Chemical Composition of Cell-Wall Preparations from Strains of Various Form-Genera of Aerobic Actinomycetes

B. BECKER, M. P. LECHEVALIER, AND H. A. LECHEVALIER

Institute of Microbiology, Rutgers, The State University, New Brunswick, New Jersey

Received for publication 9 November 1964

Abstract

BECKER, B. (Rutgers, The State University, New Brunswick, N.J.), M. P. LECHEVALIER, AND H. A. LECHEVALIER. Chemical composition of cell-wall preparations from strains of various form-genera of aerobic actinomycetes. Appl. Microbiol. 13:236-243, 1965.—Cell-wall preparations were made from more than 140 strains of aerobic actinomycetes representing most of the form-genera that have been proposed. All cell-wall preparations contained as major constituents glucoseamine, muramic acid, alanine, and glutamic acid. In addition, cell-wall preparations from various types of streptomycetes and strains of Microsporospora contained glycine and L-α,γ-diaminopimelic acid; those from strains of most Actinoplanaceae and Micromonosporaceae contained glycine and meso-α,γ-diaminopimelic acid; those from strains of Thermomonospora, Microbispora, Dermatophilus, and nocardiae of the madurae-pelletieri group contained meso-α,γ-diaminopimelic acid; and those from strains of Thermomonospora, Micropolyspora, and most nocardiae contained meso-α,γ-diaminopimelic acid, arabinose, and galactose. All the strains used were also studied morphologically.

Actinomycetes are bacteria with a fungal morphology. Unlike true bacteria, the actinomycetes form a variety of structures that can be used in their classification. These include various types of conidia on the substrate or the aerial mycelium, sporangia containing either motile or nonmotile spores, and structures that can be equated to the sclerotia, coremia, or pycnidia of fungi. A number of generic names have been proposed for various types of actinomycetes; most of these have been reviewed by Kuster (1963) and Lechevalier (1964). All the systems of classification proposed so far for the actinomycetes are artificial, and generic names refer to form-genera in all respects similar to those of imperfect fungi (Lechevalier, 1964).

The studies of Cummins and Harris (1956), Romano and Nickerson (1956), and Hoare and Work (1957) established that actinomycetes have a cell-wall composition akin to that of gram-positive true bacteria, and also indicated that the chemical composition of the cell wall might furnish practical methods of differentiating between various types of actinomycetes. As a first step in this direction, Becker and co-workers (1964) have proposed a simple method of differentiating between atypical Nocardia-like streptomycetes and atypical Streptomycetes-like nocardiae by paper chromatography of whole-cell hydrolysates.

The aim of the present study was to compare the chemical composition of cell-wall preparations made from representatives of various form-genera of aerobic actinomycetes. The results obtained might suggest other practical methods of differentiating and grouping actinomycetes.

Materials and Methods

Preparation of cell walls. The organisms from which cell-wall preparations were made are listed in Tables 1 to 4. All strains were grown in shake flasks at 28 to 50 C, and the cells were collected at maximal growth after incubation periods varying from 24 hr to 10 days. The media used were those of Becker et al. (1964). All media were used as limpid solutions, clarified by filtration whenever necessary. After incubation, pathogens were killed by shaking with 1.0% formalin overnight at 37 C (this step was omitted for nonpathogens), collected by centrifugation, and thoroughly washed with water. After treatment with ethanolic KOH (0.5%) at 37 C for 24 hr, the cells were washed with ethyl alcohol until the pH value was neutral, and were stored under ethyl alcohol at 4 C until used.

Suspensions of cells were disrupted in a Raytheon 10-ke sonic oscillator with ca. 10 g of glass beads (#812 Cataphote or equivalent, Cataphote Corp., Toledo, Ohio) for 25 ml of cell suspension. After 0.75 hr, the suspension was removed from the oscillator and centrifuged at 1,200 x g for 5 min to remove the glass beads and the unbroken cells.

236
The supernatant fluid was then centrifuged at 12,800 $\times g$, which caused a deposit of the crude broken cell walls. They were washed twice with 0.05 M phosphate buffer (pH 8.0) and twice with distilled water. The cell walls were resuspended in a freshly prepared and filtered solution of trypsin in phosphate buffer and digested at 37 C for 2 hr on a rotary shaker. Trypsin was removed by washing twice with phosphate buffer and twice with distilled water. For further purification, a freshly prepared and filtered solution of pepsin in 0.02 $n$ HCl was added to the walls, and the mixture was put on the shaker at 37 C overnight. The suspension was centrifuged, washed twice with 0.02 $n$ HCl, twice with distilled water, and twice with 95% ethyl alcohol. The purified cell walls were dried in vacuo over $H_2SO_4$.

Hydrolysis and neutralization. For amino acids and amino sugars, a 5-mg (dry weight) sample was hydrolyzed in 1 ml of 6 $n$ HCl in a sealed Pyrex tube (13 X 100 mm) in a sand bath at 100 C for 18 hr. The hydrolysate was filtered, evaporated to dryness three times on a boiling-water bath to remove the HCl, and taken up in 0.3 ml of distilled water.

For sugars, a 10-mg (dry weight) sample was hydrolyzed in 1 ml of 2 $n$ H$_2$SO$_4$ in a sealed Pyrex tube at 100 C for 2 hr. The hydrolysate was neutralized to pH 5.0 to 5.5 with a saturated solution of Ba(OH)$_2$, with methyl red as an internal indicator. The precipitate of BaSO$_4$ was centrifuged at 3,000 rev/min, and the supernatant fluid was poured off into a small beaker and evaporated to dryness in vacuo over $H_2SO_4$. The final product was redissolved in 0.4 ml of distilled water.

Chromatography. Amino acids and amino sugars were identified by spotting 10 $\mu$liters of the hydrolysate on Whatman no. 1 paper and using descending paper chromatography in a tank containing the solvent system n-butanol-pyridine-water-glacial acetic acid (60:40:30:3) (Primosigh et al., 1961) for separation. After 48 hr, the paper was removed, dried at room temperature, and sprayed with 0.4% ninhydrin in water-saturated n-butanol, followed by heating for 5 min or less at 100 C. Reference standards were run alongside the sample, and 10 $\mu$liters of a mixture of meso-$\alpha$, $\epsilon$-diaminopimelic acid, lysine, aspartic acid, glutamic acid, glycine, alanine, galactosamine, glucosamine, and muramic acid, each at a concentration of 0.01 M, were spotted on the paper for comparison. In this solvent system, clear separations were achieved except for lysine and aspartic acid, which run together. When these two compounds are present, the following high-temperature paper chromatography method was used.

The inspiration for the development of this high-temperature method came from the work of Roberts and Kolor (1957). A special apparatus was developed which permitted chromatograms to be run two-dimensionally at 60 C (Fig. 1). It consisted of a stainless-steel tray (25.4 by 30.5 by 5.1 cm), containing a trough for solvents across the width of the narrow end, and a number of stainless-steel rods running along the rest of the tray and parallel to the trough which support the chromatogram in a horizontal position. The tray was placed on a piece of plywood (38.1 by 33 cm) and was topped in succession by a rubber gasket, a sheet of single-thickness window glass, and another piece of plywood through which a slot was cut to enable one to see the solvent front. The glass was held in tight contact with the tray by means of bolts and wing nuts, which passed through the two pieces of plywood at regular intervals around their periphery. After spotting of the samples, the sheet of paper (Whatman no. 1, 26 by 22.9 cm) was irrigated in the first direction with water-dimethyl sulfoxide-n-butanol (8:72:20) for 3.5 hr at 60 C. The paper was then removed and dried overnight at room temperature in a hood. The second day, the paper was replaced in the high-temperature apparatus after having been turned 90°. In the second direction, the chromatogram was developed with a n-propanol-0.2 M acetate buffer at pH 5.6 (70:30) system for 4 hr at 60 C. Ninhydrin was used, as described above, for detection of spots.

For differentiating meso-diaminopimelic acid from L-l-diaminopimelic acid, 10 $\mu$liters of the hydrolysate were spotted on paper for descending chromatography by the method of Hoare and Work (1957) as described in a previous paper (Becker et al., 1964).

Sugars. An amount of 50 $\mu$liters of the hydrolysate was spotted on Whatman no. 1 paper for descending paper chromatography in the same solvent system as was described above for amino acid and amino sugars. A standard solution mixture (10 $\mu$liters) of galactose, glucose, arabinose,
mannose, ribose, and rhamnose, each at a concentration of 5 μg/μl, was spotted on the same paper for reference. After 24 hr, the paper was removed, dried at room temperature, sprayed with aniline acid phthalate reagent (2.0 ml of aniline, 3.3 g of phthalic acid, 100 ml of water-saturated n-butanol), and heated for 5 to 10 min at 100 C for detection of the sugar spots. This system does not separate arabinose from mannose, although pentoses are pink and hexoses are buff-colored. A solvent system, which separates all six sugars by descending paper chromatography, but which must be made up fresh weekly, consists of ethyl acetate-pyridine-water (3.6:1:1.15) (Colombo et al., 1960).

Morphology. Morphological examination of the strains was carried out as indicated by Lechevalier (1964).

RESULTS AND DISCUSSION

Cell-wall preparations were made from more than 140 strains of actinomycetes of various morphological types. Taking into account only the major components present in these cell-wall preparations, it was possible to recognize four groups (Tables 1-4). All the cell-wall preparations from these four groups of organisms contained glucosamine, muramic acid, alanine, and glutamic acid as major cell-wall components.

In Table 1 are listed the organisms of the first group: those having a "Streptomyces-type" of cell wall. In addition to the four common constituents, their cell-wall preparations contained glycine and LL-α, ε-diaminopimelic acid. In this group fall all the organisms forming long chains of conidia on the aerial mycelium. Streptomyces with verticillated chains of spores (Streptoverticillium of Baldacci, 1958), those with sclerotia (Chainia of Thirumalachar, 1955) or with pycnidia (Actinopycnidium of Krassilnikov, 1962) yielded cell-wall preparations chemically identical to those of streptomycetes without these morphological features. Cell-wall preparations from

<table>
<thead>
<tr>
<th>Table 1. Strains having a &quot;Type I&quot; or &quot;Streptomyces-type&quot; cell wall*</th>
<th>Organisms†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long chains of conidia on the aerial mycelium.</td>
<td>Streptomyces fraevis IMRU-3535; S. viridochromogenes K-94; S. antibioticus IMRU-3720; thermophilic Streptomyces spp. IMRU-3390 and IMRU-3397; Streptomyces spp. LL-SS1/4; T-12396 and RG-N-3026</td>
</tr>
<tr>
<td>Long chains of conidia on the aerial mycelium.</td>
<td>Streptomyces griseus IMRU-3475; Streptomyces spp. LL-37-37; LL-6A2; LL-W19; LL-P11/37; LL-P133; LL-27-20; LL-27-23; LL-S113; LL-27-11; LL-W65</td>
</tr>
<tr>
<td>Long chains of conidia on the aerial mycelium.</td>
<td>Streptomyces spp. LL-37-18; LL-W54; LL-37-17; LL-37-15</td>
</tr>
<tr>
<td>Verticillated chains of conidia on the aerial mycelium.</td>
<td>Streptoverticillium (Streptomyces) cinnamomeus RH-23996; S. alborectilis RH-24213; S. netropsis RH-24229; S. kentuckensis RH-24427</td>
</tr>
<tr>
<td>Long chains of conidia formed on the aerial mycelium.</td>
<td>Chainia (Streptomyces) antibiotica IMRU-3750; C. olivacea IMRU-3751; C. poonensis IMRU-3752</td>
</tr>
<tr>
<td>Long chains of conidia formed on the aerial mycelium.</td>
<td>Actinopycnidium (Streptomyces) caerulium K-CP1; K-Ae6</td>
</tr>
<tr>
<td>No conidia formed. Short chains of sporangio-spores formed on the aerial and substrate mycelia.</td>
<td>Microsclerobasidium cinere IMRU-3855; M. flavus IMRU-3857 and IMRU-3858; M. violacea IAL-795</td>
</tr>
</tbody>
</table>

* Unless otherwise indicated, the cell-wall preparations from these organisms contained only glucosamine, muramic acid, alanine, glutamic acid, glycine, and LL-diaminopimelic acid as major components.

† IMRU = Institute of Microbiology, Rutgers University; LL = authors’ collection; RG = courtesy of R. E. Gordon, Rutgers University; T = courtesy of E. Tejera, Department of Health, Caracas; RH = courtesy of R. Hütter, Eidg. Technische Hochschule, Zurich; K = courtesy of N. A. Krassilnikov, Academy of Sciences, Moscow.
Produce sporangia

Microellobosporia (Cross, Lechevalier, and Lechevalier, 1963) were also identical to those obtained from typical streptomycetes. Thus, these sporangia-bearing actinomycetes appear to form a link between the streptomycetes and the rest of the Actinoplanaceae. Macrospora violacea, described by Tsyganov, Jukova, and Timofieva (1963) is included in Table 1 as Microellobosporia violacea, the use of the generic name Macrospora having been pre-empted by its previous valid publication by Fückel (1869–1870). An effort has been made to find streptomycetes with chains of spores on the substrate mycelium (Table 1). Two groups of organisms were found to have this feature. On one group, which includes typical S. griseus strains, occasional long chains of conidia can be found on the primary mycelium. This is consistent with the observations of Orskov (1938). In the other group, the primary mycelium bears occasional single conidia, as well as short chains of spores (up to four). These last organisms might represent a link between Thermoaestromycetes (Tsiklinsky, 1899) and Micromonospora (Orskov, 1923) on the one hand, and the true Streptomycetes on the other (Waksman and Henrici, 1943).

In Table 2 are listed the organisms having a "Micromonospora-type" of cell wall. In addition to the four components common to all actinomycetes, cell-wall preparations from these organisms contain, as major constituents, meso-α, ε-diaminopimelic acid and glycine. Cummins and Harris (1958) reported that cell-wall preparations of strains of Micromonospora contained both the LL and the meso forms of diaminopimelic acid. In addition, these authors reported that the LL isomer of this amino acid was present in a larger amount than was the meso form. Our results indicate that the converse is true, namely, that when the LL isomer is present, it is always a minor component. Through an exchange of letters (and chromatograms) with C.S. Cummins, it was possible to establish that the data that we are now reporting are correct and that the previous report of Cummins and Harris was due to a clerical error. The presence of small amounts of LL-diaminopimelic acid in the cell-wall preparations of some micromonosporae is, however, an indication that these organisms may be more closely related to some of the streptomycetes than a casual observation might indicate. This hypothesis is strengthened by our report in Table 1 of some streptomycetes with single spores on their substrate mycelium. Another interesting finding is that cell-wall analyses point up a possible relationship between Actinoplanes and Micromonospora. This will probably not surprise those who have worked with these organisms. For ex-

**Table 2. Strains having a "Type II" or "Micromonospora-type" cell wall**

<table>
<thead>
<tr>
<th>Morphology</th>
<th>Organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>No aerial mycelium formed. Single conidia produced on the substrate mycelium.</td>
<td><em>Micromonospora purpurea</em> Lu-2953; <em>Micromonospora</em> spp. Cu-1013; LL-W21; LL-W22; LL 37-56; T-12328B; LL-LN-1</td>
</tr>
<tr>
<td>Produce sporangia containing several motile spores.</td>
<td><em>Actinoplanes</em> spp. LL-W13; LL-W257; LL-W57; LL-P166; LL-P128; LL-P114; LL-W256; LL-S21; LL-W179; LL-W211; LL-E3-15; LL-W178; <em>Spirillospora</em> albida ATCC 15331</td>
</tr>
<tr>
<td>Produce sporangia containing several nonmotile spores.</td>
<td><em>Amorphosporangium auranticolor</em> ATCC 15330; <em>Streptosporangium</em> spp. LL-27-4; LL-37-1; LL-W48; LL-27-2; <em>Streptosporangium</em> spp. LL-35M; LL-NCU1</td>
</tr>
</tbody>
</table>

* Unless otherwise indicated, the cell-wall preparations from these organisms contained only glucose amine, muramic acid, alanine, glutamic acid, glycine, and meso-diaminopimelic acid as major components.


* Contains also minor amounts of LL-α, ε-diaminopimelic acid.

* Contains also glucose.

* Contains also galactose.

* Contains no meso-α, ε-diaminopimelic acid but another undetermined component, probably ornithine.

* Contains no glycine.
Aerial mycelium, Couch (1954) noted, "...Indeed, a non-sporengial strain of *Actinoplanes* might easily be confused with *Micromonaspora* ...," and Jones and Bradley (1964), using different criteria, concluded that there was a relationship between members of these two genera. One should note, in addition, that the amount of glycine present in cell-wall preparations of streptosporangia was considerably less than that present in preparations from strains of *Actinoplanes* and of *Micromonaspora*, indicating that these organisms might tend to be related to some members of the next group in which glycine is not a major constituent.

In Table 3 are listed the strains in the walls of which we found, in addition to the four common constituents, only the meso isomer of \(\alpha,\epsilon\)-diaminopimelic acid. These include representatives of the genera *Thermoactinomyces* (Waksman and Corke, 1953), *Microbispora* (Nonomura and Ohara, 1957; Lechevalier and Lechevalier, 1957), *Dermatophilus* (Gordon, 1964), and a number of strains of *Nocardia*. We noted that when these nocardiae sporulated, they formed short chains of conidia on the aerial mycelium only. This is the type of sporulation that was described by Henssen (1957) for a thermophilic organism that she called *Thermopolyspora polyspora*, all strains of which have since been lost. We consider the name of this taxon illegitimate because its characterization was based on a type of sporulation produced by the organism only when it was grown in the presence of another. Henssen stated that: "Solange die Stämme rein waren, bildeten sich kein Sporen." Her characterization of the taxon was thus based on cultures contaminated with bacteria of undetermined nature, making her work completely impossible to duplicate. In our estimation, Rule 24g of the *International Code of Nomenclature of Bacteria and Viruses* (1958) should be invoked: "A name of a taxon is illegitimate ... if the characterization of the group was based upon an impure or mixed culture."

Since this time, the *T. polyspora* has been mistakenly applied by Corbaz, Gregory, and Lacey (1963) to strains of *Micropolyspora*, which form short chains of spores both on the substrate and aerial mycelia. Some of these cultures will be found in Table 4 listed as G-A91, G-A92, and G-A94, along with other strains of *Micropolyspora*. In Table 3 is included, under the name *Nocardia flexuosa*, the organisms described by Krassilnikov and Agre (1964) as *Thermopolyspora flexuosa*. This organism produces in pure culture a sporulation morphologically similar to that described by

### Table 3. Strains having a "Type III" or "madurace-type" cell wall

<table>
<thead>
<tr>
<th>Morphology</th>
<th>Organisms*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerial mycelium formed. Single conidia formed both on the aerial and the substrate myelia.</td>
<td><em>Thermoactinomyces vulgaris</em> LL-37-10A; LL-P10 LL-P11</td>
</tr>
<tr>
<td>Aerial mycelium formed. Longitudinal pairs of conidia formed on the aerial mycelium only except for strain W112 which formed these spores also on the substrate mycelium.</td>
<td><em>Microbispora rosea</em> RG-86; IMRU-3757; IMRU-3748; <em>M. amethystogenes</em> LL-37-5; <em>M. aerata</em> LL-P132; <em>Microbispora sp.</em> LL-W112; <em>M. bispora</em> IMRU-3759;</td>
</tr>
<tr>
<td>Aerial mycelium not always formed. When the aerial mycelium is formed and when sporulation occurs, short chains of conidia are formed on the aerial mycelium only.</td>
<td><em>Nocardia madurae</em> RG-1234; RG-431; RG-507; RG-1136; RG-N1070; RG-1091; IMRU-3632A; RG-1253; RG-953; <em>N. pelletieri</em> RG-408W; RG-610; RG-408; RG-513; <em>Nocardia spp.</em> LL-Sal1; LL-P56; LL-W234; LL-37-46; LL-P13; C-CF; <em>Nocardia flexuosa</em> K-1132*;</td>
</tr>
<tr>
<td>Aerial mycelium not formed. Substrate mycelium dividing in all planes to form masses of coeci that may be motile.</td>
<td><em>Dermatophilus dermatonatus</em> A-V263; A-V973B; <em>D. congoensis</em> A-V1240; <em>Dermatophilus sp.</em> A-V5355</td>
</tr>
</tbody>
</table>

*Unless otherwise indicated, the cell-wall preparations from these organisms contained only glucosamine, muramic acid, alanine, glutamic acid, and meso-diaminopimelic acid as major components.

*IMRU = Institute of Microbiology, Rutgers University; LL = authors' collection; RG = courtesy of R. E. Gordon, Rutgers University; C = courtesy of T. Cross, Bradford Institute of Technology, Bradford, England; A = courtesy of K. C. Austwick, Central Veterinary Laboratory, Weybridge, Surrey, England; K = courtesy of N. A. Krassilnikov, Academy of Sciences, Moscow.

* Contains also glucose.
2 Contains also aspartic acid.
3 Contains also mannose.
4 Contains also glycine.
Formation of aerial mycelium with chains of unusually long conidia. These are not formed on the substrate mycelium.

Aerial mycelium formed. Formation of short chains of conidia both on the aerial and the substrate mycelia.

Aerial mycelium formed. Formation of single conidia on the aerial mycelium only. Occasionally, longitudinal pairs of conidia are formed on the aerial mycelium.

Aerial mycelium not formed. Primary mycelium breaking into rod-shaped fragments that break further into coccoid elements.

---

TABLE 4. Strains having a “Type IV” or “Nocardia-type” cell wall*

<table>
<thead>
<tr>
<th>Morphology</th>
<th>Organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nocardia asteroides RG-SL107B; RG-9991; LL-102; RG-A5; N. caviae RG-1934; RG-1233; RG-1252; RG-424; RG-732; N. coeliaca IMRU-3520; RG-1124; RG-1105; N. vaccini IMRU-3589; N. brasiliensis RG-774A; RG-774B; IMRU 3487; Nocardia spp. LL-S81/1; LL-L18; LL-W45</td>
</tr>
<tr>
<td></td>
<td>Pseudonocardia sp. LL-W176, LL-27-19; PH-446</td>
</tr>
<tr>
<td></td>
<td>Micropolyspora brevicatena RG-1084; RG-1086; Micropolyspora spp. RG-1085; LL-P78A; LL-P90; LL-W68; G-A91; G-A92; G-A94</td>
</tr>
<tr>
<td></td>
<td>Thermomonospora viridis LL-S2; LL-S8; LL-S20; LL-S62; LL-S77; LL-P19; LL-P113; LL-P112; LL-37-14; LL-37-19; G-A67</td>
</tr>
<tr>
<td></td>
<td>Mycobacterium rhodochrous RG-502</td>
</tr>
</tbody>
</table>

---

* Unless otherwise indicated, the cell-wall preparations from these organisms contained only glucosamine, muramic acid, alanine, glutamic acid, meso-diaminopimelic acid, arabinose, and galactose as major components.

** IMRU = Institute of Microbiology, Rutgers University; LL = authors' collection; RG = courtesy of R. E. Gordon, Rutgers University; PH = courtesy of P. H. Gregory, Dartmouth College; G. = courtesy of P. H. Gregory, Rothamsted Experimental Station, Harpenden, Herts, England.

† Contains also mannose.

‡ Contains also aspartic acid.

§ Does not contain arabinose.

‖ Contains a component having Rf values similar to lysine or ornithine.

‖ Does not contain galactose.

Henssen (1957) for her contaminated cultures of what she called Thermopolyspora polyspora. This type of sporulation, however, is also produced by other organisms, such as sporulating strains of Nocardia madurae. Nocardia sp. C-CF, which was sent to us as a strain of Dermatophilus, might indicate the possibility of passage by mutation from a typical Dermatophilus morphology (production of masses of motile coci) to a nocardial type of morphology.

In Table 4 are listed the strains from which we obtained cell-wall preparations containing as major constituents, in addition to the four common ones, meso-α,ε-diaminopimelic acid, arabinose, and galactose. Here is included, among others, the type species of the genus Nocardia, N. asteroides (Gordon and Mihm, 1962). We feel this group deserves to be called the “Nocardia-type” even though, as we have seen (Table 3), not all organisms classified at present as Nocardia have this kind of cell wall. This is simply an indication that the concept of the genus Nocardia has not yet crystallized, and that we are dealing with a group of organisms lacking homogeneity. In Table 4 we also find members of the genus Pseudonocardia (Henssen, 1957), Micropolyspora (Lechevalier et al., 1961), Thermomonospora (Henssen, 1957), and Mycobacterium. The presence of a Mycobacterium in this group is not surprising, since Cummins and Harris (1958) have already shown that nocardiae and mycobacteria have cell walls of similar composition. The presence of members of the genus Thermomonospora in this group is of interest. First, it shows that all types of actinomyces forming single spores should not be lumped together as was done by Corbaz, Gregory, and Lacey (1963). These three types can be easily distinguished morphologically, and also yield cell-wall preparations falling in three different groups. Strain G-A67 of Thermomonospora viridis (Kuster and Locci, 1963) is the organism described by
Corbaz et al. (1963) as Thermopolyspora glauca sp. nov. Concerning the genus Micropolyspora, one should remember that it was described by Lechevalier, Solotorovsky, and McDermont (1961) to refer to aerobic actinomycetes forming short chains of conidia both on the substrate and the aerial mycelia. These organisms should not be confused with those forming short chains of spores on the aerial mycelium only, as has been done by Corbaz et al. (1963) and Krassilnikov and Agré (1964). As we have seen, the organism with short chains of spores on the aerial mycelium only are similar to nocardiae of the madurae type, and cell-wall preparations obtained from these organisms do not contain any sugar. Likewise, the statement of Thirumalachar and Sukapure (1964) that Micropolyspora is differentiated from Streptomyces "only by the fragmenting nature of the vegetative mycelium" is hardly appropriate, either from a morphological or from a chemical point of view.

One might question how, in practice, one may differentiate between a Streptomyces that produces some chains of spores on the substrate mycelium and a Micropolyspora. This problem might be serious for those who are not familiar with these organisms. How long can a short chain of conidia be? If there is any doubt in this matter, the rapid determination of the isomeric form of diaminopimelic acid (Becker et al., 1964) will give the answer.

One organism, not included in any table, that we will refer to as "Nocardia sp." LL-S81/5 yielded cell-wall preparations containing as major constituents, in addition to the four universal ones, L-α,γ-diaminopimelic acid, arabinose, galactose, and some glycine. This organism might be another link between the nocardiae and the streptomycetes.

Also unusual were Nocardia sp. LL-W45, which contained, in addition to meso-diaminopimelic acid, a component having Rf values similar to those of ornithine, and Actinoplanes sp. W178, which contained neither diaminopimelic acid nor lysine, but probably ornithine.

In addition to the major components reported in Table 2, preparations from Actinoplanes spp. LL-W57, LL-S21, and LL-W256 contained a slow-moving ninhydrin-positive component of unknown nature. A similar material was also present in cell-wall preparations of Amorphosporangium auranticolor and Spirillospora albida (Couch, 1963).

In conclusion, if one follows the methods outlined in this paper, which is in great part a modification of methods used by others (Cummins and Harris, 1958; Hoare and Work, 1957), one obtains, from aerobic actinomycetes, cell-wall preparations of four main types. We do not wish to speculate on the composition of the cell walls of these organisms; we simply wish to stress that these differences in cell-wall composition may be of great help in classifying aerobic actinomycetes. We feel that these studies permit the reliable separation of members of the broad group of "streptomycetes" from the broad group of "nocardiae" (Becker et al., 1964). Our results also indicate that there are sound reasons for considering Thermoactinomyces, Micromonospora, and Thermomonospora as three distinct genera. Also, our data show that actinomycetes with long chains of spores on the aerial mycelium (Streptomyces) should not be confused with actinomycetes with short chains of spores on the aerial mycelium (madurae-pelletierii) which in turn are different from organisms with short chains of spores both on the aerial and substrate mycelia (Micropolyspora).

Acknowledgments
We wish to thank all those who have sent us cultures (see footnotes of the tables). We also greatly appreciate the devoted technical assistance of Michele Stahl and Eva Fekete.

The chemical part of this work was supported by Public Health Service grant AI-05489 from the National Institute of Allergy and Infectious Diseases, and the morphological part by National Science Foundation grant GB-511.

Literature Cited


ÖRSKOV, J. 1923. Investigations into the morphology of the ray fungi. Levin and Munksgaard, Copenhagen.


