ANTIGENIC STRUCTURE OF *ACTINOMYCETALES*

VI. Serological Relationships Between Antigenic Fractions of *Actinomyces* and *Nocardia*

J. B. KWAPINSKI

University of New England, Armidale, New South Wales, Australia

Received for publication 20 February 1963

Abstract

Kwapinski, J. B. (University of New England, Armidale, N.S.W., Australia). Antigenic structure of *Actinomycetales*. VI. Serological relationships between antigenic fractions of *Actinomyces* and *Nocardia*. J. Bacteriol. **86**:179–186. 1963.—A total of 52 chemical fractions were obtained by a comprehensive technique of preparation from three strains of *Actinomyces* and three strains of *Nocardia*. The chemical and serological structures and specificities of disintegrated cells, cell walls, cytoplasms, and individual fractions were thoroughly studied. Cytoplasmic materials were found to be serologically alike or identical. The polysaccharide fractions, extracted from cell walls with alkali, formamide, and phenol, proved to be serologically related. Fractions prepared from the *Nocardia* by extractions in hot and concentrated solutions of acetic acid and sodium hydroxide, as well as the second protein fraction and the acetate-extracted polysaccharides of both the *Nocardia* and *Actinomyces*, proved to be genus-specific.

An apparent morphological similarity between the *Actinomyces* and *Nocardia* was regarded by certain investigators as sufficient to classify these microorganisms as one or two genera within the family *Actinomycetaceae* (Wilson and Miles, 1957; Waksman and Henrici, 1943; Breed, Murray, and Smith, 1957). Other investigators (Gordon and Mihm, 1957) divided strains of *Nocardia* into two different groups on the basis of colonial morphology, one being similar to *Streptomyces* and the other to *Mycobacterium*.

Some differences in the chemical composition of cell walls, revealed by a chromatographic technique, were used by Cummins and Harris (1958) for the proposed taxonomic separation of *Actinomyces* from *Nocardia*, with the insertion of the latter in the family *Mycobacteriaceae*. However, chemical compositions of the whole or fractionated cytoplasmic materials of these microorganisms have not been compared.

Cytoplasmic fractions of *Actinomyces* and *Mycobacterium* were found by serological techniques to be antigenically related, whereas most of the antigenic fractions isolated from cell walls were genus- or type-specific (Kwapinski and Snyder, 1961; Kwapinski, 1960). Certain serological relationships between *Actinomyces* and *Nocardia*, and among *Nocardia*, *Mycobacterium*, and *Corynebacterium*, were revealed by agglutination tests but not by the fluorescent-antibody technique (Slack et al., 1951; Slack, Winger, and Moore, 1961; Schneidau and Schaffer, 1960; Cummins, 1962).

The present studies were carried out to determine the chemical and antigenic structure of *Nocardia*, and to investigate the biological position of the *Nocardia* in relation to other genera of the *Actinomycetales*.

Materials and Methods

A total of 14 (5 nucleoprotein and 9 polysaccharide) chemical fractions of *Actinomyces israelii*, employed for this research, were isolated previously from three strains, and their chemical and serological characteristics were described (Kwapinski, 1960). Another two polysaccharide fractions (C_s and C_6) were isolated from each of the three strains of *A. israelii* by the methods cited below.

The following strains of *Nocardia* were used in the investigations: *N. asteroides* NCTC 8595, London; *N. asteroides* 5, obtained from R. V. S. Bain, University of Sydney, Sydney, Australia; and *N. rubra*, obtained from N. M. McClung, University of Georgia, Athens.

Each strain was cultivated in 5% glycerol broth or a semisynthetic medium containing 0.25% arabinose, 0.25% xylose, 1% galactose,

2% glucose, 0.5% mannose, 0.25% rhamnose, 0.5% asparagine, and 0.2% ammonium chloride dissolved in phosphate buffer (pH 6.5). Cultures in Roux bottles were incubated at 37 C for 4 to 5 days and then treated with 0.5% formalin. Cells were collected by centrifugation, washed three to five times with distilled water, and disintegrated with Ballotini no. 12 glass beads at 5 C for 10 min in a high-speed blender (Linnane and Vitols, 1962); approximately 99% of cells were disintegrated, as determined by observation in an electron microscope. Disintegration for 10 to 20 min in a Raytheon 20-kc ultrasonic oscillator produced only 20 to 30% disintegration.

Cell walls were separated from cytoplasm by centrifugation at 21,000 × *g* for 15 min in a Servall RC-2 superspeed refrigerated centrifuge and washed repeatedly with water until the washings were free from protein or carbohydrate. The first supernatant was recentrifuged for 20 min at 25,000 × *g*, passed through a no. 5 Schott glass filter, and dialyzed against water for 48 hr. Only homogeneous masses, but no particular matter, were found in smears made from the concentrated cytoplasm, as examined under a phase-contrast microscope or in Gram-stained smears. The cell-wall material was resuspended in water and centrifuged at 270 × *g* for 3 min to remove heavier particles if present. The supernatant appeared free from cytoplasmic material when examined by electron microscopy.

Chemical fractions were isolated from cell walls, and the cytoplasm was obtained by Kwapinski's method (1960) as partially modified (Fig. 1). The sulfosalicylic polysaccharide fractions were extracted from cell walls as described before (Kwapinski and Snyder, 1961). In all, 32 fractions were prepared from the strains of *Nocardia*.

Chemical examination of fractions was carried out by the following techniques: determination of nitrogen by the method of Elek and Sobotka (1925), protein by Weichselbaum's (1946) method, phosphorus by the method of Fiske and Subba-Row (1925), total carbohydrate by Dische's (1955) modification of the Molisch test, hexoses by the method of Trevelyan and Harrison (1952), pentoses by the methods of Bailey (1959) and Mejbaum (1939), and deoxyribose by Dische's (1930) method. Amino acids were identified chromatographically by the two-dimensional technique (Kwapinski and Snyder, 1961), high fatty acids as described previously (Kwapinski and Mikulaszek, 1957), and monosaccharides by Kwapinski's (1960) paper-chromatographic technique, which has been partially modified by detecting spots of sugars with 1% alcoholic solutions of cadmium chloride and aniline.

Antisera were prepared by intravenous immunization of rabbits with the disintegrated cells, or by intraperitoneal injection of cell walls, cytoplasm, or an appropriate fraction into guinea pigs. Eight intraperitoneal injections at 3-day intervals, followed in 1 week by the bleeding, sufficed for the production of antibodies with an average titer of 1:2,000.

Serological activities of fractions were examined by complement fixation (Kwapinski and Snyder, 1962). The cross-reactions were studied by (i) absorption-agglutination tests in the case of cell walls, (ii) complement fixation with the cross-absorbed antisera, (iii) complement-fixation inhibition (Kwapinski, *in preparation*), and (iv) diffusion-precipitation tests of Ouchterlony (1949) or Preer (1956), in the case of chemical fractions.

Absorption of antisera with either cell walls or cytoplasm was carried out at 37 C for 2 hr and at 4 C for 4 hr with approximately 100 to 150 mg of absorbent per ml of serum.

RESULTS

Chemical analysis of dry cells of *Nocardia* strains revealed 9.0 to 9.2% N, 1.0 to 1.1% P, and 48.8 to 50.2% carbohydrate, or about 50% protein and 50% sugar, whereas lipid was roughly 2%. The cytoplasm contained 13.6 to 14.6% N, 0.2 to 0.23% P, 82.6 to 84.0% protein, and 7.4 to 7.8% carbohydrate. Cell walls contained 6.0 to 7.1% N, 0.3 to 0.35% P, and 69.9 to 71.2% carbohydrate.

A total of 13 amino acids (cystine, aspartic acid, arginine, lysine, alanine, glycine, serine, threonine, glutamic acid, cysteine, tyrosine, valine, and leucine) were detected in the proteins by paper chromatography.

Monosaccharides were galactose, glucose, glucosamine, arabinose, rhamnose, and muramic acid (Table 1). Rhamnose, however, did not occur on the chromatograms of *N. rubra*, and glucosamine was found only in one fraction. The higher fatty acids (palmitic, stearic, linolenic, linoleic, and oleic) were revealed in all strains of *Nocardia*. Linoleic acid did not occur in the neu-

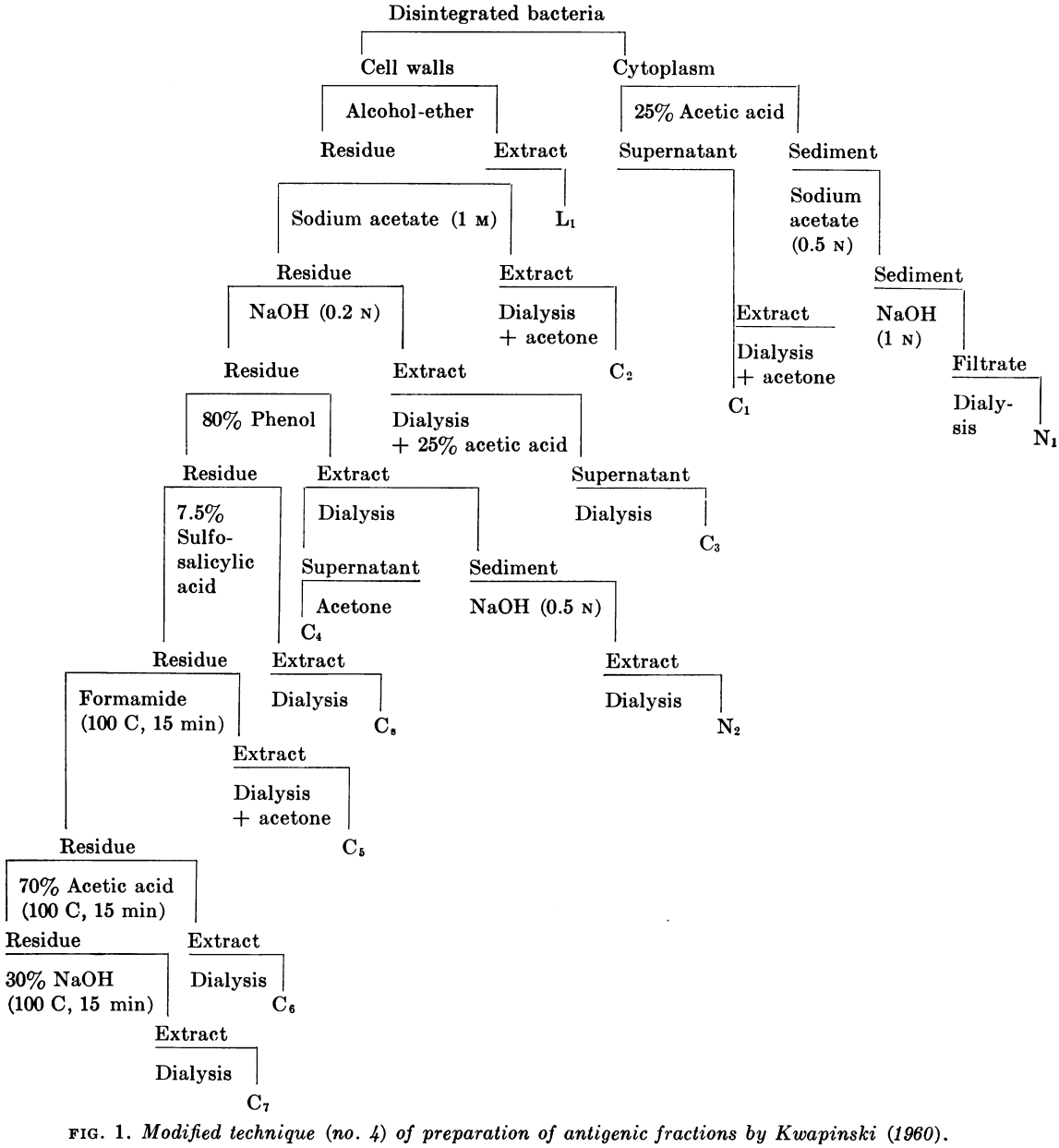

FIG. 1. *Modified technique (no. 4) of preparation of antigenic fractions by Kwapinski (1960).*

tral lipids, whereas linolenic acid could not be detected in the "firmly bound" lipids extracted with 1% ethereal HCl.

Protein fractions contained between 86 and 91% protein and no determinable amounts of deoxyribonucleic acid and ribonucleic acid (Table 2) polysaccharide fractions from 65 to 98% carbohydrate. Most fractions of *N. asteroides* occurred as galactose-arabinose-glucosides, whereas those of *N. rubra* were predominantly of the arabinose-galactoside type. Two polysaccharide fractions (C_2 and C_7) of *N. asteroides* contained rhamnose. Glucosamine was detected only in the acetic acid fraction C_6. Muramic acid was found only in *N. asteroides* in the polysaccharide fractions of cell walls isolated with cold extractants. Two cell-wall fractions (C_2 and C_7) also contained an unidentified, fast-migrating monosaccharide.

TABLE 1. *Types of monosaccharides and carbohydrate content of polysaccharide fractions of Nocardia*

Strain	Fraction	Muramic acid	Glucos-amine	Glucose	Galactose	Arabinose	Rhamnose	Monosac-charide (Rf_R 155)	Carbo-hydrate
N. asteroides	C_1	−	−	+	+	+	−	−	67
	C_2	+	−	−	+	+	+	+	79
	C_3	+	−	+	+	+	−	−	82
	C_4	+	−	+	+	+	−	−	89
	C_5	−	−	+	+	+	−	−	92
	C_6	−	+	+	+	+	−	−	88
	C_7	−	−	−	+	−	+	+	98
	C_8	−	−	−	+	−	−	−	80
N. rubra	C_1	−	−	−	+	+	−	−	65
	C_2	−	−	−	+	+	−	−	82
	C_3	−	−	−	+	+	−	−	87
	C_4	−	−	−	+	+	−	−	87
	C_5	−	−	−	+	+	−	−	93
	C_6	−	+	−	+	+	−	−	88
	C_7	−	−	−	+	−	−	−	97

TABLE 2. *Chemical constituents of the Nocardia*

Strain	Material	Percentage composition*					
		Nitrogen	Protein	Total carbohydrate	Pentose	Deoxy-ribose	Phosphorus
N. asteroides 5	Disintegrated cells	9.2	—	50.0	27.5	—	1.1
	Cell walls	6.0	—	70.6	52.5	—	0.35
	Cytoplasm	14.4	83.2	7.6	5.2	—	0.22
	Fraction N_1	15.4	85.8	0	0	0	0
	Fraction N_2	14.9	89.7	0	0	0	0
N. asteroides 8595	Disintegrated cells	9.1	—	48.8	32.2	—	1.0
	Cell walls	7.1	—	71.2	51.8	—	0.3
	Cytoplasm	13.6	82.6	87.8	7.6	—	0.2
	Fraction N_1	15.5	86.8	0	0	0	0
	Fraction N_2	16.2	90.2	0	0	0	0
N. rubra	Disintegrated cells	9.0	—	50.2	30.7	—	1.0
	Cell walls	6.8	—	69.8	50.6	—	0.32
	Cytoplasm	14.6	84.0	7.4	4.7	—	0.23
	Fraction N_1	15.2	89.1	0	0	0	0
	Fraction N_2	16.4	91.1	0	0	0	0

* Expressed in terms of dry mass.

Cell walls of *Nocardia* and *Actinomyces* reacted with homologous antisera at an average serum dilution of 1:1,600 both in agglutination and complement-fixation tests (Table 3). The *Actinomyces* antisera reacted at similar titers with cell walls of *N. asteroides* and *N. rubra*. In contrast, cell walls of *A. israelii* did not react with *Nocardia* antisera in either of the serological tests.

All carbohydrates (C) and protein (N) fractions prepared from *Nocardia* were serologically active,

TABLE 3. *Averaged complement-fixation titers of antisera versus cell walls and cytoplasms*

Prepn	Microorganism	Antisera versus					
		N. asteroides	*N. rubra*	*A. israelii*	*N. asteroides* cytoplasm	*Actinomyces* absorbed with *Nocardia* cell walls	*Actinomyces* absorbed with *Nocardia* cytoplasm
Cell walls	*Nocardia asteroides*	3,200	1,600	800	1,600	0	0
	N. rubra	1,600	3,200	3,200	1,600	0	0
	Actinomyces israelii	0	0	6,400	—	6,400	200
Cytoplasm	*N. asteroides*	3,200	3,200	3,200	1,600	0	0
	N. rubra	1,600	1,600	800	800	0	0
	A. israelii	1,600	800	1,600	800	1,600	1,600

TABLE 4. *Reactivity of chemical fractions of Actinomyces and Nocardia with antisera as determined by complement-fixation test*

Antigen	Antiserum versus				
	N. asteroides	*N. rubra*	*A. israelii*	*N. asteroides* absorbed with *Actinomyces*	*Actinomyces* absorbed with *Nocardia*
A. israelii					
C_1*	80	160	320	0	0
C_2	0	0	320		160
C_3	0	0	160		160
C_4	0	0	320		160
C_5	40	160	80	0	0
C_6	320	160	2,500		
C_8	0	0	2,500		
N_1*	40	80	160	0	20
N_2	0	0	320		320
N. asteroides					
C_1*	320	320	160	20	0
C_2	80	80	0		
C_3	80	160	80	20	0
C_4	80	80	80	40	0
C_5	160	80	160	20	0
C_6	1,000	320	0		
C_7	320	80	0		
C_8	2,500	0	320	80	40
N_1*	640	160	160	40	0
N_2	1,250	1,250	0		
L	0	0	0		
N. rubra					
C_1*	80	80	160	20	0
C_2	80	320	0		
C_3	80	80	40	10	0
C_4	80	80	80	20	40
C_5	80	80	80	20	0
C_6	320	1,000	0		
C_7	80	160	0		
N_1*	40	40	20	0	0
N_2	160	80	0	0	0
L	0	0	0		

* Fractions isolated from cytoplasm. Numbers represent highest reactive dilutions of the fractions averaged from two to three tests, expressed in thousands.

although titers in a complement-fixation test with homologous antisera ranged from 40,000 to 2,500,000 (Table 4). Lipid fractions were serologically inactive.

The carbohydrate and protein cytoplasmic fractions of both *Actinomyces* and *Nocardia* cross-reacted with heterologous antisera. In contrast, individual protein fractions (N_2) from cell walls or, more likely, from cytoplasmic membranes, as well as polysaccharide fractions (C_2) extracted with 1 M sodium acetate from cell walls, reacted only with genus-specific antisera.

All formamide polysaccharide fractions (C_5) reacted with all antisera, whereas the specificities of other polysaccharide fractions varied according to the genus. Thus, the "alkaline" fraction (C_3), the phenol-extracted fraction C_4, and the sulfosalicylic fraction C_8 of *Nocardia* gave a positive complement-fixation test in the *Actinomyces* antisera, but similar fractions of the *Actinomyces* reacted only with the genus-specific antisera. In contrast, the acetic acid-prepared fractions C_6 of each strain of *Nocardia* were reactive only in the genus-specific antisera, and the C_6 fractions of *A. israelii* strains reacted with heterologous antisera.

All *Nocardia* fractions, with the exception of the highly active sulfosalicylic fraction, cross-reacted with each other's antisera in complement

TABLE 5. *Reactivity of cell-wall and cytoplasm antisera with chemical fractions of Actinomyces and Nocardia and their cell walls and cytoplasms, as determined by complement-fixation test*

Organism	Antigen	Antiserum versus			
		N. asteroides cell walls	*N. asteroides* cytoplasm	*A. israelii* cell walls	*A. israelii* cytoplasm
A. israelii	Cell walls	400	200	400	200
	Cytoplasm	400	800	400	1,600
	C_1*	0	800	0	400
	C_2	0	0	400	400
	C_4	0	0	400	0
	C_5	1,600	1,600	400	800
	C_6	100	200	100	200
	C_8	0	0	400	0
	N_1*	0	200	0	0
	N_2	0	0	200	0
N. asteroides	Cell walls	400	1,600	200	400
	Cytoplasm	0	800	400	400
	C_1*	0	200	0	200
	C_2	400	0	0	0
	C_3	400	400	400	400
	C_4	400	400	200	100
	C_5	400	0	0	0
	C_6	400	400	0	0
	C_7	200	0	0	0
	C_8	800	0	0	0
	N_1*	0	800	0	400
	N_2	400	0	0	0
N. rubra	Cell walls	400	800	0	400
	Cytoplasm	0	800	200	400
	C_1*	0	400	0	200
	C_2	400	0	0	0
	C_3	400	400	400	200
	C_4	100	0	400	200
	N_1*	0	800	0	400
	N_2	400	0	0	0

* Fractions isolated from the cytoplasm; others isolated from cell walls. Numbers in columns represent highest reactive dilution of antisera averaged from two tests with each antigen.

fixation but only a small number cross-reacted with anticell-wall or anticytoplasm sera of heterologous genera of microorganisms (Table 5).

Antigenic fractions of *Actinomyces* and *Nocardia*, which showed serological reactivity in diffusion tests (Table 6), produced single precipitation bands and their cross-reactivities agreed with those obtained by complement fixation.

Discussion

Chemical compositions of proteins of *Nocardia* were similar, and the pattern of amino acids did not differ from that of *A. israelii* (Kwapinski, 1960).

Polysaccharides were mostly arabinose-type, as those of *Actinomyces* and *Mycobacterium*, and consisted of glucose, galactose, and arabinose, with rhamnose, glucosamine, and muramic acid confined to a few fractions. Unlike the polysaccharides of *A. israelii*, those of *Nocardia* contained only negligible amounts of glucosamine. The unidentified, fast-moving monosugar detected in the cytoplasmic polysaccharide of *N. asteroides*, and in a cell-wall fraction isolated with strong alkali at 100 C, was not found in polysaccharide fractions of *Actinomyces*.

Lipids of *Nocardia* contained a greater variety of higher fatty acids, particularly nonsaturated acids, than did lipids of *Actinomyces*.

Cytoplasmic materials of the investigated strains of *Actinomyces* and *Nocardia* were serologically closely related, in agreement with a hypothesis on the origin and development of species (Kwapinski and Snyder, 1961).

TABLE 6. *Reactivity of chemical fractions of Actinomyces and Nocardia in diffusion-precipitation tests*

Antigen	Antiserum versus	
	Actinomyces	*Nocardia*
Actinomyces		
C_1	+	+
C_2	+	−
C_5	+	+
N. asteroides		
C_1	+	+
C_3	+	+
C_4	+	+
N_1	+	+
N. rubra	+	+
C_4	+	+

TABLE 7. *Serological identification of Actinomycetaceae*

Antigen	Standard antiserum versus			
	Actinomyces	*Nocardia*	*Actinomyces*	*Nocardia*
Cytoplasm	+	+	+	+
Fraction C_6	+	+	−	+
Fraction N_2	+	−	−	+
Genus identified	+			+

Components of cell walls of the *Actinomycetaceae*, responsible for the cross reactions of cell walls within this family, seemed to be predominantly the polysaccharides extractable with cold alkali, phenol, and hot formamide.

A clearer view into the serological relationships between the *Nocardia* and *Actinomyces* was attained by examining antigenic fractions in the complement-fixation test with the anticytoplasmic and anticell-wall sera. The polysaccharide fractions of cell walls cross-reacting with the heterologous antisera were only those which proved active in both anticell-wall and anticytoplasmic homologous sera. These fractions probably contained an antigen with a specificity common to both cell walls and cytoplasm.

Despite a rather close antigenic relationship between the strains of *Actinomyces* and *Nocardia*, they could be serologically differentiated by characteristic reactions of the second protein fraction (N_2) and the 70% acetic acid-extracted fraction C_6.

A scheme proposed for further investigations on the serological identification of strains from the family *Actinomycetaceae* would consist of tests using the complement-fixation test with standard antisera of *Actinomyces* and *Nocardia* prepared with the following antigens: (i) cytoplasm or cytoplasmic fractions N_1 or C_1 and (ii) polysaccharide fraction C_6 and another with protein fraction N_2 prepared from cell walls by the extraction with a 70% acetic acid at 100 C and 80% phenol. Results of the test would be assessed as shown in Table 7.

This scheme, however, is regarded as preliminary to the planned examination of many strains of *Actinomyces* and *Nocardia* before any assess-

ment of the outlined technique of serological classification can be made.

Polysaccharide fractions isolated from both strains of *Nocardia asteroides* by the sulfosalicylic technique were the most potent of its antigens and could be tentatively regarded as species-specific, since they reacted with antisera of *N. asteroides* but not with antisera versus *N. rubra*. These fractions did not combine with antibodies to *Actinomyces* cell wall or cytoplasm and reacted only slightly with antisera to the *Actinomyces*.

ACKNOWLEDGMENTS

Appreciation is expressed to the Heads of Departments of Bacteriology, Biochemistry, and Electron Microscopy, University of Sydney, for use of the ultrasonic oscillator, high-speed blender, and electron microscope. Gratitude is expressed to J. Cailing for his technical assistance.

LITERATURE CITED

BAILEY, R. W. 1959. The reaction of pentose with anthrone. Biochem. J. **68**:669–672.

BREED, R. S., E. G. D. MURRAY, AND N. R. SMITH. 1957. Bergey's manual of determinative bacteriology. The Williams & Wilkins Co., Baltimore.

CUMMINS, C. S. 1962. Chemical composition and antigenic structure of cell walls of *Corynebacterium*, *Mycobacterium*, *Nocardia*, *Actinomyces* and *Arthrobacter*. J. Gen. Microbiol. **28**:35–50.

CUMMINS, C. S., AND H. HARRIS. 1958. Studies on the cell-wall composition and taxonomy of *Actinomycetales* and related groups. J. Gen. Microbiol. **18**:173–189.

DISCHE, Z. 1930. Über einige neue charakteristischen Farbreaktionen der Thymusnukleinsäure und eine Methode zur Bestimmung derselben in tierischen Organen mit Hilfe dieser Reaktionan. Mikrochemie **2**:4.

DISCHE, Z. 1955. New color reactions for the determination of sugars in polysaccharides. Methods Biochem. Analysis **2**:313.

ELEK, A., AND H. SOBOTKA. 1925. The Kjeldahl-Pregl method applied to nitro-compounds. J. Am. Chem. Soc. **49**:501–503.

FISKE, C. N., AND Y. SUBBAROW. 1925. The colorimetric determination of phosphorus. J. Biol. Chem. **66**:375–400.

GORDON, R. E., AND J. M. MIHM. 1957. A comparative study of some strains received as nocardiae. J. Bacteriol. **73**:15–27.

KWAPINSKI, J. B. 1960. Research on the antigenic structure of *Actinomycetales*. IV. Chemical and antigenic structure of *Actinomyces israelii*. Pathol. Microbiol. **23**:159–172.

KWAPINSKI, J. B., AND E. MIKULASZEK. 1957. Immunology of pus. Bull. Acad. Polon. Sci. **5**:47–52.

KWAPINSKI, J. B., AND M. L. SNYDER. 1961. Antigenic structure and serological relationships of *Mycobacterium*, *Actinomyces*, *Streptococcus*, and *Diplococcus*. J. Bacteriol. **82**:632–639.

KWAPINSKI, J. B., AND M. L. SNYDER. 1962. The immunology of rheumatism. Appleton-Century-Crofts, Inc., New York.

LINNANE, A. W., AND E. VITOLS. 1962. A simple high-speed blendor for the disintegration of microorganisms. Biochim. Biophys. Acta **59**:231–232.

MEJBAUM, W. 1939. Über die Bestimmung kleiner Pentose-Mengen, insbesondere in Derivaten der Adenylsäure. Z. Physiol. Chem. **253**:117.

OUCHTERLONY, Ö. 1949. Antigen-antibody reactions in gels. Acta Pathol. Microbiol. Scand. **26**:507.

PREER, J. R., JR. 1956. A qualitative study of a technique of double-diffusion in agar. J. Immunol. **77**:52.

SCHNEIDAU, J. D., AND M. F. SCHAFFER. 1960. Studies on *Nocardia* and other *Actinomycetales*. II. Antigenic relationships shown by slide agglutination tests. Am. Rev. Respirat. Diseases **82**:64.

SLACK, J. M., E. H. LUDWIG, H. H. BIRD, AND C. M. CANBY. 1951. Studies with microaerophilic actinomycetes. I. The agglutination reaction. J. Bacteriol. **61**:721–735.

SLACK, J. M., A. WINGER, AND D. W. MOORE, JR. 1961. Serological grouping of Actinomyces by means of fluorescent antibodies. J. Bacteriol. **82**:54–65.

TREVELYAN, W. E., AND J. S. HARRISON. 1952. Studies on yeast metabolism. I. Fractionation and microdetermination of cell carbohydrates. Biochem. J. **50**:298–303.

WAKSMAN, S. A., AND A. T. HENRICI. 1943. The nomenclature and classification of the actinomycetes. J. Bacteriol. **46**:337–341.

WEICHSELBAUM, R. 1946. An accurate and rapid method for the detection of proteins in small amounts of blood serum and plasma. Am. J. Clin. Pathol. **10**:40.

WILSON, G. S., AND A. A. MILES. 1957. Topley and Wilson's principles of bacteriology and immunity, 4th ed. The Williams & Wilkins Co., Baltimore.