82. *Bacillus Thiaminolyticus*, a New Thiamin-Decomposing Bacterium.

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In feces of certain subjects the presence of aneurinase, an enzyme effective to decompose thiamin, was first noticed by C. Chang¹⁾ 1947. Very many investigations on this problem were made by C. Chang, M. Fujimiya, H. Misawa, Y. Horikawa and N. Kobayashi under direction of the late Dr. T. Shibata and later of Dr. D. Matsukawa in the Niigata Medical School. It was alleged that the subjects possessing this enzyme are apt to suffer from hypothyminosis. Efforts were made to find out from feces of the concerning subjects a bacterium producing this enzyme. It was found that this enzyme appeared in the supernatant fluid of bouillon culture of a kind of spore forming bacterium obtained from feces and that this is a phenomenon never seen with any other bacteria. Taking this phenomenon as an index, D. Matsukawa and H. Misawa²⁾ repeatedly cultured feces, heated at 100°C for ten minutes, in various media under different conditions and finally obtained peculiar colonies with ciliary processes by transplanting onto an agar plate. A bacterium was thus successfully isolated in 1949. Its bacteriological properties and fermentative activities were studied. Matsukawa and Misawa concluded that this bacterium belongs to the group of *Bacillus subtilis* Cohn according to Bergey's classification. Further studies in the morphology, the method of cultivation and changes in aneurinase in and out of the bacilli during their growth were made by them. It was also noticed that 1 cc. of 1:40-80 diluted supernatant fluid of bouillon medium of these bacilli, obtained under the optimal condition, was capable to decompose 1 γ thiamin in two hours at 37°C. and pH 5.6.

In the meantime, a subcommittee on research of aneurinase was set up in this Committee, and the study on the above bacillus was taken as one of the research subjects of this Subcommittee.

A. Fujita and his co-workers: H. Saeki, T. Kishida, T. Tashiro, and M. Yamadori^{3) 4)} of the Kitasato Institute and the Kyoto Prefectural University of Medicine investigated, beside various conditions for producing aneurinase, the bacteriological properties of the bacillus supplied from Matsukawa more in detail and pointed out the following as indice to distinguish it from *bacillus subtilis*:

1. The spores are situated terminally
2. The sporangia are swollen
3. Gram-negative or slightly positive
4. Citric acid cannot be utilized as the sole carbon source
5. Xylose, arabinose and mannitol are not decomposed
6. Indole is produced.

According to the above properties, this bacillus differs from *B. circulans* in the inactivity to xylose and arabinose and from *B. laterosporus* in that to mannitol; moreover the present bacillus shows a positive Voges-Proskauer reaction. It most resembles to *B. alvei* described by Bergey (1948), but in the former the cartridge-like arrangement is not marked and fat corpuscles cannot clearly be seen in glucose-agar culture. The features on agar plate seem to be alike like as rough strain of *B. alvei*, Fermentative activities of *B. alvei* to lactose, mannose and mannitol are reported to be variable, while the present bacillus is inactive to lactose and mannitol and active to mannose. These bacilli form a white opaque coat on agar plate in 48 hours, the surface of which becomes dry and it firmly adheres to the medium in 72 hours.

From the above findings Fujita and his co-workers believed that the present bacillus is either a variety of *B. alvei*, or a new one closely resembling it.

R. Kimura and S. Aoyama of the Medical Faculty, Kyoto University and R. Hayashi of the Yamaguchi Medical School^{5) 6)} succeeded to isolate a spore-forming bacterium with the action to decompose thiamin from soil in the field near Kyoto City. They observed its bacteriological characters and compared them with those of the four strains of the bacilli in feces supplied from Matsukawa.

Later they isolated 22 strains of spore-forming thiamin-decomposing bacteria from feces of 649 healthy adults and children in Ube City, Yamaguchi Prefecture and 33 similar strains from feces of 505 patients and healthy subjects in Kyoto City.

All the strains, 60 in number altogether, showed the following characteristics and were considered to belong to one species of Genus *Bacillus*:

1. Aerobic, mesophilic, spore-forming
2. Spores ellipsoidal, terminal to subterminal; walls thick; remnants of sporangia often adhering
3. Sporangia distinctly swollen terminally, clavate
4. Gram variable
5. Acid without gas from carbohydrates
6. Good growth on ordinary media

7. Acid from glucose peptone broth after seven days
8. Starch hydrolyzed, acid from sucrose
9. No acid from xylose and arabinose in the cultures of ammoniacal or organic nitrogen source
10. The fermentation of carbohydrates resembles that of *Bacillus alvei*, described in Bergey's Manual 6th edition, 1948,
11. Most strains reduce nitrates to nitrites
12. All strains produce indole (most moderately, some weakly)
13. On nutrient agar colonies of many strains adhere remarkably to the medium
14. The supernatant fluid of 48 hour broth cultures shows a powerful thiamin-decomposing ability (1 ml. of the fluid decomposes at least 2 γ of thiamin completely within 1 hour at 37°C and pH 6.5)

From the above descriptions, it is obvious that these strains are closely related to *B. alvei*, but differ in some respects, namely in the position of spore, reduction of nitrates and formation of indole. Observations of crucial importance were therefore made by Kimura and his co-workers with each two strains of *B. circulans*, *B. alvei*, *B. laterosporus* and *B. brevis*, which were kindly sent from Dr. R. E. Gordon and Dr. N. R. Smith of the U. S. Department of Agriculture, Beltsville, Maryland. All their endeavours failed to recognize the appearance of thiamin-decomposing effect in the supernatant culture fluids of these bacteria.

The above is the outline of the investigations made by members of our Committee on the problem of thiamin-decomposing bacilli. All the members have agreed to recognize the above bacilli to belong to one new species of Genus *Bacillus* and decided to assign to them the name "*Bacillus thiaminolyticus*".

References (All in Japanese)

- 1) C. Chang: Vitamins (A Japanese Journal of Vitaminology), 2: 174, 1950.
- 2) D. Matsukawa & H. Misawa: Proceedings of the Committee on Research of Vitamin B, 31:16, 1949.
- 3) A. Fujita, H. Saeki, T. Kishida, & T. Tashiro: ditto, 35:6, 1949.
- 4) A. Fujita, H. Saeki, M. Yamadori, & T. Kishida: ditto, 36:11, 1950.
- 5) R. Kimura & R. Hayashi: ditto, 37:16, 1950; 42:12, 1950; 43:5, 1950; 45:8, 1951.
- 6) R. Kimura & S. Aoyama: ditto, 45:8, 1951.

Addendum

After this manuscript had been written, the following report was presented to the author from Dr. Ren Kimura: R. Kimura and S. Aoyama have recently obtained several strains of *Bacillus*

thiaminolyticus from feces which more or less differ in properties from the former strains. The differences are as follows:

	The former strains	The new strains
On blood agar :	darkgreen, strongly hemolytic, adhering	yellow-brown, not, or less, hemolytic, not adhering
In broth :	pellicle is formed, the fluid transparent	no pellicle, the fluid turbid, precipitated.
Spore :	terminal	central to subterminal
Gelatin :	liquefied	no liquefaction
Milk :	coagulated	no coagulation
Glycerin :	decomposed	no decomposition
Catalase :	positive	negative
Indole :	positive	negative