JOURNAL OF BACTERIOLOGY
Vol. 88, No. 5, p. 1211-1219 November, 1964

ANTIGENIC STRUCTURE OF THE ACTINOMYCETALES

VII. CHEMICAL AND SEROLOGICAL SIMILARITIES OF CELL WALLS FROM 100 ACTINOMYCETALES STRAINS[1]

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Received for publication 20 April 1964

ABSTRACT

KWAPINSKI, J. B. (The University of New England, Armidale, Australia). Antigenic structure of the Actinomycetales. VII. Chemical and serological similarities of cell walls from 100 Actinomycetales strains. J. Bacteriol. **88**:1211-1219. 1964.—Cell walls prepared mechanically from 100 strains of Actinomycetales were studied by chromatographic and serological methods. The cell walls of *Actinomyces* were found to be serologically related to those of the corynebacteria and to some strains of mycobacteria and nocardiae. The cell walls of nocardiae appeared to be more closely related to those of the mycobacteria, *Streptomyces*, *Micromonospora*, and *Waksmania*. The cell walls of *Micromonospora* and *Waksmania* showed certain serological similarities to those of *Thermoactinomyces* and nocardiae. *Micropolyspora* was antigenically different from other species of the Actinomycetales. Three serological groups of mycobacteria and four groups of nocardiae were distinguished.

The aim of this work was to investigate the chemical and antigenic similarities among cell walls of various genera and species of the Actinomycetales, before attempting the isolation of type-specific components of these microorganisms. During the 4-year period of this research, a number of papers on the chemical and serological properties of some genera of the Actinomycetales have appeared (Sohler, Romano, and Nickerson, 1958; Cummins and Harris, 1959; Davis and Baird-Parker, 1959; Schneidau and Shaffer, 1960; Slack, Winger, and Moore, 1961; Merkel, 1961; Kwapinski and Snyder, 1961; Cummins, 1962; Kwapinski, 1963). Results of these investigations have been discussed in a separate paper (Kwapinski and Seeliger, 1964).

[1] Part of this study was done in the Department of Bacteriology, The University of London, London Hospital Medical College, London, England, in cooperation with C. S. Cummins.

MATERIALS AND METHODS

The strains used in this study are listed in Table 1. Among them were 29 strains of *Mycobacterium*, 8 *Actinomyces*, 2 *Thermoactinomyces*, 1 *Jensenia*, 44 *Nocardia*, 1 *Micropolyspora*, 5 *Micromonospora*, 2 *Waksmania*, and 4 *Streptomyces*, together with 4 strains of *Corynebacterium*.

Most strains were cultivated in a 1% glycerol or glucose broth in Roux bottles or large jars at 37 or 25 C for 3 days to 8 weeks, aerobically or anaerobically, according to species. About one-half of the *Nocardia* strains were grown in Kwapinski's (1963) semisynthetic medium. One strain, *Thermoactinomyces* W-18, which failed to grow in liquid culture media, was cultivated on 5% blood-agar. The purity of the cultures, and the morphological characteristics of the microorganisms, were established by microscopic examination and by culture tests on glucose or glycerol broth or blood-agar.

Pure cultures were harvested and treated with 1% formalin at 40 C for 16 hr. After centrifugation, sediments were washed six to eight times with distilled water. Washed cells were suspended in distilled water, and were shaken with Ballotini beads (no. 12) in a Mickle disintegrator for 1 to 6 hr at 4 C. The rate of cell disintegration was approximately 95 to 99%, as determined by phase-contrast microscopy and by examination of the Gram-stained smears under a light microscope. The cell walls were separated from the cytoplasm by centrifugation at 24,150 $\times$ *g* for 10 min in a Servall refrigerated centrifuge, and were washed with distilled water five times or until no trace of protein or carbohydrate was found in the washings. The washed cell walls were resuspended in distilled water to an approximate den-

TABLE 1. *Sources of the 100 strains of actinomycetes used in this study*

Strain	Origin
Actinomyces bovis 4502, 9430	NCTC
A. israelii 233, 271, 285, 1341	P. Holm
A. israelii 6826, 10215	NCTC
Corynebacterium diphtheriae 3984, 7249	NCTC
C. hoffmanii 6981	ATCC
C. pyogenes	Kwapinski
Jensenia canicruria 57	Bisset and Moore*
Micromonospora 98, 99	Bassons 9953, 9954*
Micromonospora W-50, W-180, W-4066	H. Lechevalier
Micropolyspora bravicatena 1086	H. Lechevalier
Mycobacterium avium Sheard	Trudeau Laboratory†
M. avium Kirchberg	Communicable Disease Center (CDC)
M. balnei 11564	ATCC
M. bovis Ravenel	Trudeau†
M. bovis BCG 5692	NCTC
M. butyricum CDC	CDC
M. butyricum 357	ATCC
M. fortuitum 9820	Vallahbhai Patel Inst., Delhi
M. phlei 354	ATCC
M. phlei 8131, 8156	NCTC
M. phlei PZH	Hygiene Institute, Warsaw
M. piscium 13936	ATCC
M. rhodochrous 204	R. Gordon, 1022*
M. rhodochrous 271	ATCC
M. smegmatis 101	ATCC
M. smegmatis 237	R. Gordon, 3*
Mycobacterium P.	Med. School, Portland, Ore.
M. thamnopheos 4445, Izda	ATCC
M. tuberculosis	Trudeau
M. tuberculosis C	A. S. Paterson
Atypical mycobacteria	
scotochromogen P-6	M. S. Tarshis*
scotochromogens P-15, P-19	Salt Lake City, Utah
photochromogen P-18	M. S. Tarshis*
Battey strains An, S-7, S-10	Battey State Hosp., Rome, Ga.
Nocardia asteroides 5	R. Bain
N. asteroides 58, 60, 61	M. Gordon 103, 109, 110*
N. asteroides 72	Drake*
N. asteroides 73	NRRL 8-970*
N. asteroides 84	ATCC 3308
N. asteroides 92, 93, 94, 95	Emmons 9903, 9935, 9976, 9977*
N. asteroides 111	Institut Pasteur 504*
N. asteroides 6846	ATCC
Atypical *Nocardia* 6761	NCTC
N. blackwellii 81	ATCC 6846*
N. brasiliensis 146	McMillan 0-416*
N. brasiliensis 283, 285, 286	Barona*
N. brasiliensis 195	Institut Pasteur*
N. caprae 659	H. Lechevalier
N. caviae 91	Waksman 035*
N. corallina 78	ATCC 4273*
N. eppingeri 112	Institut Pasteur 508*
N. erythropolis 8	Waksman 3407*
N. farcinica 86	ATCC 3318*

TABLE 1.—*Continued*

Strain	Origin
N. farcinica 4524	NCTC
N. leishmanii 82	ATCC 6855*
N. lutea 192	Schneidau*
N. madurae 104	ATCC 6855*
N. madurae 1070, 5654	NCTC
N. opaca 76	ATCC 4267*
N. pelletieri 160	Schneidau*
N. pelletieri 9999	NCTC
N. polychromogenes 7	Waksman 3409*
N. polychromogenes 87	Ciferri 685*
N. protoriana 194	Schneidau*
N. ragoonensis 77	ATCC 6860*
N. rubra (*Proactinomyces* Casabo)	CBS*
N. rubra 74	NRRL S-685*
N. sebivorans 8595	NCTC
N. turbata 152, 153	Erikson*
Streptomyces griseus 8981, 9001, 9806	NCIB, Aberdeen
S. listeri	NCTC
Thermoactinomyces W-18, 37-7	H. Lechevalier
Waksmania W-17	H. Lechevalier
W. rosea 3748	H. Lechevalier

* Strains obtained from N. M. McClung, University of Georgia, Athens, Ga.

† Strains obtained from M. S. Tarshis, Veterans Administration Hospital, Alexandria, La.

sity equal to no. 2 on McFarland's scale, and were centrifuged at 122 or 278 × *g* for 2 to 3 min to remove the nondisintegrated cells. The supernatant fluids were examined microscopically as above; in all cases, complete disintegration of cells was attained by these methods.

The antisera were produced by the intravenous injection of rabbits or intraperitoneal injection of guinea pigs, according to a scheme described earlier (Kwapinski, *Methods of Serological Research, in press*). In most cases, satisfactory titers were obtained after 12 to 14 injections.

The chemical composition of the cell-wall preparations was determined qualitatively by chromatographic examination (Kwapinski, 1960; Kwapinski and Snyder, 1961) of the monosaccharides, amino acids, and higher fatty acids. A modified solvent was introduced for the chromatography of amino acids. It consisted of a 2:1 mixture of the original solvent (containing *n*-butyl alcohol, ethyl alcohol, glacial acetic acid, and distilled water, 10:5:1:2) and 20% phenol. This solvent, found to increase the separation rate of individual amino acids, was used in the unidimension and circular techniques, and as the second solvent in the double-dimension method. The first solvent applied in the double-dimension procedure consisted of propylene glycol, acetone, ethyl alcohol, and distilled water (6.5:50:23.5:20). The chromatograms were run for 12 to 16 hr in each solvent at room temperature. Amino acids were detected with 0.18% ninhydrin in ethyl alcohol.

Serological activity of the cell-wall preparations was examined by complement-fixation (Kwapinski and Snyder, 1961; Kwapinski, *in press*) with the nonabsorbed and the cross-absorbed antisera. Each test was repeated, and the results were averaged. The absorption of the sera was accomplished by adding 200 to 300 mg (wet weight) of the cell-wall sediment per ml of a serum diluted 1:3 and agitating this suspension on an electric shaker at 26 C for 1 hr, followed by 3 hr at 4 C and centrifugation. The supernatant liquid containing an absorbed antiserum was recentrifuged at 35,600 × *g* for 10 min.

RESULTS

The higher fatty acids chromatographically detected in cell walls of the microorganisms studied were palmitic, stearic, and oleic acids.

Linoleic acid was found only in *N. caprae* and *M. balnei*.

Monosaccharides revealed in the cell walls of different species or types of the Actinomycetales are presented in Table 2. Glucosamine was detected in all, and muramic acid in all but one species (*N. polychromogenes*). Arabinose could not be found in the cell walls of *Waksmania*, *Thermoactinomyces*, *S. griseus*, *N. opaca*, *N. madurae*, *N. pelletieri*, *N. rangoonensis*, *N. polychromogenes*, *N. turbata*, or in a strain of *Micromonospora*. Ribose was detected in the cell walls of *Streptomyces*, *Thermoactinomyces*, a strain of *Waksmania*, *N. caprae*, *N. erythropolis*, *M. tuberculosis*, *M. rhodochrous*, and *Jensenia*. Rhamnose was found in *M. tuberculosis*, *M. bovis*, *A. bovis*, *N. caprae*, *N. erythropolis*, *S. listeri*, and *C. diphtheriae*. Mannose occurred only in *S. griseus*, *Thermoactinomyces*, *M. tuberculosis*, *M. bovis*, and in a strain of *Waksmania*. Cell walls of all strains of *Mycobacterium*, *Actinomyces*, *Jensenia*, and *Micropolyspora* showed regularly the presence of muramic acid, glucosamine, galactose, and arabinose, with mannose, rhamnose, and ribose found only in a few strains. *N. rubra* also belonged to this group. Other strains of *Nocardia* differed considerably in the number and types of monosaccharides found in their cell walls. All strains of *Corynebacterium* proved to contain muramic acid, glucosamine, glucose, and arabinose.

A great majority of the strains showed 14 different amino acids in the hydrolysates of their cell walls (Table 3). Alanine, DL-diaminopimelic acid, glutamic acid, leucine, glycine, serine, lysine, and valine occurred as predominant ("major"), and threonine, tyrosine, cysteine, aspartic acid, arginine, and phenylalanine, as minor components. The cell walls of *Actinomyces* did not contain serine or aspartic acid; those of *N. blackwellii*, *N. caprae*, *N. eppingeri*, and *N. rubra* did not possess aspartic acid, phenylalanine, or leucine. The cell walls of *S. griseus*, *N. farcinica*, and *N. madurae* contained only five amino acids (DL-diaminopimelic acid, alanine, serine, glycine, and glutamic acid). Five amino acids (serine, glycine, glutamic acid, valine, and leucine) were also found in *N. erythropolis* and atypical (*Streptomyces*-like) nocardiae.

Spectra of serological reactions of the cell-wall preparations and heterologous antisera are presented in summary in Table 4. Detailed figures are not listed for want of space. Mimeographed copies of the detailed tables are available on request to the author.

Antisera reacted in the complement-fixation test with homologous cell-wall preparations in dilutions ranging from 800 to 6,400. Reactions with heterologous cell walls were observed at two to eight times lower titers. All antisera reactive with heterologous strains showed a complete or significant reduction of titer after absorption with the heterologous strains. Cell walls of *Actinomyces* were found serologically closely

TABLE 2. *Monosaccharides detected chromatographically in the cell walls of Actinomycetales*

Muramic acid	Glucosamine	Galactose	Glucose	Mannose	Arabinose	Ribose	Rhamnose	Cells walls from
−	+	−	−	−	−	−	−	*N. polychromogenes*
+	+	−	−	−	−	−	−	*N. opaca*, *N. pelletieri*, *N. rangoonensis*, *Waksmania rosea*
+	+	+	−	−	−	−	−	*N. madurae*, *N. turbata*, *Micromonospora* 4066
+	+	−	−	+	−	+	−	*S. griseus*, *Thermoactinomyces*, *Waksmania* W 17
+	+	+	−	±	+	±	±	*Actinomyces*, *Jensenia*, *Micropolyspora*, *N. rubra*, *Mycobacterium* (*avium*, *balnei*, *bovis*, *butyricum*, *fortuitum*, *phlei*, *piscium*, *rhodochrous*, *smegmatis*, *thamnophoes*, *tuberculosis*) and photochromogenic, scotochromogenic, and Battey strains
+	+	+	−	−	+	+	+	*N. caprae*, *N. erythropolis*, *S. listeri*
+	+	+	+	−	+	−	−	*N. asteroides*, *N. blackwellii*, *N. brasiliensis*, *N. caviae*, *N. eppingeri*, *N. lutea*, *N. farcinica*, *N. pretoriana*, *Micromonospora* 99, 180, 98, W-50
+	+	−	+	−	+	−	±	*C. diphtheriae*, *C. hoffmannii*, *C. pyogenes*

TABLE 3. *Amino acid patterns found chromatographically in cell walls of Actinomycetales*

"Major" amino acids detected								Cell-wall preparations from
Alanine	Glutamic acid	Lysine	Glycine	Serine	DL-Diaminopimelic acid	Valine	Leucine	
+	+	+	+	+	+	+	+	*Mycobacterium, Jensenia, Corynebacterium, Micromonospora, Waksmania, Thermoactinomyces, Micropolyspora, N. asteroides, N. brasiliensis, N. caviae, N. farcinica, N. lutea, N. opaca, N. polychromogenes, N. pretoriana, N. turbata*
+	+	+	+	+	+	+	−	*N. blackwellii, N. caprae, N. eppingeri, N. rubra*
+	+	+	+	−	+	+	+	*Actinomyces* spp.
+	+	−	+	+	+	−	−	*S. griseus, N. farcinica, N. madurae*
−	+	−	+	+	−	+	+	*N. erythropolis*, atypical nocardiae

related to those of corynebacteria, *M. tuberculosis*, and *M. bovis*, and less definitely to *N. farcinica*, *N. rubra*, *N. caprae*, and *Waksmania*. Cell walls of different species of *Mycobacterium* seemed to share some antigens with the cell walls of *N. asteroides*, *N. madurae*, *N. farcinica*, *N. rubra*, and *Jensenia canicruria*. Serological relationships between the cell walls of mycobacteria and those of *N. caprae*, *N. corallina*, *N. brasiliensis*, *N. pelletieri*, *N. eppingeri*, and *N. lutea* were less pronounced. The walls of *M. balnei*, *M. avium*, and *M. thamnopheos* exhibited the least serological cross-reactivity with the *Nocardia* cell-wall antisera. Practically no serological relationship was found between the cell walls of mycobacteria and *N. leishmanii*, *N. erythropolis*, *N. opaca*, *N. caviae*, *N. polychromogenes*, *N. pretoriana*, *N. rangoonensis*, and *N. blackwellii*.

The cell walls of *Streptomyces*, *Micromonospora*, and *Waksmania* seemed to share similar or identical group-specific antigens, which also reacted with *N. asteroides*, *N. madurae*, and *M. rhodochrous* antisera, but only occasionally with the sera of other nocardiae or mycobacteria. The cell walls of *Micromonospora* and *Waksmania* appeared to contain antigens common with those of *Thermoactinomyces* and corynebacteria. Cell walls of *Micropolyspora* were antigenically different from all other Actinomycetales studied, with the likely exception of *N. eppingeri* and *N. pelletieri*.

DISCUSSION

Monosaccharides revealed by these studies in the cell-wall preparations of *N. asteroides*, *N. brasiliensis*, *N. pelletieri*, and in various strains of *Mycobacterium* were generally in accordance with those detected by Cummins (1962). However, the carbohydrate patterns in the cell walls of some species of *Corynebacterium* and especially of *Actinomyces* were somewhat different. Particularly, arabinose was not found in the cell-wall preparations of *Actinomyces* by Cummins (1962), whereas it occurred regularly in the cell walls of the *Actinomyces* strains used in this work.

Different methods of preparing cell walls could also account for some discrepancies in the number of various amino acids detected by Cummins (1962). The present results correspond with those of Sohler et al. (1958) and Romano and Sohler (1956). It is possible that the treatment of cell walls with proteolytic enzymes disrupts peptide linkages between certain amino acids, and particularly those joining leucine and valine with other amino acids. These and some other amino acids are apparently lost in the digestive procedure.

The mycobacteria can be divided into three serological groups: serogroup 1, comprising strains of *M. tuberculosis*, *M. bovis*, *M. piscium*, *M. thamnopheos*, *M. fortuitum*, and *M. avium;* serogroup 2, consisting of atypical strains of *Mycobacterium* (*M. balnei*, photochromogenic, scotochromogenic, and Battey strains), which are serologically related to group 1 through some interspecies cell-wall antigens of *M. tuberculosis* and, partly, *M. fortuitum;* and serogroup 3, containing saprophytic mycobacteria (*M. butyricum*, *M. phlei*, *M. smegmatis*, and *M. rhodochrous*), which are serologically related to each

TABLE 4. *Spectra of serological reactivity of cell walls of Actinomycetales**

Cell walls of	Reactive with antisera from
A. israelii	*A. bovis, C. diphtheriae, C. hoffmannii, C. pyogenes, M. tuberculosis, M. bovis, N. asteroides, N. farcinica, N. rubra, N. caprae, Waksmania*
A. bovis	As above except *N. asteroides*
C. diphtheriae	*C. hoffmannii, C. pyogenes, A. israelii, A. bovis, M. fortuitum, M. tuberculosis, N. farcinica, Thermoactinomyces*
C. pyogenes	*C. diphtheriae, C. hoffmannii, A. israelii, A. bovis, M. fortuitum, Jensenia, Thermoactinomyces*
C. hoffmannii	*C. diphtheriae, C. pyogenes, M. fortuitum, M. tuberculosis, Jensenia, N. farcinica, Thermoactinomyces*
M. tuberculosis	*M. bovis, M. piscium, M. thamnopheos, M. fortuitum, M. phlei, M. smegmatis, M. avium, M. balnei,* Battey, photo- and scotochromogenic strains, *N. asteroides, N. farcinica, N. corallina, N. brasiliensis, N. opaca, N. blackwellii, A. israelii, A. bovis, C. diphtheriae, C. hoffmannii*
M. bovis	As above except *C. hoffmannii, N. brasiliensis, M. balnei,* photo- and scotochromogenic strains
M. piscium	*M. tuberculosis, M. bovis, M. thamnopheos, M. fortuitum, M. avium, M. phlei, M. smegmatis, M. butyricum, M. rhodochrous,* photo- and scotochromogenic strains, *N. asteroides, N. rubra, N. caprae, N. eppingeri, N. pelletieri, N. brasiliensis, S. griseus, A. israelii, A. bovis*
M. thamnopheos	*M. tuberculosis, M. bovis, M. piscium, M. phlei, M. smegmatis, M. butyricum, M. rhodochrous, M. fortuitum, Jensenia, N. madurae, N. farcinica, N. brasiliensis, N. blackwellii, S. griseus, C. hoffmannii, C. pyogenes*
Battey strains	*M. tuberculosis, M. bovis, M. phlei, M. butyricum, M. fortuitum,* photo- and scotochromogenic strains
Photochromogenic mycobacteria	Battey, scotochromogenic mycobacteria, *M. balnei, M. rhodochrous, Jensenia, N. rubra, N. caprae, N. corallina, N. eppingeri, N. erythropolis, N. turbata*
Scotochromogenic mycobacteria	Battey, photochromogenic mycobacteria, *M. balnei, M. rhodochrous, M. tuberculosis, M. fortuitum, Jensenia, N. rubra, N. corallina, N. pelletieri, N. brasiliensis, N. rangoonensis*
M. fortuitum	*M. tuberculosis, M. bovis, M. piscium, M. avium, M. thamnopheos, M. phlei, M. butyricum,* Battey and scotochromogenic strains, *N. asteroides, N. madurae, N. farcinica, N. corallina, N. brasiliensis, N. turbata, N. blackwellii, Waksmania, A. israelii, A. bovis*
M. avium	*M. tuberculosis, M. bovis, M. piscium, M. fortuitum, M. smegmatis, M. butyricum, N. rubra, N. pelletieri*
M. balnei	*M. tuberculosis, M. fortuitum, M. smegmatis,* photo and scotochromogenic mycobacteria
M. smegmatis	*M. tuberculosis, M. bovis, M. thamnopheos, M. piscium, M. phlei, M. butyricum, M. rhodochrous, M. balnei, A. israelii, A. bovis, C. hoffmannii, C. pyogenes, N. asteroides, N. farcinica, N. rubra, N. corallina, S. griseus*
M. butyricum	All mycobacteria except *M. avium, M. smegmatis,* and *M. balnei;* and *Jensenia, N. asteroides, N. farcinica, N. rubra, N. caprae, N. corallina, N. eppingeri, N. lutea, N. brasiliensis, N. erythropolis, N. opaca, N. polychromogenes, N. blackwellii, Waksmania, A. israelii, A. bovis, C. hoffmannii*
M. phlei	All mycobacteria except *M. avium, M. thamnopheos, M. balnei,* and photochromogenic strains; and *Jensenia, N. asteroides, N. madurae, N. farcinica, N. rubra, N. caprae, N. lutea, N. brasiliensis, N. opaca, N. blackwellii, C. diphtheriae, C. hoffmannii*
M. rhodochrous	All mycobacteria except *M. balnei;* and *Jensenia, N. farcinica, N. rubra, N. corallina, N. eppingeri, N. lutea, N. pelletieri, N. brasiliensis, N. rangoonensis, N. blackwellii, Micromonospora, S. griseus, S. listeri, A. israelii, A. bovis, C. hoffmannii, C. pyogenes*
J. canicruria	*M. tuberculosis, M. bovis, M. thamnopheos, M. fortuitum, M. phlei, M. butyricum,* photo- and scotochromogenic strains, *S. griseus, C. hoffmannii, C. pyogenes,* and all nocardiae except *N. eppingeri, N. pelletieri, N. leishmannii, N. polychromogenes, N. pretoriana*
N. asteroides	All mycobacteria except *M. balnei,* photochromogenic strains, *M. smegmatis,* and *M. rhodochrous;* and *Jensenia, Waksmania,* and all nocardiae except *N. pretoriana*

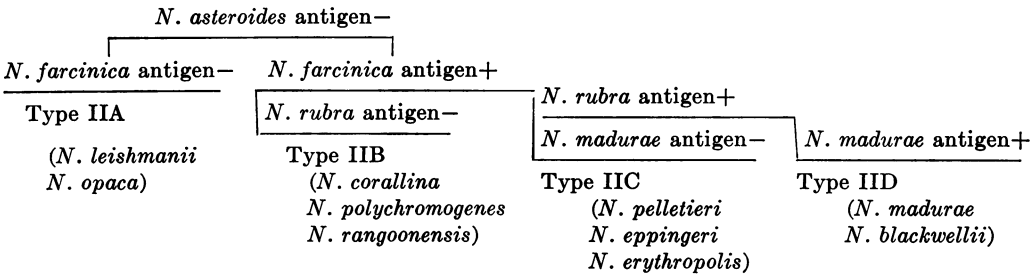
TABLE 4.—*Continued*

Cell walls of	Reactive with antisera from
N. madurae	*M. tuberculosis, M. bovis, M. fortuitum, M. piscium, M. balnei,* Battey strains, *M. phlei, M. rhodochrous, Actinomyces, C. diphtheriae, C. hoffmannii, N. asteroides, N. madurae, N. farcinica, N. rubra, N. caprae, N. pelletieri, N. brasiliensis, N. pretoriana, N. rangoonensis, Micromonospora, Streptomyces*
N. farcinica	*M. tuberculosis, M. bovis, M. fortuitum, Micromonospora,* and all nocardiae except *N. madurae, N. leishmanii, N. opaca, N. polychromogenes, N. pretoriana,* and *N. blackwellii*
N. rubra	All mycobacteria except *M. thamnopheos* and *M. balnei;* and *Jensenia, C. hoffmannii, Micromonospora, Streptomyces,* and all nocardiae except *N. brasiliensis, N. leishmanii, N. opaca, N. polychromogenes, N. pretoriana,* and *N. blackwellii*
N. caprae	*M. piscium, M. butyricum, M. phlei, Jensenia, Actinomyces, Micromonospora, Thermoactinomyces,* and all nocardiae except *N. lutea, N. leishmannii, N. erythropolis, N. opaca,* and *N. blackwellii*
N. corallina	*M. tuberculosis, M. bovis, M. fortuitum, M. phlei, M. butyricum, M. rhodochrous,* photo- and scotochromogenic strains, *Jensenia, S. griseus, N. asteroides, N. madurae, N. farcinica, N. caprae, N. eppingeri, N. lutea, N. pelletieri, N. brasiliensis*
N. eppingeri	*M. avium, M. butyricum,* photo- and scotochromogenic strains, *Micropolyspora, N. farcinica, N. rubra, N. corallina, N. brasiliensis, N. leishmanii, N. turbata, N. polychromogenes, N. blackwellii,* and atypical nocardiae
N. lutea	*M. avium, M. phlei, M. butyricum, M. rhodochrous, Jensenia, N. asteroides, N. madurae, N. farcinica, N. caprae, N. eppingeri, N. pelletieri, N. opaca, Streptomyces*
N. pelletieri	*M. avium, M. piscium, M. thamnopheos, M. butyricum, M. rhodochrous, N. asteroides,* atypical nocardiae, *N. rubra, N. caprae, N. corallina, N. brasiliensis, N. opaca, N. blackwellii, Thermoactinomyces, Micropolyspora*
N. brasiliensis	*M. fortuitum, M. piscium, M. thamnopheos, M. rhodochrous, Jensenia, N. madurae, N. farcinica, N. caprae, N. corallina, N. eppingeri, N. leishmanii, N. erythropolis, N. polychromogenes,* atypical nocardiae, *S. listeri, W. rosea*
N. turbata	*M. balnei, N. caprae, N. corallina, N. lutea, N. turbata, N. pretoriana, N. blackwellii,* atypical nocardiae, *S. griseus*
N. rangoonensis	*M. tuberculosis, N. skotochromogenes, Jensenia, N. asteroides, N. madurae, N. farcinica, N. caprae, Streptomyces*
N. blackwellii	*M. bovis, M. thamnopheos, M. butyricum, Jensenia, N. asteroides, N. madurae, N. farcinica, N. rubra, N. corallina, N. eppingeri, N. erythropolis, N. turbata, Waksmania, Thermoactinomyces, C. hoffmannii, C. pyogenes*
N. erythropolis	*M. bovis, M. thamnopheos, M. butyricum, Jensenia, N. asteroides, N. farcinica, N. rubra, N. eppingeri, N. blackwellii,* atypical nocardiae, *Waksmania*
N. opaca	*M. avium, M. piscium, Jensenia, N. asteroides, N. corallina, N. brasiliensis, N. erythropolis*
N. caviae	*Jensenia, N. asteroides, N. lutea, N. opaca*
N. polychromogenes	*N. asteroides, N. caprae, N. eppingeri*
N. leishmanii	*N. rubra, N. pelletieri, N. brasiliensis, N. rangoonensis, S. griseus*
N. pretoriana	*N. madurae, N. caprae, N. eppingeri, N. brasiliensis, N. turbata, Micromonospora, Waksmania*
Micromonospora	*M. rhodochrous, N. asteroides, N. madurae, N. rubra, N. caprae, N. pretoriana* atypical nocardiae, *S. griseus, Thermoactinomyces*
S. griseus	*M. butyricum, M. rhodochrous, N. madurae, N. farcinica, N. rubra, N. corallina, N. leishmanii, N. caviae, N. turbata, N. rangoonensis,* atypical nocardiae, *Micromonospora, Waksmania*
S. listeri	*M. rhodochrous, N. madurae, N. lutea, N. brasiliensis, N. pretoriana, N. rangoonensis, S. griseus, Waksmania*
Waksmania	*N. madurae, N. rubra, N. erythropolis, N. pretoriana, Thermoactinomyces*
Thermoactinomyces	*Actinomyces, N. rubra, N. blackwellii, Micromonospora, Waksmania*
Micropolyspora	*N. eppingeri, N. pelletieri*

* All cell-wall preparations were reactive in the homologous antisera.

TABLE 5. *Antigenic patterns in cell walls of serological group I nocardiae*

Common (interspecies) antigen of *Nocardia*									Revealed in	
pretoriana	*brasilinesis*	*pelletieri*	*atypical*	*asteroides*	*rubra*	*farcinica*	*caprae*	*madurae*	Strains of	Type
+	+	+	+	+	+	+	+	+	*N. caprae*	IA
	+	+	+	+	+	+	+	+	*N. asteroides*	IB
		+	+	+	+	+	+	+	*N. rubra*	IC
	+	+	+	+	+	+	+	+	*N. farcinica*	ID
		+	+	+	+				*N. lutea*	IE
			+	+	+				*N. caviae*	IF



Note: +, the antigen present; −, the antigen absent.

FIG. 1. *Differentiation of serological types in group II nocardiae.*

other through five or six antigens and to group 1 by some interspecies cell-wall antigens of *M. fortuitum* and *M. thamnopheos*.

The nocardiae can be serologically divided into four groups and 14 serological types. This differentiation depends on the serological detection of interspecies antigens of *N. asteroides*, *N. caprae*, *N. rubra*, *N. farcinica*, *N. madurae*, *N. brasiliensis*, *N. pelletieri*, *N. pretoriana*, and atypical *Nocardia* in the cell walls of the examined strains.

Cell walls of all strains classified in group I share three antigenic components, namely, the common (interspecies) antigen of *N. asteroides*, *N. rubra*, and atypical *Nocardia*. According to serological type, they contain none or one to six additional common factors, as shown in Table 5. Strains designated as *N. asteroides* (with the exception of atypical strains), *N. rubra*, *N. farcinica*, *N. lutea*, *N. caprae*, and *N. caviae* belong to this serological group.

All members of group II (*N. madurae*, *N. blackwellii*, *N. pelletieri*, *N. erythropolis*, *N. eppingeri*, *N. polychromogenes*, *N. rangoonensis*, and *N. opaca*) possess one antigen common with the cell walls of *N. asteroides* and share none or one to three other antigens with *N. farcinica*, *N. rubra*, and *N. madurae*, as shown in Fig. 1. Four serological types (IIA–IID) are established within this group.

Cell walls of all strains of group III contain three common antigens, all shared with *N. asteroides*, *N. caprae*, and *N. pretoriana*. Strains of *N. turbata*, *N. brasiliensis*, and atypical (*Streptomyces*-like) strains of *Nocardia* belong to this group. Strains of *N. turbata* also show a serological relationship to *N. rubra; N. brasiliensis* is related to some strains of groups I and II (*N. farcinica*, *N. madurae*, *N. corallina*, and *N. caprae*). Cell walls of all strains belonging to group III are serologically related to *S. griseus*.

Group IV comprises just one strain, *N. pretoriana*. The cell walls of this microorganism, in contrast with all other nocardiae, do not cross-react with *N. asteroides*, but are serologically related to *N. brasilienisis*, *N. madurae*, and *N. caprae*, and to some strains of *Streptomyces*.

ACKNOWLEDGMENT

Appreciation is expressed to J. N. Caling, University of New England, for the immunization of rabbits and guinea pigs.

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