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한국 근해 연안저토에서 분리한 해양 방선균이 생성하는 색소의 분리

김 기 은 · 조 문 구*
강원대학교 식품가공학과
*전주우석대학 생물공학과

Isolation of a Pigment Producing Marine *Streptomyces* sp. from Shallow-sea Muds around in Korea

Gieun Kim and Moon-Gu Cho*
Dept. of Feed Science and Technology, Kangweon National University, Chuncheon 200-701, Korea
*Dept. of Biotechnology, Chonju Woosuk University, Chonju 565-800, Korea

ABSTRACT

A marine *Streptomyces* sp., which produce water-soluble blue pigment was isolated from shallow-sea muds. The effect of various nutritional conditions on growth of isolated strain were investigated to facilite the potential use of this organism in industry. The effect of carbon source was characterized as a 10g/l of soluble starch. Growth was optimum at pH 7.0 and slightly affected with more alkaline range but was shown to decrease dramatically in acidic range. Under the optimum growth conditions, isolated strain produced substantial amounts of blue pigment and biomass.

INTRODUCTION

All most of modern industrial products even metals are coloured with natural or artificial pigments. The tar derivatives have been widely used in this purposes since their stability and processability. However, its health hazard properties natural and/or safe pigments were started to be used in foods, drugs, toys, cosmetics and many other areas from 1970s.

Among natural pigment, red and yellow are relatively abundant in nature but blue is scarce in territoral sphere, and also in order for perfection of colouring process the three primary colours (red, yellow and blue) are evitable.

In order to establish stable blue pigment production, marine microbes were taken around the sea-coast of Korea. One group of microbes, *Streptomyces* sp. produced substantial amounts of water-soluble blue pigment in a laboratory scale.

METERIALS AND METHODS

Sampling

Shallow-sea muds were taken into sterilized polyethylene container(250-ml) at 10~15m of depth in exhibited area(Fig. 1), and stored in ice-box until tested.

Marine Streptomycetes Isolation

1gm sea muds were diluted into 10ml of sterilized artificial sea water(Table 1). With a medium composition of glycerine 20, glycine 2.5, K_2HPO_4 0.1, $MgSO_4$ 0.1, $CaCO_3$ 0.1 gram per litre, agar 1.5%(w/v) in 1 litre of artificial sea water (NaCl 30, KCl 0.7, $MgSO_4$ 2.6, $MgCl_2$ 5, $CaSO_4$ 1 grams in litre of D.W.), marine streptomycetes were isolated from 0.1ml sereially diluted sample on solid medium at the conditions of 20±0.1℃ for 5~10days of incubation. Isolated colonies were Gram stained, and further tested followed by the method of ISP(1).

Isolation of Blue Pigment Producers

Among isolated streptomycetes, pigment producing strains were selected by medium colour.

Physiological Tests

Morphology and cultural characteristics of isolated strains were carried out by the methods of Waksman(2), and slide culture at 28℃ was also performed on oatmeal agar and glucose-asparagine agar. Carbon and nitrogen utilization were tested followed by Shirling and Gottlieb(3), and other physiological tests such as melanine formation(4), nitrate reduction(5), gelatin liquefaction, NaCl tolerance, milk coagulation, and enzyme activities of starch hydrolysis, protease, lipase and cellulase were examined by Bergey's manual.

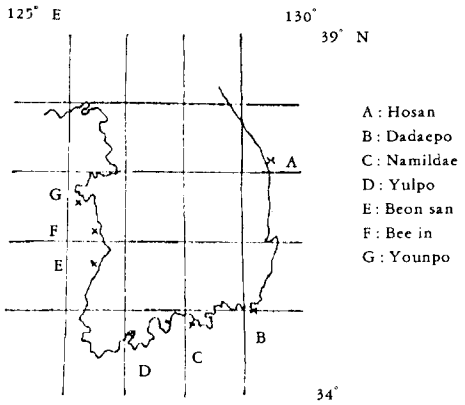

Fig. 1. Locations in the near sea of Korea for the collection of shallow-sea muds.

Table 1. Medium for isolation of marine actinomycete(g/ℓ).

Glycerine	20	$MgSO_4$	0.1
Glycine	2.5	$CaCO_3$	0.1
K_2HPO_4	0.1	Agar	15.0
Artificial sea water*	1,000ml		

*NaCl 3, KCl 0.07, $MgSO_4$ 0.26, $MgCl_2$ 0.5, $CaSO_4$ 0.1 grams in 100ml

Blue Pigment Production

Blue pigment production in small Erlenmeyer flasks(50- to 500-ml) were performed. Initially, one loopful of 2 weeks grown spores were inoculated into 10ml of liquid culture medium, and after this 0.2% of 1 week cultured broth was used for inoculation. With a 1/5th filling of production medium, medium were turn out to dark blue to blue-violet colour with the conditions described in Table 3 and 4.

Purification of Blue Pigment(6, 7)

Culture broth were centrifuged at 3,000rpm for 10min. Wet solids were used for wet weight and dry weight of biomass determination, and supernatant were purifying further through stages shown in Fig. 2. Maximum absorption spectra of crude pigment solution was examined in a range of 400~700nm.

RESULTS AND DISCUSSION

Morphology of Isolated Marine Streptomycetes

Microscopic observation(600x) of isolated ma-

Table 2. Morphological characteristics of isolated marine actinomycetes.

Hyphae	Coenocytic, branched, no vesicles formed
Aerial hyphae	Velvety
Spore	Spherical
Colony	White to greyish
Spore formation	5-7 spore linked, spiral
Soluble pigment on medium	Dark blue

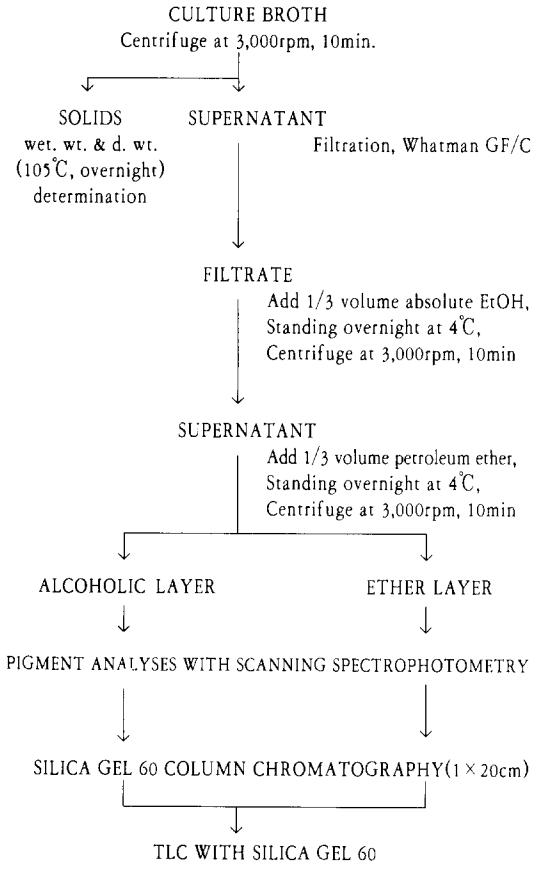

Fig. 2. Purification steps for blue pigment of a marine *Streptomyces* sp.

rine actinomycetes on oatmeal agar and glucose-asparagine agar medium at 28℃ and growth characteristics were listed in Table 2 and 3. Mycelium of isolation was coenocytic and branched but no vesicle formed. Aerial hyphae was white to greyish powderly and velvety, and 5~7 linked spherical and spiral spores were formed. Medium diffused colour was dark blue.

Effect of Carbon Source

From the results of carbon sources utilization (Fig. 3), the test strain cannot use arabinose and raffinose but 10 other sugars(glucose, sucrose, lactose, galactose, maltose, rhamnose, soluble starch, inocitol and salicin) tested as positive.

Table 3. Cultural and biochemical characteristics of isolated marine *Streptomyces* sp.

Medium Utilization:			
Glucose asparagine agar	Moderate	Starch agar	Moderate
Nutrient gelatin agar	Poor	Czapeck agar	Good
Milk agar(37℃)	Poor	Oatmeal agar	Abundant
Carbon Source:			
Glucose	+	Xylose	+
Sucrose	+	Galactose	+
Lactose	+	Maltose	+
Soluble starch	+	Arabinose	−
Inocitol	+	Raffinose	−
Rhamnose	+	Salicin	+
Physiological Characteristic:			
Melanoid pigment production	+	Nitrate reduction	+
Gelatin liquefaction	+	Starch hydrolysis	+
Protease production	+	Milk coagulation/	+
Lipase production	+	peptonization	
Cellulase production	Trace	NaCl tolerance	6%>, <9%

Table 4. Blue pigment production conditions.

Aeration	1/5th emdium filling in Erlenmeyer flask
Shaking	150 cycles per minute
Temperature	28±0.1℃ in water-bath shaker
Illumination	Room conditions
Culture peridos	5~10days

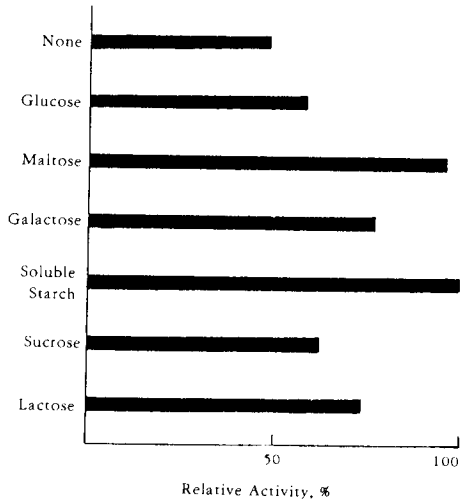

Fig. 3. Effect of carbon sources on the pigment production concentrations were fixed as 1%(w/v).

However, Soluble sterch and maltose were best when compared to glucose, and the optimum concentration of soluble starch for pigment production was determined to 1%(w/v).

Effect of Nitrogen Source

With 1%(w/v) soluble starch as carbon source, 0.1%(w/v) of each five natural nitrogen sources were tested(Fig. 4). Yeast extract was the best source of nitrogen, and the concentration was 1%(w/v).

Effect of pH

In order to determine the optimum initial pH for pigment production(Fig. 5), medium pH was adjusted 5 to 9 with buffers, and 0.2%(v/v) 1 week cultured broth was inoculated into 50ml in 250-ml baffled Erlenmeyer flask. After 7 days of shaking culture, culture broth were filtered against Whatman GF/C and optical density was measured for the selection of pH condition. Percent of relative pigment was good at pH range of 6.5 to 8.0 but pH 7.0 was optimum. Alkaline pH range was more effective for pigment production than acidic condition.

Cultural Characteristics

Most notable disadvantage of the use of fillamentous organisms in liquid suspension or submerged culture is their growth of hyphae which lead to an increase of culture broth dramatically, and this may also cause a limited oxygen transfer during culture. In this experiments, suspension culture of isolated marine *Streptomyces* sp. were formed dark-blue pellet in the bottom of flasks, and substantial viscosity increase were not observed. Pellet formation may facilitate by the addition of 0.3%(w/v) $CaCO_3$. Spontaneous carbonate buffering activity of calcium carbonate in the most media, they also has an adsorption or attachment capacity with suspended solids in liquid media. However, this effect was not examined in stirred reactor mode of operation, this may require further study.

NaCl Tolerance

Sodium chloride tolerance of teritorial microorganisms are very low, and even plants species (some salt-tolerant rice, leek, etc.) a maximum limit was 3% which require special care of irrigation and surface-soil exchange. Isolated strain showed a remarkable salt tolerance(over 6% but less than 9% of NaCl) in test conditions(Table 3).

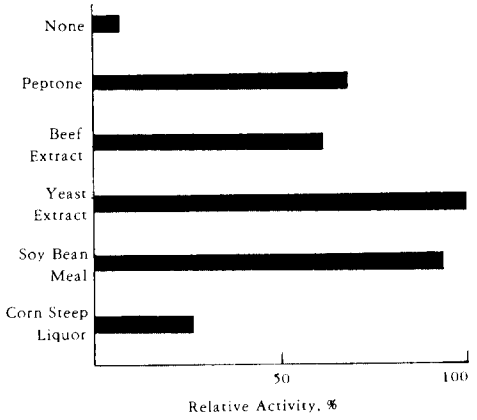

Fig. 4. Effect of natural nitrogen sources on the pigment production.

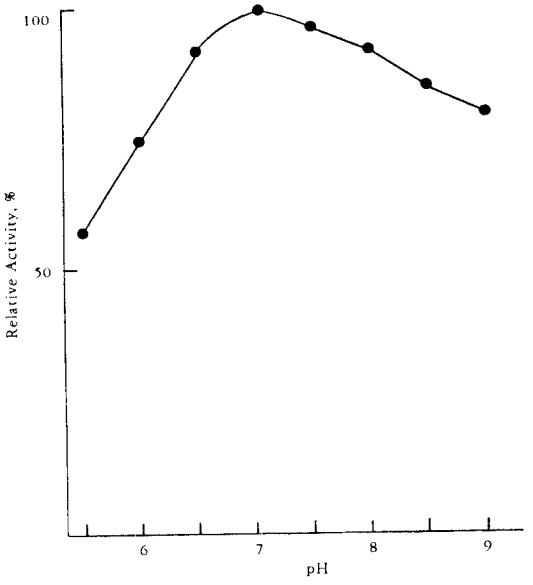

Fig. 5. Effect of initial pH on the pigment production.

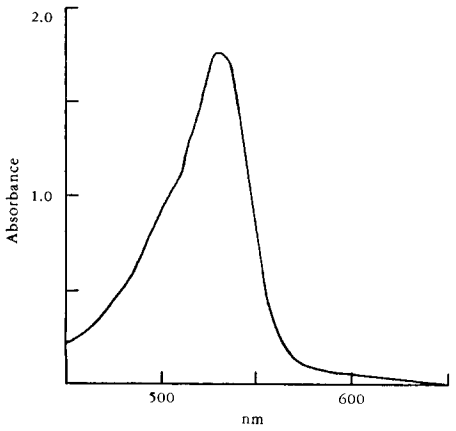

Fig. 6. Scanning spectrophotometry of purified blue pigment from isolated *Streptomyces* sp. Purification steps were summarized in Fig. 2(scan speed 120nm/min and band pass 2nm).

Probiotic era in the ancient age, the ocean would act as an important placenta of life-harbouring place. However, shallow region around in territory, especially in the depth of 20 metres, the most of microbes were originated from soil. Most of soil habitants were dead when exposed in a marine nature but some may change their physiological and metabolic patterns, and would survive as marine habitat.

This shift means that enormous changes in membrane function and cytological properties. Once soil habitant survived in marine nature, their physiological chracteristics would change slowly which adapt for absolutely new surrounding conditions(1).

Pigment Production Medium

Composition of liquid production medium for blue pigment from isolated marine *Streptomyces* sp. was: soluble starch 1, yeast extract 1, NaCl 0.5, $CaCO_3$ 0.3%(w/v), and initial pH of 7.0. The initial pH was adjusted with 1 N KOH and HCl, and other conditions are listed in Table 4.

Purification of Blue Pigment

A substantial amounts of blue pigment were produced with a marine isolated *Streptomyces* sp. When purified culture broth follow a method described in Fig. 2, its adsorption maximum in alcoholic water in ca. 530nm in 1cm of light path (Fig. 6). A simple pigment purification steps which consist with centrifuge, filtration and silica Gel 60 chromatography were designed with an economic concepts of general bioproduct.

요 약

공업적 응용 가능성을 검토하기 위하여 한국의 근해 연안저토에서 분리한 청색색소를 생성하는 *Streptomyces* sp.의 성질을 조사하였다. 탄소원은 가용성 전분으로서 1%였고, pH 7.0 이상의 약 알칼리성에서 최대성장을 하였으며, 다량의 청색색소를 생성하였다.

REFERENCES

1. M. G. Cho(1982), Mitomycin C production by *Streptomyces* sp. isolated from shallow-sea muds in Korea, M.Sc. Thesis, Kon-Kuk Univ., Seoul.
2. S. A. Waksman(1959), The Actinomycetes Vol. 1, The William's and Wilkins Co., Baltimore.
3. E. B. Shirling and D. Gottlieb(1966), *Int. J. Syst. Bact.*, **16**, 318.
4. S. T. William and T. Cross(1971), Method in microbiology, 4, 295, Academic Press, New York.
5. A. J. Holding and J. G. Collee(1971), Method in Microbiology, 6A, 19, Academic Press, New York.
6. E. Heftman(1975), Chromatography, 3rd Ed., Van Nostrand Reinhold Co., New York.
7. H. S. Kim(1978), Studies on the red pigment produced by *Monascus* sp. M. Sc. Thesis, Yon-Sei Univ., Seoul.