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# USE OF A NATURAL DYE FROM *SERRATIA MARCESCENS SUBSPECIES MARCESCENS* IN DYEING OF TEXTILE FABRICS

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Abstract: A strain of *Serratia marcescens subspecies marcescens* capable of producing a novel rose red pigment with a mass of 112 Da has been isolated from Mahim Mangroove soil. Studies regarding the growth conditions of bacteria, partial characterization of the produced pigment and use of this rose red pigment to dye natural fabrics has been studied and described. Dyeing of wool, cotton and silk fabrics with this rose red microbial pigment as natural dye indicated that the colour strength values and the dye uptake were high with satisfactory fastness properties of the dyed fabric.

Keywords: Anthocyanin compound, Bacteria, Dyeing, Fabrics, Natural dye.

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## INTRODUCTION

The textile industry discharges large proportion of effluent that mainly consists of synthetic dyes. Synthetic dyes have been extensively used in the textile industries due to their ease and cost-effectiveness in synthesis, high stability towards light, temperature and technically advanced colours covering the whole colour spectrum. However, these synthetic dyes are often toxic, mutagenic and carcinogenic leading to several human health problems such as skin cancer and allergic reactions (Gurav A. et. al., 2011; Srikanlayanukul M. et. al., 2006; Tsatsaroni and Liakopoulou, 1995). Thus, the worldwide demand for the dyes of natural origin is increasing rapidly in the textile industry. Several dye yielding plants namely Indian madder (*Rubia tinctorium*), Woad (*Isatis tinctoria*) and Weld (*Reseda luteola*) yielding red (alizarin), indigo (indigotin) and yellow (luteolin) dyes till now have been studied for their dyeing properties. The disadvantages of using plants as the source of production of natural dyes is the production of large amount of biomass, destruction of species on a large scale affecting the biodiversity, colour production is season dependent and practically low colour yields (a few grams of colour per kg of dried raw material). To overcome these drawbacks, fermentation of microorganisms such as bacteria and fungi could be a valuable source for production of natural dyes. Microorganisms produce a large variety of stable pigments and the fermentation has higher yields of pigments and lower residues compared to the use of plants (Farzaneh et. al., 2008). Microorganisms such as *Serratia sakuensis* produces red prodiogiosin pigments (Vaidyanathan et. al., 2012) *Jantinobacterium lividum*

and *Chromobacterium species* are known for production of violet pigments (Akira et. al., 2000; Adivarekar et al., 2011) *Ashbya* produces yellow riboflavin (Diana et. al., 2005) and *Fusarium oxysporum* produces anthraquinones (Nagia and Mohamedy, 2007).

The present paper mainly describes the use of a red pigment, produced by *Serratia marcescens subspecies marcescens*, as a natural dye for dyeing of natural fabrics to determine the dyeing strength of the pigment. Characterization of the pigment, evaluation of the colour strength and fastness properties of the dyed fabrics have also been carried out and discussed.

## MATERIALS AND METHODS

### Materials

Wool and Mulberry silk fabrics were purchased from local markets, Fort, Mumbai whereas Premier Mills, Mumbai, supplied cotton fabrics. Acetone extract of the rose red pigment from *Serratia marcescens subspecies marcescens* bacteria have been used as natural dye for dyeing of silk, wool and cotton fabrics.

### Methods

#### Strain Isolation and identification

A rose red pigment producing bacteria was isolated from Mahim Magroove soil sample. The culture was maintained on peptone glycerol agar medium slants and incubated at room temperature [RT] for 48 hours. The isolate was subsequently subjected to 16s rRNA identification at NCCS, Pune.

#### Production and Extraction of Rose Red pigment

50 mL of Nutrient Agar containing Peptone 1%, Beef Extract 0.3 % and Sodium chloride 3 % was poured into large petri plates and allowed to solidify. The plates were dried and 0.6mL of glycerol was spread on the surface using sterile cotton swab. The plates were incubated at RT for about 2 hours after which 24 hr culture suspension containing $10^{10}$ cfu/mL was swabbed on the plate. The plates were incubated at room temperature for 24 hrs. After incubation the cells were harvested using a scalpel washed in minimum volume of distilled water and the cell mass obtained was used for extraction of pigment. The cell mass obtained from 500mL of solidified growth medium (Modified Nutrient Agar) ( plate diameter 19 cm capable of holding 30 mL) was scrapped and suspended in 40 mL acetone and stored at $40^0$C for 6 hrs. The cell residue was removed by filtration/centrifugation and washed with small amounts of acetone till washing obtained was colourless. The filtrates were combined and the pigment from acetone was extracted into petroleum ether (40 mL) in a separating funnel. The process was repeated till the petroleum ether phase became completely colourless. The combined petroleum ether was washed with water and dried over anhydrous $Na_2SO_4$ and its yield was determined as mg/biomass (wt/wt).

#### Purification of Pigment

The pigment was purified using preparative HPTLC using a solvent system of n-Butanol: Ethanol: Water (90:10:10). 0.2 g of the dry pigment was dissolved in 2 mL of acetone. A single band having sample volume of 240µL was loaded into a silica gel TLC plate (Merck) using LINOMAT-5 applicator. The single red coloured band obtained after the run was further re-extracted in acetone. Two dimensional chromatography of the re-extracted fraction of the pigment carried out to ensure its purity using Butanol: Ethanol: Water (9:1:1) solvent system. The purified pigment thus obtained was re extracted in acidified methanol, dried and used for further characterization studies.

#### Partial Characterization of the pigment

##### pH stability

The UV visible spectra of 0.001g of extracted pigment in 2.5 ml of 0.2 M HCl –KCl (pH 4) buffer, 0.2M Phosphate Buffer (pH 7) and 0.2M glycine NaOH buffer (pH 9.2) respectively for 1hr at RT was set up and variation of the colour intensity assessed through changes in its optical density taken at its respective λmax.

### Temperature stability

The UV visible spectra of 0.002g of extracted pigment dissolved in 20 mL of respective solvents was incubated for one hour at $10^0$C, $30^0$C, $50^0$C and $100^0$C and variation of the colour intensity assessed through changes in its optical density taken at its respective lambda max.

### Classification of the Pigment

Natural pigments are categorized into various classes like carotenoids, anthocyanins, melanins, tripyrroles phenazines and riboflavins based on its aqueous and organic solvents solubility, absorption maxima and reaction with concentrated sulphuric acid, 10% NaOH, 10% Ferric chloride & $Na_2S_2O_3$. Thus, classification studies were carried out in order to check the class of the rose red pigment. For this, the pigment was dissolved in various polar and non polar solvents including water, ethanol (95%), methanol, acetone, chloroform and benzene and solubility was recorded. The dry pigment (0.1mg) was reacted with concentrated sulphuric acid to detect the presence of carotenoids whereas to detect the presence of anthocyanins the pigments were reacted with 10% NaOH solution and $Na_2S_2O_3$.

### FTIR analysis

0.005 g of dried rose red pigment was used to determine the FTIR spectrum to detect various functional groups. The pigments were placed in the sample holder and transmittance of the IR radiation recorded between the range of 4000-400 nm using the Shimadzu IR 8300 spectrophotometer.

### LC-MS analysis

The rose red pigment, purified using HPTLC was subjected to LC/MS so as to determine their mass. For this 0.1 g of dried pigment powder was suspended in 5 mL solvent namely acetone and filtered, to obtain a particle free solution. 2µL of this was injected into Varian prostar for LC and subsequently into Varian 500 MS IT Mass spectrometer. MS was carried out using APCI (Atmospheric Pressure Chemical Ionization method). The column used was Polaris 5 µ C 18 A 50*2.0 mm (stationary phase). The mobile phase was methanol water and the flow rate was 0.2 µL/min.

### Application in Dyeing of Natural Fabrics

### Dyeing Procedure

Acetone extract of the rose red pigment from *Serratia marcescens subspecies marcescens* bacteria have been used as natural dye for dyeing of silk, wool and cotton fabrics. Dye baths were prepared by diluting acetone extract of rose red dye with water [1:2]. Dyeing of silk, wool and cotton was carried out by keeping the MLR ratio as 1:25. For cotton, Glauber's salt was added to the dyebath. Dyeing experiments were conducted in Rotadyer [supplied by Rossari Labtech] at 80°C for 60 mins. After 60 mins, the rotadyer was brought to room temperature and the fabric sample was removed and given cold wash followed by soaping by using 1.5g/L nonionic detergent (Auxipon NP) at $80^o$C for 20 min. followed by cold wash. Then it was air dried and taken for the further study of; colour value and fastness properties, like wash fastness, light fastness and rubbing fastness.

### Colour Strength Properties

Dyed samples were evaluated for the depth of the colour by determining K/S and reflectance values using a Spectra flash ® SF 300, Computer Colour Matching System (Datacolour International, U.S.A). Kubelka-Munk K/S function is given by:

$$\frac{K}{S} = \frac{(1 - R)^2}{2R}$$

Where

R is the reflectance at complete opacity.
K is absorption coefficient.
S is the Scattering coefficient.

### Fastness Properties

The dyed samples were tested for their fastness properties according to the ISO standard and AATCC methods. The specific tests were as follows: Colour fastness to washing, ISO 105-CO2 (1989); Colour fastness to light, AATCC 117 using Q-Sun's Xenon Arc light fastness tester; Colour fastness to Rubbing, ISO 105-X12 (1987) using Crockmeter.

## RESULTS AND DISCUSSION

### Strain identification

The isolate was a Gram negative stubby coccobacilli isolated from Mahim mangrove soil that elaborated an intense rose red colour due to its intracellular pigment production. Biochemically it could be grouped into the Enterobacteriacea family under the genera of Serratia which was confirmed by 16s rRNA which identified it with 92% probability as *Serratia marcescens subsp. marcescens.*

### Purification of the Rose Red Pigment

Purification of the colorant undertaken through analytical HPTLC using a solvent system of butanol: ethanol: water (90:10:10) showed one pigmented band that resolved well. However, observation at 366nm under fluorescence detected impurities. Extraction of the resolved band in acetone followed by use of petroleum ether revealed a purified fraction of pigment through 2-D chromatography where in a single band under white light and fluorescent light was observed confirming the absence of any impurity.

### Partial Characterization of the Rose Red pigment

pH stability: Fig. 1 showed that the rose red pigment is stable across the pH range.
Temperature Stability: The rose red pigment was found to be temperature resistant (Fig.2) and thus can find application in high temperature treatments used in textile dyeing procedures.
Classification of the Pigment: The rose red pigment could be tentatively classified within the anthocyanin class based on its reaction with NaOH and its ability to be reduced into a colourless sulfonic acid adduct by thiosulphate such that it can be placed as a monomeric anthocyanin.

### FTIR Analysis

FTIR spectroscopy of the rose red pigment on analysis revealed the presence of functional moieties characteristic of OH group, $NH_2$ group and CH group observed through 14 prominent peaks. In contrast the spectra of HPTLC purified rose red pigment though showing similar functional moieties detected a fewer array of functional peaks prominently observed at 3292nm, 2923nm, 1731nm, 1635 nm, 1521nm, 1468nm, 1317nm, 1128nm, 1035nm and 836 nm (Fig.3 ) which though similar did not exactly match with that reported by Montaner 2005 for the Prodigiosin pigment of *S .marcescens (*2964nm, 1661nm, 1635nm, 1602nm, 1521nm, 1468nm, 1317nm, 1128nm,1035nm,1076nm, 1006nm).

### LC-MS Analysis

The FTIR analysis were further confirmed by the LC-MS spectra which on loading 2 µl of 0.1 g of colorant prepared in acetone into Varian Prostar for LC and Varian 500MSIT for MS/Ms yielded a mass of 112 (Fig. 4 ) quite different from that of reported prodiogiosin value of 324 (Farzaneh A. et. al.,

2008). The results of the LC-MS analysis strengthened the hypothesis that the rose red pigment could be an anthocyanin.

## Dyeing Performance

The microbial rose red dye showed dyeability towards all three natural fabrics namely silk, wool and cotton though to a different extent. For all the three fabrics dyeings obtained were uniform. The fabrics were dyed pink by the microbial pigment. The colour strength properties in terms of K/S and L*, a*, b* and fastness properties values of the dyed samples are given in Table 1. The highest dye uptake was found in wool followed by cotton and then silk.The light fastness properties for the dyed samples was found to be poor with moderate wash and excellent rubbing fastness properties.

Table 1: Colour Strength and Fastness Properties of Fabrics dyed with Rose red microbial dye

| Fabrics Dyed | K/S | L* | a* | b* | Fastness Properties | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Light | Wash | Rubbing | |
| | | | | | | | Dry | Wet |
| Silk | 0.4938 | 82.089 | 19.898 | -4.289 | 1 | 2 | 4-5 | 4-5 |
| Wool | 1.3759 | 65.032 | 33.194 | -3.343 | 1 | 3 | 4-5 | 4-5 |
| Cotton | 0.5129 | 86.562 | 7.955 | -0.9 | 1 | 2 | 4-5 | 4-5 |

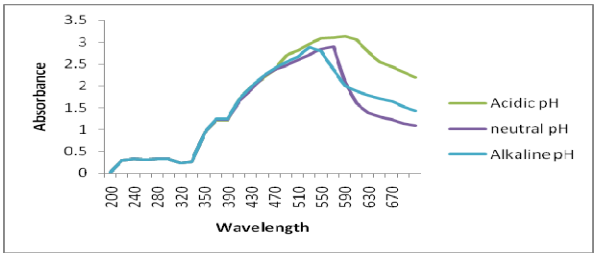


Figure 1: pH stability of Rose Red pigment

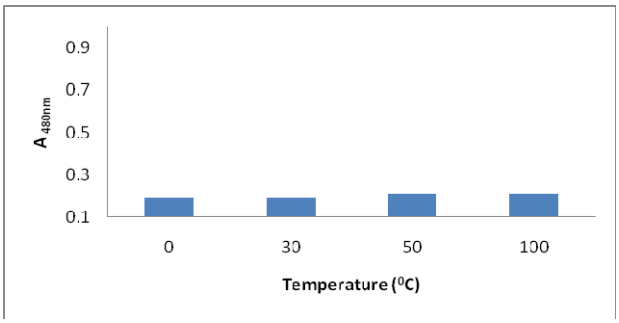


Figure 2: Temperature Stability of Rose Red pigment

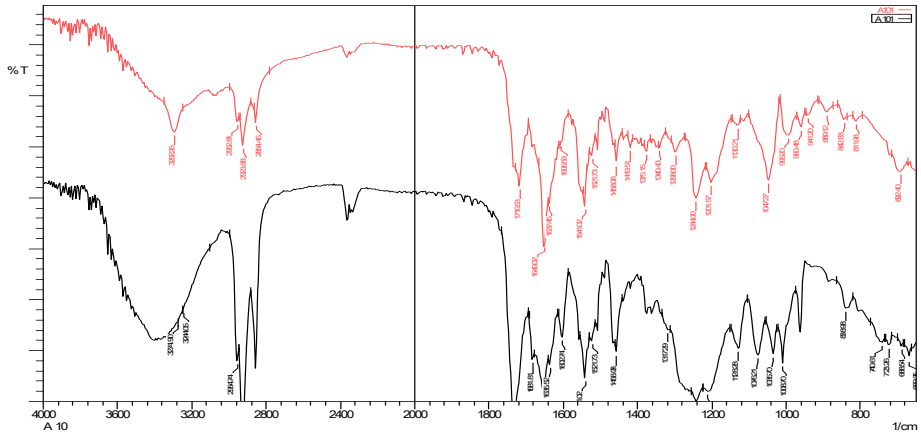


Figure 3: FTIR Spectra of Rose Red Pigment

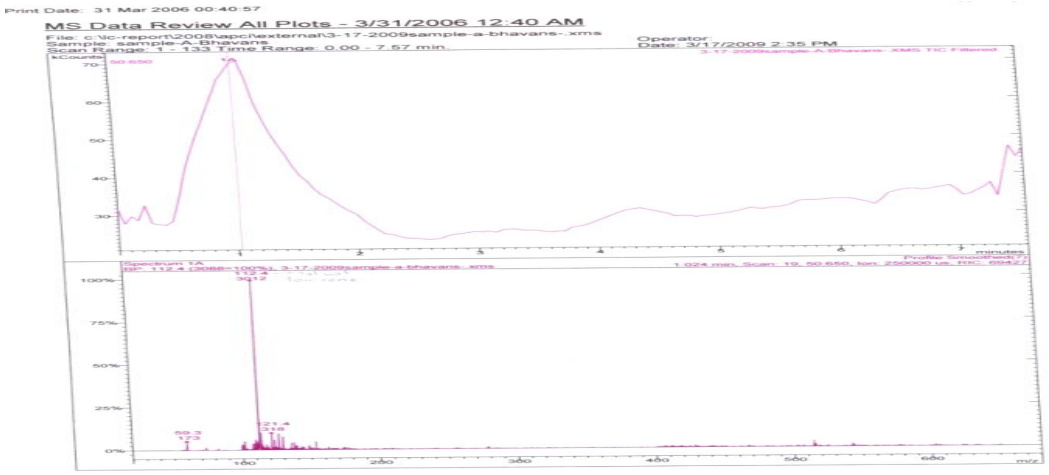


Figure 4: LC-MS Spectra of Rose Red pigment

## CONCLUSION

The rose red pigment obtained from *Serratia marcescens subspecies marcescens* can be used as a natural dye for dyeing silk, wool and cotton fabrics for pink colour. In addition to dyeability, various features of microbial dyes like their natural character, biodegradability, production being independent of seasons and large scale production using industrially controlled fermentation technologies make it a potential candidate as an alternate to the various available chemically synthesized dyes. However, the fastness properties being moderate, the future studies on either pretreatment of the fabrics or chemical modification of the microbial pigments to improve the fastness properties performance can be undertaken.

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