Kor. J. Pl. Prot. Vol. 14, No. 4. 209~213, 1975 한국식물보호학회지 제14권 제4호

Studies on the Plant Pathogenic Corynebacteria(Ⅲ)

—The amino acid composition of some plant pathogenic bacteria—

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Corynebacterium 屬 植物病原細菌에 關한 硏究 (Ⅲ)

植物病原細菌의 아미노산 組成

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(接受 1975. 6. 16)

Abstract

This paper is a report of studies to determine the amino acid composition of plant pathogenic Corynebacteria and to assess whether these characteristics can be correlated with the taxonomic position of these organisms.

The results indicated that plant pathogenic Corynebacteria contained on average less cystein, trypthophane, histidine, phenylalanine, isoleucin and total protein than did the other genera of bacteria. However, in general, the quantities of both cell protein and amino acids contained in bacterial cells were characteristics of the species or an individual strain of the organism and were not related to its classification.

Introduction

In previous paper[4] the author reported that the plant pathogenic Corynebacteria are classified into 3 groups on the basis of cultural, morphological and physiological characteristics. Since then the author has attempted to determine whether the plant pathogenic Corynebacteria can be classified according to their amino acid composition. For this purpose, the cysteine, tryptophane, histidine, phenylalanine, isoleucine and cell protein content of bacterial cells were investigated and the results of these studies are presented in this paper.

Materials and methods

(a) Strains examined

Table 1 shows the organisms used in this study. Twenty-one strains of various bacteria were used; nine species of *Corynebacterium*, four of *Pseudomonas*, two of *Erwinia*, two of *Xanthomonas*, one of *Agrobacterium* and one of *Aerobacter*. Strains 12077 and 12155 of *C. fascians*, *C. insidiosum*(12157), *C. michiganens*(12158), *C. poinsettiae*(12161), *C. flaccumfaciens*(12156) and *C. vesiculare*(12165) were purchased from the Institute for Fermentation Research,

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Osaka, Japan, and the remainder were received from National Institute of Agricultural Science, Japan.

Table 1. Organisms used in this study

Genus	Species
Corynebacterium	*insidiosum*
	spedonicum
	michiganense
	rathayi
	fascians
	poinsettiae
	flaccumfasciens
	vesiculare
	oortii
Psendomonas	*eriobotryae*
	marginalis
	lachrymans
	mori
Agrobacterium	*radiobacter*
Aerobacter	*aerogenes*
Erwinia	*aroideae*
	milletiae
	carotovora
Xanthomonas	*pruni*
	oryzae

(b) Strain maintenance

The strains were maintained in Snieszko and Bond's medium of the following composition (percentage are w/v); Bactopepton 0.3%, bacto tryptose 0.3%, bacto yeast extract 0.3%, dextrose 0.3%. The pH was adjusted to pH 7.0 using NaOH. This medium supported good growth of all strains.

(c) Preparation of dried bacterial cells[2,5]

The organisms employed in this study were shake cultured at 160rpm at 25°C in Snieszko and Bond's medium and were harvested after 24 to 72 hours at which time it was estimated from previous experience that maximum growth had occured. The cells were washed 3 times with water in a centrifuge with the rotor running at 3,000rpm for 10min, and were then suspended in a small amount of water. These suspensions were treated with 10 times there volume of acetone cooled to -20°C and filtered through filter paper on asultion pump. This process was repeated twice more using 3 times as much aceton as cell suspension and then the cell were dried in a desiccator with phosphorus pentoxide.

(d) Assay of the cell protain content.[7]

To assay the cell protein content, the nitrogen content was determined by a modification of Nessler's method of Lang(1958). One hundred mg of the acetone dried bacterial cells were treated with 4ml of an analytical reagent containing 40g of K_2SO_4 and 2ml of $SeOCl_2$ in 250ml of distilled water and 250ml of H_2SO_4 and then they were hydrolysed at 320°C for 1 hour. Standard solution of $(NH_4)_2SO_4$ were also treated as above method and then Nessler's reagent was added using the method of Koch and McMeekin (1924). The coloration of test meterial was measured at 420mμ, and the nitrogen content of the cells were calculated from standard curve prepared from standard solutions of $(NH_4)_2SO_4$. The amounts of cell protein were estimated as 100mg of protein per 16g of nitrogen.

Table 2. Preparation of the standard N-solution

Solute	Solvent	Concenturation/ml
$(NH_4)_2SO_4$	H_2SO_4	1,000μg-N

(e) Assay of amino acids[3,6,8]

Hydrolysis of the dried cell materials was carried out by the methods shown in table 3. The hydrolysates obtained by this procedure were adjusted to pH 6.8, and aliquots were then diluted to volumes appropriate for microbiological assay. A uniform medium for the microbiological determination of the amino acid consti-

Table 3. Hydrolytical treatment of materials for the assay of amino acid

Amino acid	Treatment
L-cysteine	4N HCl, 120°C, 4 hours
L-tryptophane	4N NaOH, 120°C, 9 hours
L-histidine DL-phenylalanine DL-isoleucine	4N HCl 120°C, 8 hours

tuents of cell proteins using lactic acid bacteria is given in table 5. The lactic acid bacteria used for there tests(table 4) were received from Dr. Kakuo Kidahara, Laboratory of Applied microbiology, Tokyo University of Agriculture. The double strength medium was added at the rate of 5ml per test tube containing 5ml of the hydrolysats or standard amino acid. The tubes were then autoclaved at 10 p.s.i. for 5min and inoculated with lactic acid bacteria that had been washed 3 times with 0.85% sodium chloride solution in a centrifige with the rotor running at 3,000rpm for 10min. After the cultures had been incubated at 35°C for 20 hour they were sterilized at 100°C for 30min. The turbidity was measured at 640 mμ and the amounts of cysteine. tryptophane, histidine, phenylalanine and isoleucine in the bacterial cell protein were calculated from standard curves prepared from standard amino acid solutions.

Table 4. Test organisms used for the assay of amino acid

Test organisms	Amino acid
Leuc. mesenteroides P-60 (ATCC 8042)	L-cysteine
Leuc. citrovorum (ATCC 8081)	L-histidine
Lactobacillus arabinosus	L-tryptophane DL-isoleucine DL-phenylalanine

Table 5. Double concentration basal medium for microbioassay of amino acid with lactic acid bacteria

DL-alanine	22mg	Guanine HCl	10mg
L-arginine HCl	200mg	Xanthine	10mg
DL-aspartic acid	400mg	Glucose	20mg
L-cystine	100mg	Thiamine HCl	1mg
L-glutamic acid	500mg	Riboflavin	1mg
glycine	100mg	Pyridoxine	1mg
L-histidine HCl	100mg	Pyridoxal	1mg
DL-isoleucine	200mg	Ca-pantothenate	1mg
L-leucine	100mg	Nicotinic acid	1mg
L-lysine HCl	200mg	PABA	0.2mg
DL-methionine	200mg	Biotin	0.01mg
DL-phenylalanine	200mg	Folic acid	0.01mg
L-proline	100mg	Na-acetate	20mg
DL-serine	100mg	KH_2PO_4	500mg
DL-threonine	200mg	K_2HPO_4	500mg
DL-tryptophane	10mg	NH_4Cl	3g
L-tyrosine	100mg	$MgSO_4\ 7H_2O$	200mg
DL-valine	200mg	$FeSO_4\ 7H_2O$	10mg
Adenine H_2SO_4	10mg	$MnSO_4\ 4H_2O$	10mg
Uracil	10mg	NaCl	10mg

(Amounts per 1 liter of double concentration medium, pH 6.5-6.8)

Results and Discussion

Results for the tests of 21 cultures of bacteria, representing 19 species classified in 6 genera, are presented in table 6 and 7.

In order to make comparisons between cultures the amounts of cell protein are tabulated as a percentage of the dry cell weight and the amino acid content as g of amino acid per 16g of nitrogen. This designation approximates the percentage of a particular amino acid in the protein.

The results given in table 6 and 7 show that on average, plant pathogenic Corynebacteria contained less cy stein, trypthophane ,histidine, phenylalanine, isoleucine and total protein than did the other genera of bacteria. However, the quantities of cell protein and amino acids contained in bacterial cells were characteristic of the individual species or strain of the organism and were not related to its classification. For example, in the genus *Pseudomonas* the average total protein content was 66.5 percent and in the genus *Corynebcterium* it was 55.5 percent. However, with in the genus *Corynebacterium* the protein content range from 35.9 percent (*C. fascians* ATCC 12077) to 72.2 percent (*C. michiganense* ATCC 12158). Further, large differences were found between strains of the same species in plant pathogenic Corynebacteria. For example strains of *C. michiganense* had protein contents ranging from 61.3 to 72.2 percent and *C. fascians* from 35.9 to 41.9 percent.

Similar results were obtained from the study of amino acid cell contents (table 7). The cell protein of the bacteria used in this study contained 0.14 to 0.34 percent cysteine, 0.51 to 1.14 percent tryptophane, 1.70 to 3.56 percent histidine, 4.31 to 9.56 percent phenylalanine, and 11.08 to 22.21 percent isoleucine.

Variations in the proportions of amino acids and total protein content were found between strains as well as between species, particularly. in the case of *C. fascians* and *C. michiganense.*

Similar finidings previously been reported by R.F. Anderson et al [1]., studies on lysine., methionine and tryptophane content of microorganisms.

Table 6. Protein content of bacterial cells

Organism	Protein (% of dry weight)	Organism	Protein (% of dry weight)
Corynebacterium(avg)	55.2	*P. eriobotryae*	73.8
C. insidiosum(12157)	59.7	*P. marginalis*	53.1
C. sepedonicum	48.4	*P. lachrymans*	79.8
C. michiganense(12158)	72.2	*P. mori*	59.4
C. michiganense	61.3	*Agrobacterium radiobacter*	53.2
C. rathayi	51.9	*Aerobacter aerogenes*	65.0
C. fascians(12077)	35.9	*Erwinia*(avg)	61.5
C. fascians(12155)	41.9	*E. aroideae*	50.9
C. poinsettiae(12161)	62.9	*E. milletiae*	55.6
C. flaccumfasciens(12156)	58.4	*E. carotovora*	78.1
C. vesiculare(12165)	61.3	*Xanthomonas*(avg)	60.3
C. oortii	52.5	*X. pruni*	67.5
Psendomonas(avg)	66.5	*X. oryzae*	53.1

Table 7. Amion acid content of bacterial cells

Organism	Amino acid (g per 16gN)				
	Cysteine	Tryptophane	Histidine	Phenylalanine	Isoleucine
Corynebacterium(avg)	0.22	0.63	2.73	5.90	14.29
C. insidiosum(12157)	0.20	0.77	2.32	5.70	12.18
C. sepedonicum	0.15	0.52	2.68	4.42	15.69
C. michiganene(12158)	0.15	0.51	1.70	4.60	11.08
C. michiganense	0.15	0.56	2.20	4.31	11.10
C. rathayi	0.24	0.56	2.89	4.78	17.35
C. fascians(12077)	0.24	0.65	2.78	7.51	16.70
C. fascians(12155)	0.30	0.91	3.44	7.07	15.18
C. poinsettiae(12161)	0.29	0.75	3.12	6.37	13.37
C. flaccumfasciens(12156)	0.14	0.84	2.81	4.83	13.69
C. vesiculare(12165)	0.33	0.83	2.68	7.64	14.04
C. oortii	0.21	0.78	3.56	7.62	16.76
Aerobacter aerogenes	0.23	1.14	2.49	7.82	18.15
Agrobacterium radiobacter	0.23	0.90	2.03	7.79	18.82
Xanthomonas(avg)	0.26	0.80	3.16	8.72	19.85
X. pruni	0.23	0.77	3.35	7.88	17.48
X. oryzae	0.28	0.84	2.96	9.56	22.21
Pseudomonas(avg)	0.27	0.69	2.87	6.46	17.30
P. eriobotryae	0.24	0.79	2.74	4.60	14.64

P. marginalis	0.30	0.64	3.01	7.08	18.07
P. lachrymans	0.20	0.51	2.14	6.00	15.62
P. mori	0.34	0.83	2.98	8.15	20.88
E winia(avg)	0.28	0.85	2.95	7.40	17.73
E. aroideae	0.33	0.93	3.22	7.85	20.02
E. milletiae	0.27	0.94	3.09	7.44	16.54
E. carotovora	0.23	0.67	2.56	6.91	16.64

摘 要

본실험은 *Corynebacterium*속 식물병원세균에 관한 연구의 일환으로서 *Corynebacterium*속 식물병원세균의 종 및 속으로서의 특성을 구명하기 위하여 행하여진 것으로서 균체구성단백의 정량은 Lang의 Nessler 변법을 사용하였으며 균체 구성단백의 아미노산정량은 미생물 정량에 의하였다.

*Corynebacterium*속 식물병원세균은 타속의 병원세균에 비하여 다소 적은양의 균체 구성단백을 가지고 있었으며 균체구성단백의 아미노산; Cystein, Tryptophane, Histidine. Phenylalanine 및 Isoleucin에 있어서도 같은 결과를 나타내었다. 허나 이 현상은 종적 혹은 개체의 균주의 특성으로서 분류에는 관계를 갖지않고 있음이 밝혀졌다.

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The Nature of the Variety Tongil (Suweon 213-1) in Resistance to the Striped Rice Borer, *Chilo suppressalis* W.*

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(接受 : 1976. 1. 5)

Summary

This study was conducted to evaluate the nature of the variety Tongil (Suweon 213-1) in resistance to the striped rice borer, *Chilo suppressalis* Walker, comparing with those of Rexoro (susceptible check) and TKM-6 (resistant check) selected at IRRI.

1. The striped rice borer moths much more preferred the variety Tongil for oviposition than the varieties Rexoro and TKM-6. The variety Tongil had more egg masses and more number of eggs than the varieties Rexoro and TKM-6, while TKM-6 having more egg masses and more number of eggs than Rexoro. This reaction was consistent throughout the test regardless of the number of tillers per hill.

2. In laboratory, preference of larvae for feeding was studied with 5cm of stem pieces of the varieties. The results showed, in contrast to the case of ovipositional preference, that the striped rice borer larvae least preferred the stems of Tongil among the test varieties, while larvae much more preferred the stems of Rexoro than those of TKM-6.

3. The larval weights at 20 days later infested on the 40 day-old plants were the lowest on Tongil among the test varieties. On the variety Rexoro the larvae had heavier body weights(43.0mg), higher pupation (64.9%) and higher adult emergence (83.3%) than those on Tongil (larval weights 30.3mg, pupation 60%, adult emergence 60.7%) and TKM-6 (larval weights 35.7mg, pupation 56.3%, adult emergence 51.9%). The pupal weights, however, were not consistent among the test varieties and/or sexes in comparison with the larval weights, pupation and adult emergence above mentioned.

4. Field experiments indicated that the incidence of dead hearts at 70 days after transplanting was relatively higher on the variety Tongil (11.1%) than those on Rexoro (8.9%) and TKM-6 (8.4%), and the incidence of white heads at harvest was, in contrast to the dead hearts, lower on Tongil (9.8%) than those on Rexoro (27.4%) and TKM-6 (13.9%). At harvest lower larval survival observed on Tongil (49 larvae/40 hills) than those on the susceptible variety Rexoro (104 larvae/40 hills) and on the resistant variety TKM-6(70 larvae/40 hills). The average larval weights collected from three test varieties at harvest were 80.5mg from Tongil, 83.7mg from TKM-6 and 99.6mg from Rexoro.

5. Increased nitrogen fertilizer application to the variety Tongil, the striped rice borer damage was increased. Also, preference of larvae for feeding significantly increased with the increase of nitrogen fertilizer application.

6. Any specific association between the plant characters and striped rice borer resistance could not be found. The variety Tongil even having large number of tillers, short plant height, large stem, broad leaf, etc, had still high preference of moths for oviposition, low preference of larvae for feeding, low damage, and relatively high antibiosis.

7. Resistance of the variety Tongil to the striped rice borers seemed to be associated with the low feeding preference and the relatively high antibiosis, not associated directly with the ovipositional preference.

* Ministry of Sci. & Tech. R-75-42 1975. 24p.

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