Bacillus cereus var.mycoides のCTC耐性に関する研究II

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Studies on the CTC-resistance of *Bacillus cereus var. mycoides*—II. Properties of CTC-resistant strains

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In order to resolve the antibiotic mechanism of CTC, the resistant strain obtained by repeating culture in CTC-containing medium and the sensitive strain, ie., the original, were compared on the basis of morphological and physiological properties. The results obtained were as follows;

1. No remarkable differences in characteristics or morphology were observed between the original and the resistant strains.
2. The most striking difference was observed in the nutritional requirements. That is, the original could not grow in M 10 medium, while the resistant did well.
3. Delayed spore formation was observed in the resistant compared with the original.

In the previous paper[1], the authors ascertained that *Bacillus cereus var. mycoides* adapts to CTC by repeating culture in CTC-containing medium. In the paper, isolation of CTC-resistant strain, stability of it, and its characteristics were described, especially in comparison with the original strain. It was ascertained that the resistant strain isolated from repeating culture was comparatively stable, not being reversible on its resistant character. Accordingly the authors supposed that the strain might be a mutant from the original strain.

Experimentals and Methods

Isolation of resistant strain: In order to isolate the resistant strain from repeating culture, the organisms in the culture were smeared on the penassay agar plate containing 0.1 ppm CTC. After being incubated for 24 hours at 30°C, the clumps on the agar plate similar to the preceding one, immediately being incubated. The organisms appeared on the new plate were smeared again on the similar agar plate, and the colonies appeared on such plate were transferred on the agar slant and preserved in cold room after incubated for 24 hours.

To ascertain the stability of isolates, the isolates were incubated every day for 10 days on the CTC-free agar slant. After repeating the incubation, they were inoculated on a penassay agar plate containing 0.1 ppm CTC and incubated for 24 hours. The isolates grown on such medium seems to be stable to CTC, substantially, such organisms grow well irrespective of CTC concentration within 0.1 ppm.

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Characteristics of resistant strain: The following tests were examined to compare the characteristics between the resistant and the original strains; reduction of nitrate, starch hydrolysis, Voges Proskauer test, gelatin hydrolysis, saccharides hydrolysis, spore productivity, growth in citrate medium (Koser's citrate medium), growth in Minimal 1 (M 1) and Minimal 10 (M 10) media, CTC resistance, and oxygen consumption in glucose medium (Warlburg's manometric method). The major charcateristics were examined in accordance with the methods described in the "*Laboratory Methods in Microbiology.*"[2]

The examinations marked with asterisk in table 1 were carried out as follows: *1 Saccharides hydrolysis was carried out by Hugh-Leifson system, using each glucose, xylose, arabinose, sucrose, mannose, galactose, fructose, and lactose.

Table 1. Main characteristics of test organisms.

Characteristics		Result obtained	
		Original	Resistant
Colony form		Rhizoid	Crenated
Gram's stain		+	+
Motility		−	−
*1 Saccharides hydrolysis	glucose	−	−
	xylose	−	−
	arabinose	−	−
	sucrose	−	−
	mannose	−	−
	lactose	−	−
*2 Spore formation		+	+
MR test		−	−
VP test		−	−
Gelatin hydrolysis		+	+
Growth in citrate		−	−
Starch hydrolysis		+	+
Reduction of nitrate		+	+
*3 Growth in M1 medium		−	−
*3 Growth in M10 medium		−	+
*4 Growth in 0.1 ppm CTC		−	+
*5 Oxygen consumption		±	+

For more informations, see text.

*2 Spore productivity: In this examination, modified penassay medium, prepared by supplement of $MgSO_4$ 0.05 gm/L, to the usual penassay medium was used for spore formation. The test organisms were smeared on the agar plate by grass spleader, being incubated for several days at 30°C. Microscopic observation was carried on every day during the incubation. The spore staining was by Bartholomew and Mittwer's cold method. The specimens were prepared to pick up 10 parts at random from the agar plate, and the staining spores and vegetative cells were calculated at 10 microscopic sights.

In this method, 100 sights were calculated, and the result was demonstrated in Table 2 in terms of percentage of spores in whole cells.

Table 2. Spore formation of test organisms.

Incubation day	Spore yield %						
	1	2	3	4	5	6	7
original	50	59	90	95	98	97	98
resistant	0	0.1	0.4	15	30	42	90

*3 Growth in both Minimal 1 and Minimal 10 media:[3] The examinations were subjected to the methods devised by B. Thorne and Harold B. Stull. In the study, M 1 was simplest one containing only glutamic acid, sodium citrate, and glucose for carbon sources. M 10 was modified M 1 to which were added 6 amino acids; L-leucine, L-alanine, DL-valine, L-isoleucine, L-serine, and L-threonine. The growth of the both original and resistant strains in those media was measured by turbidity after 2 days incubation.

*4 CTC-resistance: CTC resistance was demonstrated by the growth behavior of both strains on the 0.1 ppm CTC containing penassay agar plate after 24 hours incubation.

*5 Oxygen consumption at glucose oxidation: Oxygen uptake of cells was measured by Warlburg's manometric method. Examination system was as follows: in main compartment, bacterial suspension 0.5 ml (OD 0.5), M/5 phosphate buffer (pH 7.5) 0.5 ml, and distilled water 1.0 ml; in side arm, M/10 glucose 0.5 ml, and in center well 15% KOH 0.5 ml were settled respectively. After the incubation, calibrations were carried on for 60 minutes at 10 minutes intervals.

Results and Discussion

Morphological differences from microscopic observation between the original and resistant strains were not distinguishable except the colony appearance, that is, the original stain grows rhizoid-like on the penassay agar plate, while the resistant strain grows crenated on the same medium. In the cell form the former was long chain, while the latter was single rods or short chain.

Main characteristics were similar with both strains except a few observations, i.e., CTC resistance, oxygen consumption as shown in Table 1 and Fig. 1, productivity of spore in Table 2, and growth in M 10 medium in Table 3. The original strain made the spore more quickly than the resistant strain did. The production of ca. 50% was observed within 24 hours in the

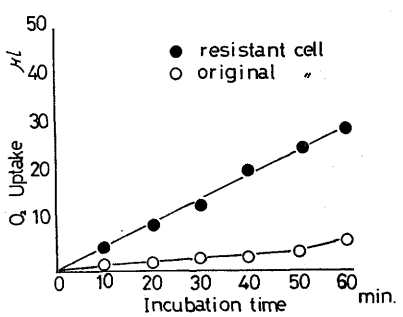

Fig. 1. O_2 uptake accompanied with glucose oxidation

Table 3. Growth in Minimal 1 and Minimal 10 media of test organisms.

	Growth in M1	(−log T) M10
Original	0	0
Resistant	0	0.9

48 hours incubation at 30°C

original, while ca. 90% in the resistant after one week. Growth in both M 1 and M 10 media was as follows: In M 1, no more growth was observed in both strains, whereas, most clear cut difference was observed on the growth in M 10 medium. The original strain did not grow in the medium for one week or more. To the contrary, the resistant strain did grow well within two days.

Concerning oxygen consumption in glucose medium, at the manometric assessment, the resistant strain seemed to demonstrate more oxygen consumption for glucose oxidation than the original strain as shown in Fig. 1. According to the results described previously, the resistant strain by repeating culture may be a mutant.

The resistant strain prepared by repeating culture was demonstrated by the features of mutant: the organism becomes more oxygen consumer, it requires different nutrient, and it sugests retardation of spore formation as compared with the original strain. The authors assume that the retardation of spore formation is due to the change of nutrient requirement.

The resistant strain did grow under the condition under which the original strain could not because of poor nutrients. It is not suprising therefore, that the original produces the spore quickly, but the resistant does slowly. Accordingly, nutrient requirement of both strains is important to study the mechanism of CTC resistance or antibiological mechanism of the antibiotics.

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