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Cell Wall Composition of *Micromonospora olivoasterospora*, *Micromonospora sagamiensis*, and Related Organisms

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Cell walls of 19 *Micromonospora* species were analyzed for their components. All the cell walls had xylose and arabinose, but the presence of glucose, galactose, mannose, or rhamnose depended on the strain. Amino acids present in the walls consisted of glycine, glutamic acid, diaminopimelic acid, and alanine, in a molar ratio of approximately 1:1:1:0.6–0.8. 3-Hydroxydiaminopimelic acid, together with *meso*-diaminopimelic acid, was found in many species and was isolated from *Micromonospora olivoasterospora* to compare the color constant in an amino acid analyzer with that of *meso*-diaminopimelic acid. The cell walls of *Micromonospora sagamiensis* and *M. olivoasterospora* contained only D-alanine and not L-alanine. All species tested except *Micromonospora globosa* contained glycolate in an almost equimolar ratio to diaminopimelic acid in their cell walls. Among 45 strains of 12 genera examined, *Actinoplanes*, *Ampullariella*, *Amorphosporangium*, and *Dactylosporangium* species had a significant amount of glycolate in the whole cells. Based on these results, the primary structure of the peptidoglycan of *Micromonospora* is discussed.

Micromonospora has attracted attention as a useful source for antibiotics since the discovery of gentamicin C complex (33). It has been gradually understood that it can produce various types of antibiotics as well as *Streptomyces* and *Nocardia* can (11). *Micromonospora*, which was proposed by Orskov (1923) as a genus of *Actinomycetales*, is characterized morphologically by the absence of true aerial mycelia and by spores borne singly on substrate mycelia. It contains glycine and *meso* (or *meso*-3-hyroxy)-diaminopimelic acid in the cell wall and is classified as cell wall type II by Lechevalier and Lechevalier (12). Since the cell wall composition is one of the important keys for the classification of *Actinomycetales*, it has been studied widely, but only qualitatively in most cases (4, 6, 10, 28, 29, 34). *Streptomyces*, a well-known producer of antibiotics, also contains glycine and diaminopimelic acid, but the configuration of the diaminopimelic acid is the LL-form. Although the primary structures of peptidoglycan of some *Streptomyces* were studied in detail (2, 13, 16, 17), nothing is known about the structure of peptidoglycan of *Micromonospora*.

This study was undertaken to examine quantitatively the amino acid composition of the cell walls from *Micromonospora* species, including *Micromonospora olivoasterospora* and *Micromonospora sagamiensis*, which were reported to produce the new aminoglycoside antibiotics fortimicin (19, 21) and sagamicin (18, 20), respectively. The determination of the configuration of alanine and the presence of glycolate in the cell wall are also reported.

MATERIALS AND METHODS

Organisms. The strains used in this study are listed in Table 1.

Cultivation. Organisms were grown at 30°C in 300-ml Erlenmeyer flasks on a rotary shaker (200 rpm) for 2 or 3 days. The flask contained 50 ml of a medium consisting of 1% glucose (Nakarai Chemicals, Ltd., Tokyo), 1% soluble starch (Kanto Chemical Co., Tokyo), 0.2% beef extract (Kyokuto Seiyaku Co., Tokyo), 0.2% yeast extract (Daigo Eiyo Chemical Co., Tokyo), 0.2% polypeptone (Daigo Eiyo Chemical Co.), and 0.1% $CaCO_3$ (Kanto Chemical Co., Tokyo). Calcium carbonate was omitted from the medium when organisms were grown for analysis using the whole cell. The medium was adjusted to pH 7.3 and autoclaved at 120°C for 15 min.

Cell wall preparation. After harvesting by centrifugation, cells were washed with water by centrifugation and then disrupted in a sonic oscillator (model UR-150P, Tomy Seico Co., Tokyo) for 15 min at 3°C. Unbroken cells were removed by centrifugation at 7,000 × *g* for 10 min. The supernatant solution was made 4% with sodium dodecyl sulfate and heated at 100°C for 40 min and, after cooling, was centrifuged at 100,000 × *g* for 30 min. The precipitates were washed with warm water by centrifugation at room temperature. The precipitates were then treated with pronase AS (Kaken Chemicals Co., Tokyo; 250,000 U of tyrosine per mg, 50 mg) at 37°C overnight in 0.1 M phosphate buffer (pH 7.5). The cell wall was then recovered and washed with water twice by centrifu-

TABLE 1. *Strains of Micromonospora used in this study*

Species	Source[a] and strain
M. carbonacea	NRRL 2972
M. chalcea	ATCC 12452
M. coerulea	ATCC 2708
M. echinospora subsp. *echinospora*	NRRL 2985
M. fusca	NRRL B-943
M. globosa	KCC A-0126
M. grisea	NRRL 3800
M. halophytica subsp. *nigra*	NRRL 3097
M. inyoensis	NRRL 3292
M. inositola	MK 41[b]
M. megalomicea subsp. *nigra*	NRRL 3275
M. melanosporea	IFO 12515
M. narashino	KCC A-0129
M. olivoasterospora	MK 70[b]
M. parva	KCC A-0127
M. purpureochromogenes	ATCC 27007
M. rosaria	NRRL 3718
M. sagamiensis subsp. *nonreducans*	MK 62[b]
M. zionensis	NRRL 5466

[a] NRRL, Northern Regional Research Laboratory, Peoria, Ill.; ATCC, American Type Culture Collection, Baltimore, Md.; KCC, Kaken Chemical Co., Tokyo, Japan; IFO, Institute for Fermentation, Osaka, Osaka, Japan.

[b] Our isolate from soil.

gation at 100,000 × *g* for 30 min and lyophilized (cell wall preparation). The cell wall preparation was suspended in 5% trichloroacetic acid and heated at 90°C for 20 min. The precipitates were collected and washed twice with water by centrifugation (peptidoglycan preparation).

Hydrolysis and neutralization. For amino acids, 5 mg of cell wall was hydrolyzed in 1 ml of 6 N HCl in a sealed Pyrex tube at 105°C for 16 h. The hydrolysate was filtered, neutralized with Dowex 44 (OH^-), lyophilized, and taken up in 0.5 ml (or 4 ml) of deionized water.

For sugars, 20 mg of cell wall was hydrolyzed in 2 ml of 2 N H_2SO_4 in a sealed Pyrex tube at 100°C for 2 h. The hydrolysate was adjusted to pH 5.0 to 5.5 with saturated $Ba(OH)_2$ and centrifuged, and the supernatant fluid was lyophilized. The dried residues were dissolved in 0.5 ml of deionized water.

Diaminopimelic acid identification. The isomers of diaminopimelic acid and its 3-hydroxy derivative were identified by thin-layer chromatography on Avicel SF plates (Funakoshi Yakuhin Co., Tokyo) with a solvent system consisting of methanol, water, 10 N HCl, and pyridine (32:7:1:4). The amount corresponding to 50 μg of cell wall was applied to each spot. Spots of amino acids were revealed with ninhydrin reagent.

Sugar identification. Descending paper chromatography was carried out for 38 h on Toyo filter paper no. 50 in the upper phase of the solvent mixture (*n*-butanol–water–pyridine–toluene, 5:3:3:4). The amount corresponding to 0.2 mg of cell wall preparation was applied to each spot. The spots of sugars were revealed with acid aniline phthalate reagent.

Quantitative amino acid analysis. An equivalent of 1.25 mg of HCl-hydrolyzed cell wall preparation was analyzed in an amino acid analyzer (model JLC-5AH, Japan Electron Optics Laboratory Co., Tokyo).

Quantitative glycolic acid analysis. The procedure followed that of Uchida and Aida (31), except that Diaion SA no. 100 (analytical grade, Mitsubishi Kasei Co., Tokyo) was used instead of Dowex 1 × 8.

Configuration of alanine. Alanine in the cell wall was purified from the acid hydrolysate by preparative paper chromatography. The purified alanine was treated with D-amino acid oxidase (Worthington Diagnostics, Freehold, N.J.) for 6 h at 37°C. The reaction mixture consisted of 30 μl of alanine solution, 50 μl of enzyme solution (1 mg of D-amino acid oxidase and 4 mg of catalase [Worthington Diagnostics] per ml of 0.1 M Tris-hydrochloride buffer, pH 8.0) and 20 μl of water. Residual L-alanine was estimated by the ninhydrin colorimetric method.

Isolation of 3-hydroxydiaminopimelic acid. The cell wall (3.85 g) of *Micromonospora olivoasterospora* was hydrolyzed in 200 ml of 6 N HCl at 105°C for 20 h. To remove hydrogen chloride, the hydrolysate was evaporated, passed through a column containing Dowex 44 (OH^-) resin, and lyophilized. The powder (2.54 g) was dissolved in 30 ml of water. After the pH of the solution was adjusted to 2.0 with 2 N HCl, it was charged to a Diaion SK no. 1B (H^+) column. The resin column was washed with deionized water and then eluted with 1 N ammonium hydroxide. Ninhydrin-positive fractions (about 100 ml) were pooled and decolorized with charcoal after removal of ammonia by evaporation and powdered by lyophilization. The powder was chromatographed on cellulose with the following solvent system: methanol–10 N HCl–pyridine–water (32:1:4:7 by volume). Eluates were monitored by thin-layer chromatography for separation of the isomers of diaminopimelic acid. Fractions containing only 3-hydroxydiaminopimelic acid were pooled and adjusted to pH 2.0 and then charged to a Diaion SK no. 1B (NH_4^+) column. The resin column was washed with deionized water and eluted with 1 N ammonium hydroxide. After lyophilization, the fractions containing the diamino acid were subjected to chromatography on an LH-20 column developed with 50% methanol. The eluates were monitored by a refractometer (model K545, Tokyo Erma Optical Works Ltd., Tokyo) and the ninhydrin reaction. 3-Hydroxydiaminopimelic acid eluted in a single sharp peak was obtained as powder by evaporation in vacuo and lyophilization and then crystallized in water-ethanol.

RESULTS

Qualitative cell wall analysis. After acid hydrolysis, amino acids and sugars in the cell walls prepared from 19 species of *Micromonospora* were qualitatively examined by thin-layer and paper chromatography, respectively. Such amino acids as alanine, glutamic acid, and glycine were found in all cell wall preparations. Diaminopimelic acid, its 3-hydroxy derivative, and sugars detected by chromatography are summarized in Table 2, where the cell wall components are expressed in relative amounts according to the sizes and intensities of their spots.

Nineteen species of *Micromonospora* appear to be divided into three groups on the basis of diamino acids in the cell wall: namely, the first

group contains only diaminopimelic acid (mostly *meso*-form), the second one contains a significant amount of both diaminopimelic acid and 3-hydroxydiaminopimelic acid, and the third one contains 3-hydroxydiaminopimelic acid and a very small amount of diaminopimelic acid. *M. olivoasterospora* and *M. sagamiensis* belong to the third group.

Such pentoses as xylose and arabinose were detected in the cell wall preparations of all the species tested, although the amounts varied to some extent. The presence of four hexoses depends on strains. Glucose and galactose were detected more frequently than mannose and rhamnose. Rhamnose was found in only three strains, *Micromonospora fusca*, *Micromonospora purpureochromogenes*, and *Micromonospora melanosporea*. None of these hexoses could be detected in *Micromonospora coerulea* and *Micromonospora inyoensis*.

Quantitative analysis of amino acid in the cell wall. In an attempt to obtain information on the peptidoglycan of *Micromonospora*, the amino acid compositions of the cell walls were quantitatively determined by an amino acid analyzer. Before this experiment, some preliminary examinations were carried out using the cell wall preparations and the peptidoglycan preparations of three strains (Table 3). Significant amounts of four amino acids such as alanine, glutamic acid, glycine, and diaminopimelic acid were detected in the hydrolysates of their preparations, and the values of other amino acids were almost less than one-twentieth of

TABLE 2. *Components in cell walls of Micromonospora species*[a]

Species	Diaminopimelic acid			Xylose	Arabinose	Glucose	Galactose	Mannose	Rhamnose
	LL-	DL-	3-hydroxy-						
M. fusca	+	++++	−	++++	++	++++	+	−	+
M. purpureochromogenes	+	++++	−	++++	++	++	+	−	++
M. melanosporea	+	++++	−	++++	++	+	+	−	+
M. chalcea	+	++++	−	++++	++	++++	+	+	−
M. narashino	+	++++	−	++++	++	−	+	+	−
M. coerulea	+	++++	−	++++	++	−	−	−	−
M. globosa	+	++++	−	+	+	++++	+	+	−
M. rosaria	±	++	+++	++++	+	++++	++	+	−
M. parva	−	++	+++	++++	++	+++	+	−	−
M. megalomicea subsp. *nigra*	−	++	+++	++++	+	++	−	−	−
M. inositola	−	++	+++	++++	++	−	−	+	−
M. olivoasteropsora	−	−	++++	++++	++	++++	+	−	−
M. halophytica subsp. *nigra*	−	±	++++	++++	++	++++	+	−	−
M. carbonacea	−	±	++++	++++	++	++++	+	−	−
M. zionensis	−	−	++++	++	++	+++	+	−	−
M. grisea	−	−	++++	++	++	++	+	−	−
M. echinospora	−	−	++++	++++	++	−	++	++	−
M. sagamiensis	−	−	++++	++++	++	−	−	++	−
M. inyoensis	−	−	++++	++	++	−	−	−	−

[a] All preparations contained major amounts of glucosamine, muramic acid, glutamic acid, glycine, and alanine. Components are expressed in relative amounts according to the sizes and intensities of their spots and graded ++++, +++, ++, +, ±, and −.

TABLE 3. *Amino acid composition in the cell walls of M. sagamiensis, M. olivoasterospora, and M. chalcea*

Organism	CCl_3COOH[a]	Amino acids (nmol per mg of cell wall)				Molar ratios[b]			
		Glutamic acid	Glycine	Alanine	A_2pm[c]	Glutamic acid	Glycine	Alanine	A_2pm
M. sagamiensis	−	585	525	365	215	1.11	1.00	0.695	0.410
	+	972	931	625	403	1.04	1.00	0.671	0.433
M. olivoasterospora	−	451	487	282	222	0.926	1.00	0.579	0.456
	+	935	919	516	387	1.02	1.00	0.561	0.421
M. chalcea	−	328	392	240	392	0.837	1.00	0.612	1.00

[a] Treatment with 5% trichloroacetic acid.
[b] Molar ratios were expressed with glycine as a unit.
[c] A_2pm, diaminopimelic acid; the contents were calculated as *meso*-diaminopimelic acid.

glycine. Hexosamines such as glucosamine and muramic acid were also detected, but the ratio between them varied, probably because muramic acid was labile in the acidic condition. For *M. sagamiensis* and *M. olivoasterospora*, the molar ratios of four amino acids were almost identical between a cell wall preparation and a peptidoglycan preparation. Thus, a direct analysis of a cell wall preparation would give correct information of amino acid composition of the peptidoglycan of *Micromonospora*.

The molar ratios of diaminopimelic acid in *M. sagamiensis* and *M. olivoasterospora* were less than a half that of *M. chalcea*. It was reported that the epimerization of 3-hydroxydiaminopimelic acid occurred during acid hydrolysis (23). Since *M. sagamiensis* and *M. olivoasterospora* contained 3-hydroxydiaminopimelic acid in the cell walls, the stability of the amino acid during hydrolysis was checked first. It was fairly stable under the condition (Fig. 1). Another possible explanation for the lower molar ratio could be the color constant used for the calculation. The values of 3-hydroxydiaminopimelic acid in Table 3 were calculated by using the same constant as that of *meso*-diaminopimelic acid. As 3-hydroxydiaminopimelic acid was not available commercially, it was isolated and purified from *M. olivoasterospora* to determine the ninhydrin color constant. The separation of the amino acid from others was successful in cellulose column chromatography. Further purification was achieved by subsequent chromatography on Sephadex LH-20. A 60-mg amount of 3-hydroxydiaminopimelic acid was obtained from 3.85 g of cell wall preparations and crystallized. The elementary analysis gave the following data: C, 39.82; H, 6.97; N, 13.39 (calculated for $C_7H_{14}N_2O_5 \cdot 1/4H_2O$: C, 39.90; H, 6.94; N, 13.29). The ninhydrin color constant of 3-hydroxydiaminopimelic acid was found to be 16.1 in an amino acid analyzer, when that of *meso*-diaminopimelic acid was 37.9 (mm^2 per μmol).

Table 4 summarizes the amino acid compositions in the cell wall preparations of 19 strains examined on the basis of the above experiments. Only four amino acids such as alanine, glutamic acid, glycine, and diaminopimelic acid (including its 3-hydroxy derivative) were detected in significant amounts, and the total contents of amino acids varied among the preparations. For example, these amino acids occupied in weight 13% of the cell wall preparation in *Micromonospora globosa* and about 26% in *M. sagamiensis*. However, the ratios among the four amino acids—glycine, glutamic acid, diaminopimelic acid (including its 3-hydroxy derivative), and alanine—are fairly constant: 1:1:1:0.6–0.8. This suggests a common primary structure in the peptidoglycan of *Micromonospora*. The other feature of *Micromonospora* peptidoglycan is that it contains one mole or less of alanine per peptide subunit and one mole of glycine per peptide subunit.

Configuration of alanine in the cell wall. The configuration of the alanine in cell walls of *M. sagamiensis* and *M. olivoasterospora* was determined by a D-amino acid oxidase method. Most of the alanine in both cell walls was found to have the D-configuration (Table 5). This indicates that the *Micromonospora* cell wall lacks L-alanine, which is usually the N-terminal amino acid in the peptide subunit of peptidoglycan, and contains only D-alanine, which is the C-terminal amino acid.

Glycolate in the cell wall. Some actinomycetes are known to have glycolyl groups, instead of acetyl groups in muramic acid. It was exam-

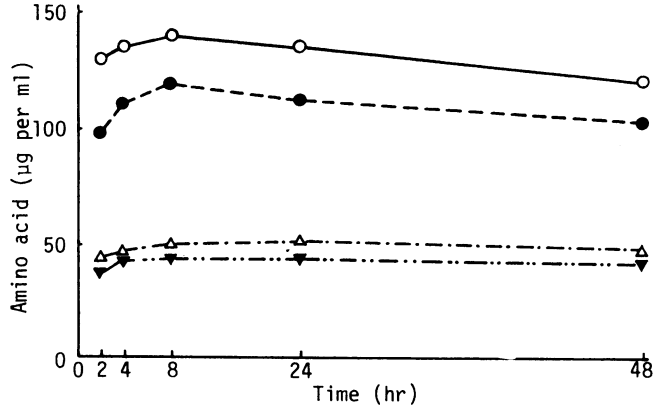

FIG. 1. *Time course of amino acids from the cell wall of M. sagamiensis during hydrolysis in 6 N HCl at 105°C. Symbols: ○, 3-hydroxydiaminopimelic acid; ●, glutamic acid; △, glycine; ▼, alanine.*

TABLE 4. *Amino acid composition in cell walls of Micromonospora species*

Species	Amino acids (nmol per mg of cell wall)					Molar ratios[a]				
	Glutamic acid	Glycine	Alanine	A_2pm[b]	3-hydroxy A_2pm[b]	Glutamic acid	Glycine	Alanine	A_2pm	3-hydroxy A_2pm
M. fusca	305	299	224	306	—[c]	1.02	1.00	0.794	1.02	—
M. purpureochromogenes	376	280	200	304	—	1.34	1.00	0.714	1.09	—
M. melanosporea	315	325	227	333	—	0.969	1.00	0.698	1.02	—
M. chalcea	328	392	240	392	—	0.837	1.00	0.612	1.00	—
M. narashino	352	384	224	376	—	0.917	1.00	0.583	0.979	—
M. coerulea	274	280	176	288	—	0.971	1.00	0.629	1.03	—
M. globosa	232	312	184	296	—	0.744	1.00	0.590	0.949	—
M. rosaria	359	339	275	58	285	1.06	1.00	0.811	0.171	0.841
M. parva	304	304	216	93	211	1.00	1.00	0.711	0.305	0.694
M. megalomicea subsp. *nigra*	443	449	367	138	315	0.987	1.00	0.817	0.307	0.702
M. inositola	327	337	253	57	276	0.970	1.00	0.751	0.167	0.819
M. olivoasterospora	451	487	282	—	565	0.926	1.00	0.580	—	1.16
M. halophytica subsp. *nigra*	304	352	256	—	283	0.864	1.00	0.727	—	1.09
M. carbonacea	528	584	408	—	665	0.904	1.00	0.699	—	1.14
M. zionensis	447	456	315	—	492	0.980	1.00	0.691	—	1.08
M. grisea	428	433	286	—	446	0.988	1.00	0.661	—	1.03
M. echinospora	448	480	296	—	461	0.933	1.00	0.617	—	0.960
M. sagamiensis subsp. *nonreducans*	585	525	365	—	504	1.11	1.00	0.645	—	0.960
M. inyoensis	496	552	336	—	587	0.899	1.00	0.609	—	1.06

[a] Molar ratios were expressed with glycine as a unit.

[b] A_2pm, diaminopimelic acid. When significant amounts of both diaminopimelic acid and 3-hydroxydiaminopimelic acid were detected by thin-layer chromatography, their contents were calculated according to their ratio by densitometric assay with a dual-wavelength TLC scanner CS-900 (Shimadzu Co., Japan) and their color constants were calculated in an amino acid analyzer.

[c] —, None.

TABLE 5. *Configuration of alanine in the cell walls of M. olivoasterospora and M. sagamiensis*

Alanine prepn	Residual alanine (mM)[a]	
	Not treated with enzyme	Treated with enzyme
Alanine from *M. olivoasterospora* cell wall	25	1.9
Alanine from *M. sagamiensis* cell wall	25	2.3
D-alanine	25	0.0
Mixture of D-alanine and L-alanine (1:1)	25	13.0

[a] Each alanine was treated with D-amino acid oxidase for 6 h at 37°C. Residual alanine was determined by the ninhydrin colorimetric method.

ined whether *Micromonospora* has glycolic acid in the cell wall. All the strains tested except *M. globosa* contained 250 to 500 nmol of glycolic acid per mg of cell wall preparation (Table 6). Its molar ratio to diaminopimelic acid (including the 3-hydroxy derivative), which is a characteristic constituent of peptidoglycan, ranges from 0.7 to 1.1. In *M. olivoasterospora* the ratio was around 0.75 irrespective of the degree of purification of the peptidoglycan. This indicates that most of the amino groups of muramic acids are acylated with a glycolyl residue. As for *M. globosa* KCC A-0126, no significant amount of glycolate was found in the cell wall preparations from cells grown on various media.

Glycolate in whole cells. The amount of glycolic acid was measured in whole cells of 44 strains (12 genera), which are classified as cell wall types I to III. *Actinoplanes*, *Amorphosporangium*, *Ampullariella*, and *Dactylosporangium* strains, which belong to cell wall type II, contained approximately 40–100 nmol of glycolic acid per mg of dried cell, although *Actinoplanes armeiacus* contained little glycolate (Table 7). Actinomycetes with no significant amount of glycolate are: (*Actinomadura*) *A. helvata* A-105, *A. pusilla* A-118, *A. roseoviolacea* A-5, *A. spadix* A-116, *A. verrucosospora* A-184; *Chainia rubra* KCC A-0131; (*Microbispora*) *M. chromogenes* M-47, *M. amethystogenes* M-9, *M. diastatica* M-5, *M. echinospora* Mb_3-1, *M. parva* M-3, *M. rosea* M-20; (*Microtetraspora*) *M. niveoa* Mt-2, *M. viridis* Mt-1; *Planobispora rosea* ATCC 23866; *Planomonospora parantospora* subsp. *antibiotica* ATCC 23864; *Streptoalloteicus hindustanus* ATCC 31158; (*Streptosporangium*) *S. album* S-16, *S. amethystogenes* S-6, *S. cinnabarium* ATCC 31213, *S. koreanum*,

TABLE 6. *Comparison of glycolyl residue content with diaminopimelic acid in cell walls of Micromonospora species*

Species	A_2-pm[a, b]	Glycolic acid[b]	Ratio of glycolic acid per A_2pm
M. carbonacea	665	390	0.586
M. chalcea	392	349	0.898
M. coerulea	288	272	0.944
M. echinospora	461	365	0.792
M. fusca	306	282	0.922
M. globosa	296	<10	<0.03
M. grisea	446	386	0.865
M. halophytica subsp. *nigra*	383	339	0.885
M. inyoensis	587	492	0.838
M. inositola	333	264	0.792
M. megalomicea subsp. *nigra*	453	404	0.842
M. melanosporea	333	328	0.985
M. narashino	376	377	1.00
M. parva	304	334	1.10
M. purpureochromogenes	304	310	1.02
M. rosaria	343	328	0.956
M. sagamiensis subsp. *nonreducans*	504	410	0.813
M. zionensis	492	395	0.803
M. olivoasterospora	565	397	0.703
M. olivoasterospora	977[c]	750[c]	0.768[c]

[a] A_2pm, diaminopimelic acid or 3-hydroxydiaminopimelic acid or both.

[b] Nanomoles per milligram of cell wall.

[c] Cell wall treated with 5% trichloroacetic acid.

TABLE 7. *Content of glycolyl residue in whole cells of Actinoplanes, Amorphosporangium, Ampullariella, and Dactylosporangium species*

Organism	Source[a] and strain	Glycolic acid (nmol per mg of dried cell)
Actinoplanes spp.		
A. armeniacus	KCC A-0070	<5
A. brasiliensis	ATCC 25844	64
A. caeruleus	NRRL 5325	47
A. deccanensis	ATCC 21983	68
A. garbadinensis	ATCC 31049	99
A. ianthiogenes	ATCC 21884	40
A. italicus	ATCC 27366	67
A. missouriensis	KCC A-0121	99
A. nipponensis	ATCC 31145	45
A. philippinensis	NRRL 5462	75
A. teichomyceticus	ATCC 31121	95
A. utahensis	KCC A-0122	57
Amorphosporangium auranticolor	ATCC 15330	71
Ampullariella digitata	ATCC 15349	75
Dactylosporangium aurantiacum	ATCC 23491	44

[a] For abbreviations of culture collections, see Table 1, footnote *a*.

ATCC 31214, *S. nondiastaticum* KCC A-0114, *S. pseudovulgare* S_2-31, *S. roseum* S-9, *S. violaceochromogenes* MK 49, *S. violaceochromogenes* subsp. *globophilum* MK 78, *S. viridialbum* NRRL B-2636, *S. viridogriseum* ATCC 25242, *S. viridogriseum* subsp. *kofuense* S_2-28.

DISCUSSION

The peptidoglycan of bacterial cell walls was reviewed by Schleifer and Kandler (26). In the usual peptide subunit, L-alanine is bound to muramic acid, followed by D-glutamic acid, which is linked by its γ-carboxy group to an L-diamino acid, and finally D-alanine is attached to the diamino acid. The cell walls of 19 *Micromonospora* strains contain glycine, glutamic acid, *meso*-diaminopimelic acid (including its 3-hydroxy derivative), and alanine in a molar ratio of 1:1:1:0.6–0.8, although the cell wall of *Micromonospora* sp. F_3 was reported to contain glycine, glutamic acid, diaminopimelic acid, and alanine in a molar ratio of 1.5:1:2:1 (32). The cell wall of *Micromonospora* contains D-alanine but not L-alanine. Thus, it seems most reasonable to assume that glycine, which has no asymmetric carbon atom, should replace L-alanine contained in the usual peptide subunit of peptidoglycan. The molar ratio of four amino acids can be explained either by the peptide moiety cross-linked directly or by the peptide moiety cross-linked by one or more peptide units having the same composition. The possibility of the latter, however, will be excluded by the presence of an almost equal amount of glycolate per diaminopimelate in the cell walls of most *Micromonospora* strains (Table 6), because such a peptide moiety has three or more moles of diaminopimelic acid per two of muramic acid, and the molar ratio of glycolate, which may bind to the amino group of muramic acid as mentioned later, should be less than 0.67 to diaminopimelic acid. A direct cross-linkage is limited to a linkage between the carboxy group of D-alanine in one peptide subunit and the ω-amino group of the diamino acid in another peptide subunit. Thus, *Micromonospora* appears to have the peptide moiety shown in Fig. 2. The somewhat lower molar ratio of alanine to glycine could be explained by the loss of C-terminal D-alanine by D-alanine carboxypeptidase.

The glycan moiety of peptidoglycan is remarkably uniform in bacterial cell walls, but some variations have been reported. The occurrence of *N*-glycolylmuramic acid has been found in *Mycobacterium, Gordona, Nocardia, Corynebacterium, Brevibacterium*, and *Microbacter-*

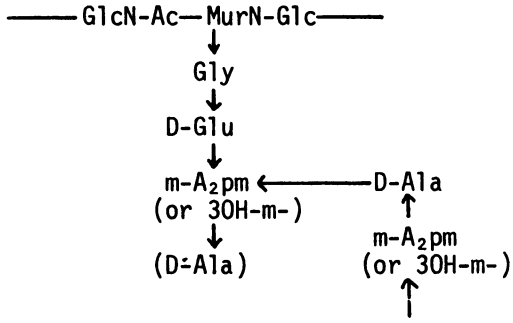

FIG. 2. *Proposed structure for the peptidoglycan in Micromonospora cell wall. Abbreviations: GlcN-Ac, acetylglucosamine; MurN-Glc, N-glycolylmuramic acid; Gly, glycine; D-Glu, D-glutamic acid; m-A2pm, meso-diaminopimelic acid; 3OH-m-, 3-hydroxy-meso-; D-Ala, D-alanine.*

ium (1, 3, 5, 9, 23, 24, 30, 31). *Micromonospora* was found to contain an almost equal molar ratio of glycolate to diaminopimelate in the cell walls. The trichloroacetic acid treatment, which is known to remove polysaccharides of bacterial cell walls, did not change the ratio between them. These suggest that in *Micromonospora* the glycolyl group replaces the acetyl group of *N*-acetylmuramic acid as in *Nocardia* and *Mycobacterium*. Lysozyme, *β-N*-acetylmuramidase, does not hydrolyze the peptidoglycan having *N*-glycolylmuramic acid. Actually, the peptidoglycans of *M. sagamiensis* and *M. olivoasterospora* were not liquified by the enzyme (unpublished data), although some *Micromonospora* strains were reported to be more sensitive to lysozyme than *Mycobacterium* (15, 28).

The peptidoglycans which have glycine bound to muramic acid have been found in such microorganisms as *Corynebacterium poinsettia* (22), *Arthrobacter* sp. (7), *Microbacterium lacticum* (27), and *Arachinia propionica* (26). These organisms also contain glycine or a diamino acid or both in the cross-linkage of the peptidoglycan. The actinomycetes having glycine in their cell walls are limited to cell wall types I and II. The glycine in the peptidoglycan of *Streptomyces* (cell wall type I) is known to connect two peptide subunits between LL-diaminopimelic acid and D-alanine (13, 17), whereas that of *Micromonospora* appears to occupy the first position (N-terminal amino acid) of the peptide subunit. Even though further investigation is required to elucidate the primary structure of the peptidoglycan in *Micromonospora*, it seems reasonable to conclude that this genus has a new type of peptidoglycan which has never been found in other microorganisms (Fig. 2).

Actinoplanes, *Amorphosporangium*, *Ampullariella*, and *Dactylosporangium* strains were found to contain a significant amount of glycolate in the whole cells. They belong to cell wall type II as does *Micromonospora*. The molar ratios of the amino acids in their cell walls reported by Szaniszlo and Gooder (30) are similar to that of *Micromonospora*, except that the molar ratios of diamino acids were less in some organisms containing 3-hydroxydiaminopimelic acid. The lower values are probably due to the ninhydrin color constant of 3-hydroxydiaminopimelic acid used for calculation. Thus, all the strains belonging to cell wall type II seem to have the primary structure of peptidoglycan shown in Fig. 2.

Draper (8) reported that the cell wall of *Mycobacterium leprae* was composed of glycine, glutamic acid, *meso*-diaminopimelic acid, and alanine in a molar ratio of 1:1:1:0.7–0.8. The peptidoglycan of *Mycobacterium* has been shown to have the peptide subunit L-alanyl-D-glutaminyl-*meso*-diaminopimelyl-(D-alanine), which is cross-linked directly between D-alanine and *meso*-diaminopimelic acid (24). Thus, *Mycobacterium leprae* may be an exception and may have a peptidoglycan similar to that of *Micromonospora*.

The cell wall composition has been widely used as one of the important keys for the classification of *Actinomycetales*. Glycolate, arabinose, and xylose were found in the cell walls of almost all of the strains of *Micromonospora* examined. Thus, these cell wall components, in addition to glycine and *meso*-diaminopimelic acid, can play an important role in the chemotaxonomy of the genus *Micromonospora*. However, it is to be determined whether neutral hexose components and 3-hydroxydiaminopimelic acid are useful for the classification of species in *Micromonospora*. A same cell wall pattern (neutral sugars and diamino acids) was observed between *M. purpureochromogenes* and *M. fusca*, the names of which were reported by Luedemann (14) to be subjective synonyms, whereas the pattern of *Micromonospora chalcea* ATCC 12452 was not identical with those of *M. chalcea* strains reported by Cummins and

Harris (6) or by Yamaguchi (34). Thus, the presence of hexoses and also 3-hydroxydiaminopimelic acid may depend upon a strain and be influenced by conditions of cultivation.

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