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Biochemical characterization of *Bacillus thuringiensis*, 23 serovars

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Bacillus thuringiensis, 23 serovars의 생화학적 특성

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The 23 serovars of *Bacillus thuringiensis* strain were commonly gram-positive and motile, formed endotoxin crystals, produced acid and alkali in the KIA media, and acid from glucose, hydrolyzed starch, and reduced nitrate, but did not produce H_2S, oxidase and indole, did not decompose lysine, ornithine, phenylalanine, malonate, lactose, dulcitol, adonitol, inositol, sorbitol, arabinose, raffinose, rhamnose, maltose, and xylose. Eighteen serovars were positive in the MR tests and 15 in the VP tests. Four serovars used citrate. Five serovars produced urease, 5 CO_2 from glucose, 2 DNase, and 15 lecithinase. Twelve serovars decomposed arginine, 11 did sucrose, 2 manitol, and 9 salicin. Serovar *tohokuensis* did not hemolyze, but the others did.

The first isolation of *Bacillus thuringiesis* was reported by Ishiwata.[1] Thereafter numerous strains of *B. thuringiensis were isolated by several investigators*[2-18] *and so far classified into 23 serovars by their peritrichous flagella antigens.*[14-22] *The B. thuringiensis* is a gram positive and rod bacillus, has flagella, and produces one or more endotoxin crystals[23, 24, 25] and extracellular β-exotoxin[26, 27, 28, 29] both of which have insecticdal properties. The properties of the toxins were reported by many investigators, but the biochemical characteristics of the bacteria have been poorly reported. Hereafter, in this paper we describe biochemical differences among 23 serovars of *B. thuringiensis* to find taxonomical keys.

Materials and Methods

Bacteria and cultural conditions

Twenty-three serovars of *Bacillus thuringiensis* strain were used for experiments, which are listed in Table 1 and 2. They were obtained from the Pasteur Institute (Dr. H. de Barjac), France, and the Bacillus Genetic Stock Center, Ohio State University (Dr. D.H. Dean), USA. The serovars were cultured at 28°C with rotary shaking.

Media

The test media used for biochemical characterizations are listed in Table 1 and 2, and were prepared by manufacturer's instructions (Difco).

Biochemical characterization of the 23 serovars

Biochemical characterizations followed Cowan and Steel's procedures[38]. Endotoxin crystals in the cells were observed with phase contrast microscope and by staining[22,25].

Results and Discussion

Common characteistics of *B. thuringiensis*, 23 serovars

Twenty-three serovars of *B. thuringiensis* were cultured in BHI media and then transferred into characterization media. All the 23 serovars were gram positive, formed spores

Key words : *Bacillus thuringiensis*, serovars. common and differential characteristics.

Table 1. Common Biochemical Characteristics of the 23 serovars of *Bacillus thuringiensis*

Bt serovars		tests and results		
thuringiensis	1 K-6	KIA test: all produced acid and alkali.	H_2S, I, lys, orn, phe, mal, lac, ado, dul, ino, sor, ara, raf, rha, mat, oxi, xyl, dul tests: all were negative.	mot, str, nit, and glc tests: all were positive.
finitimus	2 K-7			
alesti	3a K-8			
kurstaki	3a3b K-9			
sotto	4a4b K-10			
kenya	4a4c K-11			
galleriae	5a5b K-12			
subtoxicus	6 K-13			
entomocidus	6 K-14			
aizawai	7 K-15			
morrisoni	8a8b K-16			
tolworthi	9 K-17			
darmstadiensis	10 K-18			
toumanoffi	11a11b K-19			
thompsoni	12 K-20			
pakistani	13 K-21			
israelensis	14 K-22			
indianae	15 K-23			
dakota	16 K-24			
tohokuensis	17 K-29			
kumamotoensis	18 K-30			
tochigiensis	19 K-31			
colmeri	20 K-33			

Explanation of abbreviations: KIA: Kligler Iron Agar, I: indole production, lys: lysine decarboxylase, orn: ornithine, phe: phenylalanine deaminase, mal: malonate, lac: lactose, ado: adonitol, dul: dulcitol, ino: inositol, sor: sorbitol, ara: arabinose, raf: raffinose, rha: rhamnose, mat: maltose, xyl: xylose, glc: glucose, mot: motility, str: starch, nit: nitrate, oxi: oxidase.

and endotoxin crystals, produced acid and alkali in the KIA media, produced gas in the glucose media, hydrolyzed starch, reduced nitrate into nitrite, and were motile (Table 1).

All the 23 serovars did not produce H_2S, indole, oxidase, and did not decompose lysine, ornithine, phenylalanine, malonate, lactose, dulcitol, adonitol, inositol, sorbitol, arabinose, reffinose, maltose, or xylose.

Differential characteristics of *B. thuringiensis*, 23 serovars

Five serovars (*kurstaki, subtoxicus, thompsoni, pakistani, tochigiensis*) were negative in the methyl-red tests, but the others were positive. Eight serovars (*subtoxicus, alesti, kenya, pakistani, dakota, kumamotoensis, colmeri, tochigiensis*) were negative in the Voges-Proskauer tests, but the others were positive (Table 2). In the citrate utilization, 4 serovars (*thuringiensis, finitimus, subtoxicus, thompsoni*) were positive, but the others were negative. In the salicin utilization, 9 serovars (*thuringiensis, finitimus, toloworthi, thompsoni, alesti, kenya, dakota, tohokuensis, kumamotoensis*) hydrolyzed it, but the others did not (Table 2).

Five serovars (*kurstaki, galleriae, toumanoffi, thompsoni, kenya*) produced urease, but the others did not. Twelve serovars (*thuringiensis, kurstaki, galleriae, aizawai, tolworthi, darmstadiensis, toumanoffi, thompsoni, kenya, pakistani, israelensis, colmeri*) decomposed arginine, but the others did not. Five serovars (*kurstaki, sotto, subtoxicus, entomocidus, israelensis*) produced acid from glucose media, but the others did not. Also, 11 serovars (*thuringiensis, finitimus, subtoxicus, morrisoni, toloworthi, alesti, entomocidus, pakistani, dakota, indianae, kumamotoensis*) decomposed sucrose, but the others did not. Two varieties (*subtoxicus, israelensis*) decomposed manitol, but the others did not (Table 2). Two serovars (*alesi* and *tochigiensis*) produced DNase. One serovar (*israelensis*) did not produce catalase, but the others did. Eight serovars (*thuringiensis, galleriae, subtoxicus, aizawai, morrisoni, tolworthi, darmstadiensis, tohokuensis*) did not produce lecithinase, but the others did. Only one serover (*tohokuensis*) did not hemolyze, but the others did (Table 2).

De Barjac et al.[14] reported that serovar *thompsoni* produces acethylmethylcarbinol, lecithinase, and urease; decomposes salicin and starch; formed acid from sucrose and mannose. In my results, however, no acid was formed from sucrose and mannose, and the others showed the same reactions.

Serovar *alesti* by de Barjac and Lemille[28] hydrolyzes salicin, and produces urease, but in our tests it does not produce urease. DeLucca, Simonson and Larson[18] reported that serovar *dakota* is motile, produces acethylmethylcarbinol, lecithinase, acid from surcrose, does not utilize salicin, and does not produce acid from mannose or DNase, but our results showed that serovar dakota did not produce acethylmethylcarbinol, or utilize salicin. By Ohba et al.,[22] serovar *tohokuensis* does not produce acethylmethylcarbinol or catalase; is negative in the MR test; reduces nitrate to nitrite; does not utilize citrate, lysine or phenylalanine; utilizes malonate, arginine, and starch; produces lecithinase H_2S, and DNase; produces acid from glucose, maltose, fructose, and inulin; does not produce acid from rhamnose, lactose,

Table 2. Differential Biochemical Characteristics of the 23 serovars of *Bacillus thuringiensis*

Bt serovars	tests	mr	vp	c	urs	arg	gas	suc	man	sal	dna	cat	lec	hem
thuringiensis 1	K-6	+	+	+	−	+	−	+	−	+	−	+	−	+
finitimus 2	K-7	+	+	+	−	−	−	+	−	+	−	+	+	+
alesti 3a	K-8	+	−	−	−	−	−	+	−	+	+	+	+	+
kurstaki 3a3b	K-9	−	+	−	+	+	+	−	−	−	−	+	+	+
sotto 4a4b	K-10	+	+	−	−	−	+	−	−	−	−	+	+	+
kenya 4a4c	K-11	+	−	−	+	+	−	−	−	+	−	+	+	+
galleriae 5a5b	K-12	+	+	−	+	+	−	−	−	−	−	+	−	+
subtoxicus 6	K-13	−	−	+	−	−	+	+	+	−	−	+	−	+
entomocidus 6	K-14	+	+	−	−	−	+	+	−	−	−	+	+	+
aizawai 7	K-15	+	+	−	−	+	−	−	−	−	−	+	−	+
morrisoni 8a8b	K-16	+	+	−	−	−	−	+	−	−	−	+	−	+
tolworthi 9	K-17	+	+	−	−	+	−	+	−	+	−	+	−	+
darmstadiensis 10	K-18	+	+	−	−	+	−	−	−	−	−	+	−	+
toumanoffi 11a11b	K-19	+	+	−	+	+	−	−	−	−	−	+	+	+
thompsoni 12	K-20	−	+	+	+	+	−	−	−	+	−	+	+	+
pakistani 13	K-21	−	−	−	−	+	−	+	−	−	−	+	+	+
israelensis 14	K-22	+	+	−	−	+	+	−	+	−	−	−	+	+
indianae 15	K-23	+	+	−	−	−	−	+	−	−	−	+	+	+
dakota 16	K-24	+	−	−	−	−	−	+	−	+	−	+	+	+
tohokuensis 17	K-29	+	+	−	−	−	−	−	−	+	−	+	−	−
kumamotoensis 18	K-30	+	−	−	−	−	−	+	−	+	−	+	+	+
tochigiensis 19	K-31	−	−	−	−	−	−	−	−	−	+	+	+	+
colmeri 21	K-33	+	−	−	−	+	−	−	−	−	−	+	+	+

Explanation of abbreviations: mr: methylred test, vp: Voges-Proskauer test, c: citrate, urs: urease, arg: arginine decarboxylase, gas: gas production from glucose, suc: sucrose, man: mannose, sal: salicin, dna-DNase, cat: catalase, lec: lecithinase, hem: hemolysis. +: positive reaction, −: negative reaction.

arabinose, xylose, mannose, rhaffinose, sorbose, manitol, or salicin; and does not hemolyze. Our results, however, showed that the serovar produced acethylmethylcarbinol; was positive in the MR test; reduced nitrate into nitrite; did not decompose arginine or malonate; and decomposed salicin.

요 약

Bacillus thuringiensis 균주중 23 serovars의 생화학적 특성을 비교연구하였다. 23개의 혈청성 균주는 모두 그람염색에 양성반응을 나타냈고, 내독소와 아포를 형성하며, KIA배지에서 산과 알칼리를 생산하고, 포도당배지에서 깨스를 생산하며, 전분을 가수분해하고, nitrate을 환원하나, H_2S 와 indole을 생산하지 않았고, 또한 lysine, ornithine, phenylalanine, malonate, lactose, dulcitol, adonitol, inositol, sorbitol, arabinose, raffinose, rhamnose, maltose, xylose을 분해치 않고, oxidase를 생산하지 않았다.

18 serovars는 MR 검사에서 양성이였고, 15 serovars는 VP 검사에서 양성이였다. 4 serovars 는 citrate을 이용했다. 5 serovars는 포도당 배지에서 산을, 2 serovars는 DNase를, 15 serovars는 lecithinase를 생산했다. 12 serovars는 arginine을, 11 serovars는 sucrose를, 2 serovars는 manitol을, 9 serovars는 salicin을 분해했다. serovar tochigiensis 만이 hemolysis를 하지 않았다.

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