# Production, Partial Characterization, and Use of a Red Biochrome Produced by Serratia sakuensis subsp. nov Strain KRED for Dyeing Natural Fibers

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Abstract We have described a novel red biochrome, 514 Da in size, produced by solidstate cultivation of a bacterial isolate obtained from garden soil. The growth requirements of the isolate, the chemical characteristics of the biochrome produced, and the application of the biochrome in dying of silk, wool, and cotton fabrics have been studied. The biochrome obtained after 52 h of incubation and having a  $\lambda_{\text{max}}$  of 535 nm was used for dyeing the fabrics. We found that silk, wool, and cotton fabrics dyed with this new natural red compound have high color strength values and dye uptake along with good color fastness as well as antibacterial activity.

Keywords Natural dyes . Bacteria . Speckled colonies . Tripyrrol compound . Dyeing . Natural fibers · Antibacterial activity

#### **Introduction**

With the increase in the awareness about environment and ecological balance, the use of eco-friendly fibers and natural dyes has increased across the globe. This is because of the stringent environmental standards imposed by many countries in response to the toxic and allergic reactions associated with synthetic dyes [\[1\]](#page-13-0). Natural dyes are more environment friendly, exhibit better biodegradability, and have better compatibility with the environment [[2](#page-13-0)–[4](#page-13-0)] than synthetic dyes; therefore, they are a potential alternative for synthetic compounds.

Microorganisms produce various pigments like carotenoids, melanins, flavins, quinines, and, more specifically, monascins, violacein, or indigo [\[5\]](#page-13-0). Microbial production of indigo and its application in dyeing cotton fabrics have been reported [[6\]](#page-13-0). Although a large number

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of bacterial pigment producers and the pigments they produce are known, there is limited data about using these pigments as an alternative to chemical dyes [[6,](#page-13-0) [7](#page-13-0)]. Of the six classes of non-photosynthetic pigments, namely, carotenoids, anthocyanins, tripyrrols, phenazines, melanins, and riboflavins, the tripyrrol class of pigments are highly sought after as they are stable colorants and, therefore, can be uniquely adapted to the rigors of textile dyeing. The most commonly studied pigment in this class is prodigiosin.

Serratia marcescens is a pigmented enteric bacterium found in a variety of niches, including soil, water, air, plants, and animals [[8](#page-13-0)]. Serratia sakuensis subsp. nov strain KRED, however, has been reported in sewage water by Ajitkumar et al. [[9](#page-14-0)]. Although prodiginines, a family of natural red pigments characterized by a common pyrrolylpyrromethane skeleton, have been obtained from S. marcescens, their derivatives have been produced by various bacteria and actinomycetes [\[10\]](#page-14-0) (Fig. 1; Table [1\)](#page-2-0).

In addition, some uncommon prodiginines such as metacycloprodigiosin, butyl-metacycloheptylprodiginine, cycloprodigiosin, cyclononylprodiginine, and cyclomethyldocylprodiginine have also been reported [\[10\]](#page-14-0).

While many studies have assessed the antifungal, immunosuppressive, and antiproliferative activities of prodigiosins and prodigiosin-like pigments [[8](#page-13-0), [9](#page-14-0)], these reports do not discuss dyeing of fabrics, especially silk, using these types of pigments. This article, thus, primarily focuses on the optimization of the nutritional parameters for the production of the biochrome from the isolate S. sakuensis along with the structural analysis of the compound and its application as a fabric dye.



Fig. 1 The pyrrolylpyrromethane skeleton of prodigiosin

<span id="page-2-0"></span>

#### Materials and Methods

Isolation and Characterization of the Isolate by 16S rRNA

The organism was isolated from a garden soil sample procured from Aarey Milk Colony by using modified nutrient agar (peptone, 1%; beef extract, 0.3%; NaCl, 3%; and glycerol, 1 mL), and was maintained on the same medium. The isolate was partially characterized on the basis of its gram nature, colony characteristics, and biochemical and staining reactions. Further, the DNA of the isolate was extracted by phenol/isoamyl/chloroform method; it was taxonomically identified by 16S rRNA sequencing at NCCS, University of Pune.

Optimization of Parameters for Production and Extraction of Pigment

The isolate was repeatedly subcultured on modified nutrient agar medium to ensure the purity of the isolate and the reproducibility of its ability to produce the biochrome at a constant intensity. For large-scale production of the biochrome, the culture was initially grown in modified nutrient broth. Growth of the isolate as well as biochrome production was studied in half-strength modified nutrient broth, single-strength nutrient broth, half-strength nutrient broth, nutrient broth supplemented with 0.3% Tween 80, and nutrient broth with 1% agar with incubation in light, dark, and microaerophilic conditions. Moreover, the isolate was immobilized in 4% sodium alginate beads and in polystyrene rings by using an inoculum density of  $\sim$ 1×10<sup>8</sup> cfu/mL to evaluate the possibility of reusing the inoculum for continuous production of the colorant within repeated medium batches, where each batch will be used for 2 days.

Solid Substrate Fermentation for Biochrome Production

We inoculated 0.2 mL of 24-h-old culture suspension containing  $5 \times 10^{10}$  cfu/mL on modified nutrient agar plates overlaid with 0.3 mL of glycerol; these plates were incubated at 27 °C for 10 days. Cell mass yield, biochrome yield, and viable count were determined at an interval of 4 h over a period of 10 days.

For estimation of biochrome yield, the biochrome was extracted from the cell mass harvested at the requisite time interval by using the below-mentioned method and its absorbance was measured at 535 nm. Biochrome yield was calculated as follows:

Biochrome yield  $(\% ) =$  [weight of the biochrome (g)/weight of the cell mass (g)]  $\times 100$ 

Viable counts of all the 4-h aliquots were determined by the standard pour plate method using modified nutrient agar plates incubated at  $27 \degree C$  for 48 h. We did not distinguish between surface and subsurface colonies while counting.

# Effect of Nutrient Supplementation on Biochrome Synthesis

The culture retained its ability to produce biochromes when grown in solid media. However, repeated subculture invariantly gave rise to 25–30% colonies that had lost their ability for chromogenesis. Hence, supplementation studies using various organic and inorganic carbon sources (at a concentration of 1%) like glucose, mannitol, maltose, lactose, starch, xylose, arabinose, raffinose, inulin, gelatin, paraffin, glycerol, sunflower oil, coconut oil, groundnut oil, and sodium lauryl sulfate were performed using a mineral medium. Similarly, supplementation with nitrogen (0.2%), inorganic salts (0.01%), and growth factors (0.1%) through the use of yeast extract, beef extract, peptone, casein, CAS amino acids, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, ammonium sulfate, ammonium nitrate, ammonium chloride, histidine, proline, tryptophan, aspartic acid, asparagine, and methionine was carried out by the standard agar cup diffusion method, wherein 50 μL of each nutrient supplement was added into a 6-mm cup bored into the agarified mineral medium containing 0.5 mL of the culture S. sakuensis (optical density at 540 nm=0.3). The plates were incubated at room temperature for 48 h and the intensity of biochrome production around the agar cup was graded as the zone of exaltations through the determination of the said zone diameter in millimeters.

### Extraction of the Biochrome [\[4\]](#page-13-0)

S. sakuensis grown in optimized nutrient agar medium was harvested and washed with water. To this washed culture, 40 mL of acetone was added and the suspension was stored at 10  $^{\circ}$ C for 6 h. After subsequent filtration, the cell mass was repeatedly washed until the acetone extract turned colorless. The colored filtrates were then combined, and the biochrome was extracted from acetone into petroleum ether (40 mL) by using a separating funnel. The combined petroleum ether was washed with water and dried over anhydrous  $Na<sub>2</sub>SO<sub>4</sub>$ .

# Purification of the Biochrome

The biochrome was purified using preparative HPTLC with a solvent system of n-butanol/ ethanol/water in the ratio 9:1:1. We dissolved 0.2 g of the dry biochrome in 2 mL of acetone (Merck). A sample volume of 240 μL was loaded as a single band on a silica gel TLC plate (Merck) using LINOMAT-5 applicator. The single red band obtained after the run was further re-extracted in acetone. The re-extracted fraction of the biochrome was subjected to two-dimensional chromatography to ensure its purity; the solvent system used was *n*-butanol/ethanol/water (9:1:1) (110 mL). The purified biochrome thus obtained was re-extracted in acetone, dried, and used for further characterization studies.

# Partial Characterization of Biochrome

The purified biochrome was initially characterized on the basis of its color, solubility in aqueous and organic solvents, absorption maxima, and reaction with concentrated  $H_2SO_4$ , 10% NaOH, 10% FeCl<sub>3</sub>, and Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>. The UV–visible spectrum was obtained using Jasco double-beam spectrophotometer for a solution of the red biochrome in acetone. The FTIR spectrum was obtained using Shimadzu IR 8300 spectrophotometer. The molecular mass of the purified biochrome was determined using Bruker Daltonics Microtof Q electro spray– ionization mass spectrometer (ESI-QTOF), while CHN elemental analysis was undertaken using Thermofinnagan atomic absorption analyser.

 $13C$  NMR and  $1H$  NMR were obtained using Perkin Elmer NMR spectrophotometer AS1-10. The solvent used for this analysis is deuterated solvent DMSO (Merck).

Color of the Biochrome: pH and Temperature Stability

### pH Stability

The stability of the biochrome at different pH values was studied by suspending 0.5 mL of 10 mg/mL biochrome in acetone in 2.5 mL of various buffers such as 0.2 M HCl–KCl buffer (pH 4), 0.2 M phosphate buffer (pH 7), and 0.2 M glycine–NaOH buffer (pH 9.2). After incubation for 1 h at room temperature, the variation in color intensity was assessed through changes in the UV–visible spectrum. pH stability was studied for 1 h as it is the time required for dyeing procedures.

#### Temperature Stability

About 0.02 g of the biochrome was dissolved in 20 mL of acetone, and 3-mL aliquots of this solution were dispensed in four tubes. After incubation for 1 h at 10 °C, 30 °C, 50 °C, and 100 °C, the variation in color intensity was assessed through changes in the UV–visible spectrum.

### Application in Textile Dyeing

#### Dyeing of Silk, Wool, and Cotton Fabrics

Silk fabric was dyed with 15 mL of the acetone extract of the biochrome containing 0.27 g/L of the colorant by using the acid dyeing method, maintaining the material-to-liquid ratio (MLR) at 1:25 (fabric weight (g)/dyeing bath volume (mL)) for 1 h at 80 °C. Wool fabric was also dyed using the same method. For cotton fabrics, the direct dyeing method was used, maintaining the MLR at 1:25 for 1 h at 80 °C along with the addition of Glauber's salt in the dye bath. Glauber's salt helps in the exhaustion of the dye onto the fabric. For all the fabrics, dyeing was performed in Rotadyer dyeing machine (Rossari Labtech). After 1 h, the Rotadyer was brought to room temperature. The fabrics were then removed and given a cold wash under tap water. Subsequent soaping of the dyed fabrics using the non-ionic detergent Auxipon NP at 80 °C for 20 min was carried out, followed by cold washing. The fabrics were then air-dried and used for further studies for assessing color value and fastness.

#### Color Strength

Dyed samples were evaluated for the depth of the color transferred on the fabric by determining Kubelka–Munk  $(K/S)$  values using the reflectance values of the dyed samples at 510 nm using a Spectra flash® SF 300, Computer Color Matching System supplied by Datacolor International, USA. K/S value is given by the formula:

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

where R is the reflectance at complete opacity, K is absorption coefficient, and S is the scattering coefficient.

# Fastness Properties

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

# Antimicrobial Activity of Dyed Fabrics

Antimicrobial activity of the dyed fabrics was studied against Staphylococcus aureus NCTC 3578 and *Escherichia coli* ATCC 10148 by AATCC method 100, wherein the dyed fabric was incubated for 24 h with the test organism grown in a liquid nutrient medium. Percent reduction in the viable count of the organism was used to determine the antimicrobial nature of the dyed fabric.

# Results and Discussion

### Characterization of the Isolate

The isolate obtained was gram-negative stubbed bacillus, positive for catalase and negative for oxidase. It was able to ferment sugars such as glucose, fructose, and mannitol. Sequencing of the 16S rRNA subunit indicated that the isolate was S. sakuensis subsp. nov strain KRED (Genbank accession no.: AB061685).

# Optimization of the Medium for Bulk Production of Biochrome

Although the culture initially grew well and produced biochrome in the modified nutrient broth, it lost its ability for chromogenesis after three to four rounds of subculturing. Since pigmentation is a survival strategy adapted by organisms during stress, various conditions of nutrient stress were provided. The culture grew well in all the half-strength media but without biochrome production, thereby indicating loss of chromogenesis in liquid broth media under agitated conditions. In contrast, the culture was found to initiate chromogenesis under stationary conditions wherein predominance for adherence under suboptimal conditions of aeration was observed. Therefore, we studied the effect of immobilization within alginate beads and on polystyrene ring (Table 2). A

Particulars	Parameters	Yield attained $(\% )$	
<b>NA</b>	First subculture	5.26	
<b>NB</b>	After first subculture	3.4	
Single- and half-strength NB	After repeated subculture under RT/48 h light/dark/micro aerophilic	NIL.	
Solid state single strength and modified nutrient agar	After repeated subcultures	$3.42 \pm 0.25$	
Immobilization within alginate beads	Total yield after four batch runs	$9.3 \pm 0.973$	
Immobilization on polystyrene pall rings Supplementation of NA with glycerol	Total yield after two batch runs 0.3 ml glycerol	$0.5 \pm 0.210$ $7.77 \pm 0.55$	

Table 2 Yield optimization for biochrome produced by S. sakuensis

good yield of biochrome (9.3%) was obtained using immobilized alginate beads, thus indicating that this isolate exhibited a complete block in biochrome production in liquid media and required either immobilized or solid-state growth for chromogenesis.

#### Effect of Nutrient Supplementation on Biochrome Synthesis

Optimization of the medium composition through the inclusion of sugars that are known to enhance biochrome production [\[8](#page-13-0)] was attempted. Surprisingly, in this study, no biochrome synthesis was observed when minimal medium was supplemented with sugars, organic nitrogen sources, inorganic salts, and amino acid. This is similar to the finding of Quadri and Williams [[16\]](#page-14-0) who showed that inducers like methonine and proline alone did not allow biosynthesis of prodigiosin, the red pigment produced by S. *marcescens* strain Nima, and other amino acids like proline in the presence of alanine were required to induce pigment synthesis [\[11\]](#page-14-0). In the present study also, supplementation with a single amino acid does not induce pigmentation.

It has long been known that prodigiosin production is enhanced by phosphate limitation. Under conditions of low phosphate availability, pigmented strains grow to a higher density compared to non-pigmented strains [[12](#page-14-0)]. However, in this study, supplementation of minimal medium with as less as 0.01% of potassium dihydrogen phosphate and dipotassium hydrogen phosphate did not lead to any pigment exaltation.

Fatty acids are reported to enhance pigment production [[13](#page-14-0)]. In our study also, little biochrome synthesis was observed when the medium was supplemented with oils like paraffin, sunflower oil, coconut oil, and groundnut oil, although glycerol showed maximum biochrome exaltation (Fig. 2). Therefore, further studies were carried out to determine the optimum concentration of glycerol for biochrome exaltation. Maximum biochrome exaltation was observed when 15 mL of nutrient agar medium was overlaid with 0.3 mL of glycerol prior to inoculation of culture. Addition of more than 0.3 mL of glycerol led to a significant decrease in biochrome synthesis as this led to flooding on the plate and the isolate showed a complete block in biochrome production in a hydrophilic environment. In addition, although large-scale solid-state fermentation in a nutrient agar plate overlaid with 0.3 mL of glycerol



# Comparison of percentage yield of pigment from culture grown in different Nutrient Conditions

Fig. 2 Comparison of percentage yield of biochrome of S. sakuensis grown in different nutrient conditions

<span id="page-7-0"></span>

Fig. 3 Solid substrate fermentation kinetics for biochrome production by S. sakuensis

allowed biochrome production, it was not comparable to the yield obtained through alginate immobilization as a reduction of 16.8% was obtained in the yield (7.77%).

Solid Substrate Fermentation for Biochrome Production

Growth kinetic studies for this isolate indicated that production was simultaneous with growth, which was initiated within a span of 48–72 h. Minimal amount of biochrome production was observed during the logarithmic phase of growth initiated after an initial lag period of 6 h, with the maximum production occurring during the early stationary phase. The cell mass yield began to level off after 84 h, although maximum biosynthesis of biochrome occurred between 42 and 52 h after multiplication; growth had almost ceased after multiplication (Fig. 3).

Pigment being a secondary metabolite is characteristically produced by senescent cells in the stationary phase of growth. During the time of pigment production, there was no increase in viable count, establishing the fact that the cultures were nonproliferating. Bu'lock et al. called these two phases the trophophase (nourishment phase) for the period of



Fig. 4 UV-visible spectra of the biochrome produced by S. sakuensis

<span id="page-8-0"></span>105



Fig. 5 FTIR spectra of the purified biochrome produced by S. sakuensis

cellular growth and the idiophase (individual phase) for the period when secondary metabolites peculiar to individual bacteria are produced [[14](#page-14-0)].

Moreover, there is a slight reduction in the biochrome yield after 72 h of growth. This may be because the biochrome, being intracellular in nature, leaks into the medium with initiation of cell death. Another unique feature of the isolate was the development of speckled colony morphology wherein the colonies of young cells were red in color. As the culture aged, the appearance of specked colonies also increased. This occurred till the culture aged to about 7 days. After this period, the number of speckled colonies also went down and these replaced by white colonies. In the viable count done on 10-day-old culture, almost all colonies obtained were white.



Fig. 6 MS of the HPLC purified biochrome produced by S. sakuensis

<span id="page-9-0"></span>

Fig. 7 a <sup>1</sup>H NMR of *S. sakuensis* biochrome. b <sup>13</sup>C NMR of *S. sakuensis* biochrome

<span id="page-10-0"></span>

Fig. 8 Elemental analysis of S. sakuensis biochrome

#### Partial Characterization of Biochrome

Based on the color of the colony, solubility in aqueous and organic solvents, absorption maxima, and reaction with concentrated  $H_2SO_4$ , 10% NaOH, 10% FeCl<sub>3</sub>, and Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, the biochrome was classified to belong to the tripyrrol class of biochromes [\[4](#page-13-0)].



(From Lto R: pH 5, pH 4, pH 7 & pH 9.2)

Fig. 9 pH stability of biochrome produced by S. sakuensis. From left to right: (pH 5, pH 4, pH 7 and pH 9.2)

<span id="page-11-0"></span>

The UV–visible spectrum of the extracted red biochrome showed a sharp peak at 535 nm, with a spur at 565 nm (Fig. [4\)](#page-7-0).

The FTIR spectrum of the red biochrome (Fig. [5\)](#page-8-0) was dominated by a broad peak at 3,292 cm<sup>-1</sup>, indicating the presence of a primary/secondary compound. Very strong bands at 2,923 cm<sup>-1</sup> indicated the presence of many methyl, methylene, and methine groups in the molecule, while the band at  $1,728$  cm<sup>-1</sup> indicated the presence of carbonyl group, probably ester carbonyl group in the molecule. The fingerprint region for the red biochrome was characterized by bands of medium intensity at 1,456 and 1,728 cm<sup>-1</sup> and a band at 1,150 cm<sup>-1</sup> indicates the probable presence of a C–O group. However, no bands were observed in the region of 3,400–3,600 cm<sup>-1</sup>, indicating the probable absence of −NH and −NH<sub>2</sub> groups. The literature available about prodigiosin indicates the presence of −NH group in the pyrrol ring [[15\]](#page-14-0), with a mass of 324 Da [\[7\]](#page-13-0). In our study, ESI-MS analysis showed that the compound had a mass of 514 Da (Fig. [6\)](#page-8-0), indicating that this is a unique molecule showing the characteristic of prodiogiosin but with an unusual structure.

An indication of the structure of the pigment was attempted through the use of NMR spectroscopy:

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δppm: 0.83 (s, 3H, terminal methyl), 0.85 (s, 3H, terminal methyl), 0.9–2.4 (complex unresolved multiplet due to number of methylenes and methines), 3.51 (s, 3H,  $-\text{OCH}_3$ ), 3.6–4.8 (protons attached to electronegative groups such as oxygen, nitrogen, etc. and therefore shifted downfield), 5.1 (d, 2H, olefinic protons), 7.0 (broad hump, 2H, 2×−NH or −OH or 1 −OH+1>NH), 7.792 (d,

1H, =CH), 12.0 (broad hump, 2H, 2×−NH or −OH or 1 −OH+1>NH) (Fig. [7a](#page-9-0)).<br><sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>) δppm: 15.44 (terminal methyl), 22.90–32.80 (number of methylenes and methines), 55.84 (−OCH3), 62.0–71.90 (oxygen/nitrogen bonded carbons); 169.34 ( $\geq$ C=O or ArC–O), 170.58 ( $\geq$ C=O or ArC–O). The <sup>13</sup>C NMR thus indicated a total presence of 14 carbons (Fig. [7b\)](#page-9-0).

High-resolution mass spectroscopy: 537.31 (M+Na), 515.3 (M+H). Thus, the mass calculated would be 514 Da. The compound may be dimer or having cyclic ring



in terms of  $K/S$  and  $L^*$ ,  $a^*$ , and  $b^*$  of microbial dyed fabrics

Microbial dyed fabrics	Fastness properties					
	Light	Wash	Rubbing			
			Dry	Wet		
Silk		$1 - 2$	$4 - 5$	$4 - 5$		
Cotton		$1 - 2$	$4 - 5$	$4 - 5$		
Wool		$1 - 2$	3	3		

<span id="page-12-0"></span>Table 4 Colour fastness properties of microbial dyed fabrics

structure with an empirical formula of  $C_{24}H_{32}N_8O_4$  based on its CHN analysis (Fig. [8\)](#page-10-0) and NMR spectra.

A comparison of all the obtained spectroscopic data (UV–visible, FTIR, and MS) with the available literature values shows that that the compound has different spectra from those indicated in the literature. Thus, to the best of our knowledge, this may be a novel natural compound.

### Color of the Biochrome: pH and Temperature Stability

A change in the spectrum was observed in the visible range of the biochrome with variation in the values of pH. The biochrome showed a change in color from pink to yellow in alkaline conditions and reverted to pink as the pH decreased. The  $\lambda_{\text{max}}$  of the biochrome under neutral and alkaline conditions was close, i.e., 490 and 470 nm, respectively, while the  $\lambda_{\text{max}}$  of the biochrome under acidic conditions shifted to 530 nm (Fig. [9\)](#page-10-0). Similar results have been reported for prodigiosin wherein the absorption maximum for the pigment under alkaline conditions was 470 nm [[15](#page-14-0)] and the characteristic absorption maxima in acid and alkaline solutions were of 535 and 470 nm, respectively [\[16,](#page-14-0) [17](#page-14-0)]. Although the biochrome was not very stable across different pH values, it was quite heat stable (Fig. [10\)](#page-11-0); thus, it would be able to withstand the rigors of dyeing.

### Dyeing Performance

Many chemically prepared colors are considered unfavorable because of their potential toxicity and recalcitrant nature [[1](#page-13-0)]. Contrary to this, natural dyes possess antimicrobial effects and thus add value to the dyed material for controlling foul odor. Therefore, an attempt was made to dye silk, cotton, and wool with microbial pigment. The microbial pigment was found capable of dyeing all three natural fabrics, and uniform dyeing was obtained for all three fabrics. Wool and silk dyed easily and the shades obtained were deeper compared to cotton.

Culture	Control	Silk	Wool	Cotton
Staphylococcus aureus NCTC 3578		18.18	56.67	44.78
Escherichia coli ATCC 10148		56.67	48.31	59.32

Table 5 Antimicrobial property of the dyed fabric (in% reduction of growth)

<span id="page-13-0"></span>The fabrics were dyed reddish pink by the microbial biochrome. The color strength values in terms of  $K/S$  and  $L^*, a^*$ , and  $b^*$  of the dyed samples are given in Table [3](#page-11-0). The highest dye uptake was noted in wool, followed by silk and then cotton. Color fastness of all dyed samples is reported in Table [4.](#page-12-0) Although the fastness of the color with respect to exposure to light and washing was low (grades 1 for light and  $1-2$  for washing), the fastness against rubbing was grade 4–5 for silk and cotton and grade 3 for wool.

### Antimicrobial Activity of the Dyed Fabric

The results of the study of the antimicrobial activity of the fabrics using the AATCC 100 method toward S. *aureus* NCTC 3578 and E. coli ATCC 10148 are given in Table [5.](#page-12-0) The study revealed the cidal effect of this dye against these bacteria. The results showed that the dyed wool and cotton fabrics had the ability to kill about 50% of the cells of S. *aureus* and E. coli within 24 h of contact time; the activity of the dyed silk samples was lower against S. aureus. These data prove that the antibacterial activity of the colorant could be transferred to the fabrics. The lower efficacy on the dyed silk and cotton was because of the lower concentration of the colorant on these fabrics caused by less dye uptake.

### Conclusion

Though the pigment produced by S. marcescens is well known, the newly characterized strain *S. sakuensis*'s ability to produce pigment within the class of prodiginies is ill defined. We report the production of a prodigiosin-like pigment by this isolate that, though showing properties similar to prodigiosin with respect to the presence of functional groups and pH stability, has a mass very different from that reported so far to the best of our knowledge. Further characterization to elucidate its structure needs to be undertaken. The variance may confer on it properties that can make it a promising candidate for textile dyeing.

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