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CELL WALL SUGARS AND AMINO ACIDS OF THREE IMMUNOLOGICAL TYPES OF *CORYNEBACTERIUM RENALE*

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Corynebacterium renale ATCC 19412 (*C. renale* immunological type I), strain 46 Hara (*C. renale* immunological type II), and strain 42 Fukuya (*C. renale* immunological type III) were similar in that they contained arabinose, galactose, and glucose as cell wall sugars, and alanine, glutamic acid, and *meso*-diaminopimelic acid as cell wall amino acids. The results indicate that strains 46 Hara and 42 Fukuya are similar to the species of human and animals parasites and pathogens of genus *Corynebacterium* in the cell wall components.

INTRODUCTION

Corynebacterium renale immunological types II and III[6] were found to be different from *C. renale* (*C. renale* immunological type I) from the results obtained from DNA homology[3] and numerical taxonomy[5]. Whether types II and III are new species of genus *Corynebacterium* or not is under investigation (YANAGAWA & HONDA, submitted for publication).

It has been known that the organisms of species of human and animal parasites and pathogens of genus *Corynebacterium* contain arabinose and galactose in the cell wall sugars, and alanine, glutamic acid, and *meso*-diaminopimelic acid in the cell wall amino acids[1,2].

The cell wall sugars and amino acids of the three immunological types of *C. renale* are described in the present communication.

MATERIALS AND METHODS

Strains *C. renale* ATCC 19412 (*C. renale* immunological type I), strain 46 Hara (*C. renale* immunological type II), and strain 42 Fukuya (*C. renale* immunological type III), were used.

Preparation of the cell wall The cell walls of the three strains were prepared as follows. Each bacterial suspension was sonicated, using a water-cooled sonic oscillator (Kubota Co., Tokyo), at 10 kc/sec full power for 1 hr, and washed by centrifugation twice at $4,347 \times g$ for 10 min. The pellet was suspended in 3 ml of distilled water,

layered onto gradients containing 20 to 50 % sucrose, and centrifuged at 1,200 × *g* for 30 min. Of the 2 or 3 bands formed, the top band, which contained the cell wall, was collected with a pipette. The cell wall fraction was dialyzed in distilled water overnight and centrifuged at 4,347 × *g* for 10 min. The pellet was resuspended in 0.05 M phosphate buffer (pH 7.6) and digested with crystalline trypsin (Sigma, E. C. class 3. 4. 4. 4) (0.5 mg/ml), ribonuclease (Sigma, E. C. class 2. 7. 7. 16) (0.2 mg/ml), and deoxyribonuclease (Sigma, E. C. class 3. 1. 4. 5) (0.5 mg/ml) at 37°C for 2 hrs. The digested cell wall was then centrifuged at 4,347 × *g* for 10 min, and the sediment was washed twice in distilled water, resuspended in 0.02 N HCl containing crystalline pepsin (Wako Pure Chem. Indust., Osaka, E. C. class 3. 4. 4. 1) (1 mg/ml), and incubated at 37°C for 18 to 24 hrs. After the peptic digestion, the cell wall material was washed several times in distilled water. The purity of the cell wall was checked by electron microscopy using the HU-12 A electron microscope (Hitachi Co., Tokyo).

Analysis of the cell wall sugars The sugars were analyzed by gaschromatography as follows. The purified cell wall was hydrolyzed with 5% methanolic HCl at 100°C for 3 hrs. The hydrolysate was extracted with petroleum ether. Of the two phases formed, the upper phase containing fatty acid was discarded, and the lower phase containing sugar components was completely evaporated in a vacuum. The sugar components reacted at 80°C for 5 min with trimethylsilyl ether (TMS) reagents (0.65 ml of hexamethyldisilazane, 0.5 ml of pyridine, and 0.4 ml of trimethylchlorosilane) were analyzed by gaschromatograph (Shimazu GC-64 Shimazu Co., Kyoto), using 3% OV-1 on chromosorb W packed into a 2 m glass column at 170°C.

Analysis of the cell wall amino acids The amino acid components of the cell wall were analyzed by an amino acid analyzer (Hitachi KLB-3 B, Hitachi Co., Tokyo), after hydrolysis of the cell wall with 6 N HCl at 105°C for 24 hrs.

FIGURE 1 *The purified cell wall of strain 46 Hara examined electron microscopically* × 10,000

RESULTS

1 Electron microscopical examination of the purified cell wall

The purified cell walls were examined electron microscopically (fig. 1). Few whole cells of *C. renale* were found in the purified cell walls.

2 Cell wall sugars

A gaschromatogram of TMS derivatives of the methyl hexosides of the cell walls showed the presence of arabinose, galactose, and glucose

TABLE 1 *Amino acid compositions of the cell walls of C. renale*

AMINO ACID	AMINO ACID (μ MOLE) PER MG OF CELL WALL					
	ATCC 19412		46 Hara		42 Fukuya	
Lysine	0.056	2.1%	0.075	2.1%	0.075	2.1%
Histidine	0.030	1.1	0.018	0.5	0.035	1.0
Arginine	0.028	1.0	0.042	1.2	0.056	1.5
meso-Diaminopimelic acid	0.376	13.9	0.504	14.3	0.440	12.5
Aspartic acid	0.096	3.6	0.121	3.4	0.172	4.9
Threonine	0.070	2.6	0.085	2.4	0.082	2.3
Serine	0.089	3.3	0.140	4.0	0.127	3.6
Glutamic acid	0.682	25.3	0.834	23.7	0.828	23.5
Proline	Trace		Trace		Trace	
Glycine	0.119	4.4	0.136	3.8	0.172	4.9
Alanine	0.720	26.7	0.897	25.5	0.888	25.2
Half-cystine	ND		ND		ND	
Valine	0.078	2.9	0.103	2.9	0.134	3.8
Methionine	ND		ND		ND	
Isoleucine	0.029	1.1	0.056	1.6	0.073	2.0
Leucine	0.078	2.9	0.108	3.1	0.140	4.0
Tyrosine	Trace		Trace		Trace	
Phenylalanine	Trace		Trace		Trace	
Glucosamine	0.162	6.0	0.214	6.0	0.183	5.2
Galactosamine	0.110	3.7	0.162	4.6	0.119	3.4

ND: not detected

in the cell walls of the three strains. Mannose was found in the cell wall of *C. renale* ATCC 19412 and strain 46 Hara, but not in that of strain 42 Fukuya. The ratios of arabinose : galactose : glucose : mannose in the cell walls were 10 : 1 : 0.5 : 0.8 (*C. renale* ATCC 19412), 6.6 : 1 : 0.1 : 0.3 (strain 46 Hara), and 8.0 : 1 : 0.1 : 0 (strain 42 Fukuya). Arabinose was the highest in amount. The three strains were thus similar in their cell wall sugars.

3 Cell wall amino acids

The cell wall amino acids are shown in table 1. The major cell wall amino acids of the three strains were glutamic acid, alanine, and *meso*-diaminopimelic acid. The mole ratios of these amino acids relative to the moles of glutamic acid were 1 : 1.0 : 0.5 (*C. renale* ATCC 19412), 1 : 1.1 : 0.6 (strain 46 Hara), and 1 : 1.1 : 0.5 (strain 42 Fukuya). The cell walls of the three strains contained small amounts of glucosamine, glycine, galactosamine, aspartic acid, serine, valine, leucine, threonine, lysine, histidine, isoleucine,

and arginine, and traces of proline, tyrosine and phenylalanine. Half-cystine and methionine were not detected. Thus, a similarity of the cell wall amino acids was found in the three immunological types of *C. renale*.

Discussion

The three immunological types of *C. renale* were found to be similar in that they contain arabinose, galactose, and glucose as cell wall sugars, and alanine, glutamic acid, and *meso*-diaminopimelic acid as cell wall amino acids. The results agreed with those reported by CUMMINS & HARRIS for the species of human and animal parasites and pathogens of genus *Corynebacterium*. Strains 46 Hara and 42 Fukuya are thus similar to the species of human and animal parasites and pathogens of genus *Corynebacterium* in the cell wall components.

Sugars of the type specific main antigen from strain 9 (*C. renale* immunological type I), strain 35 (*C. renale* immunological type II) and strain 42 Fukuya were reported by SHINAGAWA & YANAGAWA (1970); arabinose, glucose and mannose, but not galactose, were present commonly in the antigens of these strains. In the present study, galactose but not mannose was found in the cell wall.

CUMMINS & HARRIS reported that the cell walls of the species of human and animal parasites and pathogens of genus *Corynebacterium* contained diaminopimelic acid, but not lysine. In the present study, the three strains contained a large amount of *meso*-diaminopimelic acid and only a small amount of lysine. The amino acid analyzer used in the present study may be more sensitive in detecting small amounts of amino acids than the paper chromatography used by CUMMINS & HARRIS. Whether the small amount of amino acids present in the three strains used in this study were derived from the contaminated protoplasmic substances of the organisms is not clear.

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