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Studies on the Microbial Pigment (Ⅱ)

—Inhibition of the Pigmentation—

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微生物의 色素에 關한 硏究(第 2 報)

——色素形成의 抑制——

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ABSTRACT

Glucose and galactose were the inhibitors of pigmentation of *Serratia marcescens*. Other sugars, however, even the fructose which is the structural isomer of glucose and galactose did not affect to pigmentation. The yield of pigmentation was decreased when the glucose was added to culture medium.

And it was known to that the antibiotics was roled as the inhibitors of pigmentation. The limit concentration of the inhibitors were as followings: rifampicin, 1μg/m*l*. tetracycline, 20μg/m*l*. streptomycin, 1μg/m*l* and erythromycin, 7μg/m*l*. Addition of rifampicin(1μg/m*l*) at 6 hrs cultures inhibited the formation of pigment completely.

INTRODUCTION

Recently it is known to that the red pigment of *Serratia marcescens* is the secondary metabolite(Williams, 1973) and that the formation of pigment was inhibited by some macromolecules(Qadri and Williams, 1972).

Blizzard and Peterson(1963) suggested that the formation of prodigiosin, the red pigment of *Serratia marcescens* strain Nima was inhibited by some macromolecules such as glucose and streptomycin and chloramphenicol. Qadri and Williams(1972) suggested that the pigmentation of *S. marcesens* strain Nima was also inhibited by some antimicrobials and antimetabolites such as actinomycin D, chloramphenicol, chloroquinon, cycloheximide, 5-flourouracil, mitomycin C, puromycin, 5-CH_3-tryptophan and streptomycin.

In this experiment, using the *Serratia marcescens* strain P, the inhibitors of pigmentation was determined and that conditions of the bacterial growth was observed. And this experiment was designed to

elucidate the relationship between pigmentation and protein synthesis through the culture of *S. marcescens*. To clear the relationships, 5 antibiotics which are different action mode to protein syntheis were applied to the culture system.

METHODS AND METERIALS

1. Organism and media,

The bacteria was the Serratia marcescens strain P(Ahn *et al.*, 1978), and the meia used in this experiment was nutrient medium as of solid or liquid state.

2. Bacterial count and pigment extraction,

The bacterial count and the quantitative analysis of pigment were carried out by the same methods as the previous paper (Ahn *et al.*, 1978).

3. Detection of inhibitors

The gradient methods were used for the detection of the limit concentration of inhibitors, such as sugars and antibiotios. The sugar (glucose, galactose, fructose, mannitol, sorbitol and lactose) concentration were applied as 1%, respectively and the concentration of antibiotics were as followings: penicillin 20μg/m*l*, rifampicin 1.5μg/m*l*, streptomycin 1.5μg/m*l*, tetracycline 25μg/m*l* and erythromycin 10μg/m*l* respectively.

To observe the effects of glucose on the bacterial growth and pigmentation, the non-pigmented bacteria(Ahn *et al.*, 1978) were inoculated on 500m*l* Erlenmeyer flasks each containing 1%, 0.5% and 0% of glucose in 100m*l* of liquid nutrient medium. The bacteria were cultured on water bath shaker at 30°C.

To observe the effects of antibiotics on pigmemtation, rifampicin was added to 500m*l* Erlenmeyer flasks as the concentration of 1μg/m*l* in 100m*l* of liquid nutrient medium. The treated times were at 6hrs, 12hrs, 18hrs and 24hrs after inoculation. The bacteria were also cultured on water bath shaker at 30°C. All the antibiotics were sterilized by filtration before the treatment.

RESULTS

The data in Table 1 show some relationships between sugar(glucose and galactose) and pigmentation. The glucose and galactose, each are stereoisomer, inhibited the pigmention. But the other sugars, even fructose that is the structural isomer of glucose and galactose, did not affect to the formation of pigment. Fig. 1 shows the effects of glucose on bacterial growth and pigmentation. In the bacterial growth phase, the shift-up of growth appeared at 30 hrs after inoculation by addition of glucose. The bacterial numbers in 1% glucose medium were more than that of 0.5%.

The amount of pigment was decreased by glucose and begining of pigmentation was appeared at 35 hrs in 0.5% glucose medium and 40hrs in 1% glucose medium, respectively. The amount of pigment was 31μg/m*l* at maximal peak in 0.5% glucose medium and, however, 10% of pigment was decreased by addition of 0.5% glucose. And the amount of pigment was 28μg/m*l* at maximum peak in 1% glucose medium and then 20% of pigment was decreased by addition of 0.5% glucose.

The limit concentration of antibiotics on the formation of pigment was following: rifampicin, 1μg/m*l*, tetracycline, 20μg/m*l*, streptomycin, 1μg/m*l* and erythromycin, 7μg/m*l* respectively, and penicillin was not the inhibitor of the pigmetation.

The rifampicin and streptomycin were

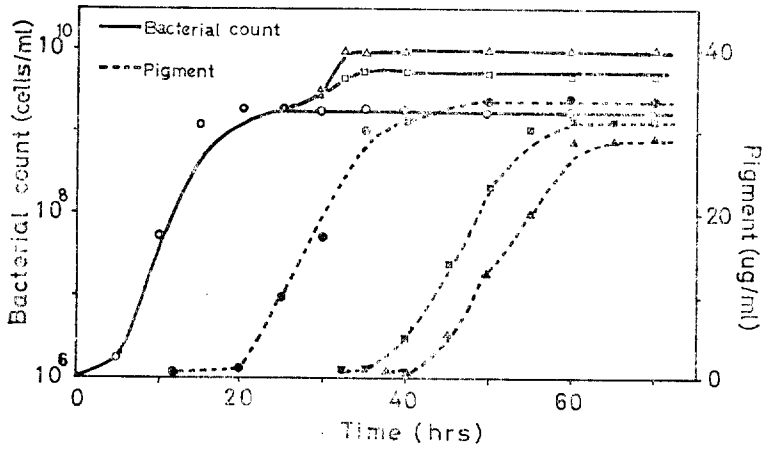

Fig. 1. The bacterial growth phase and pigmentation during the cultivation with addition of glucose. △: 1% of glucose □: 0.5% ○: 0%

Table 1. Effect of macromolecules on the pigmentation

Macromolecule	Pigmentation
None	+
Fructose	+
Galactose	−
Glucose	−
Mannitol	+
Lactose	+
Sorbitol	+
Erythromycin	− : 7μg/ml(lm)
Penicillin	+
Rifampicin	− : 1μg/ml(lm)
Streptomycin	− : 1μg/ml(lm)
Tetracycline	− : 20μg/ml(lm)

*+ : pigmentation, − : Non-pigmentation,
lm : limit concentration of pigment inhibition.

the potent inhibitors of pigmentation. According to Blizzard and Peterson(1963), the induction of pigment was inhibited by streptomycin and chloramphenicol. Qadri and Williams(1972) reported that the formation of prodigiosin was inhibited by many antibiotics such as puromycin, actinomycin D, chloramphenicol, mitomycin C, etc., especially the potent inhibitors were streptomycin, chlorampenicol and puromycin.

Table 3 shows the bacterial counts in accordance with various adding time. The rifampicin(1μg/ml) did not affected to the bacterial growth. Fig. 2 shows the effect of rifampicin(1μg/ml) on the formation of pigment with various times of addition.

Table 2. The bacterial counts of various addition times of rifampicin (1μg/ml)

Adding time / Observation time	6hr	12hr	18hr	24hr	control
5	1.8×10^6	1.9×10^6	2.1×10^6	1.7×10^6	2.2×10^6
10	7.4×10^7	7.9×10^7	8.2×10^7	7.5×10^7	8.0×10^7
15	1.2×10^9	1.4×10^9	1.4×10^9	1.4×10^9	1.4×10^9
20	1.4×10^9	1.4×10^9	1.4×10^9	1.4×10^9	1.4×10^9
25	1.4×10^9	1.4×10^9	1.4×10^9	1.4×10^9	1.4×10^9

*Initial numbers of bacteria was 8.0×10^5.

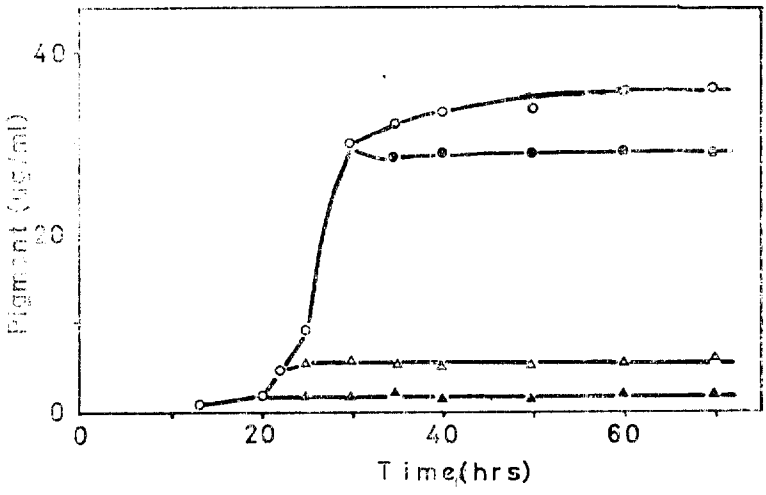

Fig. 2. The pigmentation during the cultivation with addition of rifampicin.
▲: 6th hr. △: 12th hr. ●: 18th hr. ○: 24th hr.

The pigment was not formed by the addition of rifampicin at 6 hrs, and the formation of pigment was stopped at 4—6 hrs after the addition of rifampicin. The addition of rifampicin at 24 hrs after inoculation, the amount of pigment was approximately resemble to the control. The formation of pigment approached the maximum at 28 hrs. when the pigmentation was started at 12 hrs.

Both the pigmentation and bacterial growth were not inhibited by penicillin(20 $\mu g/ml$).

DISCUSSION

Since the pigmentation was inhibited by glucose and galactose but not by fructose, it is confirmed that the materials which are the stereoisomers of glucose are the inhibitors of pigmentation. It is well known that the yield of penicillin is decreased by adding of glucose.

By the results of the effects of adding the glucose(Fig. 1), the shift-up period appeared when glucose was used as carbon source and the pigmentation was occured when the content of glucose was decreased. This phenomenon is explained the catabolite repression.

Reffering to the effects of antibiotics as inhibitor, the streptomycin and rifampicin were the potent inhibitors. It is believed that the m-RNA level and ribosomal level are probably required for initiation of pigmentation. The inhibition of pigment formation by addition of certain antibiotics may indicate that macromolecular synthesis are involved in biosynthesis(Qadri and Williams, 1972). Rifampicin was the potent inhibitor, and the inhibition of pigmentation occured 4—6 hrs after the addition (Fig. 2). This result means that the macromolecules for the pigmentation are ready made before the begining. Really, the enzymes of synthesizing prodigiosin are formed just before maximal production of pigment occurs(Qadri and Williams, 1972). Since the bacterial growth was not affected by the antibiotics which concentrations actually affected on pigmentation, pigment formation is more sensitive to inhibition than growth of cells.

적 요

Glucose와 galactose는 *Serratia marcescens*의 색소형상을 억제하였다. glucose와 galactose의 구조이성질체인 fructose와 그 외의 당류는 전혀 영향을 미치지 못했다. 배양액에 glucose를 첨가해준 결과 색소의 형성량은 현저히 저하되었다.

항생제 역시 색소형성의 억제물질이였으며 억제의 최저 농도는 rifampicin 1μg/m*l*, tetracycline 20 μg/m*l*, streptomycin 1μg/m*l*, erythromyin 7μg/m*l*이였다. 배양후 6시간후에 rifampicin을 처리한 결과, 색소의 형성은 전혀 일어나지 않았다.

REFERENCES

1. Ahn. T.S., Y.K. Choi and S.W. Hong, 1977. Sudies on the microbial pigment The effects of invironmental factors to the pigmentation. *Kor. J. Microbiol.*
2. Blizzard, J.L. and G.E. Peterson, 1963. Selective inhibition of proline induced pigmenlation in washed cells of *Serratia marcescens*. *J. Bacteriol.* **85**, 1136—1140.
3. Hammond, R.K. and D.C. White, 1970 a. Inhibition of carotenoid hydroxylation ib *Staphylococcus* by mixed-function oxidase inhibitors. *J. Bacteriol.* **103**, 607—610.
4. Hammond, R.K. and D.C. White, 1970 b. Inhibitors of v itaminK$_2$ and carotenoid synthesis in *Staphylococcus aureus* by Diphenylamine. *J. Bacteriol.* **103**, 611—615.
5. Qadri, S. M.H. and R.P. Williams, 1972, Biosynthesis of The tripyrrol bacterial pigment, prodigiosin, by ninproliferating cells of*Serratia marcescens*. *Texas rept. Bio. Med.* **30**, 73—83.
6. Rapoport, H. and K.G. Holden, 1962. The synthesis of prodigiosin. *J.Amer. Chem. Soc.* **84**, 635—642.
7. Williams, R.P., 1973. Biosynthesis of pro digiosin, a secondary metabolite of *Serratia marcescens*. *Appl. Microbiol.* **25**, 396—402.
8. Williams, R.P., R.H. Scott, D.V. Lim and S.M.H. Qadri, 1976. Macromolecular synth esis during biosynthesis of prodigiosin by *Serratia marcescens*.*Appl. Environ. Microbiol.* **31**, 70—77.