Studies of the CTC-resistance of *Bacillus cereus* var. *mycoides*—III.

Comparative Study of Nutritional Requirement of strains both CTC-sensitive and CTC-resistant

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In the examination various organic compounds, which would seem to be the growth factors of strains both CTC-sensitive and CTC-resistant or either strain of them, were tested in terms of the additives to the Minimal 10 (M10) medium. The results obtained were as follows; 1) CTC-resistant strain grown in the M10 medium was not affected by supplement of any single amino acid, but grew best on casamino acids. The growth of it was not accelerated only by B-vitamins, but also by various bases of nucleic acid; 2) On the contrary, the CTC-sensitive strain could not grow in the M10 medium on supplement of either casamino acids, B-vitamins, or the combination of both components. However, it grow on purine bases of either adenine or of guanine. Additionally, the supplement of casamino acids and pyrimidine bases were more stimulative for its growth, 3) Comparatively growth of the CTC-sensitive strain in the medium fortified by the purine bases and the casamino acids were roughly identical to that of basal medium supplemented with yeast extract.

In the previous paper, the authors suggested that the differences of nutritional requirement between the CTC-sensitive strain and the CTC-resistant strain of *Bacillus cereus* var. *mycoides*, in the THORN's Minimal 10 (M10) medium, play a part of a key to solve the antibiological and the resistant mechanisms of CTC. In this paper, it was ascertained that the CTC-sensitive strain required the purine bases for their growth, but the CTC-resistant strain did not the purine bases. In other words the CTC-resistant strain may be able to synthesize the purine bases from the constituents, presumably glucose and some amino acids in the M10 medium. Since the growth of bacteria is initiated with DNA biosynthesis, the different requirement of the bases, observed in the both strains, is due to the different path way of DNA biosynthesis. If the CTC inhibits only the path way of CTC-sensitive strain, it is not so surprising to make the hypothesis that in the medium containing CTC in the bacteriostatic level of the sensitive strain, the sensitive strain does not grow but the resistant strain grows.

Experimental

Effect of the additives on the growth of both CTC-sensitive and resistant strains: In the examination, the authors expected that the difference of nutritional requirement

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between the both strains might be observed in the M10 medium by supplement of various additives. The additives and their amounts added to 10 ml of the M10 medium were as follows: 1) single amino acid; 1.6 mg of each amino acid, in addition to those namely, glutamic acid, alanine, valine, leucine, isoleucine, serine, and threonine was added into the M10 medium: 2) all amino acids; all amino acids besides those in the M10 medium were added into the M10 medium with 0.8 mg of each amino acid, therefore, such medium was considered as all amino acids medium. The supplement of 0.1 mg of casamino acids in the M10 medium was also examined in comparison with the supplement of all amino acids: 3) vitamins, single vitamin was added into 10 ml of the M10 medium with the following amount; thiamine 100 mr, pantothenic acid 200 mr, pyridoxal 100 mr, niacin 100 mr, folic acid 2 mr, biotin 1 mr, and cyanocobalamine 1 mr, respectively. All vitamins were added into 10 ml of the M10 medium at the concentration of half amount of individual vitamin mentioned above: 4) bases; 50 r of each base of guanine, adenine, cytosine, thymine, and uracil were added individually in the M10 medium fortified with all vitamins and all amino acids or casamino acids: 5) 25 r of each purine and pyrimidine bases were added into the M10 medium: 7) 30 mg of yeast extract was added into M10 medium. Growth experiment was carried out by using the different two strains of Bacillus cereus var. mycoides, one being CTC-resistant and another CTC-sensitive. The subculture was made in the aquatic penassay medium at 30°C for 24 hours incubation. The light inoculum was used in this examination. The growth effect of the additives was measured by the turbidity at 540 mμ in comparison with the M10 medium only.

Results

The effect of amino acids on the growth of bacteria was shown in Fig. 1. As shown in the figure, the CTC-sensitive strain did not grow by any supplement of amino acids, even by that of casamino acids, while the growth of the CTC-resistant strain was observed in the M10 medium without any supplements. On the other hand, this organism grew fully by supplement of the casamino acids or all amino acids. The supplement of any single amino acid did not give any growing effect on the CTC-resistant strain as comparison with the growth in the M10 medium only. However, its growth was markedly increased by the supplement of either casamino acids or all amino acids. The result of the vitamin-supplement was shown in Fig. 2. The supplement of any vitamins, or even the mixture of all vitamins was not able to contribute on the growth of the CTC-resistant strain as well as on that of the CTC-sensitive strain. The highest effect was obtained in the supplement of yeast extract in the both strains. The yeast extract was suitable for the growth of both strains, however, the effect-difference of yeast supplement between the both strains was only observed in the 24 hours incubation, nevertheless, it was not
Fig. 1. Growth behavior of both CTC-sensitive strain and CTC-resistant strain in the M10 medium supplementing various amino acids

- CTC-sensitive strain
- CTC-resistant strain

* 1 Each amino acid was supplemented in M10 medium
* 2 All amino acids, casamino acids, and each amino acid were individually supplemented in the M10 medium

Fig. 2. Growth behavior of both CTC-sensitive strain and CTC-resistant strain in the M10 medium supplementing various vitamins

- CTC-sensitive strain
- CTC-resistant strain

* Each vitamin was supplemented in the M10 medium

Fig. 3. Growth behavior of both CTC-sensitive strain and CTC-resistant strain in various bases

- CTC-sensitive strain
- CTC-resistant strain

* Each base was supplemented in the M10 medium

Ascertained in the 48 hours incubation. Finally, the growth effect by bases was demonstrated in the Fig. 3. As shown in the figure, the CTC-sensitive strain did grow only by the supplement of either guanine or adenine (purine bases), but it did not grow by the other bases, cytosine, uracil, and thymine (pyrimidine bases). When the both purines were compared, the growth by guanine was slightly higher than that by adenine. Whenever the M10 medium was fortified with adenine, guanine, and casamino acids, the growth of the CTC-sensitive strain seemed to reach the maximal growth obtained in the penassay medium, however, the growth fell considerably, compared with that in the M10 medium supplementing the yeast extract. The growth in the M10 medium supplemented with purine bases, pyrimidine bases, and casamino acids was identical to that in the M10 medium containing yeast extract.
Discussion

The growth of both CTC-sensitive and CTC-resistant strains in the M10 medium was already published as a difference in which CTC-resistant strain grew, but CTC-sensitive strain did not. In this study, it was the M10 medium depended mainly upon the bases requirement of the both strains, namely that the CTC-sensitive strain did not grow without the purine bases, while the CTC-resistant strain did grow. Since these bases are one of the important compounds to build up the DNA in the growth of microorganism, the authors assumed that the CTC-sensitive strain may be devoid of the capacity to biosynthesize DNA from the organic compounds, presumably, glucose and some amino acids contained in the M10 medium; whereas the CTC-resistant strain seems to have such capacity. The repeating incubation of the sensitive strain in the CTC-containing medium presumably make a mutant capable of biosynthesis of DNA from glucose and some amino acids. As another interpretation, it is considered that the mutant having the capacity of DNA biosynthesis might have increased during the repeating cultivation. The authors demonstrate that the CTC may inhibit the DNA biosynthesis from the purine bases, accordingly the CTC-sensitive strain having such path way may cease their multiplication in the CTC-containing medium. On the other hand, the CTC-resistant strain may take another path way for DNA biosynthesis, therefore CTC may not interfere the path way of the CTC-resistant strain growing in the CTC-containing medium.

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Reference

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