Journal of Marine Biosciences Vol. 1 (1), 11–19, 2015
Print ISSN 2454–3519 Online ISSN 2454–3527

JMBS

Antibacterial Activity of Textile Fabrics Treated with Red Pigments from Marine Bacteria

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Abstract

The present study was carried out to investigate the antimicrobial properties of different textile materials using the bacterial red pigment as natural dye. The antimicrobial activity of pigment after application on textile materials was tested against pathogens like *Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Salmonella typhi*. The pigment proved to be very effective in inhibiting microbial growth after application on textile material. Present study strongly suggests that the pigment produced has the dyeing and antibacterial property and could be used in different textile industries. Further, the wash performance studies with the textile materials treated with pigment and revealed that thiourea, is a safe and effective mordant, and can be used in wound dressing cloth.

Keywords: Antibacterial activity, Bacterial pigment, textile materials

INTRODUCTION

Natural pigments have been extensively used in painting, cloth, cosmetics, food, pharmaceuticals and plastic products. In the cosmetics, food and pharmaceutical industries, due to the serious environment and safety problems caused by many artificial synthetic pigments, research has focused on processes for the production of safe and natural pigments from natural resources (Cho et al., 2002). Production and biosynthesis of colorants for textile dye applications has attracted increased interests in past few years. Nature produces different types of biocolorants from various resources like plants, animals, and microorganisms, which are possible alternatives to synthetic dyes and pigments currently employed (Mapari *et al.*, 2005). The term natural dye is usually associated with colours obtained from plant or animal sources. The skin infections are spread by one person-to-other person and also by surface contact with, clothes, hands, and hospital devices like bedclothes (Ayliff and Lowbury, 1982). These colorants are exclusively made from non renewable resources such as fossil oil. The production of the synthetic colorants is economically efficient and technically advanced with colors covering the whole color spectrum. However, synthetic colorants are on non-renewable and environmental toxicity, and human health concerns of some synthetic dyes. So searching renewable and environmentally friendly resources for

production of colorants is an urgent need. Antibacterial fabrics are important not only in medical applications but also in terms of daily life usage. The application of antimicrobial finishes to textiles can prevent bacterial growth on textiles (Jakimiak et al., 2006). Usually, antimicrobial properties can be acquired to textile materials by chemically or physically incorporating functional agents onto fibers or fabrics (Gouda, 2006; Jantas and Górna, 2006). To protect the mankind and to avoid cross contamination, a special finish like antimicrobial finish has become necessary. As consumers have become more aware of hygiene and potentially harmful effects of microbes, the demand for antimicrobial finished clothing is increasing. Textiles material are considered to be very capable of carrying bacteria for the transmission of skin infection **.** Microbes – bacteria, fungi and yeast – are present almost everywhere. Whereas human beings have an immune system to protect against accumulation of micro-organisms, materials such as textiles can easily be colonized by high numbers of microbes. Textiles are carriers of micro-organisms such as pathogenic bacteria, odor-generating bacteria, mould. These micro-organisms adhere to the textile and may cause (in good growth conditions of temperature and humidity) offensive odors, discoloration, cross-infection or transmission of diseases (i.e. hospital textile). These factors are the driving force behind the development of antimicrobial treatments.Therefore, antimicrobial textiles may be of great help in the recovery process of transplant patients, people with immunodeficiency diseases, low immunity patients (Tinker, 2010).These textile materials may also be useful for individuals coming in to contact with patients, such as visitors, nurses, doctors and other healthcare workers.

The present study was aimed to determine the antibacterial activity of textile fabrics applied with red pigment against *Staphylococcus aureus,* and *Escherichia coli*. Since it is important to maintain the antibacterial activity of fabrics in conditions of use, the antibacterial activity of unwashed fabrics was also compared alongside washed fabrics.

MATERIALS AND METHODS

Isolation of marine bacteria

Seawater samples were collected in the intertidal zone at coastal locations of Nellore Krishnapatnam. The samples were spread on the entire surface of Marine Agar (g/L) consisting of Peptone - 5.0, Yeast Extract - 1.0, Ferric Citrate - 0.1, Sodium Chloride - 19.45, Magnesium Chloride - 8.8, Sodium Sulfate - 3.24, Calcium Chloride - 1.8, Potassium Chloride - 0.55, Sodium Bicarbonate - 0.16, Potassium Bromide - 0.08, Strontium Chloride – 0.034, Boric Acid – 0.022, Sodium Silicate – 0.004, Sodium Fluoride – 0.0024, Ammonium Nitrate – 0.0016, Disodium Phosphate – 0.008, Agar. 15.0). After incubation at 25 °C for 2 days, all colonies with different pigmentation and morphology were selected for bacterial isolation.

Identification of bacteria

To study the biochemical characterization of the isolated strain, different tests such as temperature tolerance, pH tolerance and NaCl concentration (minimum, optimum and maximum) were performed. Specific tests like Gram staining, spore staining,

motility, indole production, MR-VP test, gelatin hydrolysis, citrate utilization, triple sugar iron agar, oxidase test, catalase production, nitrate reduction, urease test and starch hydrolysis were performed according classical methodology. Fermentation of different sugars as sole carbon sources such as arabinose, sucrose, glucose, fructose, rhamnose, xylose, raffinose and mannitol etc was evaluated for isolated strains. To identify species level, the molecular identification of 16S rRNA by PCR amplification of the 16S rRNA gene, BLAST analysis, and comparison with sequences in the GenBank nucleotide database was performed at the Microbial Type Culture Collection Centre, IMTECH, Chandigarh, India.

Fermentation process

A loop full of culture was inoculated to the pre-sterilized medium 50 ml (Zobell marine broth, g/L). The flask was kept incubation for 16 – 18 h or till the absorbance of culture reached to 1.0 optical density at 600 nm at 28^{o}C at stationary conditions. After the incubation time is completed 1 ml of the inoculum is transferred to the production medium 100 ml Zobell marine broth in 250 ml Erlenmeyer flask. The flask was incubated at 28^{o}C for 72 h.

Extraction and measurement of pigment production

Extraction of pigment was done according to (Slater et al. 2003) with following modification. The 10 ml culture broth was centrifuged (10,000 rpm, 10 min) and methanol was added to colored pellet and incubated at 60^{o}C for 20 min. The reactants were centrifuged at 10,000 rpm for 10 min and the colored supernatant was analyzed by scanning in UV –Visible spectrophotometer for detecting the absorption maximum (λ max) by scanning in the range of 400-600 nm. Pigment produced by bacterium was purified by few steps, the microbial pigmented compound from the crude methanol extracts were filtered (whatman filter paper) to remove residual biomass and then concentrated by using rotary evaporator. A chloroform/water liquid – liquid extraction was used to remove hydrophilic impurities. The organic phase, containing the biopigment, was concentrated. In the next step the dried extract was dissolved in chloroform and then applied to silica gel column (18 cm height and 20 mm width) previously packed with silica gel (column chromatography grade) and pre-washed twice with n-hexane. The separation initiated with the addition of ethylacetate as eluting medium. Different fractions consisting of different colors ranging from light yellow to bright red color and the red colored fraction was collected from the column and subjected to TLC.The product was identified as prodigiosin by mass spectrometry using methanol.

Dying of textile materials:

The application of dye was evaluated for different grades of textile materials available in the market and cut in to piece of equal one cm2 of each fabric of Bakrum**,** Silk**,** Local olyeste**,** Polyester, Long Cloth**,** Cotton, Satin, Micro, and 2/1 were soaked in 2 mL methanolic extract of pigment taken in different test tubes and incubated for 1h at room temperature and the thiourea is used as mordant in another set of experiment. For all the experiments

white cloth material were taken as a control.

Washing performance

The dyed textile materials were dipped in detergent solution for 20 min at room temperature and the materials were washed with tap water and allowed to dry for 30 min .The absorbance of the detergent solution were taken at 535nm in a UV-visible spectrophotometer. The same experiment was repeated for dyed textile materials treated with thiourea as a mordant.

Antibacterial Activity of Dyed Fabrics.

Pigment-dyed textile material were analyzed for their antimicrobial activity .The textile fabric sample was cut and placed within a sterile Petri dish, and 1.0 mL of *Escherichia coli* (K-12) or *Staphlococcus aureus* was added onto the surfaces. Fabrics were then incubated at 37 °C for 16 h, placed into 10 mL of sterile water, and shaken vigorously for 5 min. The solution was serially diluted to 10^2, 10^3, and 10^4 concentrations, and 100 *í*L of each diluted sample was placed onto agar plates. The plates were incubated at 37 °C for 24 h. The numbers of CFU were enumerated between dyed and undyed fabrics and were compared to determine antibacterial activity of the textile fabric samples:

$$\text{reduction of bacteria (\%)} = \frac{A - B}{A} \times 100$$

where *A* and *B* are the number of bacteria counted from the undyed and the dyed fabrics, respectively.

RESULT AND DISCUSSION

Screening and isolation

With advent of new technologies, the growing needs of the consumer in the wake of health and hygiene can be fulfilled without compromising the issues related to safety, human health and environment. Taping new potential antimicrobial substances, such as, red pigment from bacteria can considerably minimize the undesirable activities of the antimicrobial products Many natural pigments are reported to be most suitable finishing agents for medical wears with barriers against micro organisms. To carve a niche for textile materials, this kind of value adding finishes are the need of the hour.

The marine samples collected from Nellore marine area were used to isolate pigment producing microbial strains using zobell marine agar plates. After incubation at 30^{o}C several colonies were developed, however, bacterial colonies which showed pigmentation were picked and purified in subsequent experimentation.Such different colored pigment producing microbial strains all the isolated strains were screened further for their ability to produce antibacterial activity (antagonistic) by and agar well diffusion method using test cultures such as *E. coli* and *B. subtilis.* A total of 70 different bacterial strains were isolated, purified and preserved. Among isolated strains, the strain which produces red colored pigment with good antibacterial activity against both gram positive and gram negative bacteria was selected for further studies and designated as SKMASRSP9. (Fig 1)

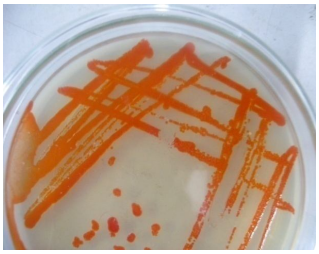

Figure 1: Pure culture of the red pigment producing marine bacterium *Vibrio* sp. SKMASRSP9.

Identification of characterization of isolated strain

Microscopic analysis of the selected isolate revealed that it is spore forming and produces red pigment when grown on Zobell marine agar medium. The gram test denoted that it is strain belongs to gram negative bacteria. The isolate is rod shaped which is slightly curved when observed under microscope. To confirm further, characterization of the isolated strain was done by biochemical studies Table1 and 16S rRNA sequencing studies. The different biochemical properties as per Bergey's Manual of Determinative Bacteriology (Holt et al., 1994) of the isolated strain SKMASRSP9 and the presented identification key of various morphological and biochemical characteristics further suggest that this strain could be identified *Vibrio* sp. To confirm the identity of the isolated marine strain, 16sRNA sequencing analysis was performed based on maximum identity revealed that strain is closely related to *Vibrio* sp.and the sequence of this strain was deposited in NCBI under the accession No. HE798514.

Extraction of pigment:

In view of the pigment hydrophobic nature, different solvents such as ethanol, methanol, and Distiled water, chloroform, ethyl acetate, petroleum ether and acetone have been considered to find the suitable solvent for effective extraction from isolated marine *Vibrio* sp. biomass. Identically grown *Vibrio* sp. biomass was used and extracted pigment concentration was estimated by measuring the absorbance at 536 nm. The pigment extract had the same *Rf* value (0.9) as a prodigiosin reported by Nakashima *et al*. The pigment was analyzed by UV spectrophotometry and mass spectrometry. With results for prodigiosin purified from *Serratia* sp KH – 95 by Song et al (2006) . Mass spectrometry revealed a molecular weight of 324 Da for the purified pigment (Figure 2) for prodigiosin

Table 1. The biochemical characteristics of isolated marine bacteriaum *Vibrio* sp. SKMASRSP9.

Biochemical test	Result
Colour	Red
Cell Shape	Comma
Cell size	Small
Gram staining	- ve
Carbohydrate utilization test	+ve
H_2S production test (TSI)	+ve
Urease test	+ve
Phenyl di-aminase test	-ve
Indole test	-ve
MR test	-ve
VP test	-ve
Citrate utilization test	+ve
Nitrate reduction test	+ve
Starch hydrolysis	+ve
Phenol red fermentation test	-ve
Gelatin hydrolysis	-ve
Catalase test	+ve
Lipase test	+ve
Casein hydrolysis (protease test)	+ve
Chitinase activity	-ve

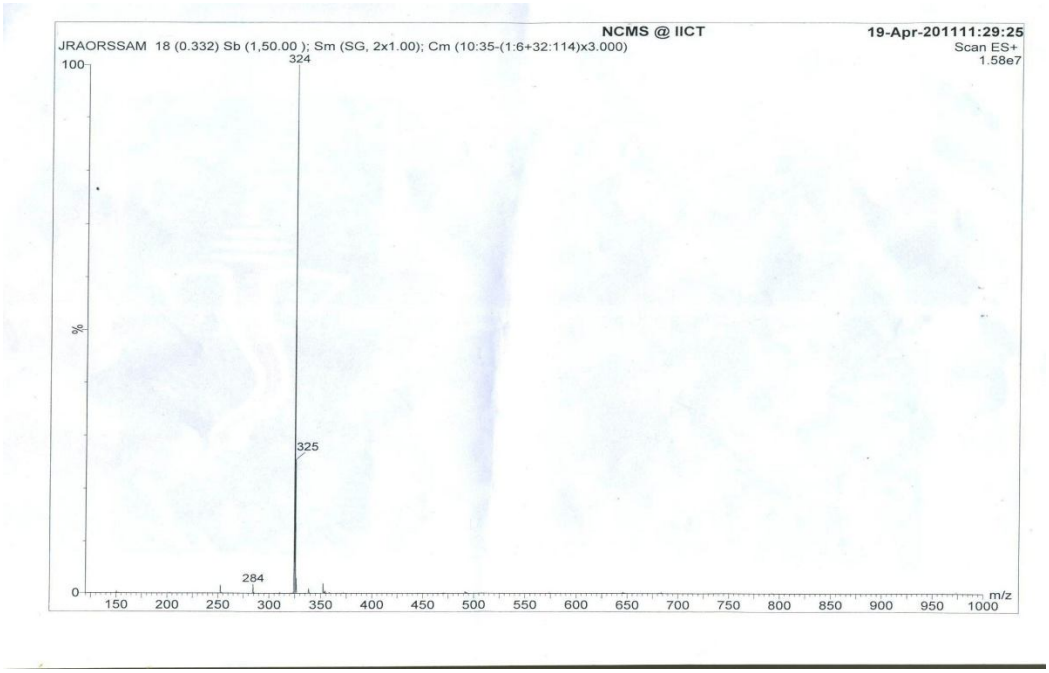

Figure 2 : Mass spectrum of purified red pigment extracted from *Vibrio* sp. SKMASRSP9.

Figure 3: Textile fabric materials dyed with pigment, the concentration of the pigment used 200 µ and 100 µ were applied in warm surface to the cloth material and white cloth as a control and allowed to dry at room temperature for 1h. 1. Bakrum, 2.Silk, 3.Local Polyester, 4.Polyester, 5.Long Cloth, 6.Cotton, 7.Satin, 8.Micro, 9.2/1.

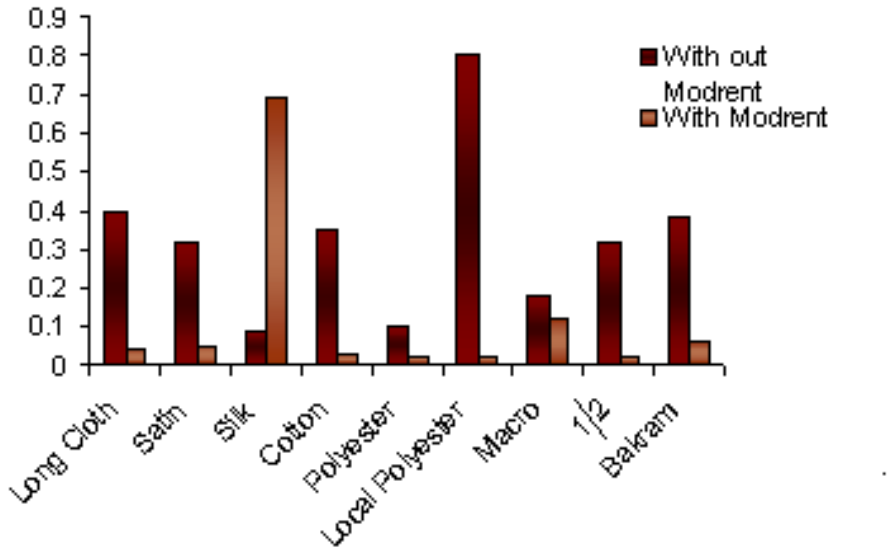

Figure 4 : The washing performance of different textile materials

Dying and wash performances

The microbial red pigment showed effective dyeness towards all fabrics in different extent (Figure 3). The fabric textile materials treated with pigment showed that the pigment lost from the cloth after washing with warm water solution at room temperature. (Figure 4). The loss of pigment from the same textile materials treated with mordant was also found to be less. The results suggest clearly shown that thiourea is an effective mordant for treating the dyed textile materials and it can withstand wash conditions.

Antibacterial activity

To evaluate the antibacterial activities of the dyed fabric, a bactericidal activity test was carried out. The Bakrum**,**Silk**,**Local Polyeste**,**Polyester,Long Cloth**,**Cotton, Satin,Micro, 2/1 samples dyed with the colorant extract were used as model samples. The results showed that the dyed silk fabrics material had ability to kill about 50% of the *S. aureus* and *E. coli* bacteria within 16 h of contact time; the activity of the dyed fabrics antibacterial function was shown in Table 2. The data on the antibacterial activities of the colorants could be obtained on the fabrics. The dyed fabrics showed antimicrobial properties against *E. coli* and *S. aureus* bacteria within a contact time of 16h

Table 2. Antimicrobial Activity of Textile Materials stained with red pigments extracted from *Vibrio* sp. SKMASRSP9.

Cloths Types	**Zone of Inhibition (mm)**	
	E.coli	***S .aureus***
Bakrum	01	0
Silk	48	52
Local Polyesters	09	0
Polyester	10	0
Long Cloth	15	20
Cotton	19	13
Satin	08	05
Micro	16	03

Conclusion

The textile fabrics treated with the red pigment of the isolated bacteria is a good alternative for the wound dressing muslin cloth. It not only fixes well to the wound but also provides antibacterial principle for the efficient treatment of the wounds.

Acknowledgements

The authors thank the Authortities of Kakatiya University and Indian Institute of Chemical Technology for facilities and support.

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