# Antibacterial Colorants: Characterization of Prodiginines and Their Applications on Textile Materials

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A strain of *Vibrio* sp. isolated from marine sediments produced large quantities of bright red pigments that could be used to dye many fibers including wool, nylon, acrylics, and silk. Characterization of the pigments by electrospray ionization mass spectrometry (ESI-MS) and nuclear magnetic resonance (NMR) revealed three prodiginine-like structures with nonpolar characteristics and low molecular mass. UV−visible spectra of the major constituent in methanol solution showed absorbance at $\lambda_{max}$ 530 nm wavelength. The accurate mass result showed that the main isolated product has a molecular mass of *m*/*z* 323.1997. Further analysis using mass fragmentation (MS/MS), $^1$H NMR, COSY, HMQC NMR and DEPT confirmed the detailed structure of the pigment with an elementary composition of $C_{20}H_{25}N_3O$. Fabrics dyed with the microbial prodiginines demonstrated antibacterial activity.

## 1. Introduction

Biosynthesis of colorants for food and textile applications has attracted increased interests in recent years. Nature produces many biocolorants from various resources including plants, animals, and microorganisms, which are possible alternatives to synthetic dyes and pigments currently employed (*1*). The currently used colorants are almost 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 facing the following challenges: dependence on non-renewable oil resources and sustainability of current operation, environmental toxicity, and human health concerns of some synthetic dyes. Thus, searching renewable and environmentally friendly resources for production of colorants is an urgent need. Plants could produce and had been employed in production of natural colorants before synthetic dyes were invented, but in very low yields and low eco-efficiency (*2*). In fact, using plants in producing colorants is not environmentally friendly and sustainable due to the large amount of biomasses produced. Practically, fermentation of microorganisms such as fungi and bacteria could be a valuable source of manufacturing colorants. Microorganisms produce a large variety of stable pugments such as carotenoids, flavonoids, quinones, and rubramines, and the fermentation has higher yields in pigments and lower residues compared to the use of plants and animals (*3*). Thus, biosynthesis of dyes and pigments via fermentation processes has attracted more attention in recent years (*3, 4*). Technically speaking, biosynthesized pigments can serve as major chromophores for further chemical modifications, which could lead to colorants with a broad spectrum of colors (*4*). Besides, some natural colorants, especially anthraquinone type compounds, have shown remarkable antibacterial activity in addition to providing bright colors (*5*), which could serve as functional dyes in producing colored antimicrobial textiles.

As an effort to investigate natural pigments produced by fermentation processes, this paper will discuss the characterization of pigments obtained from a bacterial strain isolated from marine sediments using mass spectrometry and nuclear magnetic resonance (NMR) and explore their applications in dyeing different fibers.

## 2. Materials and Methods

**2.1. Media and Growth Conditions.** Seawater-rich media (SRM; 0.25 g yeast extract and 0.25 g tryptone per liter filtered seawater) was used in initial isolation and characterization of bacteria from marine sediments. Seawater-base rich media (SBRM) containing 15 g tryptone and 5 g yeast extract per liter of seawater base (SB; 20 g NaCl, 3 g $MgSO_4 \cdot 7H_2O$, 0.15 g $CaCl_2 \cdot 2H_2O$ per liter distilled water) was used for all subsequent culturing at 30 °C unless otherwise stated. For agar plates, SRM and SBRM were solidified with 1.8% (w/v) Bacto agar (DifcoTM, Sparks, MD).

**2.2. Strain Isolation and Identification.** One gram of marine sediment from the shore of Eel Pond in Woods Hole, MA was added to 10 mL of SB and vortexed vigorously for 10 min, and the suspension was serially diluted from $10^{-1}$ to $10^{-7}$. Then, 50 $\mu$L of each dilution was plated onto SRM agar plates and incubated at 25 °C. The 16S rDNA gene from candidate colonies were amplified by colony PCR using universal primers 8F and 1492R (*6*), a Promega PCR kit, and run on an Eppendorf Mastercycler PCR machine, under the following regime: initial 5 min at 95 °C, followed by 30 cycles at 95 °C for 30 s, 50 °C for 30 s, 72 °C for 2 min, a final extension at 72 °C, and then 4 °C infinity. PCR fidelity was verified by the presence of a single ~1.5 kb band on 1.3% agarose TBE gels by electrophoresis (*7*). Partial sequence of the 16S rDNA gene was obtained by sequencing the above PCR amplified product at the Josephine Bay Paul Center in Comparative Molecular Biology and Evolution at the Marine Biological Laboratory,

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Woods Hole, MA, and deposited under EU583479 and EU583480 in GenBank.

**2.3. Production of Microbial Prodiginines.** Batch fermentations were run to obtain large quantities of prodiginine-producing microbial biomass. Starter cultures were grown by inoculating single colonies grown on SBRM agar plates into 250 or 500 mL Fernbach flasks containing 50 or 100 mL of SBRM liquid media, respectfully, and grown overnight in a shaking incubator set at 30 °C and 200 RPM. The next day, 2 L Erlenmeyer flasks, 2.8 L Fernbach flasks, and a 10 L New Brunswick BioFlow 3000 bioreactor containing 0.8, 1, and 8 L of SBRM, were inoculated with 20, 25, and 800 mL of the overnight cultures to initiate fermentations, respectively. Erlenmeyer and Fernbach shake-flask cultures were grown at 30 °C and 225 rpm. The bioreactor culture was grown with an agitation of 500 rpm, 2 L/min rate of aeration at 30 °C, and a few drops of Antifoam A (Sigma-Aldrich, Milwaukee WI). After 24 h, fermentation cultures were harvested, and cells were collected by centrifugation for 10 min at 10,000 rpm. Biomass from 16 L of culture was transferred into a 2 L Erlenmeyer flask and extracted with 1 L of methanol in the dark at 20 °C on a rotary shaker at 150 rpm. After 12 h, the mixture was centrifuged for 10 min at 10,000 rpm to recover the solvent phase, and the biomass was extracted twice again with 1 L of methanol. These crude methanol extracts were stored in amber glass bottles at 4 °C until further purification or use in dye tests of different textiles.

**2.4. Purification of Prodiginines.** A three-step purification scheme was applied to separate the microbial prodiginines from the crude methanol extracts. In the first step, the crude methanol extracts were filtered (Whatman, GF/A, 15 cm, England) to remove any residual biomass and then concentrated by using a rotary evaporator (Buchi Rotavapor, R-114, Germany). A chloroform/water liquid−liquid extraction was carried out to remove hydrophilic impurities. The organic phase, containing the prodiginines, was concentrated by using the rotary evaporator. In the second step, the dried extract was dissolved in chloroform and then applied to a silica gel column (20 cm × 20 cm; silica gel 60; Merck, EMD, Darmstadt, Germany). Compounds were separated using a chloroform/ethyl acetate (95:5) solvent system, and fractions containing visible pigments were collected, concentrated, and redissolved in methanol.

The final step of purification was achieved using an Agilent 1100 series HPLC equipped with photodiode array detector (Agilent Technologies, Palo Alto, CA). A Phenomenex Luna C-18(2) semipreparative column (250 mm × 10 mm, 5 $\mu$m) was used (Phenomenex, Torrance, CA) for separation of components. The separation was achieved by using water (A) and acetonitrile/methanol (1:1) (B) mobile phases, and a gradient elution program at 3 mL/min with the following parameters: 0−25 min 15−100% B (linear gradient), 25−35 min 100% B (isocratic), and 35−40 min 15% B (isocratic) to re-equilibrate the column. Fractions containing targeted compounds were combined and concentrated by solvent evaporation.

**2.5. Identification of Prodiginines.** Nuclear magnetic resonance (NMR), liquid chromatography mass spectrometry (LC−MS), and Fourier-transform mass spectrometry (FT−MS) structure elucidation methods were applied to characterize and identify the purified compounds.

***2.5.1. LC-MS Analysis.*** LC−MS/MS analysis was performed with reversed-phase (RP) chromatography by using a solvent system of acetonitrile/methanol 50/50 (LC−MS grade, J T. Baker, Phillipsburg, NJ) as solvent B, and 13 mM ammonium acetate buffer (pH 5.5, adjusted by acetic acid) as solvent A, at a flow rate of 0.3 mL/min at 40 °C. RP separations were accomplished by using an ACQUITY UPLC system (Waters, Milford, MA). An ACQUITY UPLC BEH shield C18 column (150 mm × 2.1 mm, 1.7 $\mu$m particle size, Waters) was used. After a 0.5-min isocratic run at 15% B, a gradient to 100% B was completed at 20 min. HPLC columns were connected to the electrospray interface of a Finnigan LTQ (Thermo Electron Co., Waltham, MA) linear ion trap mass spectrometer. Nitrogen sheath gas pressure was set to 7 bar at a flow rate of 2−3 L/min. Spray voltage was set to 5 kV. The temperature of the heated transfer capillary was maintained at 350 °C. Full scan mass spectra were acquired from 100 to 1200 Da and unit mass resolution in both positive and negative modes.

The accurate mass measurements were performed on a Finnigan LTQ FT, FT−MS instrument (Thermo Electron Co., Waltham, MA).

***2.5.2. NMR Spectrometry.*** All NMR spectra were measured using Avance DRX-500 MHz and Avance DRX-600 MHz spectrometers (Bruker BioSpin, Billerica, MA) running XWIN-NMR software, version 3.5. $^{13}$C and DEPT spectra were obtained on the DRX-500 with broadband decoupling of $^{1}$H and $^{1}$H NOE for the standard $^{13}$C observe spectra. COSY and HSQC spectra were obtained on the DRX-600 instrument using a 5 mm TXI cryoprobe. COSY spectra were obtained in magnitude mode, and HSQC spectra were phase-sensitive. All spectra were acquired at 20 or 23 °C and referenced through the residual solvent peak to TMS (tetramethylsilane) at 0.0 ppm in $^{1}$H and $^{13}$C.

**2.6. Dyeing of Textile Materials.** Fabric samples were purchased from TestFabrics Inc. (West Pittiston, PA). The crude pigment solution of methanol/water, 1/1, v/v with 1% solid mass was used as a dyeing bath. All dyeing experiments were carried out using fabric samples (1 g) that were wetted in hot water and sealed in 150 mL stainless steel dyeing containers. The containers were housed in an Ahiba-Polymat Laboratory scale dyeing machine (SDL ATLAS, Stockport, England). The dyeing solution to fabric (w/w) ratio (liquor ratio) was 50:1. Multifiber test cloths were dyed under both neutral and acidic pH conditions. Wool, silk, and nylon were dyed under acidic conditions in a methanol/water solution of the extract that was first adjusted to pH 3.5 by using 5% sulfuric acid solution. Acrylic fabrics were dyed in a methanol/water solution of the extract that was adjusted to pH 4.5 using 5% acetic acid solution. A standard dyeing procedure (60 min at 80 °C) was performed in the ATLAS dyeing machine. The dyed fabrics were rinsed in warm water and detergent solution, then rinsed in cold water, and dried at room temperature. Dye concentration in solution was measured by a spectrophotometer. The dye contents on dyed fabrics were evaluated by measurements of *K*/*S* (Kubelka−Munk) value in a Color-Eye 7000A Spectrophotometer (Gretag-Macbeth, New Windersor, NY). *K*/*S* was reported according to an equation $K/S = (1 - 2R)^2/2R$ where $R$ is the reflectance reading of dyed fabric, $K$ is the absorbance coefficient, and $S$ is the scatter coefficient. According to the Kubelka−Munk theory, *K*/*S* is directly proportional to the color strength (content) of a solid surface. Five measurements were carried out at five different positions for each dyed fabric.

**2.7. Antibacterial Activity of Prodiginine-Dyed Fabrics.** Prodiginine-dyed fabrics were analyzed for their antimicrobial activity following American Association of Textile Chemist and Colorists (AATCC) test method 100-1999 (*8*). One gram of fabric sample was cut and placed within a sterile Petri dish, and 1.0 mL of *Escherichia coli* (K-12) or *Staphlococcus aureus* (ATCC 12600) grown in 10 mL of nutrient broth (approximate formula per liter: beef extract, 3.0 g; peptone, 5.0 g; distilled

**Table 1. Cultured Bacterial Strains Most Closely Related to *Vibrio* sp. Strain KSJ45 by 16S rDNA Analysis**

| strain | isolation source | % identity with KSJ45 | accession no. | reference |
|---|---|---|---|---|
| *V. gazogenes* strain ATCC 29988T | saltwater marsh sediments, Woods Hole, MA | 98 | X74705 | 9−10 |
| *V. rhizoshpaerae* strain MSSRF3 | mangrove rhizosphere, Pichavaram, India | 97 | DQ847123 | 11 |
| *V. ruber* strain VR1 | coastal seawater, Keelung, Taiwan | 97 | AF462458 | 12 |
| *V. mangrovensis* strain MSSRF10 | mangrove rhizosphere, Pichavaram, India | 97 | DQ273663 | 11 |

water, 1000 mL; final pH 6.8 ± 0.2) at $0^5-10^6$ CFU/mL 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 $\mu$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 fabric samples:

$$\text{reduction of bacteria (\%)} = \frac{A - B}{A} \times 100 \qquad (1)$$

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

## 3. Results and Discussion

**3.1. Isolation of a Marine Prodiginine-Producing Bacterium.** During measurement of the bacterial titer in marine sediment collected from Eel Pond in Woods Hole, MA, a single crimson red colony lightly tinged in green was observed. Culturing of this strain resulted in colonies that were slightly raised, with smooth and entire edges and a light glossy sheen. Examination of the cells under phase contrast microscope revealed a population of highly motile bacteria slightly vibroid in shape. The morphological characteristics, intense red pigmentation, and marine origin suggested the strain may be related to *Vibrio gazogenes* (ATCC 29988T), a bacterium species that was first isolated by Harwood C.S., 1978 (as *Beneckea gazogenes*) and a known producer of a red, tripyrrole-type structure of prodigiosin. Analysis of the partial 16S rDNA gene PCR amplified from this Woods Hole isolate revealed 98% sequence identity to the *V. gazogenes* type-strain ATC29988T (NCBI accession x74705), and it was thus designated as *Vibrio* sp. strain KSJ45 (Table 1**).**

**3.2. Production and Purification of Prodiginines.** To identify the prodiginines produced by *Vibrio* sp. strain KSJ45 and characterize their potential functions as antimicrobial pigments, batch fermentations were run to obtain large quantities of cell materials. Cultures were routinely grown for 24 h in shake flasks and a New Brunswick BioFlow 3000 bioreactor with SBRM, producing intensely crimson-red cultures. The red prodiginine pigments were only associated with the cell material and were absent in clarified culture supernatants. A solvent screening test on methanol, ethanol, and acetone revealed that methanol was the best reagent in extracting the red pigments from the cell materials. The extract was dried under reduced pressure and concentrated. The pigment was separated by liquid−liquid separation by chloroform/water. The chloroform extract was concentrated and purified by TLC. The major band which had a dark pink color was removed by acetone and dried. The HPLC results showed that the extract contains three main components as C1, C2, and C3 (Figure 1a). UV−vis spectra of the pigment revealed that compound C2 had an absorbance maximum at 530 nm in methanol solution at pH 3, which was in agreement with published value for prodigiosin purified from *Serratia* sp. (Figure 1b) (*13*).

**3.3. Structural Identification of the Main Pigment (C2).** One of the main components with a retention time of 11 min (Figure 1a) was analyzed using ESI−MS/MS analytical technique. This component was characterized with a molecular mass of *m*/*z* 324 Th, $[M + H]^+$, which indicates an odd number of nitrogen atoms in the molecule (Figure 1b). The accurate mass measurement resulted a mass of 323.1991 Da (molecular mass) by FT−MS, which was adequate to a molecule with an elementary composition of $C_{20}H_{25}N_3O$ ($\Delta m = 0.7$ mDa). The accurate mass measurement experiment was followed by an MS/MS fragmentation study in product mode. The product spectra showed the following major fragments: an ion at *m*/*z* 252 Th, a product of losing an alkyl chain ($C_5H_{14}$), an ion at *m*/*z* 309 Th, a product of deduction of a methyl group, and another ion of *m*/*z* 293 Th, a product of losing a methoxyl group from the parent compound (Figure 1c). To support the structure of compound (C2), $^1$H NMR and $^{13}$C NMR analyses along with H-COSY and HMQC were carried out. The $^1$H and $^{13}$C NMR chemical shifts and signal assignments are shown in Table 2. The proton of methoxyl group has a shift of 4.02 ppm with signal intensity of 3 protons. Aromatic protons appear in the region of 6−7.2 ppm. From $^{13}$C COSY results the relation between protons on alkyl chain (C9−C13) and the position of neighboring protons in the first pyrrole ring (H1, H2, and H3) were proved. Signals in the very low magnetic field in $^1$H NMR spectrum (~12.5 ppm, 2 signals) showed the existence of N−H protons of the pyrrole rings (Table 2). To confirm the pattern of all the carbon atoms, $^{13}$C NMR, DEPT90, and DEPT135 spectra were generated (Table 2). On the basis of these spectra all the quaternary carbon, CH, $CH_2$ and $CH_3$ carbons were identified, and the $^{13}$C NMR data were found to be identical to literature values (*14*, *15*), which supported the chemical structure of the compound (C2).

**3.5. Minor Pigment Identifications.** Additionally, two lower intensity chromatographic peaks (C1, C3) with retention times of 10.5 and 11.9 min and maximum UV absorbances $\lambda_{max}$ at 535 and 530 nm, respectively, were detected (Figure 1a). Identification of the chemical structures of these compounds helps to understand their relationship to the C2 compound and to recognize any possible reactions among these compounds. Also, the chemical structures and specific functional groups of the colorants play an important role in their adsorption onto different textile materials, which is critical in the dyeing process. Mass spectrometric analysis showed parent ions of C1, *m*/*z* 322 Th $(M + H)^+$ and of C3, *m*/*z* 352 Th $(M + H)^+$ (Figure 2). The accurate mass measurement for the compound C3 was $M = 351.2303$ Da which adequately matches that of a molecule with the elementary composition of $C_{22}H_{29}N_3O$ ($\Delta m = 0.8$ mDa). This compound has a similar fragmentation pattern to compound C2 with a fragment ion at *m*/*z* 252 Th, which was related to the loss of an alkyl chain ($C_7H_{15}$), and an ion at *m*/*z* 337 Th for the loss of a methyl group. In addition to the mass spectrometric data, similar UV−vis absorbance characteristics were found. Basically, both components (C2 and C3) showed a strong signal at $\lambda_{max}$ of 535 nm, which can be related to an identical core structure but in different lengths of aliphatic side chains. We have to emphasize that compound C3 is more hydrophobic as a result of the presence of a longer aliphatic side chain, which is in good agreement with the higher retention

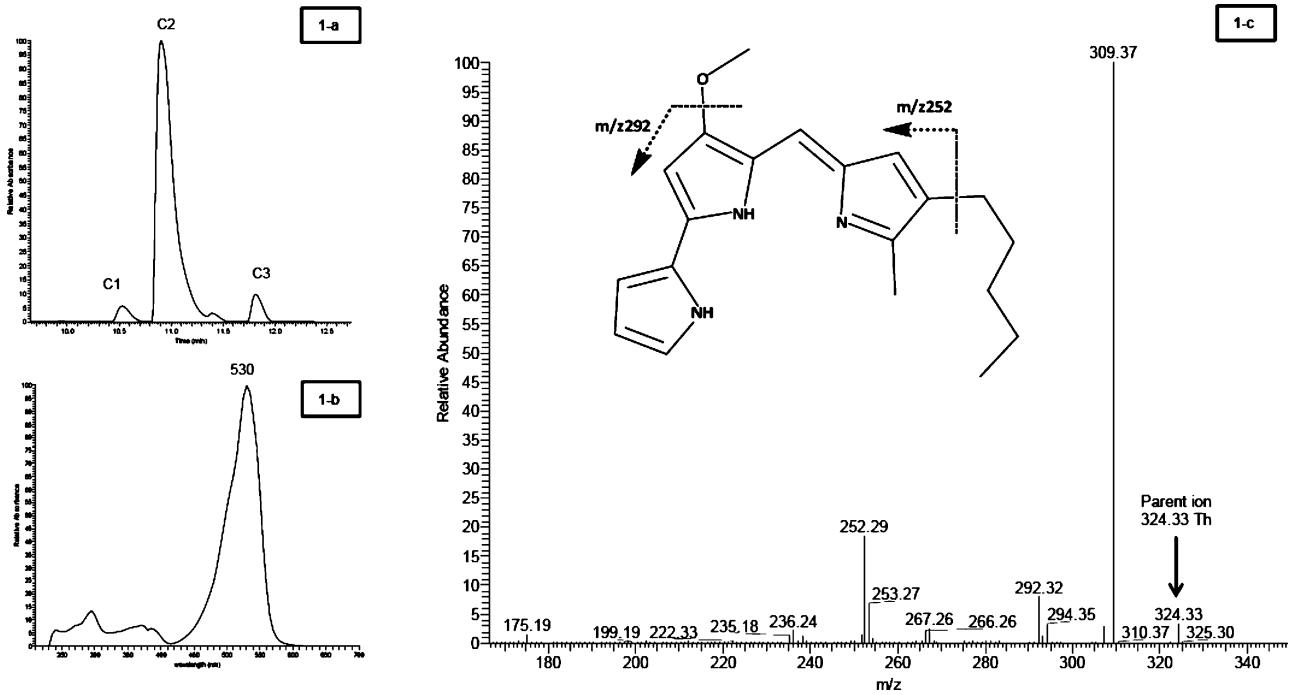
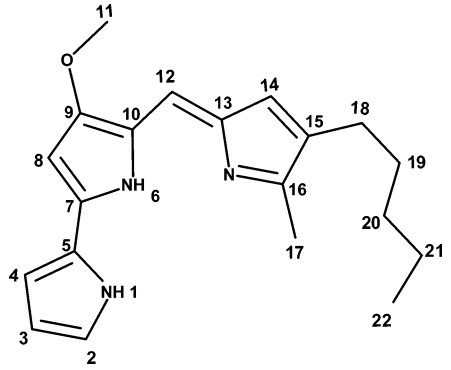



**Figure 1.** Structural analysis of the red pigments: (a) chromatogram of the red pigment extract, (b) UV−vis absorbance spectra of a methanol extract, (c) ESI-MS/MS spectra.

**Table 2. $^1$H and $^{13}$C NMR Chemical Shifts and Signal Assignments of Component C2**




| | $^1$H | | |
|---|---|---|---|
| assignation | $\sigma$ (ppm) | $J$ (Hz) | $\sigma$ $^{13}$C |
| NH1 | 12.44 (b,1H) | | |
| NH6 | 12.54 (b,1H) | | |
| C9 | | | 165.83 |
| C7 | | | 147.78 |
| C16 | | | 146.86 |
| C15 | | | 128.57 |
| *C*2 | 7.23 (m,1H) | | 127.15 |
| C13 | | | 125.18 |
| C5 | | | 122.08 |
| C10 | | | 120.72 |
| C12 | 6.95 (s, 1H) | | 115.99 |
| C4 | 6.92(m,1H) | | 117.24 |
| C14 | 6.69 (s, 1H) | | 128.50 |
| C3 | 6.35 (m,1H) | | 111.81 |
| C8 | 6.08 (s, 1H) | | 92.94 |
| C11 | 4.01 (s, 3H) | | 58.72 |
| C17 | 2.76 (s, 3H) | | 12.35 |
| C18 | 2.39 (t, 2H), | 7.58 | 25.29 |
| C19 | 1.53 (tt,2H), | 7.10 | 29.68 |
| C21 | 1.32 (m,2H) | | 22.49 |
| C20 | 1.30 (m,2H) | | 29.78 |
| C22 | 0.89 (t, 3H) | 7.02 | 14.01 |

time value compared to that of C2 using identical chromatographic conditions.

The other minor component C1 showed an accurate mass of *m*/*z* 321.1833 Da, indicating a structure of $C_{20}H_{23}N_3O$ ($\Delta m$ = 0.8 mDa) similar to prodigiosin but short two protons. Its maximum absorbance was found at $\lambda_{max}$ = 530 nm. The fragmentation pattern revealed a loss of methyl group (*m*/*z* 307 Th) but did not show a significant loss of the other alkyl group like the other components, C2 and C3. The molecular mass, mass fragmentation patterns, and UV−vis absorbance of this compound suggested it as a cycloprodigiosin, which was earlier reported (*16*). The results indicated that both prodigiosin and cycloprodigiosin, which belong to the same class of pigments and do not have significant difference in their functional groups, were produced by the same strain of microorganisms. It is predicted that these compounds would not show different dyeing characteristics on the fabrics.

**3.6. Dyeing Process.** The extracted colorant was dissolved in methanol and water and then was used to dye multifiber fabric at 80 °C and pH 4.5. The dyed fabric was washed with 1% detergent solution to remove any physically absorbed colorant on the surface. Results indicated that wool, silk, nylon 66, acrylic, and modacrylic fabrics were colored to a pretty deep shade, whereas cotton, viscose, and polypropylene could only be stained by the same dyeing solution (Figure 3).

As an attempt to test the structural features, the colorants were directly used as disperse dyes to color multifiber fabric with an assistance of a dispersing agent. Polyester component was dyed in a deeper color than most other fibers under this condition. However, the overall dyeing performance was lower under this condition than that of the acidic condition, indicating characteristics of the colorants more similar to those of ionic dyes. Considering the presence of the -NH functional group, these colorants could behave as cationic dyes under acidic condition, which could dye acrylics, wool, and nylon fabrics. To further confirm the speculation, wool and acrylic fabrics were dyed under varied acidic conditions (sulfuric acid, pH 3.5, acetic acid pH 4.5), respectively, and the dye uptakes were compared using their *K*/*S* values, an indication of dye concentration on fabrics. The *K*/*S* values of dyed acrylic fabric decreased from

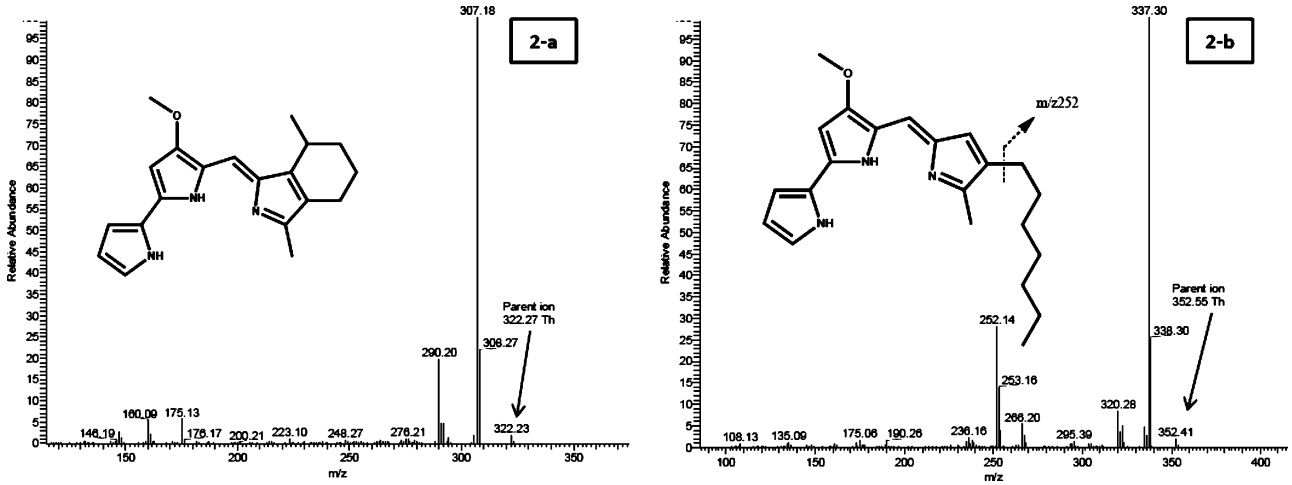



**Figure 2.** Mass spectrometric analysis (MS/MS in product mode) of the red pigments: (a) C1 component and (b) C3 component.

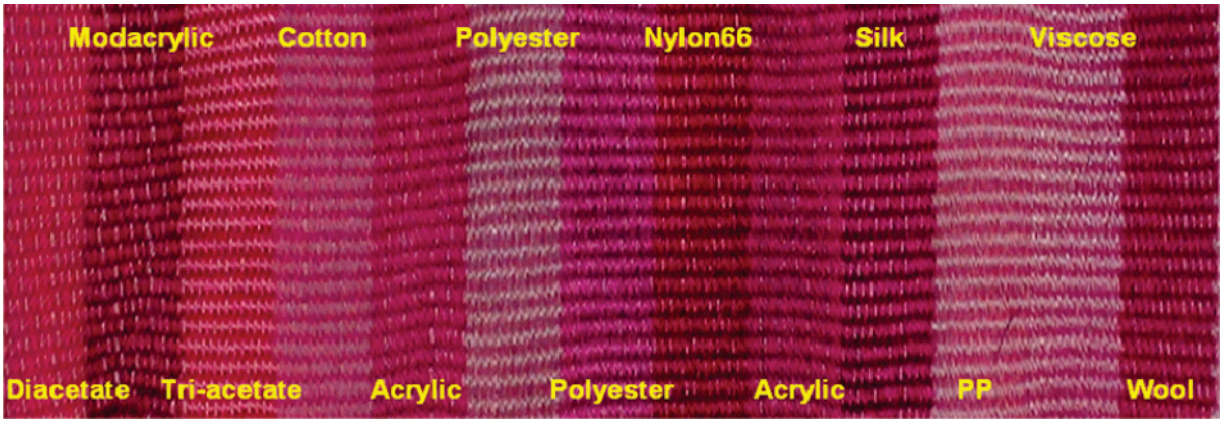



**Figure 3.** Colored multifibers fabric with the red pigments from *Vibrio* sp. strain KSJ45.

8.85 to 7.21, whereas that of dyed wool fabric dyed varied from 3.43 to 3.25, as dye bath pH changed from 4.5 to 3.5. This result is in general agreement with the basic structure of prodiginines identified above.

**3.7. Colorant Stability.** Dyeing textiles was mostly conducted under high temperature with low pH, so the stability of the colorant under the above condition is critical. A dyeing exhaustion measurement of the colorant solution (mixture of C1, C2, and C3) before and after heating at 80 °C and pH 4.5 was carried out by UV−vis spectrometry. The results showed a 15% reduction in concentration in the solution after 60 min of the treatment (Figure 4), which reveals that the colorant is not highly stable in acidic solution at elevated temperature. Thus, increasing the stability is critical for textile applications of the biocolorants.

**3.8. Antibacterial Result.** It was reported that prodigiosin has antimicrobial activities (*15*, *17*). However, the antibacterial properties could be reduced or lost when the colorants are bound to the fabrics. To evaluate the antibacterial activities of the dyed fabric, a bactericidal activity test was carried out. The wool, nylon, and cotton samples dyed with the colorant extract (mixture of C1, C2, and C3) were used as model samples. The results showed that the dyed wool fabrics had ability to kill about 50% of the *S. aureus* and *E. coli* bacteria within a 16 h of contact time; the activity of the dyed silk samples was lower and cotton did not show any antibacterial function (Table 3). These data prove that the antibacterial activities of the colorants could be obtained on the fabrics. The lower efficacy on the dyed silk and cotton was due to lower concentration of the colorants on those fabrics caused by lower dye uptakes. In fact, the dyed cotton fabric exhibited the lightest color (Figure 3).

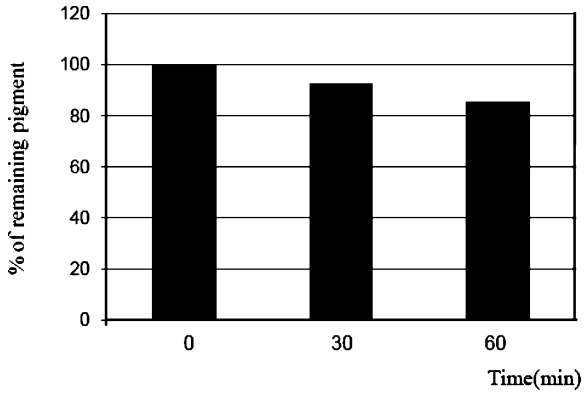



**Figure 4.** Stability of pigments under dyeing conditions.

**Table 3. Antimicrobial Activity of Textile Materials Dyed with *Vibrio* sp. Strain KSJ45 Extracts**

| | reduction of bacteria (%) | |
|---|---|---|
| fabrics | *E. coli* | *S. aureus* |
| wool | 52 | 46 |
| silk | 35 | 29 |
| cotton | 0 | 0 |

## 4. Conclusion

A bacterial strain isolated from marine sediments produced copious amount of bright red colorants. A16S rDNA sequence analysis identified the strain as *Vibrio* sp., with the greatest

sequence identity to *Vibrio gazogenes*. The structures of colorants were characterized by NMR and LC−MS and identified as a mixture of prodigiosin, cycloprodigiosin, and heptaprodigiosin. The textile dyeing tests showed that these colorants can dye wool, silk, nylon, and acrylic fabrics and have characteristics similar to those of ionic and disperse dyes, which is consistent with the identified structures. Dyeing stability of the colorant showed 15% reduction of its content after heating. The dyed fabrics showed antimicrobial properties against *E. coli* and *S. aureus* bacteria within a contact time of 16 h.

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