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Effect of Triol Fraction of Ginseng Saponin on Filamentous Formation through Reduction in C-AMP Concentration in *Bacillus subtilis*.

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*Bacillus subtilis*에서 인삼 saponin의 triol fraction에 의한 C-AMP 농도 감소가 filament생성에 미치는 효과

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Abstract

Bacillus subtilis grown on medium containing 0.1% triol fraction, 0.005% sodium deoxycholate, and 0.05% taurocholate respectively was shown to grow as filamentous form and contain 2.3×10^{-5}M, 1×10^{-5}M, and 2.3×10^{-5}M of intracellular C-AMP, respectively. The concentration was 3 or 4 times lower than that of the control. But concentrations of extracellular C-AMP were similar to that of the control. Such decrease in C-AMP concentration was shown to parallel with decrease in autolysin activity indicating 40% and 20% lower activity of *Bacillus subtilis* grown on medium containing 0.05% and 0.1% triol fraction respectively than that of the control. The activity was also shown to be in inverse proportion with the formation of filament.

I. Introduction

It is frequently observed that certain bacteria grow as long filament under conditions such as raising temperature[7] and pH[14], addition of C-AMP to medium[16] and ginseng saponin fraction[4] as well as in certain bacteria defective in autolysin production [8 9]. Autolysin with several autolytic anzymes is believed to participated in wall growth division and shape maintenance of growing cells but their true physiological role is controversial [7 9 12].

We have reported ealier that *B. subtilis* grows as long filament, and septum formation is uncomplete and irregular under electron microscope[4]. We have also reported that there is reduction in C-AMP amount in *E. coil.* K-12 by addition of ginseng saponin[5]. Such observations have prompted us to eamine whether there are any changes in amount of C-AMP and autolysin activity in filamentous *B. subtilis* which can be induced by triol fraction of saponin.

II. Materials and Methods

All materials and methods are same as described elsewhere[2 4 5] with following additions.

Preparation of autolysin and autolysinless cell wall

Crude autolysin for enzyme source and autolysinless cell wall for substrate were obtained from *B. subtilis* (ATCC 6651) as described elsewhere[6]. Isolation of extracellular autolysin was made as described[15].

Addition of C-AMP, triol fraction of saponin, sodium deoxycholate, and taurocholate to medium

The amount of C-AMP was added to medium as described[16] and even larger and lower. The concentrations of triol and diol fraction were from 0.5% to 10^{-2}%. The concentrations of sodium deoxycholate and taurocholate were from 0.05% to 0.1%.

III. Results

The long filamentous *B. subtilis* was not observed and even short as expected when even higher and lower amount of C-AMP applied by Utsumi *et al.*[16] was used. However, under same condition except adding C-AMP, saponin fraction (0.05-0.1%) and saponin structure-like compounds such as deoxycholate (0.005%) and taurocholate (0.05-0.1%) were shown to induce filament formation (Fig. 1) as reported elsewhere[4]. However, the effect of saponin structure-like compounds was less than that of sapon in fraction. However, no filament was observed by diol fraction.

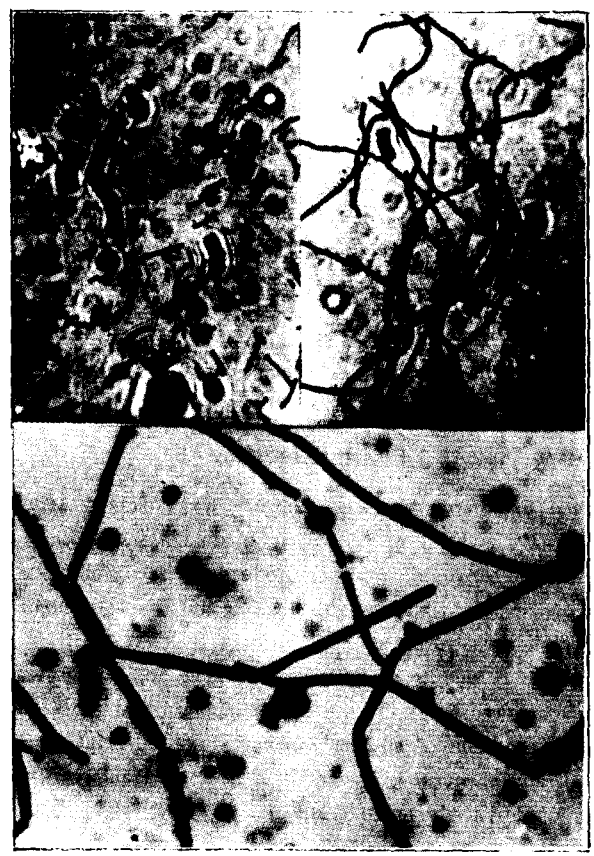

Fig. 1. Photographs of *B. subtilis* grown on medium containing triol fraction of ginseng saponin, and sodium deoxycholate(X600) and without(X1000)(anticlockwise from bottom).

The autolysin activity of filamentous and non-filamentous B. subtilis

Crude autolysin was obtained from filamentous cells grown on medium containing saponin and non-filamentous cells grown on medium without saponin under same condition and activity of both was compared (Fig. 2). Initial velocity of autolysin in filamentous cells was 2-3 times greater than that in non-filamentous cells. However, decrease in autolysin activity was not proportional to increase in concentrations of saponin (all data not given). Deoxycholate and taurocholate were shown to have less effect on autolysin activity at very limited concentration. Extracellular autolysin activity was not observed even when larger amount of supernatant[15] was used.

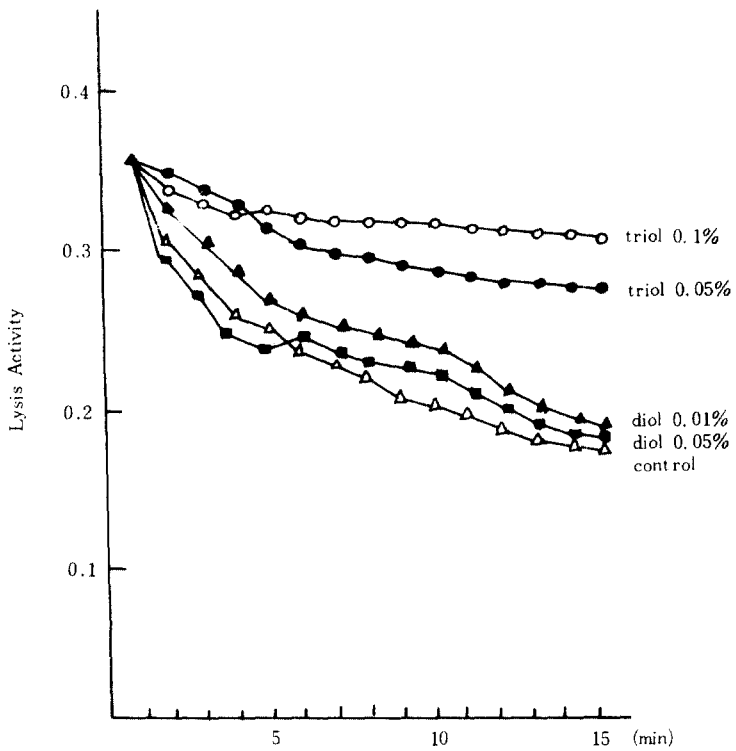

Fig. 2. Comparison of autolysins obtained from *B. subtilis* grown on mediun containing diol and triol fraction of ginseng saponin, and without saponin.

The level of C-AMP in filamentous and non-filamentous B. subtilis

The amounts of C-AMP from filamentous and non-filamentous *B. subtilis* was shown (Fig. 3). The amount of intracellular C-AMP in filamentous *B. subtilis* was significantly smaller than that of non-filamentous *B. subtilis* (one third). Such trends held true for deoxycholate and taurocholate. However, there was no big difference in extracellular C-AMP (Fig. 4). Such trends in the amount of C-AMP is in good agreement with previous results[5].

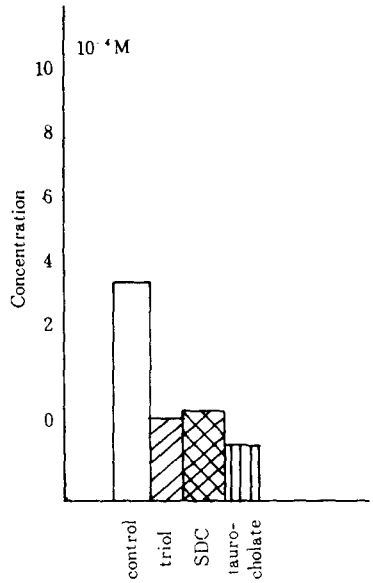

Fig. 3. Comparison of intracellular C-AMP extracted from *B. subtilis* grown on medium containing triol fraction of ginseng saponin, sodium deoxy-cholate, taurocholate, and without them.

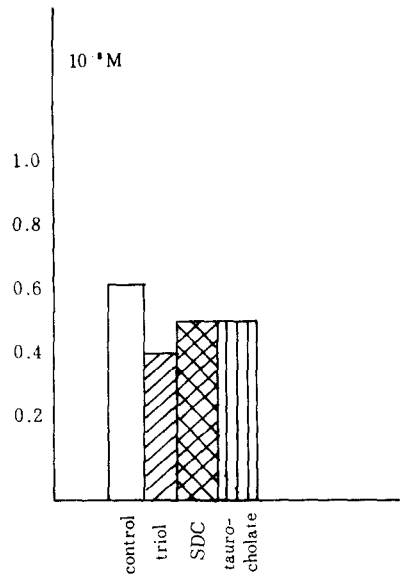

Fig. 4. Comparison of extracellular C-AMP excreted by *B. subtilis* grown on medium containing triol fraction of ginseng saponin, sodium deoxy-cholate, taurocholate, and without them.

IV. Discussion

As synthesis of number of protein in microorganism is controlled by C-AMP, it is possible that filamentous formation of microorganisms including *E. coil.* is induced by C-AMP[3] or/and reduction in the total amount of protein[1], especially autolysin. In this sense, the expected results seem to be obtained in some strain but not in others[3]. Our results belongs to the latter because of no evidence of filamentous formation in *B. subtilis* induced by saponin, deoxycholate, and taurocholate respectively has lower concentration of intracellular C-AMP than that of non-filamentous *B. subtilis.* However, concentration of extracellular C-AMP is almost similar between filamentous and non-filamentous *B. subtilis* and lower than that of intracellular C-AMP. The mechanism by which the cellular concentration of C-AMP is regulated on is not understood at present. Evidently the activities of at least two enzymes, i.e., the synthetic adenylate cyclase and the degradative C-AMP phosphodiesterase, affect the cellular concentration of the cyclic nucleotide. It has been suggested that the release of C-AMP into medium also play regulatory role[13] and C-AMP phosphodiesterase can not be the primary site of regulation[3]. Concentrations of extracellular C-AMP in all medium free of cell are 10^{-6}M order, which seems to be regulatory concentration in *B. subtilis* as suggested[13] and 10^{-5}M order appears to be critical for protein synthesis. If it is true, we may suggest that saponin fraction reduce concentration of intracellular C-AMP through unknown mechanism, which produce less autolysin which in turn seems to be critical factor for filament formation. Preliminary as results are, several microorganisms are shown to have lower concentration of C-AMP by addition of saponin fraction (unpublished results). But the mechanisms are to been seen.

요 약

0.1% triol fraction, 0.005% sodium deoxycholate 및 0.05% taurocholate를 각각 함유하는 배지에서 *Bacillus subtilis*를 생육시 filament를 형성 하였으며, intracellular C-AMP 의 농도는 각각 2.3×10^{-5}M, 1.5×10^{-5}M, 2.3×10^{-5}M이었다. 이 농도는 대조군에 비하여 3~4배가 낮은 것이다. 반면에 extracellular C-AMP의 농도는 각각 0.4×10^{-8}M, 0.5×10^{-8}M, 0.5×10^{-8}M로 대조군과 유사한 농도였다. 이러한 intracellular C-AMP의 농도의 감소는 autolysin의 감소를 동반하여 0.05% 및 0.1% triol fraction을 함유한 배지에서 생육한 *Bacillus subtilis*의 효소 활성은 대조군에 비하여 각각 40% 및 20% 였다. 그리고 이 활성도와 filament의 길이는 반비례하는 경향을 보였다.

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