcescens</i>	1.08
<i>Micrococcus luteus</i>	0.433
<i>Micrococcus roseus</i>	0.615
<i>Micrococcus rubrum</i>	0.412
<i>Staphylococcus aureus</i>	0.452

Fig.2: Spectroscopic absorbance of isolates

Table 2: UV Absorbance of each isolate

Isolates	No: of samples	Distance travelled by solvent (cm)	Distance travelled by compound (cm)	colour	RF value
<i>Pseudomonas aeruginosa</i>	1	10	8.1	Green	0.81
<i>Serratia marcescens</i>	1	10	7.8	Red	0.78
<i>Micrococcus luteus</i>	1	10	4.1	Yellow	0.41
<i>Micrococcus roseus</i>	1	10	4.2	Yellow	0.42
<i>Micrococcus rubrum</i>	1	10	4.0	Yellow	0.40
<i>Staphylococcus aureus</i>	1	10	4.5	Yellow	0.45

Table 3: Separation along with RF value using solvent-1(protein)

Sample	Standard value	RF value
Bacterial Red pigment	Prodigiosin (0.85)	0.78
Bacterial Yellow pigment	Staphyloxanthin (0.44)	0.43
Bacterial Green pigment	Pyoverdine (0.95)	0.81

Table 4: Comparison of Standard value and RF value of bacterial pigment

Nature is rich in colour and pigment producing microbes (S.Babitha, 2009). From Thin Layer Chromatographic results, the RF value of the pigment produced by *Serratia* species was 0.78 which was close to the standard RF value 0.85 of Bacterial Red Pigment Prodigiosin. From the study of Srimathy *et.al.* (2017), Prodigiosin and related pigments can be considered as alternatives for textile dye due to its antibacterial and antifungal properties. Similar observations were obtained for *Pseudomonas*, *Micrococcus* and *Staphylococcus* species (Table.3, Table.4). The extracted bacterial pigments when used to dye clothes showed maximum absorbance and the microbial dyeing was not readily altered by washing.

Since bacteria are strict aerobes, their growth and pigment production can be increased by shaking. Maximum growth and pigment production occurred in 100ml of medium taken in Erlenmeyer flask. Increase the volume of the medium above 100 ml resulted in decreased growth and pigment production. Asker and Ohta (1999) have reported similar observation. Clothes dyed with natural dyes have good UV protection properties. It protects skin from 80% UV rays and thus helps to prevent sunburns, skin damage and skin cancer. Feng *et.al.*,(2007) reported similar observation.

## Conclusion

Synthetic colouring agents being used in textile industry are toxic and carcinogenic. Natural pigments are known to be antioxidant and supposed to be harmless for human. Plants and microorganisms are the most important natural source for pigments.

Estimation of bacterial population using dilution plate technique showed the highest population count in the soil taken from the place Kanjiram while lowest in Kidangur. The isolated pigment producers include *Pseudomonas aeruginosa*, *Serratia marcescens*, *Micrococcus luteus*, *Micrococcus roseus*, *Micrococcus rubrum* and *Staphylococcus aureus*. Produced pigments were used as a dye for colouring cotton clothes. Pigmented bacteria appear in good numbers in light absorbing surface soil, mainly because pigments are generally produced as a shield against severe sunlight and heat in order to protect the sensitive internal organelles of microorganisms.

## Reference

- CK, Venil., & P, Lakshmanaperumalsamy, 2009. An insightful overview on microbial pigment: prodigiosin, journal Biology 5:49-61
- D, Asker., & Y, Ohta., 1999. Production of canthaxanthin by extremely halophilic bacteria, Journal of Bioscience Bioengineering 88:617-623.
- D, Cristea., & G.Vilarem., 2006. Improving light fastness of natural dyes on cotton yarn. Dyes and pigments: 70,267-272.
- F, Delgado Vargas., A.R Jimenez., & O, Paredes-Lopez, 2000. Natural pigments: carotenoids, anthocyanins and betalainins-characteristics, biosynthesis, processing and stability 3:173-289.
- F, Delgado Vargas, 2002. Natural Colorants for food and Nutraceutical Uses.
- L, Dufosse, 2006. Microbial Production of Food Grade pigments. Journal of Food technology and Biotechnology 44 (3): 313-321.
- R, Srimathi., R, Priya., M, Nirmala., & Malarvizhi, 2017 Isolation, Identification, Optimization of Prodigiosin Pigment Produced by *Serratia marcescens* and its Applications IJLEMRO9:11-21
- S, Babitha., 2009 Microbial pigments, Biotechnology for agro-industrial residues utilization, 147-162.
- T, Bechtold., & R, Mussak, 2009. Hand book of Natural Colorants
- XX, Feng., LL, Zhang., JY, Chen., & JC, Zhang, 2007. New insights into solar UV-protective properties of natural dye : Journal of Cleaner Production: Volume 15, Issue 4: 366-372