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Isolation of Bacteria Producing Bluish-Purple Pigment and Use for Dyeing

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Abstract

Janthinobacteriun lividum was isolated from wet silk thread whose color became bluish-purple [7,8]. This bacterium produced large amounts of bluish-purple pigment on some media containing amino acids, such as Wakimoto medium. The pigment was extracted with methanol and was identified as a mixture of violacein and deoxyviolacein. This pigment could be used to dye not only natural fibers like silk, cotton and wool, but also synthetic synthetic fibers like nylon and vinylon, and generally gave a good color tone. The shade depended on the material. Silk, cotton and wool showed a bluish-purple color, nylon a dark blue color, and acetate a purple color. Dyeing could be performed by a simple procedure consisting of either dipping in the pigment extract or boiling with the bacterial cells. By changing the dipping time and the temperature of the dye bath, shades ranging from light purple to deep bluish-purple could be selected. The color fastness of the dyed material was about the same as that materials dyed with vegetable dyes, but the color faded easily when the material was exposed to sunlight. However, since the pigment can be mass-produced by culturing, if these shortcomings could be overcome, the dye may become promising. The pigment displayed an antimicrobial activity against phytopathogenic fungi like Rosellinia necatrix which causes white root rot of mulberry 7). It could also be used as a bio-fungicide.

Discipline: Sericulture
Additional key words: Janthinobacterium lividum, violacein, natural pigment, bluish-purple color

Introduction

At present, fabrics are dyed mainly with synthetic pigments. However, natural pigments are still valuable because of their natural color tones. People have had a strong liking for natural purple pigments since ancient times. Among the natural purple pigments, that obtained from shell fish belonging to the genus *Murex* is very famous. But it is very expensive because mass production is difficult. About 10 years ago, one of the present authors (Kojima) observed that parts of wet silk threads, that had been left unattended, had turned bluish-purple. Since then, this color change of silk thread waste was observed every year in winter[7]. In the course of studying this phenomenon, one of the present authors (Shirata) isolated *Janthinobacterium lividum* from such silk threads[7,8]. The bluish-purple pigment produced by this bacterium dyes not only natural fibers like silk, wool and cotton, but also synthetic fibers like nylon and acetate fairly well. The main component of this pigment was found to be violacein, which could be obtained in large amounts by culturing the bacterium. Since the fabric dyed with this pigment has a mild feel, it can be successfully used as a fabric dye[6].

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Materials and methods

1) Culture medium

The following 6 types of culture media were used.

(1) Semi-synthetic potato agar medium (Wakimoto medium): 1,000 mL of boiled extract of 300 g potato tubers, 0.5 g of $Ca(NO_3)_2 \cdot 4H_2O$, 2 g of $Na_2HPO_4 \cdot 12H_2O$, 5 g of peptone, 15 g of sucrose and 15 g of agar.

(2) Potato sucrose agar medium (PSA medium): 1,000 mL of boiled extract of 250 g potato tubers, 20 g of sucrose and 15 g of agar.

(3) King B medium: 20 g of proteoe peptone No. 3, 1.5 g of K_2HPO_4, 1.5 g of $MgSO_4 \cdot 7H_2O$, 10 mL of glycerol, 15 g of agar and 1,000 mL of water.

(4) Modified King B medium: King B medium without K_2HPO_4 and $MgSO_4 \cdot 7H_2O$.

(5) Peptone agar medium: 20 g of peptone, 1,000 mL of water and 15 g of agar.

(6) Silk extract agar medium: 1,000 mL of boiled extract of 20 g of silk thread waste and 15 g of agar.

Unless otherwise specified, the semi-synthetic potato agar medium(a) was used for all the experiments.

2) Isolation and identification of the bacterium

The bacterium was isolated using standard methods from the bluish-purple parts of silk thread waste. Fresh bacteria that had been cultured for one day at 25°C were used for identification. The morphology of the bacterium and the colonies, the extent of multiplication at 37°C, motility, Gram reaction, and production of water-insoluble pigments and fluorescent pigments were examined by standard methods[9]. Other bacteriological properties were determined by identical sets of bacteria, API20E and API20NE, using *Janthinobacterium lividum* strain IAM13948 (=ATCC 12473 type) as the standard.

3) Bioassay of physiological activity

(1)Effect on cultured insect cells

A cultured cell line originating from the ovaries of *Antheraea eucalypti*, a silkworm of the family Saturniidae, has been established[2]. We used *A. eucalypti* cells that had been successively cultured in the Silkworm Disease Laboratory of the National Institute of Sericultural and Entomological Science to study the effect of the pigment on cultured cells. These cultured cells are known to be highly sensitive to toxic components. In our experiments, the cells were cultured at 26°C in a culture solution prepared by the addition of 5% each of silkworm serum and bovine embryo serum to the medium used by Grace[2]. For examining the effects, 0.1 mL of the pigment suspension containing 0.3% of the dried methanol extract of the pigment was added to 3 mL of the culture solution and the culture was maintained for 48 h. The cells were then observed through an inverted microscope to detect abnormalities.

(2) Toxicity to silkworms

First instar larvae of the silkworm variety Habataki were fed with mulberry leaves that had been treated with 0.1 g of the dried methanol extract of the pigment per 1.4 g of leaf, and the growth during the first instar stage was observed.

(3) Anti-microbial activity

Five bacteria and 8 fungi, all of which are plant pathogens, were used for testing the anti-microbial activity (Table 3).

In the case of bacteria, 15 mL of King B medium that had been first dissolved and then cooled to 55°C and 2 mL of a bacterial suspension containing about 10^9 cells per mL were mixed and poured into a 9 cm diameter petri dish to prepare flats containing the bacteria. Separately, 1 g of dried methanol extract of the pigment was dissolved in 50 mL of acetone to prepare a stock solution. This stock solution was mixed with an equal amount of distilled water to prepare a diluted 1/2 strength solution, which was then diluted successively, to half strength each time, using 50% acetone. A 10 μL of this diluted solution was dropped onto the medium containing the bacteria. After evaporation of the acetone and water in a clean bench, the bacteria were cultured for 1–2 days and the diameter of the growth inhibition zone of the bacteria, that appeared as a transparent circular area in the region where the diluted pigment solution had been added, was measured.

In the case of fungi, a small piece of fungal mass (about 0.5×0.5×0.5 mm) was inoculated onto PSA medium and 10 mL of the diluted pigment solution prepared above was dropped over the fungal mass. After culturing for 3–4 days, the diameter of the fungal colonies that had developed was measured.

(4) Color fastness

Silk and cotton fabrics that had been dyed with the methanol extract of the bacteria, and those dyed by boiling with the bacterial cells, were used to examine the color fastness, according to the JIS method[4]. The color depth of the silk fabric used in the experiment corresponded to No. 3 of the the standard color depth chart.

Results and discussion

1) Characteristics of the bacterium and pigment

(1) Isolation of the bacterium

Considering that the blue color of the silk thread waste was due to a microbe, we attempted the isolation of

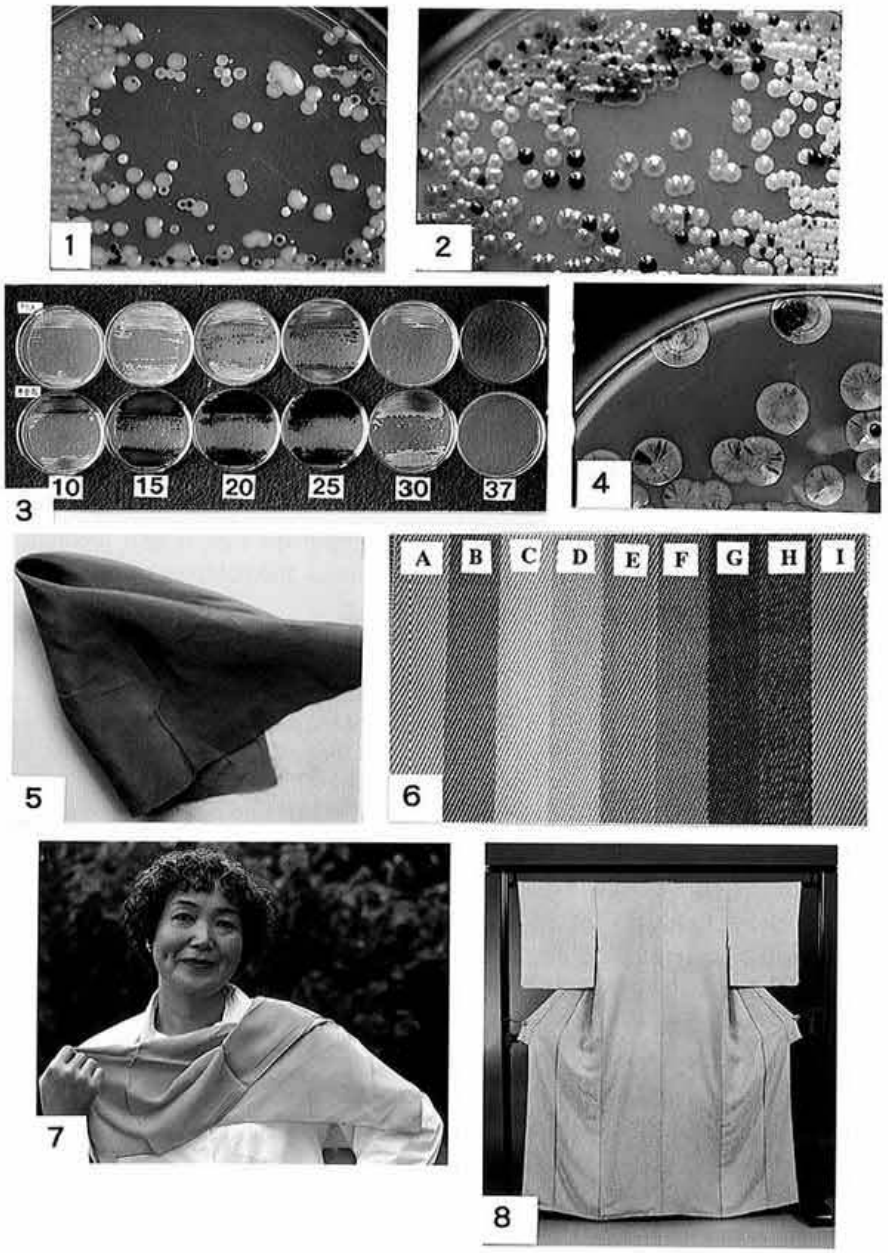


Fig. 1. *Janthinobacterium lividum* which produces a bluish-purple pigment and samples dyed with the pigment

1: Isolation of bacteria on King B medium. Bacteria that produced bluish-purple pigment could be seen in 4-day-old colonies.

2: Isolation of bacteria that produced bluish-purple pigment on semi-synthetic potato agar medium. The light and dark purple colonies all consist of *J. lividum*, the dark colored colonies belonging to strains with high pigment production.

3: Multiplication of *J. lividum* and pigment production on potato sucrose agar medium (PSA, upper) and semi-synthetic potato agar medium (lower) at different temperatures (10–37°C). More pigment was produced on the semi-synthetic potato agar medium and the optimum temperature was 25°C.

4: Emergence of pigment-producing mutant bacteria. Reverse mutation from non-pigment-producing bacteria to pigment-producing bacteria could be seen.

5: Silk fabric dyed and showing a bright bluish-purple color in a concentrated methanol extract of the pigment.

6: Dyeing performance of the pigment with 9 different materials (multifiber test cloth).
A: polyester, B: raw silk, C: acrylic fiber, D: rayon, E: wool, F: acetate, G: vinylon, H: nylon, I: cotton.
Nylon and vinylon showed a deep bluish-purple color, whereas acetate showed a purple color.

7: Scarf showing a light bluish-purple color in a relatively dilute methanol extract solution of the pigment.

8: Kimono dyed by boiling with the bacterial cells.

the bacteria on various media[8]. During the first 2 days of culture, a number of yellow and greyish white bacterial colonies appeared, but there were no bluish-purple colonies. After 4 days, however, small bluish-purple colonies appeared on the King B medium (Fig. 1–1). This culture was mixed with sterile water and subjected to pure culturing on the semi-synthetic potato agar medium. Dark bluish-purple colonies and light purple colonies developed within 3 days (Fig. 1–2). When silk thread waste was inoculated with the dark bluish-purple colonies, the thread developed a bluish-purple color within a few days.

(2) Pigment production by the isolated bacterium under different conditions

The production of the bluish-purple pigment differed considerably, depending on the medium used. The media, listed in decreasing order of pigment production, were ①semi-synthetic potato agar medium, ②silk extract agar medium, ③King B medium and peptone agar medium, and ④ potato sucrose agar (PSA) medium. The bacteria multiplied fairly well in the PSA medium but pigment production was low (Fig. 1–3). The media listed under ① to ③ above are rich in peptone or amino acids unlike medium ④.

When shake culture in a liquid semi-synthetic potato medium was compared with culture on the solid agar medium, for pigment production, culture on solid medium was found to be better. Extraction of the pigment was also easier from the bacterium on the solid medium. However, the bacterium cultured in the liquid medium also produced a fairly large amount of pigment.

As for the effect of the culture temperature, the bacterium grew well in the range of 5–30°C, with optimum growth at 25°C. No growth occurred at 37°C. Maximum pigment production occurred at 25°C, followed by 20°C. At temperatures lower than 10°C and higher than 30°C, pigment production was very low (Fig. 1–3).

Bacterial cells cultured at different temperatures were extracted with methanol to determine the dyeing performance of the extracts for silk fabric. The darkest color was obtained with extracts of bacteria cultured at 25°C, followed by bacteria cultured at 20, 15, 10, 30 and 5°C. The order of the temperature for pigment production, was about the same as that of the growth and color of the bacteria on the medium.

When the isolated bacteria were cultured at temperatures higher than 20°C, particularly in the range of 27–30°C, a mutant of light purple or white color bacteria often appeared (Fig. 1–4).

On the basis of the above results, the semi-synthetic potato agar medium was selected as the optimum medium for pigment production. The optimum temperature for culture was 20–25°C. All the subsequent culture experiments were conducted using this medium and this temperature range. When the bacterium was cultured under these conditions for 5 days, 0.25 g of bacterial cells were produced per 9 cm diameter petri dish. By extracting these cells with methanol, 47 mg of dry extract was obtained which was dissolved in 16 mL of methanol to prepare a stock solution for dyeing. Thus the extract content of this stock solution was 0.3%.

(3) Identification of the bacterium

The bacterial strain that showed the highest pigment production was selected and single colony separation was performed three times to obtain a pure strain. The selected strain S-9601 was used for the identification of the isolated bacterial cells. The colonies of this strain initially showed a greyish-white color when grown on the semi-synthetic potato agar medium. After several days of culture, the colonies showed a light bluish-purple and

Table 1. Bacterial characteristics of the isolated strain S9601

Shape of bacterial cells	Bacillus
Colony shape	Flat
Growth at 37°C	–
Motility	+
Gram reaction	–
Fluorescent pigment	–
Production of water-insoluble pigment	+ (bluish-purple)
Aerobic / anaerobic	Aerobic
Nitrate-reducing activity	+
Gas production from nitrate	+
Oxidase activity	+
Hydrolysis of esculin	+
Hydrolysis of gelatin	–
Arginine dihydrolase activity	–
Urease activity	–
β-Galactosidase activity	–
Lysine decarboxylase activity	–
Ornithine decarboxylase activity	–
Tryptophan deaminase activity	–
Indole production	–
Hydrogen sulfide production	–
Acetone production	–
Utilization of sugars and organic acids	
Glucose	+
L-Arabinose	+
D-Mannose	+
D-Mannitol	+
Maltose	+
N-Acetyl-D-glucosamine	–
Sodium citrate	+
Adipic acid	+
dl-Malic acid	+
Potassium gluconate	–
n-Capric acid	–
Phenyl acetate	–

Note: *J. lividum* IAM13948 gave the same results.

then a dark bluish-purple color. The colonies were flat with entire margins. The bacteria were Gram negative, aerobic, bacilli, having flagella and motility. The bacteriological characteristics are shown in Table 1.

The bacteriological characteristics of strain S-9601 agreed with the reported properties of *Janthinobacterium lividum*[1]. When the isolated strain and the standard *Janthinobacterium lividum* were tested together, the 2 were identical in terms of the production of the bluish-purple pigment and all bacteriological characteristics. Therefore, the bacterium was identified as *Janthinobacterium lividum*.

(4) Extraction of pigment

The bacterium was inoculated onto the semi-synthetic potato agar medium and cultured for one week. The bacterial cells that had become dark bluish-purple were placed in a small beaker and extracted with various organic solvents. Tetrahydrofuran was found to be the most efficient solvent for extracting the pigment, followed by methanol. Acetone, ethyl acetate and ether did not extract the pigment well. Almost no pigment was extracted with water (Table 2).

(5) Separation of the pigment components and their structure

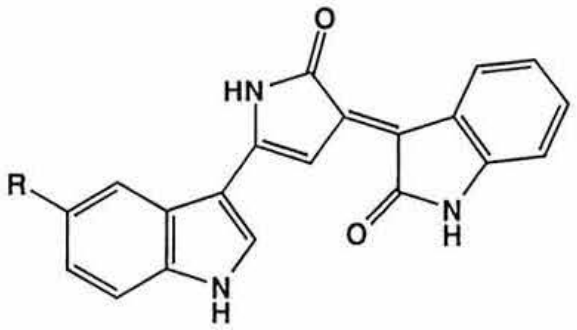
The bacterial cells were extracted with tetrahydrofuran for one day and the extract was filtered. Then, after concentrating the extract under reduced pressure, it was fractionated by silica gel chromatography (hexane : tetrahydrofuran: acetone = 4 : 2 : 1) and reversed phase high performance liquid chromatography (70% methanol), recrystallized, and 2 bluish-purple pigment components were separated. Component 1 (which was the main component and had a higher polarity than the other component) and Component 2 were subjected to different types of instrumental analysis, to determine their chemical structure.

Component 1 had a molecular weight of 343 when estimated by FAB-MS: m/z344$(M+H)^+$ and the presence of nitrogen was inferred. The IR spectrum suggested the presence of amide also. ^1H and ^{13}C NMR spectra showed that Component 1 has a total of 4 NH or phenol OH, 11 quaternary carbons and 9 tertiary carbons, and no secondary or primary carbons. Based on these data and after detailed examination of the MS spectrum, etc., Component 1 was identified as violacein (Fig. 2).

Table 2. Extraction of the pigment by organic solvents

Organic solvent	Concentration (%)	Pigment extraction[a)]
Tetrahydrofuran	100	++++
Methanol	100	+++
Methanol	50	+++
Ethanol	100	++
Acetone	100	++
Acetone	50	+
Ethyl acetate	100	++
Diethyl ether	100	+
Control (water)		±

a): The color depth of the extracted solution was classified into 5 grades, from colorless (–) to dark (++++).



R=OH : Violacein
R=H : Deoxyviolacein

Fig. 2. Chemical structure of the bluish-purple pigment
The main component violacein is a kind of antibiotic.

Component 2 had a molecular weight of 327 when estimated by FAB-MS:m/z344$(M+H)^+$ and the presence of nitrogen was inferred. The IR spectrum was similar to that of Component 1. ^1H and ^{13}C NMR spectra did not show any signal originating from phenol OH. The carbon signal that appeared at the base of the phenol OH was shifted to a considerably higher magnetic field. Based on these data and other changes in the spectral pattern, Component 2 was identified as deoxyviolacein (Fig. 2), the deoxy form of Component 1 without the phenol group. Various spectral data of these 2 components of the pigment agreed with reported data[3, 6].

(6) Safety and antimicrobial activity of the pigment

a: Safety

When the effect of the extracted pigment on cultured insect cells was studied, no adverse effect could be detected. It was thus concluded that the pigment was not toxic or exhibited a negligible toxicity to insect cells. The toxicity to silkworm cells was then examined. Twenty silkworms were made to ingest a total of about 2 g of mulberry leaves on which the pigment had been applied during their 1st instar stage. All 20 of them developed normally, without any signs of abnormality.

b: Antimicrobial activity

A study of the antimicrobial activity of the pigment

Table 3. Antimicrobial activity of the bluish-purple pigment against phytopathogens

Phytopathogen	Dilution of pigment solution[a]					
	2	4	8	16	22	64
(Bacterium)	Diameter of growth free zone (mm)[b]					
Bacillus subtilis	13	13	10	8 ±	8 ±	8 ±
Clavibacter michiganensis pv. *michiganensis*	21	17	9	8	8 ±	0 ±
Erwinia carotovora subsp. *carotovora*	0	0	0	0	0	0
Pseudomonas cichorii	0	0	0	0	0	0
Xanthomonas campestris pv. *oryzae*	15	13	12	8	8 ±	0 ±
(Fungus)	Growth inhibition(%)[c]					
Bipolaris leersiae	70	67	64	44	18	3
Botrytis cinerea	83	81	72	49	15	2
Colletotrichum demantium	100	100	100	100	100	100
Diaporthe nomurai	100	95	84	52	40	16
Fusarium lateritium f. sp. *mori*	63	56	33	11	4	0
Fusarium solani f. sp. *mori*	63	58	54	29	17	13
Rosellinia necatrix	100	100	100	100	100	58
Sclerotinia sclerotiorum	95	94	83	23	8	0

a): The pigment was extracted from bacterial cells with methanol and dried. 1 g of this dried extract was dissolved in 50 mL of acetone which was taken as the full strength solution. The solution prepared by the addition of an equal amount of distilled water to the full strength solution was taken as the 2 dilution solution. In the subsequent dilution steps, an equal amount of 50% acetone was used for the dilution. A 50% acetone solution was used as the control.

b): A 10 μL aliquot of the diluted solution was dropped onto King B medium that had been mixed with the bacterium. After 2 days of culture, the diameter of the area where there was no growth was measured. The ± symbol indicates that the antibacterial activity was very low.

c): Small pieces of fungal colonies were placed on PSA medium and 10 μL of the diluted solution was dropped onto them. The diameter of the fungal colonies was measured 3–5 days later and the inhibition rate of the growth of each fungal colony compared to the diameter of the fungal colonies in the control, was calculated.

toward plant pathogens revealed that it had such an activity against 11 of the plant pathogenic microorganisms tested, excluding *Erwinia carotovora* and *Pseudomonas cichorii* (Table 3). The inhibitory activity was particularly strong against *Colletotrichum dematium* and *Rosellinia necatrix* which cause anthracnose and white root rot diseases of mulberry, respectively (Fig. 3).

2) Dyeing and characteristics of the pigment

(1) Method of dyeing

a: Dyeing with the extract solution

Dyeing was attempted using an organic solvent solution of the pigment. After extraction of the pigment with tetrahydrofuran and air-drying of the extract, a fixed amount of dry pigment was dissolved in a certain amount of various organic solvents. Silk and cotton fabrics were immersed in the various solutions for half a day and the extent of dyeing was compared. The cotton and silk fabrics were dyed very well both in the methanol and the ethanol solutions (Table 4).

In the next experiment, the bacterial colonies were extracted with methanol and the fabric was directly immersed in the extract. The fabric developed a bluish-purple color, indicating that methanol could extract the pigment well, and also enabled the pigment to become attached to the fabric.

The dyeing method used was very simple and consisted of 3 steps: ①immersion of the silk fabric in the methanol extract overnight; ②washing with water; and ③drying in the shade.

b: Boiling dye with bacterialcells

The bacterial cells were transferred to a vessel along with the medium and boiled after the addition several times of more than 10 times the amount of water. After bringing the solution to boiling, the fabric to be dyed was dipped in it and the bath was allowed to simmer for about 20 min. The fabric was then washed with water and dried in the shade. If a deep color was not required, immersion of the fabric for 3–5 min in the bath, after boiling and cooling to 80–90°C, was sufficient.

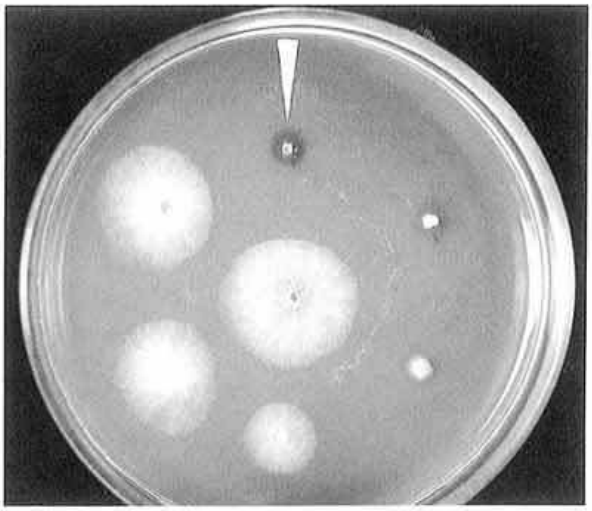


Fig. 3. Anti-fungal activity of the bluish-purple pigment
Seven small pieces of a colony of *Diaporthe nomurai*, which causes die-back of mulberry, were placed on PSA medium. Each 10 μL of the diluted pigment solution with 50% acetone was dropped onto them. The twofold diluted solution was placed in a 12 o'clock position and the strength was 1/4, 1/8, 1/16, 1/32, and 1/64 in the clock-wise direction. The control, 50% acetone alone, was located in the center of the dish. The photograph shows the growth after 7 days.

The characteristics of the 2 methods of dyeing used here were as follows: ①the procedure was very simple, ②satisfactory dyeing could be achieved without using mordants, and ③dyeing occured uniformly. Fabric made from the silk of the silkworm variety Akebono is considered to be generally prone to uneven dyeing and to require special procedures for dyeing. However, even Akebono silk could be uniformly dyed by an unskilled person using the dyeing methods described above when the liquor ratio of the bath was sufficiently high. The shade of the dyed fabric varied depending on the amount of dye present in the bath in the range of light blue, violet, bluish-purple and dark bluish-purple. Fig.1–5, 1–7 and 1–8, respectively show the silk fabric dyed to a deep color in the extract solution, a silk scarf dyed to a comparatively light color with the extract, and a kimono dyed to a very light color by boiling with the bacterial cells.

Table 4. Dyeability in different organic solvents

Organic solvent	Concentration used (%)	Extent of dyeing[a)]
Methanol	100	++++
Methanol	50	++
Ethanol	100	++++
Ethanol	50	++
Acetone	100	++
Acetone	50	+
Ethyl acetate	100	+
Ethyl acetate	50	+
Control (water)		+

a): The color depth of the silk fabric dyed at room temperature was classified into 5 grades, from colorless (–) to dark (++++).

(2) Dyeing performance

a: Dyeability of different fibers

Since we had found that the pigment was capable of dyeing not only natural fibers but also synthetic fibers, we compared the dyeing performance of 9 different fibers, using a multi-fiber test cloth (Fig. 1–6). The dyeing performance differed depending on the type of fiber. Nylon was the easiest to dye, followed by vinylon, acetate, raw silk, cotton and wool. Rayon could be dyed only slightly and almost no dyeing occurred with acrylic fiber and polyester. Although Fig. 1–6 shows that polyester also had been dyed to a moderate extent, almost no dyeing occurred when the pigment concentration was low. Most of the fibers showed a color range from light purple to bluish-purple. However, the shade differed slightly depending on the fiber, for example, nylon showed a deep bluish-purple color and acetate a purple color.

b: Duration of immersion and extent of dyeing

The extent of dyeing differed with the duration of immersion in the dye bath. At room temperature using the methanol extract, silk and cotton become dyed within 5–10 min of immersion. The color became deeper within 1–10 h and dye uptake continued even after, reaching a peak in about 2 days. On the contrary, nylon and acetate required only a very short time for dyeing. Dyeing occurred within several seconds after the start of immersion and within more than 10 h. Dyeing of acetate peaked after about 1 h. With nylon, the shade could be varied freely from pale lavender to dark bluish-purple by changing the dyeing time.

c: Temperature and dyeing rate

The dyeing rate was significantly affected by the temperature of the dye bath. For example, when silk was dyed in the methanol solution of the pigment, the immersion time required for the color depth achieved in 15 min of dyeing at 20°C, was 5 min at 40°C, 1 min at 60°C, and 30 s at 70°C. For achieving the color depth obtained after 6 h at 20°C, it took one day at 10°C and 3 days at 0°C.

(3) Color fastness

Silk samples dyed by the 2 methods were tested for their color fastness[4)]. The method of dyeing did not affect appreciably the color fastness (Table 5). Although the fastness of the color against light was somewhat low

Table 5. Color fastness of silk fabric showing a bluish-purple color[a)]

Color fastness test against	Staining[b)] Fading	Evaluation[c)]	JIS Standard
Light		1	L 0841 Day-night method
Washing	Staining	2	L 0844
	Fading	2–3	A No. 1 method
Hot water	Staining	3–4	L 0845
	Fading	4	(50°C×10 min)
Acidic sweat	Staining	3–4	L 0848
	Fading	3–4	A method
Alkaline sweat	Staining	3	L 0848
	Fading	3	A method
Rubbing	Staining (dry)	5	L 0848 II type
	(wet)	5	
	Fading (dry)	3	
	(wet)	2–3	

a): About the same results were obtained with different methods of dyeing.
b): The extent of staining was estimated by the amount of color transferred to colorless fabrics when the dyed piece of fabric was sandwiched between colorless pieces of silk and cotton fabric, and treated.
c): Color fastness was graded into 5 categories, 1–5, in the order of increasing fastness.

(Grade 1), the fastness against washing was of Grade 2–3, and 3–5 in other tests. Overall, the color fastness was about the same as that of vegetable dyes. In other words, although the color fastness was not very high, it was in the practically tolerable range, except for the fastness against light[4)]. About the same results were obtained with cotton fabric in all the tests. The color fastness against light of deeply dyed nylon and vinylon was slightly higher than that of silk or cotton(Grade 1–2).

(4) Improvement of color fastness against light

In practice, when a bluish-purple dye is used to dye fabrics, the major problem is the photosensitivity of the color. The bluish-purple color fades very rapidly in the presence of sunlight, and such dyeing is therefore of limited practical use[4)]. As a result, we examined various treatments for improving the resistance to light of textiles dyed with the bluish-purple pigment[5)]. We had examined various treatments for improving the color fastness against light of fiber and textile products dyed with this bluish-purple pigment. It was found that if the dyed material was subjected to post-treatment with a thiourea solution, the fading of color due to light was considerably reduced[5)].

A piece of silk fabric (plain Habutae with Mezuke weight 14) was dyed in a methanol solution of the bluish-purple pigment extracted from the bacterium, at room temperature by dipping in the dye bath at a liquor ratio of about 1:100 for 2 days. The dyed material was then

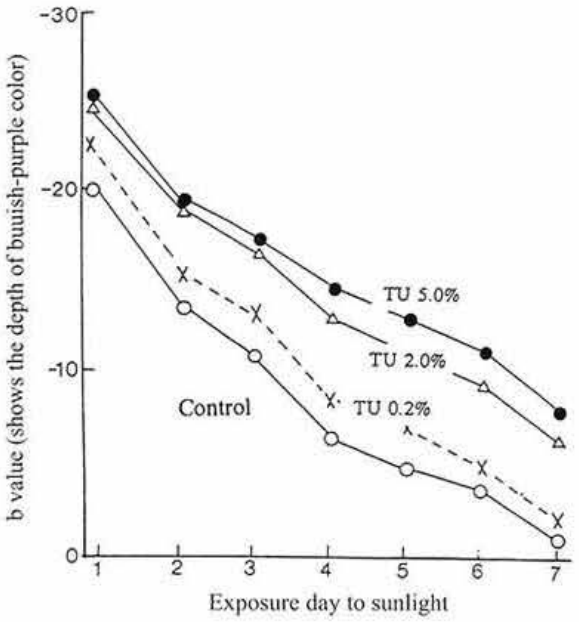


Fig. 4. Fading of the bluish-purple color after exposure to sunlight
Samples of TU.0.2%, TU.2.0% and TU.5.0% were treated with 0.2%, 2.0% and 5.0% thiourea solution for about 1 min, respectively.

Table 6. Effect of thiourea treatment on fading of bluish-purple dyed fabric after exposure to sunlight

Frequency of treatment with thiourea	Color coefficient b value[a] of fabric after exposure	Reduction rate after exposure (%)[b]
0 (Control)	−13.65	45.2
1	−18.61	25.4
3	−19.00	23.8
5	−20.96	15.9

a): The more negative the b value, the deeper the bluish-purple color. B value of unexposed fabric as control was −24.93.
b): The reduction is obtained by comparing the b value of the original dyed fabric.

immersed in a 0% ~ saturated thiourea solution (liquor ratio about 1:300) for about 1 min at room temperature. It was then drained and air-dried. The color fastness of the dried material against light was determined by the direct sunlight method (JIS L 0841). The thiourea-treated fabric showed a slower rate of color fading, suggesting that the color fastness had improved (Fig. 4). The effect of suppression of fading under light became more pronounced with the increase of the thiourea concentration. When the dyed samples were treated with a 5% thiourea solution and a saturated thiourea solution, the former showed a color fastness of Grade 2 and the latter of Grade 3. The color fastness of the untreated material was lower than Grade 1. The bluish-purple dyed material which had been treated with the thiourea solution did not cause any change in shade, nor did it have any adverse effect on the feel of the material.

Next, we treated the bluish-purple dyed material once, 2 or 5 times with a saturated thiourea solution, through the steps described below.

① Dipping in a saturated thiourea solution for 3 min at room temperature, draining and air-drying.
② Washing with lukewarm water (deionized water at 40°C) for 2 min.
③ Dipping in a saturated thiourea solution for 1 min at room temperature, draining and air-drying.

Only the steps ② and ③ were repeated when the treatment was applied several times. The fading of color under light was suppressed even more with repeated thiourea treatment (Table 6).

Conclusion

About 10 years ago, one of the present authors (Kojima) discovered that the color of some thread dust left in a bucket had become bluish-purple. We were able to isolate a bacterium which was identified as *Janthinobacterium lividum*.

The bacterial cells were extracted with tetrahydrofuran for one day and the extract was filtered. Then, after concentrating the extract under reduced pressure, it was fractionated by silica gel chromatography (hexane : tetrahydrofuran : acetone = 4 : 2 : 1) and reversed phase high performance liquid chromatography (70% methanol), recrystallized, and 2 bluish-purple pigment components were separated. The color was derived from a pigment called violacein stored by the bacteria. The bacteria are not pathogenic and violacein is not toxic.

The pigment from these bacteria can be used to dye natural fibers, providing good color tone and stability. The results of the color fastness tests against washing, hot-water, sweat and rubbing were about the same as those of vegetable dye and were in the practically tolerable range, except for the fastness against light (Grade 1). We examined various treatments for improving the resistance to light of textiles dyed with the bluish-purple pigment. It was found that when the dyed material was subjected to post-treatment with a thiourea solution, the fading of the bluish-purple color due to light was considerably reduced. When the dyed samples were treated with a 5% thiourea solution and a saturated thiourea solution, color fastness of Grade 2 and Grade 3 was obtained with the former or the latter, respectively. Also, the bluish-purple dyed material which had been treated with the thiourea solution did not show any change in shade, nor did it have any adverse effect on the feel of the material. Moreover, since mass-culture of the bacteria is possible, the pigment and dye can be produced cheaply.

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