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Studies on the Microbial Pigment (V)

The effect of some detergent on pigment formation in *Serratia marcescens* strain P

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微生物의 色素에 關한 硏究(第 5 報)

—色素形成에 미치는 界面活性劑의 영향—

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ABSTRACT

In order to study on the pigment formation of *Serratia marcescens*, the synthesis of prodigiosin was examined in the presence of a wide range of concentration of detergents. A high elevation of pigment formation was obtained in case of the treatment with SDC and SAP. And the population growth of the bacteria was increased by SDC and SAP, in the concentration of optimum concentration of pigment formation. The alkaline phosphatase activity was also increased in the treatment of SAP, SDC and SDS. The possible mechanism of the detergents on enhancement of pigment formation could be explained by an increase of enzyme activity and membrane transport.

INTRODUCTION

Serratia marcescens produces the red pigment prodigiosin which has some antimicrobial activity(Williams, 1973). Prodigiosin is the secondary metabolite(Williams, 1973; Williams *et al.*, 1976) and the formation of prodigiosin is inhibited by some macromolecules(Blizzard and Peterson, 1963) and the precursor of prodigiosin is proline(Lim *et al.*, 1977). The enhancement of pigment formation was obtained in the presence of low concentration of inorganic phosphate (Witney *et al.*, 1976) and sodium dodecyl sulfate(Feng *et al.*, 1982). Its biosynthesis is influenced by various environmental and nutritional factors(Ahn *et al.*, 1977; Ahn *et al.*, 1978) but the complete biosynthetic pathway of prodigiosin in *Serratia marcescens* remains uncertain.

Recently, we observed that the population growth of *Escherichia coli* was increased by some detergents(Lee *et al.*, 1983). In this study, the bacterial growth and pigment formation were observed for determing the pigment as secondary metabolite by the use of detergents. And the effect of detergent on alkaline phosphatase that was realted to synthesis of secondary metabolites(Witney *et al.*, 1977) was examined.

MATERIALS AND METHODS

Chemicals:

Ginseng saponin was purified by the use of methanol(Lee *et al.*, 1983). Sodium dodecyl sulfate(SDS), sodium deoxycholate(SDC) and Triton X-100(TX-100) were purchased from Sigma chemical Co. And other reagents were analytical grade.

Organism and culture:

Serratia marcescens strain P, isolated in our laboratory(Ahn *et al.*, 1977), was used in this experiment. The organisms were grown at 30°C on nutrient broth(Difco Co.). For experiments with detergents, various concentrations of detergents were added to media after filtered(millipore, 0.45μm), aseptically.

Bacterial count:

Bacterial counts were determined by optical density in a spectrophotometer(Schmadju UV-150-02) at 350nm. It was checked at an interval of four hours until 40hrs cultured.

Extraction of pigment:

Ten milliliters of liquid culture, 0.1ml of 1N H_2SO_4, and 5ml of *n*-buthanol were added to vials. After the solutions were well shaken with tube stirrer, the solutions were taken carefully and determined the absorbancy of the supernatant with spectrophotometer at 540nm. The amount of pigment was checked at an interval of four hours until 70hrs, after 10 hours.

Preparation of the enzyme:

After 10hrs incubation, the culture were centrifuged at 3,000×g for 30min in a high speed centrifuge(Beckman JA-21). The cells were washed twice with 10mM Tris-HCl buffer, pH 7.2. The suspended cells were sonicated for 30 min, and the homogenate obtained was centrifuged at 30,000×g for 30min. The membrane fraction was used to determine enzyme activity.

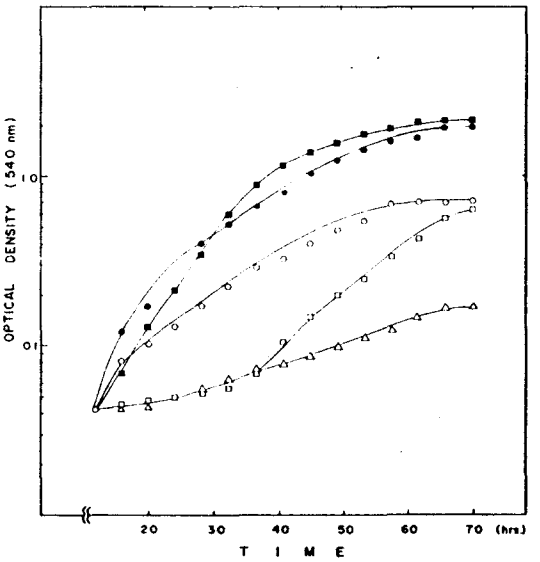

Fig. 1. Effect of detergent on pigment formation
—○—: Control —●—: SDC
—□—: SDS —■—: SAP
—△—: TX-100

Enzyme assay:

The activity of alkaline phosphatase was determined by the method of Torriani(1960), with *p*-nitrophenyl phosphate. The enzyme activity was measured by spectrophotometer at 400nm. One unit of enzyme activity is equivalent to the change of 0.01 OD change per minute per mg of protein, in 0.1M glycine buffer, pH 9.8.

RESULTS

1. Effect of detergents on pigment formation.

When cells were grown in the various concentration of detergents, the red pigment prodigiosin was significant enhanced by SDC and saponin(SAP). In the treatment with SDS, final amount of pigment was increased as much as control group, but the pigment formation was inhibited by TX-100(Fig. 1). The optimum

Table 1. Effect on Detergents on Pigment Formation

Amount of detergents	Optical density
Control	0.740
SAP 0.1%	1.131
0.08%	1.725
0.05%	1.217
0.03%	1.055
TX-100 0.1%	0.131
0.08%	0.180
0.05%	0.143
0.03%	0.121
SDC 0.1%	1.121
0.08%	1.214
0.05%	2.010
0.03%	1.340
SDS 0.1%	0.254
0.08%	0.478
0.05%	0.685
0.03%	0.520

concentration of detergents on pigment formation was SDS: 0.05%, SDC: 0.05%, TX-100: 0.05%, SAP: 0.08%, respectively (Table 1).

2. Effect of detergents on bacterial growth. In the treatment of detergents, bacterial population was increased by SDC and SAP, but decreased in the treatment with TX-100 and SDS. The concentration of detergents, applied to media, is the optimum concentration of pigment formation (Fig. 2).

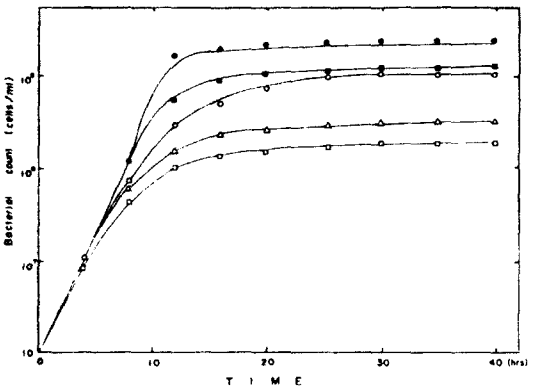

Fig. 2. Effect of detergent on bacterial growth
—o—: Control —•—: SDC —□—: SDS
—▪—: SAP —△—: TX-100

3. Effect of detergents on alkaline phosphatase. The effects of some detergents on the enzyme activity were examined (Table 2). SDS and SDC were applied in the concentration of 0.1μM to 0.5μM, and SAP and TX-100 were 10^{-4}% to 10^{-1}%, respectively. It was found that SAP was the most effective activator on alkaline phosphatase. And SDS and SDC were also increased up to about 20% as compared with control.

Table 2. Effect of Detergents on Alkaline phosphatase

Amount of detergents	Enzyme activity (Unit)*	% of increase
Control	1.63	0
SAP 10^{-4}%	1.63	0
10^{-3}%	1.63	0
10^{-2}%	1.97	20.9
10^{-1}%	2.56	57.1
TX-100 10^{-4}%	1.56	−4.3
10^{-3}%	1.69	3.7
10^{-2}%	1.53	−6.1
10^{-1}%	1.09	−33.8
SDC 0.1μM	1.81	11.0
0.2μM	1.91	17.2
0.3μM	1.91	17.2
0.4μM	1.94	19.0
0.5μM	1.88	15.3
SDS 0.1μM	1.81	11.0
0.2μM	1.84	12.9
0.3μM	1.88	15.3
0.4μM	1.94	19.0
0.5μM	1.91	17.2

* Unit means the change of 0.01 OD change per minute per mg of protein.

DISCUSSION

Prodigiosin considered to be secondary metabolite according to the fact that the pigment occured when the bacterial growth was in stationary state. Almost the microbial pigments are the secondary metabolite (Anderews *et al.*, 1973; Gerber, 1975), and secondary metabolites may be located in the cell envelope of microorganisms, as is true of prodigiosin (Williams, 1973). Some nutrient factors, expecially tyrosine (Ahn *et al.*, 1978) and methionine (Qadri and Williams, 1973), are needed for optimal synthesis of prodigiosin. The antibiotics such as streptomycin and rifampicin are the potent inhibitors. And the inhibition of pigment formation by addition of certain antibiotics indicates that macromolecular synthesis is involved in biosynthesis (Qadri and Williams, 1972; Williams *et al.*, 1976). The anionic detergent SDS enhances the production of prodigiosin in *Serratia marcescens* 08 (Feng *et al.*, 1982).

S. marcescens synthesize the pigment under alkaline conditions (Williams, 1973). And the alkaline conditions were characteristics for production of other secondary metabolites.

In this report, SDC and SAP increased bacterial population growth and pigment formation. Lee *et al.* (1983) suggested that these detergents were used as an energy source or stimulator of the membrane transport in *Escherichia coli*.

The alkaline phosphatase activity was also increased in the treatment of SDS, SDS, and SAP. Alkaline phosphatase activity is related to secondary metabolism (Witney *et al.*, 1977). Es-

pecially, alkaline phosphatase activity is increased just before the synthesis of the secondary metabolite (Gerber, 1975).

When SDS is in direct contact with a gram-negative bacterial cell, the detergent may interact with the cell wall lipoprotein and lipopolysaccharides (Shafa and Salton, 1960). And TX-100 and SDC also usually bind to membrane proteins (Clarke, 1975; Makino *et al.*, 1973). Feng *et al.* (1982) suggested that SDS may bind certain components in the cell envelope of *S. marcescens* 08. This binding may increase an extra negative macromolecular site needed for the binding and/or synthesis of the positively charged prodigiosin or for its precursors to condense. And in the previous study (Kim *et al.*, 1984), palmitoylcarnitine and saponin represented a significant increase of nutrient uptake.

By the result of above, we suggested that the enhancement of pigment is related not only to the effect of extra negative macromolecular site but also to the effect of detergents on enzyme activity and membrane transport.

적 요

*Serratia marcescens*를 계면 활성제 처리하에서 배양한 결과 SDC와 SAP의 처리군에서 색소형성의 증가를 보였으며, 색소 형성에 있어 가장 효과가 좋았던 처리 농도에서 개체군의 성장을 관찰한 결과 SDC와 SAP의 처리군에서 역시 그 성장효과가 잘 나타났다. 또한 2차 대사과정에 있어 그 역활이 큰것으로 밝혀진 alkaline phosphatase의 효소 활성도에 미치는 이들 계면활성제의 역활을 실험한 결과 SAP처리군에서 매우 높은 증가 효과를 보였으며 SDC와 SDS의 처리군에서도 20%정도의 높은 증가효과를 나타내었다.

이상의 결과로 고찰하여 볼때 *Serratia marcescens*의 색소형성의 증가현상은 이들 계면활성제들이 세포막에 있어 영양물질의 투과를 돕거나 효소활성도를 증가시킴으로 나타난다고 판단되었다.

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