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Bio- Colouration Of Textile Substrates With Microbial Dye

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ABSTRACT
Natural colours are generally extracted from plants and microorganisms and are often called bio-colours because of their biological origin. There is an increasing demand for natural colors in the food, pharmaceutical, cosmetics, textile, printing and dye industry.After ban on certain synthetic azo dyes there is great deal of emphasis on the screening of newer natural sources of dyes like microbes. Since, bacteria are very fast growing, from the economic perspective, bacterial origin dyes have best potential as commercial sources of dyes. Natural dyes having antibacterial property may find use in the dyeing of textile materials specially Medtech like sheets and gowns for hospital use and on articles, which are less suitable for laundering such as mattresses and upholstery. Some strains of Pseudomonas fluorescens,non-pathogenic bacteriaproduce beautiful colours and same may be explored for their possible use as natural dye for textile materials. Pseudomonas fluorescensare abundantly present in soil and thus can be readily isolated.In present study, Pseudomonas fluorescens, (Strain, Pf-24),was cultivated onto Modified Kings' B agar. The slimy growth of bacterial cells that had become dark red was scraped out from the plate and dried in air and directly used for dyeing.Antibacterial activity of dyed fabrics against pathogenic bacteria (Staphylococcus aureus and E. coli) was assessed to ascertain the extent of resistance of dyed fabrics for the growth of these bacteria. Different assessment methods namely qualitative and quantitative assessments were used to assess antimicrobial activity of dyed cotton, silk and wool samples.Findings of the study revealed that dye is more effective against gram positive bacteria than gram negative bacteria. Apart from colouring, this bio-dyeing also imparted anti-microbial finish to the fabric andthus dyed fabric can be categorized under smart textiles.
Keywords: *Natural dye, bacterial dye, antibacterial activity, microbial dye*

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INTRODUCTION

Microbial pigments are of great structural diversity. They may be derivatives of the material classes of carotenoids, phenazine dyes, pyrrole dyes, azaquinones etc. [1]. Pigments from bacteria like *Chromobacterium violaceum*, *Janthinobacterium lividum*, *Chromobacterium lividum* and *Pseudoalteromonas luteoviolacea* have been used for dyeing textiles and good dyeing results have not only been obtained in connection with natural fibers such as silk, wool and cotton, but also with synthetic fibers such as nylon [2, 3]. These studies suggest that bacteria may be as good source for dyeing textile fibers. Several bacteria especially strains of *Pseudomonas fluorescens* produce beautiful colours in culture media and these colours may be explored for their possible use as dye for textile materials.
In recent decades, there has been an increasing tendency towards the prevention of microbial attack on textiles. Natural fibres have keratin and cellulose, etc., that provide important requirements such as oxygen, moisture, nutrients and temperature for the bacterial growth [4, 5]. Natural dyes having antibacterial activity would be valuable for the dyeing of sheets and gowns for hospital use and on articles, which are less suitable for laundering such as mattresses and upholstery. The dyes exhibiting

good wash fastness have durable antibacterial effect [6].When a product has a negative influence on the vitality of a micro-organism it is generally termed as 'antimicrobial. At present plants are principal source of natural dyes and extracts from roots, stem, leaves, flowers, fruits and seeds of diverse species of plants exhibit antibacterial properties [7]. Phenomenal growth of textile industry has increased the demand of dyes including natural dyes to manifold and it is not possible to meet this demand from plant origin dyes because of shrinking land availability owing to various factors, hence alternative sources of natural dyes needs to be explored. Microbial dyes can be a good alternative of natural dyes as less time and space is required for production. Present study describes the preparation of natural dye from *Pseudomonas fluorescens* (strain pf-24) and its application on natural textile substrates like silk, wool and cotton and antibacterial properties exhibited by dyed fabrics.

MATERIAL AND METHODS

Dye source

Pigment producing, non-pathogenic strain of *Pseudomonas fluorescens,* (Strain, Pf-24) isolated from soil, were obtained from Bio-control Laboratory, Department of Plant Pathology, College of Agriculture, G.B.P.U.A.&T., Pantnagar, Uttarakhand. This strain was used for production of natural dye, which was also tested for mammalian toxicity and was found completely safe [8].

Textile substrates

Cotton, silk and wool were selected as natural textile substrate to find out compatibility of dyes with these fibers and effect of dyes in the present study. Tasar silk and Merino wool and cotton fabric were procured from Gandhi Ashram, Pantnagar, Uttarakhand, India. The constructional details of all the experimental fabrics are given in Table- 1.

Cultivation of the bacteriaand Preparation of dye

Prior to attempt the cultivation of bacteria for dye preparation, the growth conditions, which favoured pigment production, like type of medium (broth or agar), pH of medium, incubation temperature, shake or stationary culture, incubation time were determined [8]. Strain pf-24 of *Pseudomonas fluorescens* was grown on Modified King's B agar medium (pH7.0), plates were incubated in stationary condition in a B.O.D. incubator at 25^{0}C for 48 hrs [9]. After 48 hrs of incubation the slimy/mucous growth of bacterial cells that had become dark red was scraped out with the help of flat spatula from the plate, placed in a glass plate and dried in air. This dried dark red powder soluble in water was directly used as dye for textile material.

Dyeing

All the experimental natural fabrics has their own extent to absorb the dye, therefore, all the fabrics were dyed with optimized dyeing conditions, which produced good colours on fabrics that were colourfast. Dyeing of silk and wool fabric with extract was carried out at 10% of (on weight of fabric) at 1:30 (material to liquor ratio) for 60 minutes at acidic medium (pH-5) at 70ºC. In case of cotton fabric, alkaline medium (pH-9) at 90ºC for 75 minutes was selected as optimum dyeing condition. In case of dyeing of cotton fabrics 0.02g tartaric acid was added to dye bath as simultaneous mordant for facilitating even dye, brightness in colour and better fixation. Tartaric acid used as a mordant which did not change the basic hue of dye, it only imparted brightness and even dyeing to cotton yarns. Dyed samples were rinsed in cold water and dried under shade [8].

Assessment of antibacterial activity

Antibacterial activity of all fabric samples dyed with natural dye extracted from pf-24 strain was assessed both qualitatively and quantitatively.*S. aureus* ATCC 25923 and *E. coli* ATCC 35218 were used as test organisms to determine the antibacterial properties of dyed fabric samples. *Staphylococcus aureus* and *Escherichia coli* are human pathogens and are representative of gram positive and gram negative bacteria respectively. Standard pure cultures of the bacteria were procured from the Institute of Microbial Technology (IMTECH), Chandigarh, India as Microbial Type Culture Collection (MTCC) and successively cultured in the Department of Veterinary Microbiology, College of Animal and Veterinary Sciences, G. B. P. U. A. & T., Pantnagar.

a. Qualitative assessment

AATCC test method 90-1974 was used as agar diffusion plate method for qualitative assessment of antibacterial activity of dyed samples. Circular test specimens having a diameter not greater than 28.6 mm (11/8in.) from silk, wool and cotton fabric dyed with dyes from Pf-24 were prepared. Test organism culture was grown overnight at 37ºC and 120rpm in 10ml nutrient broth. Nutrient broth and nutrient agar 1.5% (Hi Media laboratories Ltd, Mumbai) were used. Nutrient agar was melted, cooled at 45ºC and inoculated with 1ml culture of the test organism per 150ml. Then 15ml agar was poured into a 100mm diameter flat bottom petridish and allowed 15 minute lapse of time before adding the test specimen. All the dyed and control specimens were gently pressed into intimate contact with the seeded agar, using sterile forceps in triplicates. All the plates were incubated for 24 hours at 37ºC. Then a clear zone around the fabric through the bottom of the plate was measured which is a measure of diffusbility as well as of antibacterial activity. The width of the clear zone was calculated as follows:

$$W = \frac{T - D}{2}$$

Where, W = width of clear zone in mm

T = total diameter of fabric specimen and clear zone
D = diameter of the fabric specimen

b. Quantitative assessment

The ASTM: E 2149-01 procedure was used for quantitative analysis. Fabric swatches of dimension 4 x 4.8cm ± 0.1cm from wool, silk and cotton fabric dyed with dye Pf-24 was prepared. Control and dyed swathes were kept with 1.0 ± 0.1ml of bacterial inoculum in a 250ml container. The inoculum was a nutrient broth culture containing 2.7 x 10^5/ml colony forming units (CFU) of bacteria. All swatches were transferred to 100ml of saline water and kept on a shaker for 30 minutes followed by serial dilutions upto 10^3. The test was performed in triplicate for two sets of samples. One ml from each dilution were placed on nutrient agar and incubated for 48 hours at 37ºC. Viable colonies of bacteria on the agar plate were counted and the reduction in number of bacteria was calculated using following formula:

$$R = \frac{B - A}{B} x100$$

Where, R= % reduction in bacteria by the treatment

B = the number of CFU of bacteria recovered from the inoculated treated test swatches in the jar immediately after inoculation (at '0' contact time)

A = the number of CFU of bacteria recovered from the inoculated treated test swatches in the jar incubated over 48 hours.

RESULTS AND DISCUSSION

The dye from Pf-24 has dyeing property which is accomplished from our studies that can have a great potential in textile coloration. Colonies on MKB agar plate of strain Pf-24 produced excessive plum red pigment. They were raised, whole, circular, convex and smooth and ranged from 1 to 3mm in diameter after 48 h of incubation. The colonies have a mucoid appearance and tacky consistency (Fig.1). Strain Pf-24 incubated on King's B agar medium at pH-7.0 was optimized which incubated at 25ºC for 2 days for getting maximum pigment of desired colour. Intracellular red pigment was scraped, dried and was directly used for dyeing

Antimicrobial activity of dyed samples

Metabolic products of many bacteria possess antibacterial activity against other bacteria. As the dye pigment was extracted from the bacteria, it is important to conduct appropriate laboratory test for antibacterial activity imparted to dyed fabrics. Antibacterial activity of dyed fabrics against pathogenic bacteria (*Staphylococcus aureus* and *E. coli*) was assessed to ascertain the extent of resistance of dyed fabrics for the growth of these bacteria. Different assessment methods namely qualitative and quantitative assessment were used to assess antimicrobial activity of silk, wool and cotton samples dyed with dye extracted from Pf-24.

a. Qualitative assessment

No inhibition zone was observed against *E. coli* and *Staphylococcus aureus* in control swatches. Profuse growth of bacteria in and around the fabric swatches was observed in case of control samples. Dyedswatches of silk, wool and cotton exhibited a clear inhibition zone of 7.55, 8,0 and 4.5 mm, respectively against *E. coli.* The inhibition zone against *Staphylococcus aureus* was 9.5mm (silk), 8.0mm (wool) and 5.0mm (cotton). Comparison of test samples with control samples clearly indicated that all dyed fabric samples hindered the growth of the test pathogens up to various extents (Table-2, Fig. 2).

It was also observed that the antibacterial activity of dyed samples was more against *S. aureus* when compared with the activity against *E.* coli that is good result for textile materials as fabric remains in direct contact of body and *S. aureus* is the bacterium, mainly present on the skin surface and causes skin infections and also a cause of body odour. Bacteria such as *S. aureus*, *S. epidermidis* are established in the human skin and Staphylococcus, coryneforms, micrococcus bacteria have been isolated from head, legs and arms of the human body[10, 11].

The variation in antimicrobial activity (zone size) may be due to different dyeing behaviour of samples, dye content present in the samples and extent of diffusion of dye in the agar medium, which resists the bacterial growth [12].'Zone of inhibition' can be an indication of the level of treatment, although the size of the zone is also dependent on the type of fabric being tested. Zones can only be compared between similar samples. The zone does not indicate that any area outside of the sample itself is protected. Having a zone does not mean that there is a 'halo' of protection in the air around the sample [13].

Gupta *et al.* [6] studied the antimicrobial properties of eleven natural dyes against three types of Gram-negative bacteria. Seven of the dyes showed activity against one or more of the bacteria. Their results demonstrate that certain dyes are able to reduce microbial growth almost completely in the case of *E.coli* and *Proteus vulgaris*. Shirata*et al.* [14] isolated a bluish purple pigment from a bacterium, *Janthinobacteriumlividum*, and used it for dyeing. This pigment showed antibacterial activity against many plant pathogens. Siva [15] stated that some natural dyes by themselves have medicinal properties.

b. Quantitative assessment

After qualitative assessment of all the experimental specimens, the quantitative assessment of antibacterial activity was done by using the ASTM: E 2149-01 method. Results of the experiments are shown in Table-3.

It is evident from the Table that percent reduction in CFU (Colony Forming Units) of both bacteria was found much more in case of silk and wool samples as compared to cotton sample due to the less absorption of the dye. However, dye showed less reduction in CFU of gram negative bacteria (*E. coli*)in contrast to more reduction in gram positive bacteria (*S. aureus*). This shows that dye is more effective against gram positive bacteria than gram negative bacteria. Thus, quantitative results support qualitative assessment.In fact,Haynes *et al*. [16] and Toohey*et al*. [17] also found in their study that phenazine derivatives are bacteriostatic to *S. aureus*.

Table 1: Constructional details of fabrics

Textile Material	Constructional Details		
	Fabric count		Weave
	Warp	Weft	
Silk	104	100	Plain
Cotton	84	72	Plain
Wool	76	65	Plain

Table 2: Inhibition zone exhibited by dyed samples

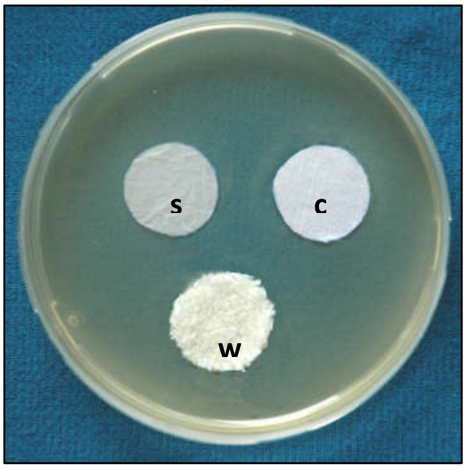
Test pathogens	Inhibition Zone (mm)					
	Silk		Wool		Cotton	
	Control	Dyed	Control	Dyed	Control	Dyed
***E. coli* (Gram –ve)**	Nil	7.5	Nil	8.0	Nil	4.5
***S. aureus* (Gram +ve)**	Nil	9.5	Nil	8.0	Nil	5.0

Table 3: Antimicrobial activity of the dyed fabric by percentage reduction test

Test pathogens	% reduction in CFU		
	Silk	Wool	Cotton
***E. coli* (gram –ve)**	28.95	16.35	15.26
***S. aureus* (gram+ve)**	37.46	25.28	21.48

Fig.1 Pigmented growth of *Pseudomonas fluorescens (Pf-24)*

Zone against *E. coli*   **Zone against *S. aureus***



Control

S: Silk, W: Wool, C: Cotton

Fig.2 Inhibition zone exhibited by dyed fabric samples

CONCLUSIONS

Findings of the study conclude that all the dyed samples are inhibitory to micro-organisms and thus offer medicinal properties. Dyeing with dye prepared from Pf-24 imparted anti-microbial finish to the fabric. This additional medicinal finish imparted to dyed fabrics makes the fabrics categorized under smart textiles. Natural dyes having antibacterial property may find use in the dyeing of sheets and gowns for hospital use and on articles, which are less suitable for laundering such as mattresses and upholstery.

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