〈Article〉 KOR. JOUR. MICROBIOL. Vol. 16, No. 1 p. 16~20 (1978)

Studies on the Microbial Pigment (Ⅲ)

—The Effect of Tyrosine.—

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微生物의 色素에 關한 硏究(第3報)

—Tyrosine의 영향—

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ABSTRACT

Of the eight single acids, only the tyrosine induced the pigmentation. By dint of tracer works of labelled tyrosine with wild type and two mutants, 82% of carboxyl-tyrosine was incorporated into the primary cellular metabolite and 19% of this was inverted to the red pigment. And 11% was incorporated into the red pigment directly.

INTRODUCTION

Serratia marcescens did not form the pigment when it cultured at 37°C or cultured on the minimal medium(Ahn *et al.*, 1977 a). This phenomenon suggested that the pigmentation might require some amino acids, and in really many hypotheses were explained about the pigment pathway from the various amino acids(Williams *et al.*, 1971; Calhoun *et al.*, 1972; Scott *et al.*, 1976; Qadri and Williams, 1973; Cushly *et al.*, 1971; Wasserman *et al.*, 1973).

In this study, the single amino acids were treated for the determining of the pigmentation inducer. And using the tracer work, the incorporation ratio into the pigment and primary cellular metabolite was determined.

METHODS AND MATERIALS

1. Microorganisms and media

The bacterial strain was the *Serratia marcens* strain P from soil(Ahn *et al.*, 1977 a). The modified basal medium of Qadri and Williams(1971): NH_4NO_3 10g, K_2HPO_4 10g, NaCl 8g and $MgSO_4$ 0.5g in 1,000m*l* of distilled water, was used for dteermining the effect of single amino acids as a sole carbon source. The minimal medium containing 0.5% sodium citrate and 1% glycerol with the basal medium was used for mutagenesis and tracer into work. Agar was added above medium to solidify as the concentration of 1.5%.

2. Bacterial count and pigment extraction

The bacterial count and the quantitative analysis of pigment were caried out using

the same methods of Ahn *et al.*, (1978 a).

3. Effects of single amino acids

To observe the effects of single amino acids as a sole carbon source, the non-pigmented bacteria (Ahn *et al.*, 1978 a) were inoculated on 5ml of liquid basal medium in test tube. Eight amino acids that were known to pigmentation in microbes, L-histidine, L-proline, L-alanine, L-cysteine, L-tyrosine, L-tryptophan, L-glutamic acid and L-phenylalanine (Williams and Qadri, 1971; Calhoun *et al.*, 1972) were added respectively to the liquid basal medium to the final concentration of 5mg/m*l*, except tyrosine of 0.5mg/m*l*, because of its solubility. The formation of pigment had been observed for 3 days after inoculation.

4. Tracer work of radio isotope

Since tyrosine was confirmed as significant material in pigmentation during preliminary research, L-(carboxyl-^{14}C-) tyrosine was used for determining the incorporation ratio into pigment. Specific activity of the labeled tyrosine (manufactured by The Radiochemical Centre, England) was 322 μCi/mg. The isotope was added to 500 ml Erlenmeyer flasks containing 100ml of liquidminial medium plus tyrosine to the final concentration of 22,077cpm/m*l*.

5. Mutagenesis

Mutants deficient in catabolism of tyrosine and in pigmentation were isolated by treatment of wild type, strain P, with N-methyl-N'-nitro-N-nitroguanidine by the procedure of Lim *et al.* (1976). Bacteria were grown in liquid minimal medium at 37°C on the water bath shaker for 12hrs. After centrifugation of cultures at 3,000 rpm, the pellet of bacteria was washed three times in buffer: Tris·HCl 0.6g, $(NH_4)_2SO_4$ 0.1g, $MgSO_4 \cdot 7H_2O$ 0.01g, $CaNO_3$ 0.0005g and Fe $SO_4 \cdot 7H_2O$0. 00025g in 100ml of distilled water. The pellet resuspended in this buffer to 1/5 of the original volume of culture, and NTG was added to the suspension to a final concentration of 30μg/ml. After 3hrs of incubation at 37°C, the suspension was added to an equal volume of cold 0.5M phosphate buffer(pH 7.0). The treated bacteria were centrifuged, washed 3 times with phosphate buffer, and suspended in liquid minimal medium. And then the bacteria were incubated at 37°C with shaking for 12hrs prior to being streaked on plates. Mutant of non-pigmentation was isolated by streaking on solid minimal medium containing tyrosine. The strain of non-pigmentation was reinoculated on fresh medium and described as strain 4.

A mutant deficient in catabolism of tyrosine was isolated by using replica plates of basal medium containing tyrosine as a sole carbon source. The bacteria unable to catabolize the tyrosine was marked as strain B.

6. Measurement of radioactivity

The extraction of pigment from bacteria (Ahn *et al.*, 1977 a) was applied to all of 3 strains, wild type strain P and two mutants strain B and 4. Bacterial cells were gathered by filtration on Millipore filter paper (pore size 0.45μm). And 0.1m*l* sample was added to 14 m*l* of scintillation cocktail solution and 4 ml of ethanol and well mixed.

The radio-activites were measured by Scintillation counter (Alloska LSB) for two minutes.

RESULTS

Of the eight single amino acid treatments, only the tyrosine induced the red pigment. Williams *et al.* (1971) reported that the prodigiosin was induced by L-proline, L-alanine, L-asparatic acid, L-glutamic acid,

L-serine and ornithine, among which the proline was the most effective for the pigmentation. In the paper of Calhoun *et al.*(1972), it was explained that skimate and some amino acids such as phenylalanine, tyrosine, tryptophan and anthranillic acid were closely related with the formation of pyocyanine produced by *Pseudomonas aeruginosa.*

The data in Table 1 show the characteristics of wild type and two mutants. Although the strain 4 was deficient in formation of pigment on minimal medium plus tyrosine and the strain B was deficient in catabolism of tyrosine, the two mutant strains produced the pigment in solid nutrient medium at 30°C. This may be interpreted that other nutrient materials except tyrosine were also related to the formation of pigment.

Table 1. The characteristics of 3 strains

Medium / Strain	minimal	minimal + tyrosine	basal + tyrosine	N.A
P	+np	+p	+p	+p
B	+np	+p	−	+p
4	+np	+np	+np	+p

*+ : growth, − : non-growth,
p : pigmentaaion np : non-pigmentation

Incorporation ratio of labled tyrosine and the radioactivities of cells and pigments are shown in Table 2. The radio-activity of cell, strain P, was approximately equal to that of strain 4. It may be explained that the pigment was formed through the primary cellular metabolite. But the radio-activity of the pigment of strain B was 2,610 cpm/m*l*, and that of cell was 5,360 cpm/m*l*, which was 1/5 of the strain P. Reffering to the radio-activities of strain B, about 1/3 of the labled tyrosine was incorporated into the pigment directly. The incorporation ratio of labelled tyrosine into pigment was 19% through the primary cellular metabolite and 11% directly.

Table 2. The radio-activities in each strains (cpm/m*l*)

Strain	Cell	pigment
P	18,250	6,970
B	5,360	2,610
4	18,130	0

*Total radio-activity is 22,077 cpm/ml.
The incorporation ratio into
cell=18,250/22,077=82.6%
*P.C.M.=18,130/22,077=82.1%
Pigment=6,970/22,077=31.5%
=2,610/22,077=11.8%
*P.C.M.=primary cellular metabolite

DISCUSSION

It is new fact that the tyrosine played a role on the pigmentation of *Serratia marcescens.* Scott *et al.*(1976) postulated that the proline was incorporated directly into the prodigiosin and alanine, histidine were the incorporating materials into the prodigiosin. Qadri and williams (1973) described that the methionine enhanced the pigmentation and that 2-C and carboxyl of methionine were incorporated into the prodigiosin. Cushly *et al.*(1971) found out that the 1-C and 2-C of acetate incorporated incorporated into the prodigiosin. Wasserman *et al.*(1973) suggested that the amino acids such as glycin, alanine, proline, methionine and serine, and acetate were incorporated into the prodigiosin.

Tyrosin is known as the precursor of melanoid pigment, but the pigment in this experiment was not the melanoid pigment because precipitation was not appeared by adding a drop of $FeCl_3$.

The data in Table 1 means that the other organic materials are related with the pigmentation because of strain B and strain 4 could form the pigment on the nutrient agar medium. The nutrient medium induced the pigmentation complexly.

With the result of tracer work(Table 2), the pathways of carboxyl-tyrosine into pigment might be postulated as the Fig. 1.

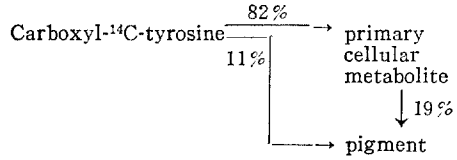

Fig. 1. The postulated pathways of tyrosine into pigmentation

摘 要

미생물의 색소형성에 관계한다고 보고된 8종의 amino acid中에서 tyrosine처리구에서만 색소가 형성되었다. 방사선동위원소로 표시된 tyrosine과 2종의 돌연변이체를 사용하여 색소로의 전환 비율을 알아본 결과 tyrosine의 carboxyl기가 82%는 초기에 세포 대사물질로 쓰이며, 19%는 색소로 직접 전환이 되는 것을 알았다.

초기에 대사물질로 들어간 82%중 11%는 다시 색소로 전환된 것으로 측정되었다.

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