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Cultural Conditions of *Streptomyces californicus* KS-89 for the Production of Bluish Purple Pigment

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Abstract

The optimal cultural conditions for production of the bluish purple pigment by the cultivation of *Streptomyces californicus* KS-89 were determined with various substrates. The carbon and nitrogen sources on the production of pigment indicated that soluble starch and glycerol as carbon sources and sodium glutamate, sodium nitrate as nitrogen sources given a maximum yield of the pigment at 30℃ for 7 days. The addition of ferrous sulfate was essential. The highest production of pigment was observed with cultivation in a medium containing 2.0% soluble starch, 1% glycerol, 0.5% sodium glutamate, 0.05% sodim nitrate, 0.001% L-proline, 0.025% K_2HPO_4, 0.005% $MgSO_4 \cdot 7H_2O$, 0.04% $FeSO_4 \cdot 7H_2O$, 0.001% thiamine · HCl and pH 7.0.

Introduction

Natural colors found throughout the vegetable, plant kingdom, are especially widespread and its plays an important role in our enjoyment of foodstuffs and in our assessment of their quality. These compounds are responsible for many of the brilliant red, oranges, yellow, blue colors of edible fruit and berries vegetables and mushrooms as well as flowers, insects, birds, marine algaes, fishes and other animals. Nevertheless colors cannot be used for food processing because of these natural colors were easily decolorized during food processing, storages and transportation and pH, heat, ultraviolet and oxygens. Therefore, attempts were strongly required to produce the natural colors production through economic synthetic methods. In the previous paper [1], strain KS-89 isolated form soil collected in Pusan area was found to produce a bluish purple pigment in glycerol-starch-glutamate medium[2]. Strain KS-89 was identified as a *Streptomyces* by taxonomic studies[3-7] and named *Streptomyces californicus* KS-89.

Streptomyces californicus KS-89 produced water soluble and bluish purple pigment in basal medium. Particularly, microbial pigment found from *Monascus* sp. [8-11] and *Streptomyces* sp. [12-13] produced much more under optimal condtions such as pH, substrates, inoculum sizes of strain and fermentation times. Those factors seemed to be important to increase yield of bluish purple pigment for given strain. The present paper describes to establish the optimal conditions for efficient production of bluish purple pigment.

Materials and Methods

Microorganism

Streptomyces californicus KS-89[1] was used in this experiment. The strain was maintained on gly-

cerol-starch-glutamate (GSG) agar slants.

Media

The glycerol-starch-glutamate(GSG) medium was contained 1.0% soluble starch, 1.0% glycerol, 0.1% sodium glutamate, 0.05% $NaNO_3$, 0.025% L-proline, 0.025% K_2HPO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.001% $FeSO_4 \cdot 7H_2O$ and 0.001% thiamine·HCl. The pH was adjusted to pH 7.0.

Assay for bluish purple pigment prouduced in culture broth

For bluish purple pigment fermentation, a preculture (1mℓ) of *Streptomyces californicus* KS-89 was inoculated into a 300mℓ shake flask containing 50mℓ of medium. After incubation at 30℃ for 7~10 days, bluish purple pigment is excreted into the 10mℓ culture broth. Broth was sampled periodically for analysis. After centrifugation at 3,000rpm for 10min to seperate the mycelium, the supernatant was determined colorimetically by measuring its absorbance at 575nm.

Dry cell weight

Cells were collected on the filter paper and washed with distilled water and then dried in vacuum desiccator at 30℃ for 3~4 days.

Results and Discussions

Effect of carbon source

Various mono, di and polysaccharides, sugar alcohols and organic acids were checked to determine the most suitable carbon sources for bluish purple pigment production. The medium was prepared by replacing starch in GSG medium with other carbon sources listed in Table 1. Table 1 showed the results of pigment production by various carbon sources examined at individual consideration. Soluble starch, sucrose and maltose were found to be the most suitable substrates for bluish purple pigment production, but sugar alcohol produced very low level of the pigment. From this result, soluble starch serves a superior to carbon sources. The effect of soluble starch concentration on bluish purple pigment production was investigated in the range of 1.0% to 5.0%. In this case,

Table 1. Effect of carbon sources on bluish purple pigment production by *Streptomyces californicus* KS-89 at 30℃ for 7 days

Carbon sources	Amount added(%)	Dry cells weight (mg/mℓ)	Pigment (OD 575nm)
Glucose	1.0	0.31	0.974
Fructose	1.0	0.10	0.227
Galactose	1.0	0.12	0.320
Arabinose	1.0	0.08	0.137
Xylose	1.0	0.20	0.396
Sucrose	1.0	0.68	2.228
Maltose	1.0	0.70	2.470
Mannose	1.0	0.10	0.258
Lactose	1.0	0.37	0.930
Soluble starch	1.0	0.72	2.478
Sorbitol	1.0	0.14	0.428
Inositol	1.0	0.07	0.113
Mannitol	1.0	0.16	0.247

Cultivation was carried out as described in *Materials and Methods* except carbon source in basal medium.

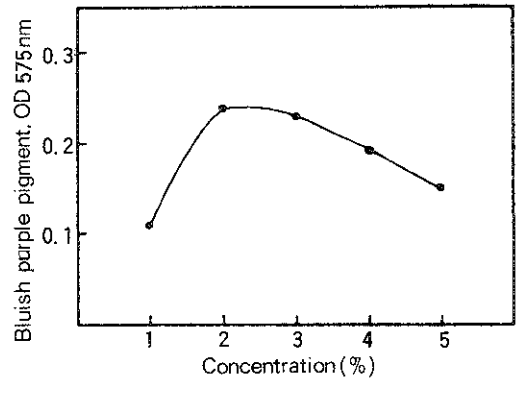

Fig. 1. Effect of concentration of soluble starch as carbon sources for production of bluish purple pigment.
Cultivation was carried out as described in *Materials and Methods* except that the test medium substituted the soluble starch in basal medium with the indicated carbon sources.

bluish purple pigment production was maximum at a concentration of 2.0% of soluble starch (Fig. 1).

Effect of nitrogen sources

In preliminary experiments, it was found that natural nitrogen sources were essential for bluish purple pigment production. Table 2 shows the effect of various nitrogen sources on the pigment production by *Steptomyces californicus* KS-89. Monosodium glutamate and sodium nitrate were proved to be the best of the various nitrogen sources tested. Therefore, in this experiment, the effect of the concentrations and combination of these two nitrogen sources on bluish purple pigment production were investigated in more detail. As shown in Fig. 2, in medium containing one or the other as sole nitrogen source, monosodium glutamate was superior to sodium nitrate.

In both cases, the most suitable concentration was 0.5% monosodium glutamate, and 0.05% sodium nitrate. When both substrates indicated over than concentration described, those concentration led to decrease the bluish purple pigment production. In medium containing both nitrogen sources at a total concentration of less than 0.5%, much more bluish purple pigment was produced than in medium containing only one nitrogen source.

The combination of 0.5% monosodium glutamate and 0.05% sodium nitrate led to maximum production of bluish purple pigment. On the other hand, Table 3 indicates the promoting effect of different nitrogen substrates with amino acid in the following basal medium, among them, proline were found to be the most effective substrates promoting pigment accumulation.

Effect of sodium chloride, calcium carbonate, ferrous sulfate and other salts

The effects of minerals described above were investigated for the production of bluish purple

Table 2. Effect of nitrogen sources on bluish purple pigment production by *Streptomyces californicus* KS-89 at 30℃ for 7 days

Nitrogen sources	Amount added(%)	Dry cells weight (mg/mℓ)	Pigment (OD at 575nm)
Organic nitrogen			
Beef extract	0.1	0.14	0.258
Tryptone	0.1	0.06	0.187
Peptone	0.1	0.06	0.181
Yeast extract	0.1	0.04	0.159
Monosodium glutamate	0.1	0.72	2.478
Urea	0.1	0.10	0.340
Casein	0.1	0.09	0.236
Inorganic nitrogen			
$(NH_4)_2SO_2$	0.1	0.26	0.500
$(NH_4)_2HPO_4$	0.1	0.25	0.470
$NaNO_3$	0.1	0.72	2.478
$(NH_4)_2CO_3$	0.1	0.29	0.406

Cultivation was carried out as described in *Materials and Methods* except that the medium substituted nitrogen sources instead of amino acids.

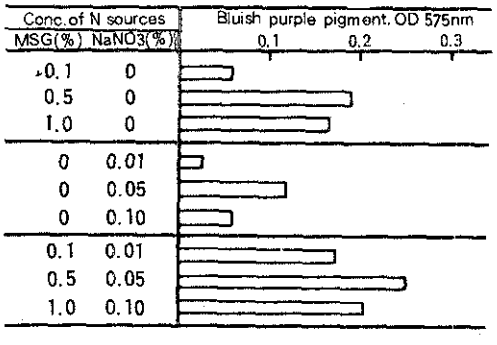

Fig. 2. Effect of concentration of monosodium glutamate and sodium nitrate alone and together on bluish purple pigment production in the culture broth.
Cultivation were carried out for 7 days as described in *Materials and Methods* except that the nitrogen concentration in the basal medium was modified as indicated, and directly assayed value in the supernatant.

pigment. The addition of 0.1~0.4% sodium chloride to the medium led to decrease bluish purple pigment production. The addition of 0.05% magnesium sulfate did not affect bluish purple pigment production. Further the addition of trace amounts (10~20 ppm) of the metal ions Fe^{3+}, Na^{+}, Co^{2+}, Mn^{2+}, Cu^{2+} and Zn^{2+} did not produced the bluish purple pigment.

But the presence of ferrous sulfate was found to be most effective for production of bluish purple pigment(Table 4).

Effect of concentration of ferrous sulfate

Some microbial pigment is chelated with ferrous complex[12], several kinds of mineral and salts sources were examined to find out suitable substates than other minerals for pigment production. Fig. 3 showed the results of pigment production from various concentration of ferrous sulfate. Bluish purple pigment increased gradually with increasing concentrations of ferrous sulfate. The pigment showed the highest level of productivity when 0.04 % ferrous sulfate added in culture were almost not changed. When the concentration of ferrous sulfate was added less them 0.02% and over than 0.2% in the medium, its produced a small amount of the pigment. These result suggested that ferrous ion may be considered convert such as complex compound of the pigment when ferrous sulfate released into the culture broth[13].

Table 3. Effect of amino acids on bluish purple pigment production by *Streptomyces californicus* KS-89

Amino acid	Amount added (%)	Pigment (OD at 575nm)
Leucine	0.025	0.360
Alanine	0.025	0.175
Phenylalanine	0.025	0.156
Histidine	0.025	1.620
Threonine	0.025	0.146
Tyrosine	0.025	0.258
Aspartic acid	0.025	0.166
Cysteine	0.025	0.102
Methionine	0.025	0.440
Valine	0.025	0.565
Arginine	0.025	0.828
Proline	0.025	2.478
Tryptophan	0.025	0.181
Lysine	0.025	1.320

Cultivation was carried out described in *Materials and Methods* except that the test medium substituted amino acid in basal medium.

Table 4. Effect of minerals on bluish purple pigment production by *Streptomyces californicus* KS-89

Mineral sources	Amount added (%)	Pigment (OD at 575nm)
Control	0.000	0.400
$CaCl_2$	0.001	0.428
$CoCl_2$	0.001	0.748
$ZnCl_2$	0.001	0.040
$MnSO_4$	0.001	0.790
$MgSO_4$	0.001	0.046
$CuSO_4$	0.001	0.050
$Al_2(SO_4)_3$	0.001	0.241
$FeSO_4 \cdot 7H_2O$	0.001	2.478

Cultivation was carried out described in *Materials and Methods* except that the test medium substituted minerals in basal medium.

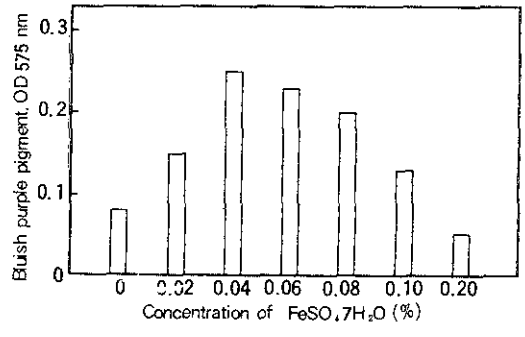

Fig. 3. Effect of concentration of ferrous sulfate on bluish purple pigment production in the culture broth.
The pigment was measured at 575nm after diluted 10 times with distilled water.

Effect of vitamin B group

Six vitamin B group were tested for their stimulation effect on the bluish purple pigment formation. 0.001% pyridoxine used in the pigment production medium increased the productivity of the pigment, but other could not caused any enhancing effect of the pigment production(Table 5).

Effect of pH and temperatures

The initial pH of the medium was varied from 4 to 9 with 1N-hydrochloric acid or sodium hydroxide. The pigment production at various pH were shown in Table 6. The maximum yield of bluish purple pigment was obtained when the initial pH of the medium was adjusted to pH 7.0 for 7 days cultivation. An shown in Table 7, the pigment production by *Streptomyces californicus* KS-89 was not correlated with cell growth.

The maximum bluish purple pigment was produced at pH 7.0. On the other hand, to find out maximum temperatures, *Streptomyces californicus* KS-89 were incubated at temperature ranges of 10℃ to 40℃. As shown in Fig. 4, the bluish purple pigment production increased with rising temperature and reached a maximum at 30℃, but the production of the pigment markedly decreased at temperature of higher than 30℃.

Table 5. Effect of vitamin B group on the bluish purple pigment production by *Streptomyces californicus* KS-89

Vitamin B	Amount added (%)	Pigment (OD at 575nm)
Control	0.000	1.100
Thiamine	0.001	0.270
Riboflavin	0.001	1.138
Biotin	0.001	0.250
Niacin	0.001	1.138
Ca-pantothenate	0.001	1.034
Pyridoxine	0.001	2.478

Cultivation was carried out described in *Materials and Methods* except that the test medium substituted vitamin in basal medium.

Table 6. Effect of the initial pH of the medium on the production of the pigment by *Streptomyces californicus* KS-89

Initial pH	Final pH	Pigment (OD at 575nm)
4	4.4	0.022
5	5.6	0.522
6	6.8	1.247
7	7.4	2.478
8	8.6	2.330
9	9.8	0.428

Cultivation was carried out on a rotary shaker at 30℃ for 7days in pigment producing medium described in *Materials and Methods.*

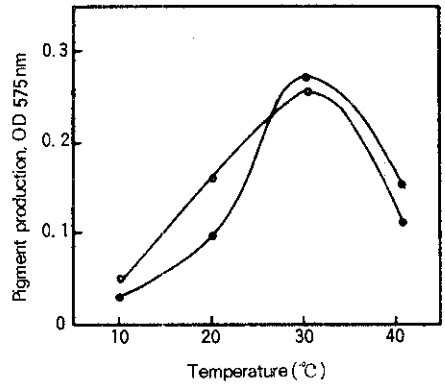

Fig. 4. Effect of incubation temperature on the pigment production by *Streptomyces californicus* KS-89.
○——○ : pigment production
●——● : dry cells weight
Cultivation was carried out described in *Materials and Methods* at the indicated temperature for 7 days in the pigment production. The pigment was measured at 575 nm after diluted 10 times with distilled water.

References

1. Ryu, B. H. and Chi, Y. E. : Isolation and identification of strain KS-89 which produced bluish purple pigment. *Kor. J. Appl. Microbiol. Bioeng,* in press.
2. Shinobu, R. : Memoirs of Osaka University of

the Liberal Arts and Education, B. *Natural Science*, No. 7, 63(1953)
3. Shirling, E. B. and Gottlieb, D. : Methods for characterization of *Streptomyces* species. *Internat. J. Syst. Bacteriol.*, **16**, 313(1966)
4. Waksman, S. A. : *Actinomycetes*, 2, 330 : The Williams and Wilkins, Co., Baltimore, (1961)
5. Buchanan, R. and Gibbons, N. E. : Bergey's Manual of Determinative Bacteriology, 8th Ed., The Williams and Wilkins Co., Baltimore (1989)
6. Nonomura, H. : Key for classification and identification of 458 species of *Streptomyces* included in ISP. *J. Ferment. Technol.*, **52**, 78(1974)
7. Pridham, T. G. and Lyons. J. : Progress in classification of the taxonomic and nomenclatural status of some problem *Actinomycetes*, (1969)
8. Hitol, T., Shima, T., Suzuki, T., Tsukioka, M. and Osagaswara, S. : Hyperpigment productive mutant of *Monascus anka* for solid culture. *Agric. Biol. Chem.*, **43**(9), 1975(1979)
9. Lin, C. F. : Isolation and cultural conditions of *Monascus* sp. for the production of pigment in a submerged culture. *J. Ferment. Technol.*, **51**(6), 407(1973)
10. Yoshimura, M., Yamanaka, S., Mitsugi, K. and Hirose, Y. : Production of *Monascus*-pigment in a subemerged culture. *Agric. Biol. Chem.*, **39**(9), 1789(1975)
11. Ryu, B. H., Lee, B. H., Park, B. G., Kim, D. S. and Roh, M. H. : Production of red pigment by using protoplast fusion of *Monascus anka*. *Kor. J. Food Sci. Technol.*, **21**(1), 37(1989)
12. Oshima, M., Ishizaki, N., Hansdo, A., Tonooka, Y. and Kanda, N. : Isolation and properties of Neopurpuratin, a purplish-red pigment produced by mixed culture. *J. Ferment. Technol.*, **59**(5), 335(1981)
13. Oshima, M., Ishizaki, N., Handa, A. and Tonooka, Y. : Cultural conditions for production of neopurpuratin, a purplish-red pigment, by mixed culture of *Streptomyces propurpuratus* with *Bacillus* sp. *J. Ferment. Technol.*, **61**(1), 31(1983)

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Streptomyces californicus KS-89에 의한 청자색 색소의 생산조건

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요 약

Streptomyces californicus KS-89에서 생산되는 청자색 색소를 생산하기 위한 기질별 최적조건을 구하였다. 청자색 색소를 생산할 때 요구되는 기질로서는 탄소원으로 가용성 전분과 glycerol이, 질소원으로 글루타민산소다와 질산나트륨으로서 30℃에서 7일 동안에 생산능이 가장 우수하였다. 이때, 황산철은 필수적인 무기질이었다. 결론적으로 청자색 색소의 생산을 위한 최적 배양조건은 2.0% soluble starch, 1% glycerol, 0.5% sodium glutamate, 0.05% sodium nitrate, 0.001% L-proline, 0.025% K_2HPO_4, 0.005% $MgSO_4 \cdot 7H_2O$, 0.04% $FeSO_4 \cdot 7H_2O$, 0.001% thiamine · HCl과 pH는 7.0이었다.