Taxonomic Significance of Arabinose in The Family *Pseudonocardiaceae*

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Sugar composition of whole cells and cell wall preparations of the family *Pseudonocardiaceae*, mycolate-less wall chemotype IV actinomycetes, was examined to clarify the taxonomic significance of cell-wall sugars. Among the strains of the genera examined, cell wall types of *Actinopolyspora*, *Saccharopolyspora* and *Amycolata* were confirmed to be chemotype IV/A, however, those of *Pseudonocardia*, *Amycolatopsis*, *Saccharomonospora*, *Pseudoamycolata*, *Actinokineospora* and *Kibdelosporangium* were found to be III/A, and that of *Saccharothrix* to be III/A or III/C. Thus, the family *Pseudonocardiaceae*, which was proposed for the closely related genera on the basis of 16S rRNA sequencing studies, was indicated to include organisms with wall chemotypes III and IV, and the description of the family should therefore be emended.

On the basis of 16S ribosomal RNA (rRNA) sequences and chemotaxonomic features, Embley *et al.*¹⁾ proposed the new family *Pseudonocardiaceae* for the mycolate-less wall chemotype IV actinomycetes^{2,3)}, *Actinopolyspora*, *Amycolatopsis*, *Saccharopolyspora*, *Saccharomonospora* and *Pseudonocardia*. Subsequently, Bowen *et al.*^{4,5)} proposed from the results of 16S rRNA sequencing analyses that the genera *Amycolata*, *Kibdelosporangium*⁶⁾, *Saccharothrix* and *Actinokineospora*⁷⁾ should also be included in this family. These 10 genera form distinct sublines within the phylogenetic tree of the family *Pseudonocardiaceae* and share a number of chemotaxonomic features⁸⁻¹¹⁾. All the current members of the family, with exception of the genus *Saccharothrix*, have been reported to have a chemotype IV wall comprising *meso*-diaminopimelic acid (DAP), arabinose and galactose as diagnostic markers. *Saccharothrix australiensis* is known to contain a chemotype III/C wall^{12,13)} as defined by the presence of *meso*-DAP, galactose and rhamnose and the absence of arabinose in the whole cells and cell walls.

In this paper, we report on the sugar composition of whole cells and cell wall preparations from the mycolate-less wall chemotype IV

actinomycetes of the family *Pseudonocardiaceae* including a new genus *Pseudoamycolata*¹⁴⁾, which is closely related to the family, and discuss the taxonomic significance of arabinose in the cell wall and of cell wall types and whole cell sugar patterns according to Lechevalier and Lechevalier¹³⁾.

MATERIALS AND METHODS

Microorganisms and culture conditions

The bacterial strains used in this study are shown in Table 1. *Actinopolyspora halophila*¹⁵⁾ and *Actinopolyspora mortivallis*¹⁶⁾ were cultured in IFO culture medium No. 284 containing 0.75% casamino acids (Difco), 1% yeast extract (Difco), 2% magnesium sulfate heptahydrate, 0.3% trisodium citrate dihydrate, 0.3% potassium chloride, 15% sodium chloride and 49.8 mg/L of ferric sulfate heptahydrate at 37°C and 45°C for 3 days, respectively. *Saccharomonospora glauca*¹⁷⁾ was cultured in a medium containing 1% peptone, 0.2% yeast extract, 0.2% sodium chloride and 0.2% glucose, pH 7.1 (PY medium) at 45°C for 3 days. *Pseudoamycolata halophobica*¹⁴⁾ was cultured in PY medium containing 15% sodium chloride at 28°C for 3 days. The microorganisms other

Table 1. Strains studied.

Genus	Species	IFO number ^{a)}	Other strain designations ^{b)}
<i>Actinokineospora</i>	<i>A. riparia</i>	14541 ^T	NRRL B-16432, JCM 7471
<i>Actinopolyspora</i>	<i>A. halophila</i>	14106 ^T	ATCC 27976
	<i>A. mortivallis</i>	15162 ^T	JCM 7550
	" <i>A. thermovinacea</i> "	14334 ^T	ATCC 35862
<i>Amycolata</i>	<i>A. alni</i>	14991 ^T	VKM Ac-901
	<i>A. autotrophica</i>	12743 ^T	ATCC 19727
	<i>A. hydrocarbonoxydans</i>	14498 ^T	ATCC 15104
	<i>A. saturnea</i>	14499 ^T	NRRL B-16172
<i>Amycolatopsis</i>	<i>A. azurea</i>	14573 ^T	NRRL 11412
	<i>A. fastidiosa</i>	14105 ^T	ATCC 31181
	<i>A. mediterranei</i>	13415 ^T	ATCC 13685
	<i>A. methanolica</i>	15065 ^T	LMD 8032
	<i>A. orientalis</i> subsp. <i>lurida</i>	14500 ^T	ATCC 14930, NRRL 2430
	<i>A. orientalis</i> subsp. <i>orientalis</i>	12806 ^T	ATCC 19795
	<i>A. rugosa</i>	14506 ^T	ATCC 43014
	<i>A. sulphurea</i>	13270 ^T	NRRL 2822
	" <i>A. tolypophorus</i> "	14664	ATCC 21177
<i>Kibdelosporangium</i>	<i>K. aridum</i> subsp. <i>aridum</i>	14493 ^T	ATCC 39323
	<i>K. aridum</i> subsp. <i>largum</i>	15152 ^T	ATCC 39922
	<i>K. philippinense</i>	14839 ^T	NRRL 18198
<i>Pseudoamycolata</i>	<i>P. halophobica</i>	15048 ^T	DSM 43089
	<i>P. halophobica</i>	15047	DSM 43092
<i>Pseudonocardia</i>	<i>P. compacta</i>	14343 ^T	ATCC 35407
	<i>P. thermophila</i>	12133	KCC A-0032
<i>Saccharomonospora</i>	<i>S. azurea</i>	14651 ^T	ATCC 43670
	" <i>S. caesia</i> "	12990 ^T	KCC A-0098
	<i>S. cyanea</i>	14841 ^T	ATCC 43724
	<i>S. glauca</i>	14831 ^T	DSM 43769
	<i>S. viridis</i>	12207 ^T	ATCC 15386
<i>Saccharopolyspora</i>	<i>S. erythraea</i>	13426 ^T	ATCC 11635
	<i>S. gregorii</i>	15045 ^T	NCIB 12823
	<i>S. hirsuta</i> subsp. <i>hirsuta</i>	13919 ^T	ATCC 27875, KCC A-0170
	<i>S. hirsuta</i> subsp. <i>kobensis</i>	15151 ^T	ATCC 20501
	<i>S. hordei</i>	15046 ^T	NCIB 12824
	<i>S. rectivirgula</i>	12464 ^T	KCC A-0057
	<i>S. spinosa</i>	15153 ^T	NRRL 18395
	<i>S. taberi</i>	15061 ^T	NRRL B-16173
<i>Saccharothrix</i>	<i>S. aerocolonigenes</i>	13195 ^T	ATCC 23870, NRRL B-3298
	<i>S. australiensis</i>	14444 ^T	ATCC 31497, NRRL 11239
	<i>S. coeruleofusca</i>	14520 ^T	DSM 43679
	<i>S. coeruleoviolacea</i>	14988 ^T	VKM Ac-1083
	<i>S. cryophilis</i>	14475 ^T	NRRL B-16238
	<i>S. espanaensis</i>	15066 ^T	NRRL 15764
	<i>S. flava</i>	14521 ^T	ATCC 29533
	<i>S. longispora</i>	14522 ^T	DSM 43749
	<i>S. mutabilis</i> subsp. <i>capreolus</i>	12847 ^T	ATCC 23892
	<i>S. mutabilis</i> subsp. <i>mutabilis</i>	14310 ^T	ATCC 31520
	<i>S. syringae</i>	14523 ^T	DSM 43886
	<i>S. texasensis</i>	14971 ^T	NRRL B-16134
	<i>S. waywayandensis</i>	14970 ^T	NRRL B-16159

(continued)

Genus	Species	IFO number ^{a)}	Other strain designations ^{b)}
<i>Corynebacterium</i>	<i>C. ammoniagenes</i>	12612 ^T	ATCC 6871
	<i>C. ammoniagenes</i>	12072	ATCC 6872
	<i>C. amycolatum</i>	15207 ^T	ATCC 49368, JCM 7447
	<i>C. callunae</i>	15359 ^T	ATCC 15991
	<i>C. cystitidis</i>	15284 ^T	ATCC 29593, JCM 3715
	<i>C. flavescens</i>	14136 ^T	ATCC 10340
	<i>C. glutamicum</i>	12168 ^T	ATCC 13032
	<i>C. jeikeium</i>	15298 ^T	ATCC 43734
	<i>C. kutscheri</i>	15288 ^T	ATCC 15677
	<i>C. matruchotii</i>	15360 ^T	ATCC 14266
	<i>C. minutissimum</i>	15361 ^T	ATCC 23348
	<i>C. mycetoides</i>	15289 ^T	ATCC 43995
	<i>C. pilosum</i>	15285 ^T	ATCC 29592, JCM 3714
	<i>C. pseudodiphtheriticum</i>	15362 ^T	ATCC 10700
	<i>C. pseudotuberculosis</i>	15363 ^T	ATCC 19410
	<i>C. renale</i>	15290 ^T	ATCC 19412
	" <i>C. stationis</i> "	12144	ATCC 14403
	<i>C. striatum</i>	15291 ^T	ATCC 6940
	<i>C. variabilis</i>	15286 ^T	ATCC 15753, JCM 2154
	<i>C. variabilis</i>	14757	ATCC 33010
	<i>C. vitrumen</i>	12143 ^T	ATCC 10234
	<i>C. xerosis</i>	15287 ^T	ATCC 373, JCM 1971
	<i>C. xerosis</i>	12684	ATCC 7094
<i>Mycobacterium</i>	<i>M. diernhoferi</i>	14756 ^T	ATCC 19340, JCM 6371
<i>Nocardia</i>	<i>N. brasiliensis</i>	14402 ^T	ATCC 19296, JCM 3374
<i>Rhodococcus</i>	<i>R. equi</i>	14956 ^T	ATCC 6939, JCM 1311
<i>Tsukamurella</i>	<i>T. paurometabola</i>	12160 ^T	ATCC 8368
	" <i>T. wratislaviensis</i> "	15274 ^T	NCIB 13082

a) T: Type strain.

b) Abbreviations for culture collections: ATCC, American Type Culture Collection, Rockville, U.S.A.; DSM, Deutsche Sammlung von Mikroorganismen, Braunschweig, FRG; JCM, Japan Collection of Microorganisms, Institute of Physical and Chemical Research (RIKEN), Wako, Japan; KCC, Kaken Chemical Co. Ltd., Tokyo, Japan; LMD, Laboratorium voor Microbiologie, Delft, The Netherlands; NCIB, National Collection of Industrial Bacteria, Torry Research Station, Aberdeen, Scotland; NRRL, ARS Culture Collection, Northern Regional Research Center, U.S. Department of Agriculture, Peoria, U.S.A.; VKM, All Union Culture Collection of Microorganisms, Institute of Biochemistry and Physiology, USSR Academy of Sciences, Moscow, Russia.

28°C for 3 days in PY medium. For the comparison of cell-wall polysaccharide, 23 strains (20 species) of the genus *Corynebacterium*, type strains of the genera *Mycobacterium*, *Nocardia* and *Rhodococcus* and two strains (2 species) of the genus *Tsukamurella*¹⁸⁾ were used. Of these strains, *Corynebacterium amycolatum* is a mycolate-less *Corynebacterium* species¹⁹⁾.

Preparation of cell walls and cell-wall polysaccharide and their chemical analysis

Cell walls were prepared by disrupting the cells (wet wt. ca. 10 g) with glass beads (0.11–0.12 mm in diameter) using a Kubota sonic oscillator at 180 W for 30 min, followed by treatment with RNase A and pronase E, and then lyophilized as described by Schleifer and Kandler²⁰⁾.

Table 2. Molar ratio of whole-cell and cell-wall sugar of the species in the family *Pseudonocardiaceae*.

Species	IFO number	Whole-cell sugar composition ^{a)}							Cell-wall sugar composition ^{a)}					
		Rib	Amino sugar	Rha	Man	Ara	Gal	Glc	Amino sugar	Rha	Man	Ara	Gal	Glc
<i>Actinokineospora</i>														
<i>A. riparia</i>	14541 ^T	0.2	1.7	0.3	0.1	0.3	1.0	1.2	2.3	0.2	—	—	1.0	0.5
<i>Actinopolyspora</i>														
<i>A. halophila</i>	14106 ^T	0.1	0.4	—	—	0.8	1.0	—	0.6	0.1	—	0.9	1.0	—
<i>A. mortivallis</i>	15162 ^T	1.4	2.0	—	0.3	2.6	1.0	—	1.3	—	—	1.1	1.0	—
" <i>A. thermovinacea</i> "	14334 ^T	0.4	0.1	0.1	1.5	3.4	1.0	0.4	1.0	0.2	0.7	3.1	1.0	—
<i>Amycolata</i>														
<i>A. alni</i>	14991 ^T	0.1	0.7	—	0.5	0.9	1.0	1.0	0.7	—	0.5	0.5	1.0	0.3
<i>A. autotrophica</i>	12743 ^T	—	1.0	—	0.4	0.8	1.0	0.8	1.5	—	—	1.0	1.0	0.2
<i>A. hydrocarbonoxydans</i>	14498 ^T	0.1	1.2	—	0.3	0.3	1.0	2.3	2.5	—	—	—	1.0	0.2
<i>A. saturnea</i>	14499 ^T	—	1.0	—	0.3	0.8	1.0	0.9	2.3	—	—	0.9	1.0	0.6
<i>Amycolatopsis</i>														
<i>A. azurea</i>	14573 ^T	0.2	1.9	—	0.1	0.6	1.0	0.1	2.9	—	—	0.3	1.0	0.3
<i>A. fastidiosa</i>	14105 ^T	0.1	1.5	0.1	0.2	0.5	1.0	0.9	1.0	0.1	—	0.3	1.0	0.3
<i>A. mediterranei</i>	13415 ^T	0.1	0.4	—	0.1	0.3	1.0	0.3	0.5	—	—	0.1	1.0	0.1
<i>A. methanolica</i>	15065 ^T	—	0.1	—	0.4	1.3	1.0	0.1	0.8	0.2	0.4	1.7	1.0	0.2
<i>A. orientalis</i> subsp. <i>lurida</i>	14500 ^T	0.2	2.0	—	0.2	0.7	1.0	0.7	1.3	—	—	—	1.0	0.7
<i>A. orientalis</i> subsp. <i>orientalis</i>	12806 ^T	0.2	1.4	—	0.1	0.7	1.0	—	2.1	—	—	0.4	1.0	—
<i>A. rugosa</i>	14506 ^T	0.1	1.0	0.1	—	0.6	1.0	0.4	0.6	—	—	0.4	1.0	—
<i>A. sulphurea</i>	13270 ^T	0.2	0.3	—	0.2	0.6	1.0	0.1	2.4	0.2	—	0.4	1.0	0.2
" <i>A. tolypophorus</i> "	14664	0.3	1.1	0.1	0.3	0.9	1.0	0.8	1.8	0.1	—	0.2	1.0	0.3
<i>Kibdelosporangium</i>														
<i>K. aridum</i> subsp. <i>aridum</i>	14493 ^T	0.2	1.3	—	0.3	0.4	1.0	0.3	3.1	—	—	—	1.0	—
<i>K. aridum</i> subsp. <i>largum</i>	15152 ^T	0.2	1.5	0.1	0.4	0.4	1.0	0.4	3.7	—	—	—	1.0	1.1
<i>K. philippinense</i>	14839 ^T	1.7	1.7	3.7	3.7	—	1.0	5.3	1.7	0.1	0.6	—	1.0	0.2
<i>Pseudoamycolata</i>														
<i>P. halophobica</i>	15048 ^T	2.0	4.6	—	1.6	0.2	1.0	4.6	5.2	—	1.2	—	1.0	3.4
<i>P. halophobica</i>	15047	0.1	0.4	—	0.3	0.4	1.0	0.3	0.7	—	0.3	0.4	1.0	0.2
<i>Pseudonocardia</i>														
<i>P. compacta</i>	14343 ^T	—	1.8	—	0.3	0.2	1.0	1.1	1.4	—	0.2	0.1	1.0	0.5
<i>P. thermophila</i>	12133	—	1.9	—	0.2	0.4	1.0	1.0	0.3	—	0.1	0.2	1.0	0.5
<i>Saccharomonospora</i>														
<i>S. azurea</i>	14651 ^T	0.2	0.6	0.1	0.2	0.5	1.0	0.1	0.9	—	—	0.2	1.0	0.2
" <i>S. caesia</i> "	12990 ^T	0.1	1.0	0.1	0.1	0.3	1.0	0.2	0.6	0.1	—	0.1	1.0	—
<i>S. cyanea</i>	14841 ^T	0.2	5.6	—	0.3	—	1.0	7.1	1.3	—	—	—	1.0	—
<i>S. glauca</i>	14831 ^T	0.4	0.5	0.2	0.2	0.7	1.0	—	0.7	0.1	—	0.3	1.0	0.1
<i>S. viridis</i>	12207 ^T	0.1	3.4	—	—	0.3	1.0	0.2	0.6	0.1	—	0.1	1.0	—
<i>Saccharopolyspora</i>														
<i>S. erythraea</i>	13426 ^T	0.1	0.7	—	—	2.5	1.0	0.1	0.8	0.3	—	1.9	1.0	—
<i>S. gregorii</i>	15045 ^T	0.7	1.9	—	0.5	0.9	1.0	0.5	2.5	—	0.3	0.8	1.0	—
<i>S. hirsuta</i> subsp. <i>hirsuta</i>	13919 ^T	0.3	0.9	0.2	—	1.6	1.0	0.2	0.9	0.1	—	1.5	1.0	—
<i>S. hirsuta</i> subsp. <i>kobensis</i>	15151 ^T	0.2	0.7	0.1	—	0.5	1.0	0.1	0.7	0.1	—	0.2	1.0	—
<i>S. hordei</i>	15046 ^T	0.2	0.8	0.1	—	1.5	1.0	—	1.2	0.3	—	1.3	1.0	—
<i>S. rectivirgula</i>	12464 ^T	0.5	2.7	—	—	1.1	1.0	0.7	1.2	0.2	—	1.2	1.0	—
<i>S. spinosa</i>	15153 ^T	0.3	0.8	0.1	0.1	1.3	1.0	0.5	0.5	—	—	1.0	1.0	—
<i>S. taberi</i>	15061 ^T	0.2	0.6	—	—	2.4	1.0	—	0.7	—	—	2.1	1.0	—

(continued)

Species	IFO number	Whole-cell sugar composition ^{a)}							Cell-wall sugar composition ^{a)}					
		Rib	Amino sugar	Rha	Man	Ara	Gal	Glc	Amino sugar	Rha	Man	Ara	Gal	Glc
<i>Saccharothrix</i>														
<i>S. aerocolonigenes</i>	13195 ^T	0.2	1.7	0.1	0.7	—	1.0	0.6	2.9	0.1	—	—	1.0	—
<i>S. australiensis</i>	14444 ^T	—	0.5	0.1	0.3	0.1	1.0	0.9	1.3	0.1	0.1	—	1.0	0.6
<i>S. coeruleofusca</i>	14520 ^T	0.1	0.2	0.1	0.1	—	1.0	0.2	0.3	0.1	—	—	1.0	0.2
<i>S. coeruleoviolacea</i>	14988 ^T	0.1	0.7	—	0.4	—	1.0	0.3	0.3	—	—	—	1.0	0.3
<i>S. cryophilis</i>	14775 ^T	0.1	1.6	0.3	0.6	—	1.0	0.5	0.9	0.1	—	—	1.0	0.2
<i>S. espanaensis</i>	15066 ^T	0.1	1.8	0.1	0.3	—	1.0	2.7	9.1	0.6	—	—	1.0	1.2
<i>S. flava</i>	14521 ^T	0.2	1.2	—	0.4	—	1.0	0.9	1.9	—	—	—	1.0	—
<i>S. longispora</i>	14522 ^T	0.2	1.4	—	0.3	1.9	1.0	0.7	5.6	—	—	—	1.0	0.3
<i>S. mutabilis</i> subsp. <i>capreolus</i>	14847 ^T	1.3	4.6	0.4	1.3	—	1.0	3.9	3.4	0.1	—	—	1.0	—
<i>S. mutabilis</i> subsp. <i>mutabilis</i>	14310 ^T	0.4	1.0	0.2	0.9	—	1.0	3.4	2.1	0.3	—	—	1.0	—
<i>S. syringae</i>	14523 ^T	2.5	27.2	3.0	12.0	1.3	1.0	1.8	2.1	0.1	—	—	1.0	0.1
<i>S. texasensis</i>	14971 ^T	0.1	0.8	0.1	0.5	0.1	1.0	0.6	3.9	0.1	—	—	1.0	—
<i>S. waywayandensis</i>	14970 ^T	1.0	4.8	0.4	1.2	—	1.0	1.0	6.2	—	—	—	1.0	—

a) Rib: Ribose, Rha: Rhamnose, Man: Mannose, Gal: Galactose, Glc: Glucose.

The polysaccharide was isolated from cell wall with 0.5N NaOH at 100°C for 30 min to 1 hr, as previously described²¹⁾. After cooling to room temperature and neutralizing with acetic acid, the supernatant was concentrated to a small volume *in vacuo*. Polysaccharide was precipitated by adding 2 volumes of cold ethanol and the precipitate was dissolved in water, dialyzed using distilled water and then lyophilized.

For quantitative determination of sugar composition, whole cells (wet wt. ca. 10 mg), cell wall (dry wt. ca. 2 mg) and polysaccharide (dry wt. ca. 2 mg) were hydrolyzed with 2N HCl at 100°C for 3 hr. After removal of HCl, the hydrolysate was analyzed using a high-performance liquid chromatography (HPLC) apparatus (Shimadzu LC-5A) equipped with a Shim-pack ISA 07/S2504 column (250 × 4 mm) and a Shimadzu RE-530 spectro-fluorometer as described previously²¹⁾.

For the analysis of uronic acid, polysaccharides were hydrolyzed with 2N TFA at 100°C for 1 hr, and uronic acid was measured by the carbazole method of Bitter and Muir²²⁾. Phosphorus was measured by the method of

Ames²³⁾. Fractionation of polysaccharide on DEAE-Toyopearl and Sephadex G-100.

Samples were loaded on a column (20 × 1 cm) of DEAE-Toyopearl (TSK gel 650M, Tosoh, Corporation, Japan) equilibrated with 0.01 M Tris-HCl buffer, pH 7.1 (21, 24). Polysaccharide was eluted with a linear gradient of NaCl from 0 M to 0.5 M after elution with 50 ml of Tris-HCl buffer. Total sugars were detected by the phenol-sulfuric acid method. Fractions (2 ml each) containing carbohydrates were pooled and lyophilized after dialyzing with distilled water.

For further purification of polysaccharide, the preparation was loaded onto a Sephadex G-100 column (4.3 × 62 cm) and eluted with distilled water. The molecular weight of the polysaccharide was measured by gel filtration with Dextran T10 (m.w. 9,000) as a marker. Fractions of 3 ml each were collected, and total sugars were detected by the phenol-sulfuric acid method. Fractions containing carbohydrate were combined and lyophilized, and the sugar composition was analyzed by HPLC after 2N HCl hydrolysis (100°C, 3 hr).

RESULTS

Sugar composition of whole cells and cell wall

Molar ratio of sugars in whole cells and cell-wall preparations from the strains in the family *Pseudonocardiaceae* is shown in Table 2 and 3. Table 2 shows the molar ratio of whole cell and cell-wall sugars relative to galactose. Whole cells and cell walls of many strains in the family *Pseudonocardiaceae* contained arabinose and galactose as diagnostic sugars, and other sugars such as glucose, rhamnose and mannose were minor components (chemotype IV/A). In the present study, when the molar ratio of sugars to galactose in the cell-wall preparations or whole cell are above 0.5, the sugar was expressed as diagnostic or major component. Among the 10 genera examined, strains of all the species of three genera, *Actinopolyspora*, *Amycolata* and *Saccharopolyspora*, except for *Amycolata hydrocarbonoxydans* IFO 14498^T (T=type strain) and *Saccharopolyspora hirsuta* subsp. *kobensis* IFO 15151^T, were found to contain arabinose and galactose as diagnostic sugars in whole cells or cell-wall preparations, while the

strains and the strains of the species of the other genera, *Amycolatopsis*, *Pseudoamycolata*, *Pseudonocardia*, *Saccharomonospora*, *Actinokineospora*, *Kibdelosporangium* and *Saccharothrix*, except for *Amycolatopsis methanolica* IFO 15065^T, did not contain arabinose as a major component either in whole cells or cell-wall preparations. Especially, *Amycolatopsis orientalis* subsp. *lurida* IFO 14500^T, *Pseudoamycolata halophobica* IFO 15048^T, *Saccharomonospora cyanea* IFO 14841^T, *Kibdelosporangium aridum* subsp. *aridum* IFO 14493^T, *K. aridum* subsp. *largum* IFO 15152^T, *K. philippinense* IFO 14839^T and almost all the strains of the genus *Saccharothrix*, contained arabinose only as a minor component or did not contain any either in the whole cells or cell-wall preparations. But, *A. methanolica* IFO 15065^T contained arabinose as a diagnostic sugar either in whole cell or cell-wall preparations.

On the other hand, as shown in Table 3, none of the species of *Saccharothrix* except for *S. syringae* IFO 14523^T contained rhamnose as a major component, a diagnostic sugar of this genus^{3,12,13}, either in their whole cells or cell walls. Whereas this genus is reported to have

Table 3. Molar ratio of rhamnose to galactose in the whole cells and cell-wall preparations of the species in the genus *Saccharothrix*.

Species	IFO number	Molar ratio of Rha to Gal ^{a)} in	
		whole cells	cell-wall
<i>S. aerocolonigenes</i>	13195 ^T	0.06	0.13
<i>S. australiensis</i>	14444 ^T	0.06	0.11
<i>S. coeruleofusca</i>	14520 ^T	0.07	0.09
<i>S. coeruleoviolaceae</i>	14988 ^T	0	0
<i>S. cryophilis</i>	14475 ^T	0.33	0.14
<i>S. espanaensis</i>	15066 ^T	0.09	0.56
<i>S. flava</i>	14521 ^T	0.02	0
<i>S. longispora</i>	14522 ^T	0	0
<i>S. mutabilis</i> subsp. <i>capreolus</i>	12847 ^T	0.34	0.14
<i>S. mutabilis</i> subsp. <i>mutabilis</i>	14310 ^T	0.18	0.31
<i>S. syringae</i>	14523 ^T	3.0	0.11
<i>S. texasensis</i>	14971 ^T	0.11	0.11
<i>S. waywayandensis</i>	14970 ^T	0.04	0

a) Abbreviations for sugars: See Table 2.

cell-wall type III and whole cell sugar pattern (12,13), arabinose was detected in the whole cells of *S. longispora* IFO 14522^T and *S. syringae* IFO 14523^T (Table 2).

Cell-wall polysaccharide

To clarify the kind of cell-wall polymer in actinomycetes, whether it is a neutral polysaccharide, teichoic acid or acidic polysaccharide or arabinogalactan, we isolated the cell-wall polysaccharides from the cell walls of the representative strains in the family *Pseudonocardia* and related genera used in the present study, with alkali treatment and examined the chemical composition.

Figure 1 shows the chromatographic profile

of the polysaccharide from four strains, *Saccharothrix australiensis* IFO 14444^T, *Kibdelosporangium aridum* subsp. *aridum* IFO 14493^T, "*Saccharomonospora caesia*" IFO 12990^T and *Corynebacterium amycolatum* IFO 15207^T. As shown in the figure, polysaccharides from these strains were separated into two fractions on DEAE-Toyopearl chromatography. Fraction I (PS-I), which passed through the column, was suggested to be a neutral polysaccharide, and fraction II (PS-II), which was adsorbed on the DEAE-Toyopearl but was eluted with a linear gradient of NaCl from 0 to 0.3 M, was suggested to be an acidic polysaccharide. Table 4 shows the chemical composition of PS-I and PS-II and a comparison of the molar ratio of sugar composi-

Fig. 1. Chromatography of alkali extract of the cell walls on DEAE-Toyopearl. Fractions of 2 ml each were corrected. A: *Saccharothrix australiensis* IFO 14444^T, B: *Kibdelosporangium aridum* subsp. *aridum* IFO 14493^T, C: "*Saccharomonospora caesia*" IFO 12990^T, D: *Corynebacterium amycolatum* IFO 15207^T. Symbol: ⊗, carbohydrate [A₄₉₀]

Table 4. Sugar composition of the polysaccharides isolated from the cell walls.

Species	IFO	PS	Molar ratio of sugars ^{a)}					Uronic acid	Phosphorus
			Rha	Ara	Man	Gal	Glc		
<i>Actinokineospora riparia</i>	14541 ^T	CW ^{b)}	—	0.18	—	1.00	0.40	—	—
		I	—	0.16	—	1.00	0.23	tr ^{c)}	—
		II	—	0.16	—	1.00	0.20	+	—
<i>Kibdelosporangium aridum</i> subsp. <i>aridum</i>	14493 ^T	CW	tr	tr	—	1.00	tr	—	—
		I	—	tr	—	1.00	—	tr	—
		II	—	—	—	1.00	—	+	—
" <i>Saccharomonospora caesia</i> "	12990 ^T	CW	0.07	0.06	—	1.00	—	—	—
		I	—	0.06	—	1.00	—	tr	—
		II	0.04	0.02	—	1.00	—	+	—
<i>Saccharothrix australiensis</i>	14444 ^T	CW	0.11	—	0.12	1.00	0.62	—	—
		I	0.13	—	0.18	1.00	0.13	tr	—
		II	0.08	—	0.11	1.00	0.10	+	—
<i>Corynebacterium amycolata</i>	15207 ^T	CW	—	1.15	—	1.00	—	—	—
		I	—	3.64	—	1.00	—	tr	—
		II	—	3.27	—	1.00	—	+	—
<i>Corynebacterium xerosis</i>	12684	CW	0.03	1.92	0.77	1.00	—	—	—
		I	0.06	2.45	0.90	1.00	—	tr	—
		II	0.06	1.87	0.67	1.00	—	+	—
<i>Nocardia brasiliensis</i>	14402 ^T	I	—	3.18	—	1.00	—	tr	—
		II	—	3.37	—	1.00	—	+	—
<i>Rhodococcus equi</i>	14956 ^T	I	—	3.45	—	1.00	—	tr	—
		II	—	2.20	—	1.00	—	+	—

a) Abbreviations for sugars: See Table 2. b) Cell wall preparation. c) A trace amount was detected.

tions relative to galactose in these polysaccharides. The results demonstrate that sugar composition and molar ratio of PS-I and PS-II of the strains are similar.

As phosphorus and uronic acids were not detected in any fraction, both PS-I and PS-II were considered to be a neutral heteropolysaccharide containing arabinose and a small amount of uronic acid.

On the contrary, cell wall polysaccharides of *C. amycolatum* IFO 15207^T, *Nocardia brasiliensis* IFO 14402^T and *Rhodococcus equi* IFO 14956^T were composed of arabinose and galactose, while that of *Corynebacterium xerosis* IFO 12684 had a small amount of rhamnose and mannose in addition. Abou-Zeid²⁵⁾ reported that *Corynebacterium pseudodiphtheriticum* contains arabinogalactan with mannose and glu-

cose. To determine whether this is a peculiar feature of a single species or a property of the entire genus *Corynebacterium*, we examined the cell wall sugar composition of 23 strains (20 species) of the genus *Corynebacterium*, one species each of *Nocardia*, *Mycobacterium* and *Rhodococcus* and 2 strains (2 species) of the genus *Tsukamurella*. As shown in Table 5, 18 species of the genus *Corynebacterium* and 2 species of the genus *Tsukamurella*, cell walls were composed of arabinose and galactose as diagnostic sugars, and of rhamnose, mannose, glucose and xylose as minor components. Cell wall of *C. amycolatum* IFO 15207^T, *C. vitrumen* IFO 12143^T and type strains of the species of the genera *Mycobacterium*, *Nocardia* and *Rhodococcus*, were composed of arabinose and galactose only. Further purification of the poly-

saccharides was attempted with gel filtration on Sephadex G-100 to rule out the possibility of contamination, and it was clarified that polysaccharides of *C. amycolatum* IFO 15207^T and *C. xerosis* IFO 12684 were separated into two fractions, but sugar composition of each fraction was the same as that of cell walls, and mannose was present in a constant ratio in the purified fractions of *C. xerosis* IFO 12684. Similarly, cell-wall polysaccharide of *A. riparius* IFO 14541^T, purified on Sephadex G-100

column, was composed of galactose and a small amount of arabinose.

DISCUSSION

Wall chemotype and whole cell sugar patterns^{8,13} are taxonomically significant markers for the actinomycetes. Especially, the presence or absence of arabinose in the whole cells and cell walls is used as an useful taxonomic criterion for classifying actinomycetes at a generic

Table 5. Cell-wall sugars in the species of the genus *Corynebacterium*, *Nocardia*, *Mycobacterium*, *Rhodococcus* and *Tsukamurella*.

Species	IFO	Molar ratio of sugars ^{a)}						
		Rha	Ara	Man	Gal	Glc	Xyl	
<i>Corynebacterium</i>	<i>C. ammoniagenes</i>	12612 ^T	—	2.19	0.65	1.0	—	—
	<i>C. ammoniagenes</i>	12072	—	2.96	0.39	1.0	—	—
	<i>C. amycolatum</i>	15207 ^T	—	3.27	—	1.0	—	—
	<i>C. callunae</i>	15359 ^T	0.60	1.89	—	1.0	—	—
	<i>C. cystitidis</i>	15284 ^T	—	1.10	0.24	1.0	—	—
	<i>C. flavescens</i>	14136 ^T	—	2.11	1.47	1.0	—	—
	<i>C. glutamicum</i>	12168 ^T	0.42	2.16	1.07	1.0	1.56	—
	<i>C. jeikeium</i>	15298 ^T	—	0.43	—	1.0	0.14	—
	<i>C. kutscheri</i>	15288 ^T	0.92	2.76	—	1.0	—	—
	<i>C. matruchotii</i>	15360 ^T	0.94	3.15	—	1.0	—	—
	<i>C. minutissimum</i>	15361 ^T	—	2.69	1.74	1.0	—	—
	<i>C. mycetoides</i>	15289 ^T	0.14	1.68	0.90	1.0	0.28	—
	<i>C. pilosum</i>	15285 ^T	0.27	1.10	0.30	1.0	0.36	—
	<i>C. pseudodiphtheriticum</i>	15362 ^T	1.04	3.47	—	1.0	—	—
	<i>C. pseudotuberculosis</i>	15363 ^T	0.91	3.37	—	1.0	—	—
	<i>C. renale</i>	15290 ^T	0.22	1.80	1.00	1.0	0.16	—
	<i>C. stationis</i>	12144	0.25	4.44	0.56	1.0	—	—
	<i>C. striatum</i>	15291 ^T	—	2.32	0.89	1.0	—	—
	<i>C. variabilis</i>	15286 ^T	0.08	1.52	0.41	1.0	—	—
	<i>C. variabilis</i>	14757	0.06	0.97	0.21	1.0	0.35	—
	<i>C. vitarumen</i>	12143 ^T	—	0.56	—	1.0	—	—
	<i>C. xerosis</i>	15287 ^T	—	2.22	0.85	1.0	1.03	—
	<i>C. xerosis</i>	12684	—	1.87	0.67	1.0	—	—
<i>Mycobacterium</i>	<i>M. diernhoferi</i>	14756 ^T	—	1.95	—	1.0	—	—
<i>Nocardia</i>	<i>N. brasiliensis</i>	14402 ^T	—	3.18	—	1.0	—	—
<i>Rhodococcus</i>	<i>R. equi</i>	14956 ^T	—	3.45	—	1.0	—	—
<i>Tsukamurella</i>	<i>T. paurometabola</i>	12160 ^T	0.62	1.25	0.69	1.0	0.46	0.28
	" <i>T. wratislaviensis</i> "	15274 ^T	0.33	1.00	0.47	1.0	0.14	0.19

a) Abbreviations for sugars: See Table 2.

level. The family *Pseudonocardiaceae*, proposed on the basis of nucleotide sequence of 16S RNA, is characterized by the mycolate-less cell wall chemotype IV¹⁾. However, the genus *Saccharothrix* with a chemotype III/C wall¹²⁾ has been included in this family. Moreover, in the strains of the genus *Kibdelosporangium*⁶⁾ and *Actinokineospora*⁷⁾, arabinose is present in the whole cells, while its amount was reduced or not found in the purified cell walls. Therefore, Bowen *et al.*⁴⁾ proposed that the family description be emended to include organisms with cell wall chemotype III.

In this study, we quantitatively analyzed the sugar compositions of whole-cells and cell-wall preparations of the 78 strains (74 species, 15 genera) of the family *Pseudonocardiaceae* and the genera *Corynebacterium*, *Mycobacterium*, *Rhodococcus*, *Nocardia* and *Tsukamurella*. As summarized in Table 6, some differences in sugar composition can be found between our results and the reported descriptions^{2,3)}. Three genera (*Actinopolyspora*, *Saccharopolyspora* and *Amycolata*) of the family contain arabinose as a major component of the whole cell and the

cell wall and mannose, rhamnose and glucose as minor components in the cell wall (wall chemotype IV/A). *A. hydrocarbonoxydans* IFO 14498^T did not contain arabinose in the cell wall preparation and *S. hirsuta* subsp. *kobensis* did it as a minor component. Therefore, the taxonomic position of these strains should be re-examined.

From our present results, it is not clear whether the cell-wall polysaccharides of these chemotype IV actinomycetes are arabinogalactan or not. Cell-wall polysaccharide of the genera *Mycobacterium*, *Nocardia*, *Rhodococcus*, *Tsukamurella*²⁶⁾ and *Corynebacterium* has been recognized to be arabinogalactan. However, the structural studies are limited only to those on *Mycobacterium*²⁷⁻³¹⁾. As shown in this study, sugar components of cell-wall polysaccharides of these genera, except for the genera *Corynebacterium* and *Tsukamurella*, are only arabinose and galactose. Cell walls of the species of the genera *Corynebacterium* and *Tsukamurella* were composed of arabinose, galactose, rhamnose, mannose, glucose and/or xylose.

When arabinose is present as a major component in the cell walls, the organisms could be

Table 6. Cell-wall polymers in the family *Pseudonocardiaceae*.

Genus	This study			Literature ^{b)}	
	Whole cell sugar ^{a)} pattern	Cell wall type	Polysaccharide	Cell wall type	Whole cell sugar ^{a)} pattern
<i>Actinopolyspora</i>	A (Ara, Gal)	IV	Heteropolysaccharide with arabinose as a major component	IV	A (Ara, Gal)
<i>Amycolata</i>	A (Ara, Gal)	IV		IV	A (Ara, Gal)
<i>Saccharopolyspora</i>	A (Ara, Gal)	IV		IV	A (Ara, Gal)
<i>Actinokineospora</i>	A (Ara, Gal)	III	Heteropolysaccharide with arabinose as a minor component	IV	A (Ara, Gal)
<i>Amycolatopsis</i>	A (Ara, Gal)	III		IV	A (Ara, Gal)
<i>Kibdelosporangium</i>	A (Ara, Gal)	III		IV	A (Ara, Gal)
<i>Pseudoamycolata</i>	A (Ara, Gal)	III		IV	A (Ara, Gal)
<i>Pseudonocardia</i>	A (Ara, Gal)	III		IV	A (Ara, Gal)
<i>Saccharomonospora</i>	A (Ara, Gal)	III		IV	A (Ara, Gal)
<i>Saccharothrix</i>	C/A (Ara, Gal)	III	Heteropolysaccharide with no arabinose	III	C (Rha, Gal)

a) Abbreviations for sugars: See Table 2. b) Data from references 2) and 3).

considered to have cell wall chemotype IV, even if it is not clear whether the cell-wall polysaccharide is arabinogalactan or not. Hence, the cell wall chemotype of *Actinopolyspora*, *Saccharopolyspora* and *Amycolata* should be IV.

On the other hand, within the six genera *Pseudonocardia*, *Amycolatopsis*, *Saccharomonospora*, *Pseudoamycolata*, *Actinokineospora* and *Kibdelosporangium*, except for *A. methanolicus* IFO 15065^T, arabinose was present in the cell walls, but the molar ratio of arabinose to galactose in the cell wall preparations was below 0.50. Of the strains in these genera, *A. arvensis* subsp. *lurida* IFO 14500^T, *K. aridum* subsp. *aridum* IFO 14493^T and *K. aridum* subsp. *aridum* IFO 15152^T were found not to contain arabinose in their cell walls, although the sugar was present in the whole cells. In the cell wall and whole cells of *S. cyanea* IFO 14841^T and *K. philippinense* IFO 14839^T, arabinose was not detected. Therefore, the results indicate that arabinose is not a major component of the cell walls of these strains. In the case of *A. methanolicus* IFO 15065^T, arabinose is a diagnostic sugar in whole cell and cell wall, and hence, the taxonomic position should be studied.

The genus *Saccharothrix* has been reported to be cell wall chemotype III/C^{12,13}, and the cell wall sugar compositions in our results agree with those in the literatures. However, as arabinose was found in the whole cells of *S. longispora* IFO 14522^T and *S. syringae* IFO 14523^T, the whole cell sugar pattern of these two species is thought to be pattern A.

From the present studies, it became clear that arabinose is a significant marker for the taxonomy of actinomycetes. We concluded that even if arabinose is present in the cell walls, as long as it is not a major component, the cell wall should be considered to be chemotype III, and we propose that six genera, *Actinokineospora*, *Amycolatopsis*, *Kibdelosporangium*, *Pseudoamycolata*, *Pseudonocardia* and *Saccharomo-*

nospora, are organisms with cell wall chemotype III and whole cell sugar pattern A and that the genus *Saccharothrix* is one with cell wall chemotype III/C or III/A. Cell wall chemotype IV by Lechevalier and Lechevalier¹³) should be restricted to the actinomycetes with arabinogalactan or polysaccharide in their cell walls where arabinose is a major component.

For the reason mentioned above, the description of the family *Pseudonocardiaceae* should be emended to include actinomycetes with chemotype III as Bowen *et al.*⁴) have suggested on the basis of phylogenetic analysis.

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