



## DYEING OF SILK/WOOL USING CRUDE PIGMENT EXTRACT FROM AN ISOLATE *KOCURIA FLAVA* SP. HO-9041

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**Abstract:** The prolonged use of natural colours has not only had an adverse effect on environment but also human health. In view of these concerns, natural colours/pigments are now becoming alternatives to synthetic ones. The present study involved isolation, identification, and characterization of the natural pigment producing bacteria and its applicability in textile field. Various microbes screened from the garden soil of our institute (ICT Mumbai) resulted in isolation of a yellow-pigment-producing bacterium. The 16s-rRNA results disclosed that these bacteria had 99% similarity to *Kocuria flava* sp. HO-9041. The dyeing of fabric (silk and wool) with the extracted pigment showed a good dye uptake, and the colour strength given by K/S (ratio of absorption/scattering) increased satisfactorily upon dyeing with bacterial pigment. These tests showed the ability of the pigment to be used as colorant for fabrics, enhanced more so by the dyeing in absence of a mordant (substance used for attaching dye to fabric). Apart from its applicability in textiles, the pigment showed antibacterial activity also. This may give rise to potential applications of such dyed fabrics in apparels, children-ware, and medical textiles, where hygienic conditions are required.

**Keywords:** *Kocuria flava*, Yellow pigment, Wool, K/S value, Silk, Medical textiles.

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## INTRODUCTION

Human eye does not see only in black and white, which is what gives colour one of the most important place in human lives. Recognition and acceptance of any article or product depends on its appearance, of which colour forms one of the important attribute. Many times, colour is the first test used by consumers to validate a product. Therefore, along with the product quality, our focus should also be on its colour to make it more attractive. The textile dyeing business has existed for over 4000 years. Almost till 1850's, the dyes obtained were of natural origin. However, with the synthesis of organic chemical dye mauve by

Perkin in 1856, the synthetic dye industry has grown at a steady rate, eliminating the use of natural dyes (Ferreira *et al.* 2004). Today approximately 1.3 million tonnes of dyes, pigments and dye precursors (valued at around \$23 billion), produced by textile industry is almost completely manufactured synthetically (Sengupta and Singh, 2003). However, repeated and prolonged use of synthetic colours have thrown up many issues like (i) Requirement of hazardous chemicals for production process, (ii) petroleum as the feedstock of all synthetic dyes, (iii) Allergic and toxic reactions to humans and (iv) Hazardous waste generation, the disposal of which is a

major environmental and economic challenge (Sengupta and Singh, 2003). Therefore, many countries have imposed stringent environmental standards in response to the various ecological and economic challenges associated with synthetic dyes. These constraints have caused a growing interest in natural pigments not only in foods, cosmetics, pharmaceuticals, but also in textiles. This has led to an upsurge in demand of natural colours, resulting in an annual growth rate of ~7% for natural colours, the market of which has already reached US\$ 0.66 Billion in 2010 (Transparency Market Research, 2011). Earlier natural pigments used to be isolated from ores, insects, plants, and animals. However, recently, genetically engineered plants, cell cultures, and microorganisms, are gaining increasing interest as sources of natural pigments as they offer certain distinct advantages over colours produced from plants and animals (Gupta *et al.* 2011). The advantages of less time for cultivation and independence from season and geographical conditions are what make the microorganisms an exciting source of natural colours. Some common examples of such natural pigments are red pigment extracted from *Serratia sakuensis*, applied to dyeing of natural fibres (Vaidyanathan *et al.* 2011). Along with red pigment from *Serratia marcescens*, the use of violet pigment from *Chromobacterium violaceum* in dyeing of natural fibre (Ahmad *et al.* 2012) and blue and red pigments isolated from *Streptomyces* sp. have been reported for dyeing of natural fibre (Kramar *et al.* 2014). Therefore, as the market of natural colours becomes intense, the manufacturers are now resorting to the use of natural colours in more and more products. This has given impetus to the production of highly stable natural colours and the exploration of new natural extracts, hence providing an opportunity to explore microorganisms as a sustainable source of natural colours/pigments.

## EXPERIMENTAL

**Materials:** Soil sample was collected from the gardens of our institute (Institute of Chemical Technology ICT), Mumbai. Ready-for-dye (RFD) wool/silk fabrics used in dyeing were procured

locally. Nutrient broth, nutrient agar, soybean case digest media, and methanol (AR grade), were purchased from Hi-media Laboratories, Mumbai. Standard bacterial culture of *Bacillus cereus* and *E.coli* were procured from National Collection of Industrial Microorganisms (NCIM), Pune.

**Isolation and screening of the pigment producing Organism:** Various Samples from soil, air, and water were screened for isolation of pigmented bacteria. A bright yellow-pigment-producing bacterium was isolated from soil sample collected from the Institute of Chemical Technology (ICT) garden area. The protocol adopted for the same was as follows:

1 gm of soil sample mixed with 100 ml of 0.90% saline solution was thoroughly homogenised to get soil suspension. Serial dilutions up to  $10^{-8}$  were made. On the nutrient agar plates, 200  $\mu$ l of samples from the last three dilutions were plated. The plates were incubated for 48 hours at 37 °C. The colony of interest (producing bright yellow pigment) was isolated from the rest. The isolate was sub cultured repeatedly to maintain the pure form of yellow colony. Soybean casein digest media was used for liquid media, while soybean casein digest agar was used for solid media to preserve the culture for future.

**Identification and characterisation of bacteria:** Bacteriological examination of the strain was carried out by gram staining while identification of the isolate was done using 16sr-RNA technique. The base sequence of 16s r-RNA gene of the isolate was amplified using standard universal 16s r-RNA primers (James. 2010) and the nucleotide sequence of the isolated gene was analysed by Scigenome Laboratories, Mumbai. The base sequence data obtained was examined for homology with 16s r-RNA sequences data of NCBI using BLAST.

**Partial Characterization of pigment:** A good understanding of the physical and chemical properties of a pigment is important for determining their applications and quantifying them with greater reliability and ease. Hence, the pigment characterization in terms of its solubility, UV/Vis spectra and IR spectra were determined. The visible spectra of the pigment was obtained using a UV-Vis spectrophotometer

(UV 2550), while the IR prestige-21, Shimadzu was used for the IR curve of the isolated pigment.

**Dyeing of Wool:** Dyeing of fabric carried out in ROTA (supplied by Rossari Labtech, Mumbai) was done using the methanolic extract of pigment. Acid dyeing method was used for dyeing of the fabric with liquor ratio of 20:1 at 100°C for 1 hour. The fabric was rinsed and air dried. Colour strength and fastness properties of fabric were further tested.

**Evaluation of Colour Measurement:** Dyed wool fabrics were evaluated for the depth of colour by determining K/S values using a Spectraflash Ò SF 300 computer colour matching system supplied by Data color International, U.S.A. Average of four readings, taken at four different sample areas, was used to calculate the reflectance values. The Kubelka Munk (K/S) function was found using equation 1 given below.

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

where,  $R$  = reflectance at complete opacity

$K$  = absorption coefficient

$S$  = scattering coefficient

Tone of the colour in terms of CIE  $L^*$ ,  $a^*$  and  $b^*$  values was also measured on the same machine. Parameter  $L^*$ , which is always positive stands for the brightness and the values are from 0 (black) to 100 (white). Parameter  $a^*$  stands for the red-green axis while parameter  $b^*$  for yellow-blue axis.

**Fastness testing:** Of the entire colour testing of fabrics, the light fastness, the washing fastness, and rub fastness are considered important (Sharma *et al.*, 2012). Therefore, Standard methods of AATCC and ISO were used to check the fastness property of the dyed fabric. The tests performed were:

- Washing Fastness carried out by ISO 105-CO2 (1989).
- Light Fastness carried out by AATCC 117 using Q-Sun's Xenon Arc light fastness tester.
- Rubbing Fastness carried out by ISO 105-X12(1987) using Crockmeter.

**Antibacterial activity of the pigment:** A gram-positive strain *Bacillus cereus* and a gram-negative strain *E. coli* were used for antibacterial activity testing of the pigment. 1 ml of inoculum from broth having  $10^9$  CFU/ml test organisms was spread using glass spreader on six agar plates to get results in triplicate (three gram-positive and three gram-negative). Two wells were cut into agar layer with stainless steel bore of approximately 4-mm diameter. One well was as blank containing methanol as solvent, since the pigment was prepared in methanol. The other bore having 70 µl of pigment sample was loaded and the plates kept for 24 h of incubation. The average diameter of the zone of inhibition was recorded around the well. The final readings subtracted from the blank reading gave the actual readings.

## RESULTS AND DISCUSSION

**Selection of pigmented bacteria:** The bright yellow-pigment-producing bacterium was isolated from the ICT garden from the rest of non-pigmented ones (Figure 1). The bacteria did not lose the pigmentation property in spite of repeated sub culturing.



Figure 1. Photograph of yellow-pigmented isolate from soil

**Production and Extraction of the Pigment:** Repeated culturing of isolate was done to ensure consistent pigment production. The isolate showed pigment production on both solid media that include petri plates and in liquid media. Considering large-scale production, the focus was on liquid media preparation since solid media preparation and extraction of pigments from the same was tedious. The isolate, grown on 100 ml of soyabean case digest media for 48 h at 37°C was centrifuged at

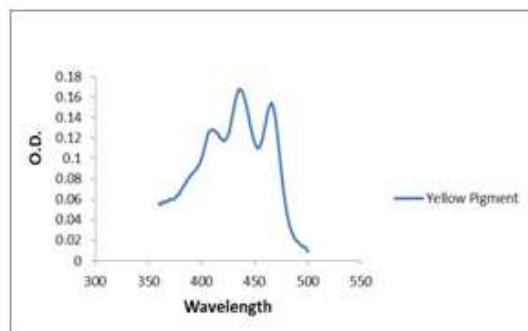
a stirring speed of 7000 rev/min for about 10 minutes at 4°C. After centrifugation, the supernatant was discarded while the yellow coloured pigment was washed with water to remove any impurities. 20 ml of methanol was added to the coloured cell pellet and the mixture was re-centrifuged at 7000 rpm for 20 minutes, to extract all the yellow pigment into methanol. Fresh methanol was added until the cells became visually colourless.

**Characterisation of bacteria:** The isolate was gram-positive, coccoid in shape, and aerobic. Apart from morphological studies, 16s r-RNA studies revealed that the bacteria belonged to *Kocuria flava* sp., recently discovered under genus *Kocuria* (Zhou et al. 2010).

#### **Partial characterization of isolated pigment**

**Solubility:** The yellow pigment was highly soluble in Ethanol and methanol and insoluble in solvents like hexane, toluene and chloroform. Alcoholic carotenoids or Xanthophyll's are generally more soluble in ethanol and methanol (Rodriguez, 1999).

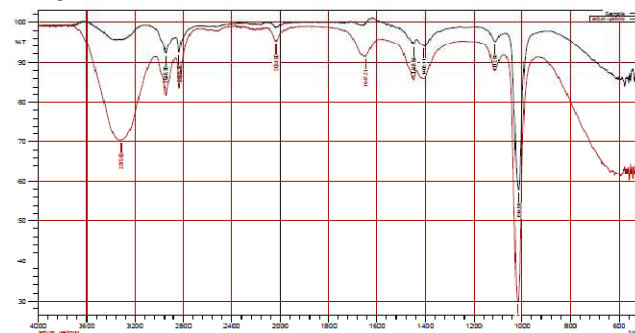
**Light Absorption spectra:** The conjugated double bond system constitutes the light-absorbing chromophore and is what gives pigments their attractive colour and light absorption spectrum, which can also help serve as a guide for its identification and quantification. The yellow pigment (extracted in methanol and ethanol both) was scanned in the visible region and was seen to have a three peak spectra shown in Figure 2. The three-peak spectrum is a characteristic feature of a carotenoid.



**Figure 2: Light absorption spectra of pigment in methanol.**

**IR analysis:** The yellow pigment was analysed using IR for determining its functional groups.

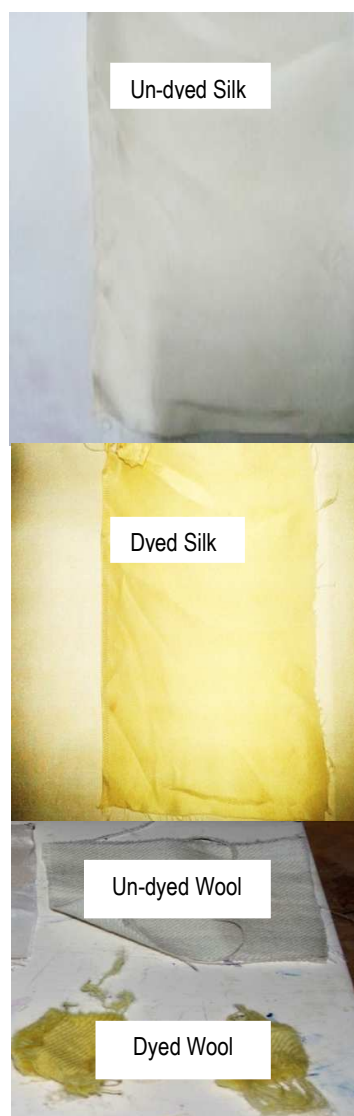
The IR spectra shown in Figure 3, shows a broad peak in the range of 3200-3500  $\text{cm}^{-1}$ , indicative of OH group, which can also help explain the reason for high solubility of pigment in ethanol and methanol. Alkane stretching peaks were present in the range of 2800-2950  $\text{cm}^{-1}$ . The C=C bonding, a characteristic feature of pigment was also observed with a peak at 1600-1680  $\text{cm}^{-1}$ . A strong peak at 1014  $\text{cm}^{-1}$  shows the presence of an alkoxy group (C-O) linkage.



**Figure 3: IR spectra of yellow pigment**

**Application of pigment in textiles:** Attempts made to dye wool and silk with the microbial pigment produced in this work resulted in uniform dyeing of wool. The dyeing of wool/silk was carried out at 100°C for 60 min; the results of which are shown in Figure 4, and the scientific basis of colour uptake compiled in Table 1.





**Figure 4. Photograph of Dyed fabric (silk/wool) along with un-dyed fabric**

**Table 1: Colorant strength values of wool substrate dyed with microbial dye**

Fabric	L*	a*	b*	K/S
Wool BD)	82.79	-0.444	11.416	0.6854
Wool AD)	82.246	-1.286	25.924	2.6786
Silk (BD)	91.46	-0.736	3.085	0.0537
Silk (AD)	93.56	-0.742	6.861	0.8962

AD= After Dyeing; BD= Before Dyeing

The wool fabric dyed with microbial dye showed uniformity and levelness. From Table 1 it was observed that the colour strength and K/S value increased when wool and silk were dyed with microbial dye; indicating a good dye uptake of this microbial chrome on substrate. Also seen from Table 1, was the value of a\* (indicator of

redness and greenness) of microbial dyed fabric was lower when compared with that of un-dyed wool/silk. This means that the microbial dyed wool's greenness characteristic is higher. Also observed was the value of b\* (indicator of yellowness and blueness) of microbial dyed wool/silk was higher when compared with that of un-dyed wool/silk. This means that the dyed wool substrate is more yellow than RFD wool substrate. The dyed fabrics were also tested for assessment of different fastness properties such as, washing fastness, light fastness, and rubbing fastness. The results of which are compiled in Table 2. The rubbing fastness for wool/silk was excellent, though the light fastness and washing fastness was average.

**Table 2: Fastness properties of silk and wool substrate dyed with microbial dye**

Fabric	Washing Fastness	Light Fastness	Rubbing Fastness	
			Dry	Wet
Wool	1-2	2	3-4	3
Silk	2-3	2	5	4-5

The results obtained for dyeing are in the absence of any mordant (substance used for attaching dyes to fabric). Hence, with the use of such substances (which is a common practice for dyeing of fabrics with natural colours), the fastness values is very likely to increase to a level of 4 or 5. Therefore, these fastness values in the absence of any mordant indicate good potential of this pigment extracted from *K. flava*. Thus, this bacterial yellow pigment can be used to dye wool fabric by exhaust process. Such a dyed fabric could have possible applications in apparels, baby wear, and in any other product, that requires hygienic conditions.

**Antibacterial studies:** The pigment apart from its applicability in textiles was also investigated for its antibacterial activity. Gram-positive *Bacillus subtilis* sp. and gram-negative *E. coli* sp., were used as standard to check the antibacterial activity. After incubation period, 10.4±0.2 mm cleared zone of inhibition was observed in case of gram-positive bacteria. From this result, it was conclusive that the pigment was effective against gram-positive bacteria, whereas no significant activity was found against gram-negative bacteria. This may be due to the additional cell membrane present

in gram-negative bacteria making them more resistant to antibacterial substances.

Today textile dyeing and treatment is not only a growing industry in terms of market but also services offered. The desire for eye catching fancy colourful clothing is what drives this market. However, the textile dyeing industry causes an estimated 17-20% of the industrial freshwater pollution. Moreover 10-15% of the total dyestuff (equivalent to 280,000 tonnes of dyestuff) used during the manufacturing of textile products is released into the environment annually (Gad, 2011). These staggering numbers is what has prompted the textile industry to look for alternative textile dyes that are not only produced using a greener route but are also less damaging and biodegradable when released into the environment. Recent studies are now focussing on microbial dyes/colorants as an alternative to synthetic chemical dyes. In our study, we report isolation of an organism producing one of the three primary colours. The *Kocuria flava* organism isolated has not only shown a characteristic yellow colour but also the capability to produce the colour continuously. Moreover, the ability of *K. flava* to produce the pigment in good quantity even in minimal media adds to the desirable features of the yellow colour from this organism. The uniform application of the extracted pigment on natural fabrics in the absence of any mordant shows the ability of the colour as a textile colorant. The usage of the crude extract for dyeing can also help reduce the cost of downstream processing in industry. In addition, the antibacterial activity of the isolated colour adds to the advantages of the extracted colour. Hence, the yellow colour extracted from the organism has great potential as colorant. Though the known advantages of usage of natural colours over synthetic ones are high, the high cost of the bacterial pigment production process and the purification of these compounds remain a bottleneck in the area of pigment application in textile industry. However, bacterial pigment production and its application in various industries is one of the emerging fields of research. Hence as work on pigment production is intensified especially in pigment

production using cheap and suitable growth medium (Venil et al., 2013), there is hope that bacterial colour/dye application in textile industry can be made truly worthwhile.

## CONCLUSION

The yellow-pigment-producing bacteria from ICT garden showed significantly good application as a natural dye for colouring of silk and wool. The pigment also showed medication property enhancing its business worth. Such a dyed fabric might have an application within medical textiles. Pigment yield can be maximised with optimisation of media and extraction parameters. Pigment can be further analysed and characterised to elucidate its structure successively, which shall facilitate in describing the most effective application for that pigment in foods, cosmetics, paper, or any other such industries.

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