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Research Paper

A novel natural dye from *Pseudomonas fluorescens* imparts antibacterial finish and ultraviolet radiation resistance to textiles

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■ **ABSTRACT :** The present study was taken up as an exploratory study to test if natural dye containing *Pseudomonas fluorescens* pigments may be used to develop protective clothing. This microbial dye was used to dye cotton, wool and silk fabrics and these fabrics were tested for antimicrobial finish against common human pathogens *Escherichia coli* and *Staphylococcus aureus* and their resistance to UV radiation. Wool samples exhibited best antimicrobial finish against *E. coli* while same was observed for silk samples against *Staphylococcus aureus*. Quantitatively, inhibition rate was highest for dyed wool samples (23.77% and 49.47%) followed by silk (22.22% and 42.55%) and cotton (16.17% and 32.37%) for *E. coli* and *Staphylococcus aureus*, respectively. Among wool, silk and cotton fabrics, wool samples exhibited best UV protection factor. Study concluded that all the dyed samples exhibited antibacterial and UV protection properties. Textile materials with antibacterial finish may find use in preparation of sheets and gowns for hospital use and articles, which are less suitable for laundering such as mattresses and upholstery. UV protective textiles can be used in various apparels and accessories such as hats, shoes, umbrellas, baby-carrier covers, tents and beach cannabis etc.

■ **KEY WORDS:** Bacterial pigment, Natural dye, Antibacterial finish, Textiles, UV protection, Protective textiles

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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 (Singh *et al.*, 2005). A variety of antimicrobial textile agents such as organometalics, phenols, quaternary ammonium salts and organo-silicones have been reported (Yang *et al.*, 2000). Synthetic compounds are more complex and it will take a long time for them to complete their natural cycles and return to nature; thus causing a lot of environmental pollution. Due to the fact that natural dyes can often inhibit the growth of micro-organisms traditionally, different plants have been used as natural dyes in textile and carpet industries and it is believed that these dyes are less allergic and more stable

than the chemical ones (Calis *et al.,* 2009).The interest in these natural dyestuffs is increasing because of recently discovered useful functions such as antioxidant effects and antibacterial effects in addition to the positive feelings people have about their safety (Kato *et al.,* 2004).

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. UV protective textiles can be used in various apparels and accessories such as hats, shoes, umbrellas, baby-carrier covers, tents and beach cannabis etc.The dyes exhibiting good wash fastness have durable antibacterial effect (Gupta *et al.,* 2004).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 (Thilagavathi *et al.,* 2007). Several sources of plant dyes rich in tannins, napthoquinones are reported to exhibit antibacterial and antifungal activities (Siva, 2007). 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.

Microbial pigments are of great structural diversity. They may be derivatives of the material classes of carotenoids, phenazine dyes, pyrrole dyes, azaquinones etc. (Duran *et al.,* 2016). The use of bacteria to permanently dye or stain cloth is not new. Fermentation processes use bacteria and enzymes to digest, transform and synthesize natural materials from one form to another which is essential in few cases of natural dyeing. These processes have been dated back to over 5,000 BC. With the advent of synthetic dyes and industrial manufacturing, many of these processes became obsolete (Veni *et al.,* 2013). In 1882 a blue-black pigment was isolated from *Chromobacterium violaceum*. This pigment was found to be violacein and deoxyviolacein later on this pigment could be isolated from bacterial strains of *Janthinobacterium lividum, Chromobacterium lividum* and *Pseudoalteromonas luteoviolacea*. This pigment has been used for dyeing textiles and good dyeing results have not only been obtained in connection with natural fibres such as silk, wool and cotton, but also with synthetic fibres such as nylon (Tan *et al.,* 2011). These studies suggest that bacteria may be as good source for dyeing textile fibres. Several bacteria like 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.

Present study describes the preparation of natural dye from *Pseudomonas fluorescens* (strain pf-27) and its application on natural textile substrates like silk, wool and cotton and antibacterial and UV protection propertiesexhibited by dyed fabrics.

■ RESEARCH METHODS

Pigment producing, non-pathogenic strain of *Pseudomonas fluorescens,* (Strain, Pf-27) 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 (Kato *et al.,* 2004).

Cotton andprotein yarns *viz*., silk and wool, were selected as natural textile substrate to see effect of dyes in the present study. Tasar silk and Merino wool and cotton fabric (Table A) were procured from certified outlets in open market.

Table A : 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

Cultivation of the bacteriaand extraction of pigment for dyeing :

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 (Mishra, 2007). Strainpf-27 of *Pseudomonas fluorescens* was grown on Modified King's B broth

(pH7.0), flasks were incubated in stationary condition in a B.O.D. incubator at 25^0C for 48 hrs.

The extracellular pigment producing cell mass was harvested by and colourless cell mass pellet was discarded and the blue-green pigmented cell-free supernatant was concentrated and dried in air. Water was added to dried powder and used as a dye solution.

Dyeing of textile materials :

The dyeing experiment was carried out on all the selected textile substrates of 10 x 10 cm by the standard method prescribed for naturaldyes.All the experimental fabrics (silk, wool and cotton) were dyed using the optimized dyeing conditions, *i.e.* pH of dye liquor, concentration of dye, M: L ratio, dyeing time and temperature (Mishra, 2007). In case of dyeing of cotton fabrics 0.02g tartaric acid was addedto 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.

Test bacteria :

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. and T., Pantnagar.

Assessment of antibacterial activity :

Antibacterial activity of all fabric samples dyed with natural dye extracted from pf-27 strain was assessed both qualitatively and quantitatively.

Qualitative assessment :

AATCC test method 90-1974 (AATCC, 2001) 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 dye from Pf-27 without using mordents 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 of inhibition in mm

T = Total diameter of test specimen and clear zone in mm

D = Diameter of the test specimen in mm

Quantitative assessment :

The ASTM: E 2149-01 (ASTM, 2001) 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-27 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 (CFU/ml) 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.

Resistance to ultraviolet radiation :

Ultra-violet protection factor (UPF) of all the dyed experimental fabrics was determined according to test method described in UVR TRANSMISSION AATCC-183: 2004(AATCC, 2004). The UPF is computed as the ratio of the erythemally weighted ultraviolet radiation (UV-R) irradiance at the detector with no specimen to the erythemally weighted UV-R irradiance at the detector with a specimen present. Four specimens from each sample were tested for the dry testing. Specimen of 50x50mm (2x2 inches) was cut from each sample. Conditioned specimens under standard atmosphere were placed against the sample transmission port opening in the sphere. UV transmission was taken with the specimen oriented in one direction, a second measurement at 0.79 rad (45º) to the first and a third at 0.79 rad (45º) to the second. Individual measurements were recorded. Average spectral transmittance was calculated for the three measurements per specimens with a total of five specimens representing each fabric sample. UPF was computed using mean per cent transmission in the UVA region (320-400nm) and mean per cent transmission in the UVB region (280-320nm). UPF of each specimen was calculated using following equation:

$$UPF = \frac{\Sigma E\,\lambda \times S\,\lambda \times \Delta\,\lambda}{\Sigma E\,\lambda \times S\,\lambda \times T\,\lambda \times \Delta\,\lambda}$$

where, E_λ = relative erythemal spectral effectiveness

S_λ = Solar spectral irradiance

T_λ =average spectral transmittance of the specimen (measured)

$\Delta\lambda$ = Measured wavelength interval (nm)

The UV protection category was determined by the UPF values (Table B) described by Standards Australia/ New Zealand AS/NZS 4399 (1996) (AS/NZS, 1996).

Table B : UV Protection category for fabrics (Standards Australia/ New Zealand AS/NZS 4399)

UPF range	UVR Protection category
15 - 24	Good protection
25 - 39	Very good protection
40 - 50, 50+	Excellent protection

■ RESEARCH FINDINGS AND DISCUSSION

The dye from Pf-27 has dyeing property which is evident from our studies that can have a great potential in textile coloration (Mishra, 2007). Young culture of Pf-27 on agar plate produced fair blue-green pigment along the both edges of streaking region only (Fig. 1).

Broth culture of Pf-27 was centrifuged at 5000 rpm for 30 minutes. Bacterial cells were observed in the form of colourless pellet whereas supernatant was green in colour (Fig. 1). Thus, pigment produced by Pf-27 was in cell free supernatant and out of bacterial cell, they gave colour in media *i.e.* extracellular blue-green pigment, which cannot be separated from agar plate therefore, it was obtained from broth (Sergeeva *et al.*, 1974).

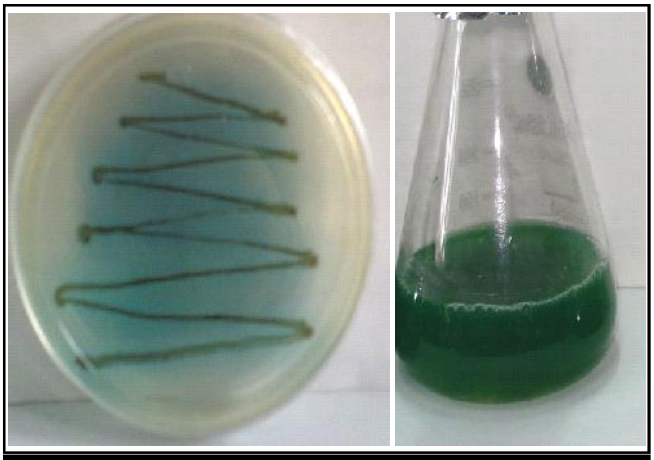


Fig. 1 : Pigmented growth of *Pseudomonas fluorescens (Pf-27)*

Dyeing of textile materials :

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 (Mishra, 2007). 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. Dyed samples were rinsed in cold water and dried under shade.

Antibacterial activity of dyed samples :

Metabolic products of many bacteria possess

antibacterial activity against other bacteria (Veni *et al.*, 2013 and Gupta *et al.*, 2004). 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.

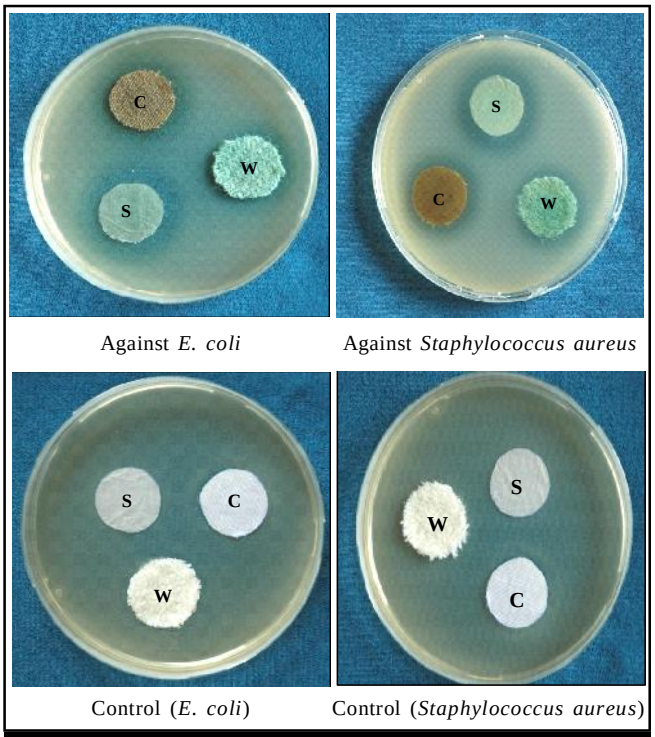
Qualitative assessment :

Dyedswatches of silk, wool and cotton exhibited a clear inhibition zone against test organisms. 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.The largest inhibition zone was observed for dyed silk samples against both *E. coli* and *Staphylococcus aureus*.Comparison of test samples with control samples clearly indicated that all dyed fabric samples hindered the growth of the test pathogens upto various extents (Table 1 and Fig. 2).

It was also observed that the antibacterial activity of dyed samples was more against *S. aureus* than *E. coli* that is good 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 (Doshi, 2006; Yamazaki *et al.*, 2010 and Uzeh *et al.*, 2012).

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 (Achwal, 2003).'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 (Clemo, 2003).

Gupta *et al.* (2005) and Clemo (2005) 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.* (2000) isolated a bluish purple pigment from a bacterium, *Janthinobacterium lividum*, and used it for dyeing. This pigment showed antibacterial activity against many plant pathogens. Siva (2007) stated that some natural dyes by themselves have medicinal properties.



Against *E. coli* | Against *Staphylococcus aureus*

Control (*E. coli*) | Control (*Staphylococcus aureus*)

Fig. 2 : Inhibition zone exhibited by fabric samples dyed with pigment of *Pseudomonas fluorescence (Pf-27)* S= Silk, C= Cotton, W= Wool

Table 1 : Inhibition zone exhibited by fabric samples dyed with pigment from *Pseudomonas fluorescens* (Pf-27)

Test pathogens	Inhibition Zone (mm)					
	Silk		Wool		Cotton	
	Control	Dyed	Control	Dyed	Control	Dyed
E. coli (gram –ve)	Nil	0.60	Nil	0.25	Nil	0.48
S. aureus (gram+ve)	Nil	0.90	Nil	0.45	Nil	0.25

Table 2 : Quantitative assessment of antimicrobial activity of the dyed with pigment from *Pseudomonas fluorescens* (Pf-27)

Test pathogens	% reduction in CFU		
	Silk	Wool	Cotton
E. coli (gram negative)	28.95	16.35	15.26
S. aureus (gram positive)	37.46	25.28	21.48

Table 3 : Mean UPF and protection categories of fabricsdyed with pigment from *Pseudomonas fluorescens* (Pf-27)

Textile material		Mean UPF	UV protection category
Silk	Control	9.94	-
	Dyed	15.86	Good protection
Wool	Control	117.7	Excellent protection
	Dyed	964.57	Excellent protection
Cotton	Control	10.88	-
	Dyed	15.08	Good protection

Quantitative assessment :

Quantitative assessment of antibacterial activity was done by using the ASTM: E 2149-01 method. Per cent reduction in CFU (Colony Forming Units) of both bacteria was found much more in case of silk and wool than cotton samplesdue to the less absorption of the dye. However, dye showed less reduction in CFU of *E. coli* in contrast to more reduction in gram *S. aureus* (Table 2). This shows that dye is more effective against gram positive bacteria than gram negative bacteria. Thus, quantitative results support qualitative assessment.Other investigators have also reported that phenazine derivatives are bacteriostatic to *S. aureus* (Haynes *et al.*, 1956 and Toohey *et al.*, 1965).

Resistance to ultraviolet radiation (UVR) :

According to standards AS/NZS: 4399 (AS/NZS, 1996),only wool fabric is under classified category whereas silk and cotton fabrics are in unclassified categories. Among three fabrics wool had maximum UPF showing excellent UV protection (Table 3). Among the natural fibres, wool has more UPF than silk and cotton (Sunder and Kumar, 2005). The construction of fabrics and the fibre types have great influence on protection from ultraviolet transmittance. The UPF of textiles depends on their construction, the spaces between the yarns and their fibre types. Good skin protection is achieved by the textile itself with a sufficient weight and covering power of the fabric). UPF is considered to be closely related to the fibre properties and the dye fibre interactions and the same dyes can give very different results on different fibres. Woolfibre exhibits more dye-fibre interaction than silk and cottonfibres,hence on wool fabric dye imparted maximum degree of sun protection with UPF more than 50 (Sivaramakrishnan, 2007; Gupta and Ruchi, 2007). Therefore, dyed wool fabric may be used to produce high UV protective fabrics, which can be used in various apparels and accessories such as hats, shoes, umbrellas, baby-carrier covers, tents and beach cannabis etc. (Sivaramakrishanan, 2007).

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-27 imparted anti-microbial finish and resistance to UV radiation to the fabric samples. These additional properties acquired by dyed fabrics categorize them 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. UV protective textiles can be used in various apparels and accessories such as hats, shoes, umbrellas, baby-carrier covers, tents and beach cannabis etc.

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