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SEROLOGICAL RELATIONSHIPS AND ISOLATION OF A GROUP-SPECIFIC ANTIGEN OF THE CORYNEFORM BACTERIA

by

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ABSTRACT

Immunodiffusion tests of absorbed and unabsorbed antisera were used to determine the types and distribution of antigens among human pathogens Corynebacterium diphtheriae and C. xerosis, animal pathogen C. pyogenes, phytopathogen C. fascians and soil types Arthrobacter globiformis and A. tumescens. Representative species from the four ecological groups were tested with absorbed and unabsorbed antisera. Fourteen antigen which were distributed among the species, showed varying degrees of antigenic relationship based on the type, number and amount of antigens shared.

Of the antigens distributed among the coryneforms tested, only the antigen designated G was found to be present in every one of the species. The G antigen was isolated from cell sonicates of A. tumescens by hot HCl, DEAE cellulose and Sephadex G-200 column chromatography. The purity was confirmed by disc electrophoresis which showed a single protein band. Biochemical composition of the purified antigen was estimated to be 46.86% carbohydrate, 44.29% protein, 2.52% nucleic acid and 3.36% hexosamine. Seven of the antigens were found to be dispersed among the coryneforms tested whereas the other six were restricted to specific species. Since at least 5 of the antigens were shared by the two Arthrobacter spp., and three with the other coryneforms, it is proposed that the two Arthrobacter spp. be transferred to the genus Corynebacterium.

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INTRODUCTION

The coryneform group of bacteria are primarily characterized on a morphological basis and include the genera Corynebacterium, Arthrobacter, Brevibacterium, Microbacterium, Cellulomonas, Listeria, Erysipelothrix, Mycobacterium and certain species of Nocardia. In recent years, the term 'coryneform' has come to be used for a wide variety of Gram-positive, non-sporing rods but etymologically, 'coryneform' means club-like. Originally, this designation referred to those species belonging to the genus Corynebacterium. Coryneform cells are rods, often club-shaped or, if short, pear-shaped and even coccal in old cultures. The cells are usually Gram-positive except the Arthrobacter spp. which are Gram-negative in young cultures. A snapping or bending post-fision movement characteristically results in the formation of V, Y and other angular arrangements of the cells. The species are generally non-motile but plant pathogens and some of soil coryneforms are motile. The coryneform group of bacteria are widely distributed among diverse ecological groups such as human, animal, plant and soil habitats. Since species in soil are ubiquitous (39,44,45) and active in decomposing complex organic substances (41), interest in this group of bacteria has been stimulated in recent years.

Studies of the relationships among the various species and

groups are few and controversial. Conn and Dimmick questioned the inclusion of plant pathogens within the genus Corynebacterium on the basis of morphological and physiological studies (9). The reclassification of C. pyogenes, an animal pathogen, was also suggested on the basis of cell wall composition (13). In the past, most of the work has been carried out on C. diphtheriae, the human pathogen which is the type species of genus Corynebacterium. Later on, attention has been paid to other coryneforms and their relationships. In 1962, Katznelson et al suggested that A. tumescens, a soil coryneform occupies an ancestral position among the group (21). More recently Lazar has proposed that C. fascians, one of the plant pathogenic corynebacteria occupies an intermediate position among human, plant and animal strains.(29).

A few comparative studies have been done on immunodiffusion of coryneforms from human, plant and animal sources but not on the soil coryneforms. In this study, coryneform bacteria were selected from four ecological groups including the human strains Corynebacterium diphtheriae and Corynebacterium xerosis, the animal pathogen Corynebacterium pyogenes, the phytopathogen Corynebacterium fascians and Arthrobacter globiformis and Arthrobacter tumescens as representative of soil isolates. Immunodiffusion tests were used to study the antigenic relationships and to reveal common antigens. A group-specific antigen was isolated from cell sonicates of A. tumescens.

HISTORICAL

Various studies associated with the taxonomy of coryneform bacteria have been too limited in scope to provide a basis for a satisfactory classification; and some of the findings have been controversial.

The coryneform bacteria are grouped together by morphological, cultural and biochemical characters (6). The GC base ratios of 114 strains of corynebacteria selected on morphological and physiological criteria showed that the genera of coryneform bacteria, with the exception of Cellulomonasm, were taxonomically heterogenous (49). The GC base ratio of Arthrobacter globiformis strains bordered on, or overlapped the GC range of Corynebacterium, Mycobacterium, and Nocardia (19).

Immunochemical, serological and chromatographic analyses of some coryneform bacteria suggest the reclassification of these microorganisms is needed. In a study using numerical taxonomy, Harrington (17) brought out the complexity of their interrelationship. C. pyogenes showed little similarity to any of the other corynebacteria and the phytopathogen C. fascians was most closely related to the animal pathogens. His findings, together with the results of Kwapinski (24,26), Cummins and Harris (12), Cummins (14) and the morphology of genus Corynebacterium, Mycobacterium,

and Nocardia, led him to support the suggestion of Cummins (14) that these genera should be merged together and that there was no reason to separate them on the basis of acid-fastness (17).

Biochemical analysis of the cell walls by Cummins (1956) showed that Corynebacterium strains, except for strains of C. pyogenes, have a distinctive pattern of sugars and amino acid components, namely: arabinose, galactose as sugars, and alanine, glutamic acid and DL-diaminopimelic acid (DAP) as principal amino acids (11). Furthermore, this pattern of cell wall components was shared by strains of Mycobacterium, Nocardia (14) and Actinomyces (25). From an analysis of cell wall composition of seven strains of Arthrobacter spp., no close relationships were evident between these organisms and the Corynebacterium proper (13). According to the recent work of Yamada and Komagata (49), the coryneform bacteria were divided into 3 groups by the presence of each of DL-, L- DAP or lysine in the cell wall. C. diphtheriae, C. xerosis and C. fascians with cell wall containing DL-DAP and a snapping mode of division were placed in one group and A. globiformis and A. tumescens with cell wall containing lysine and a bending mode of division were in another group.

Most of the early serological studies have been carried out on C. diphtheriae strains by agglutination techniques and were handicapped because the organism had not been classified into the gravis,

intermedius and mitis groups (1). Ewing later investigated the serological relationships of the gravis group, and found five distinct types designated A, B, C, D and X (16). These findings were confirmed by Robinson and Peeney (43). Tarnoski found 11 gravis types which included the five ones already mentioned, five intermedius and 13 mitis types (51). Hewitt described 13 gravis types, of which nine were identical with the ones previously described, two intermedius types and 40 mitis types (20).

Using a precipitin technique, a group-specific polysaccharide of C. diphtheriae was demonstrated by Wong and Tung (48). Polysaccharide preparations from cells of all serogroups of C. diphtheriae, as well as from C. xerosis, C. pyogenes and C. murium proved to be serological related to one another (3,4). The first separation of a group-specific polysaccharide from C. diphtheriae by modern methods was achieved by Holdworth (1952). The carbohydrate was isolated from the purified cell wall preparation and contained D-arabinose, D-galactose and D-mannose in a molar ratio 3:2:1. The nucleoprotein-polysaccharide fractions extracted by mild acid hydrolysis from the sonicates of C. hofmanii proved to be species-specific by Banach and Hawirko (2).

Cell wall antigens of the coryneform group have been investigated by Cummins (12). Two antigens were isolated from the cell walls of C. diphtheriae and proved to be species-specific (10). Since cross-reactions did not occur with whole cells, the

antigens appeared to be deep within the cell wall. In a later study, Cummins reported that agglutinin titres of C. diphtheriae antiserum with cell wall suspensions of C. xerosis and C. renale did not differ significantly from those given by homologous strains (14). Hoyle obtained species-specific antigens from alcohol extracts of C. diphtheriae and a group-specific antigen common to the gravis, intermedius and mitis strains as well as to C. hofmanii (20).

Two kinds of antigens, designated O and K, were present in C. diphtheria cells (27,34). The group-specific, heat stable, somatic O antigen, found in deeper layers of the cells, was resistant to heat at 127C for 2 hr. The type-specific, heat-labile K antigen, located at the superficial layer of the cell, was mainly responsible for agglutination of intact cells (27,34). Chemical analysis showed that K antigen was protein whereas the O antigen was polysaccharide (48). The group-specific O antigen was responsible for cross-reactions between cell walls of C. diphtheriae, C. fascians, C. ovis, C. xerosis and C. hofmannii, (Kwapinski 24, 25).

Antigens present in the cytoplasm of C. diphtheriae and C. hofmannii proved to be closely related (Kwapinski, 25). The cytoplasm of corynebacteria contained one large antigenic complex which was a nucleopolysaccharide protein and produced one, or seldom two, precipitin bands with homologous antisera by immunodiffusion tests (Kwapinski, 25).

A few investigations have attempted to show serological relationships among coryneform bacteria. Double gel diffusion technique was used to show that C. diphtheriae, C. pyogenes, C. ovis, C. renale, C. murium and C. equi were serologically related (5). C. diphtheriae was found to be most closely related with C. equi and C. fascians with the phytopathogenic group. Barber et al (3, 4) and Saragea et al (39), in contrast with Wong and Tung (48) and Lazar (29), showed that C. diphtheriae and the other human corynebacterium species were related to each other only through their nucleoprotein antigens, and that their polysaccharide antigens were species-specific. Gorlenko (16) suggested that C. fascians might be an intermediate form of Corynebacterium species . His proposal was supported by enzyme study of Robinson (37) in which C. fascians produced a pattern of enzymes similar to that produced by some plant, human and animal pathogenic corynebacteria. The peroxidase pattern of C. fascians was similar to that of the plant pathogens but the esterase and catalase pattern resembled that of animal pathogens. Using tube precipitin, double gel diffusion and immunoelectrophoretic techniques, Lazar found that corynebacteria from human, animal and plant sources were antigenically related (29). The plant pathogens C. fascians, C. flascumfaciens, C. poinsettiae and C. betae showed cross reactivity with the corynebacteria from man and animals.

C. fascians showed the greatest degree of cross reactivity with C. diphtheriae and a moderate degree with the animal pathogens C. equi, C. renale and C. kutscheri and the phytopathogens mentioned above. On the basis of this finding, Lazar considered that C. fascians occupied an intermediate position among the corynebacteria of the three ecological groups (29). This is not in agreement either with the proposal that C. fascians should be classified as a member of the genus Nocardia (7, 4) or that it formed a boundary with the genera Corynebacterium, Mycobacterium and Nocardia (31). Lazar recommended that additional serological studies of members of the genus Corynebacterium, particularly C. fascians, and representative species of the genera Arthrobacter and Nocardia would be required in order to elucidate the understanding of the inter-relationships of coryneform bacteria (29).

The comparatively few studies of Arthrobacter species carried out on carbohydrate metabolisms (50), nutritional requirement (23, 45) and cell wall composition (23) have clearly indicated that the group was heterogeneous. However, serological data of Arthrobacter spp. are few. Katznelson et al reported that Arthrobacter antiserum reacted with most of the Arthrobacter strains and with C. poinsettiae, a plant pathogen; and suggested that A. tumescens occupied an ancestral position among coryneform bacteria (21).

MATERIALS AND METHODS

Cultures

Strains of cultures of Corynebacterium xerosis ATCC 373, Corynebacterium fascians ATCC 13000, Corynebacterium diphtheriae ATCC 19409, Corynebacterium pyogenes laboratory strain, Arthrobacter globiformis ATCC 8010 and Arthrobacter tumescens ATCC 6947 were obtained in a lyophilized state.

Media and Growth Conditions

Trypticase soy broth (TSB, Baltimore Biological Laboratories) was used for the growth of the Corynebacterium spp. The medium was supplemented with 1% yeast extract (TSBY) for Arthrobacter spp.

Cultures of Corynebacterium spp. were incubated at 37 C for 24 hr and those of Arthrobacter at 28 C for 36 hr.

Stock cultures were maintained on TSB or TSBY containing 2% agar and 0.5% $CaCO_3$ and stored at 4 C.

Buffer Solutions

Phosphate buffer 0.02 M, pH 7.2 was used throughout this study except that the buffer was adjusted to pH 7.0 for polyacrylamide gel electrophoresis.

Preparation of Cell Sonicates

Agar media (240 ml in Roux bottles) were seeded with 5 ml inocula of 24-36 hr broth cultures showing a heavy turbidity. After incubation, the surface cell mass was harvested in about 20 ml phosphate buffer and centrifuged 15 min at 10,000 g. The cells were collected and washed a few times in phosphate buffer. Five gm cell mass, wet weight, was then suspended in a small amt of buffer and 2 vol of 0.45 - 0.50 mm glass beads, and sonicated in a Bronwill homogenizer for five 90 sec periods or until all the cells were broken as observed by phase contrast microscope. The cell sonicate was centrifuged twice at 15,000 g for 15 min, stored at - 20 C, and used as antigens for immunodiffusion tests and for the preparation of vaccines.

Preparation of Cell Sonicate of Arthrobacter tumescens

Arthrobacter tumescens cells, grown in 800 ml vol, were inoculated into carboys containing 10 litre TSBY and incubated with aeration at 28 C for 36 hr. The cells from 20 litres were collected by using a Sharples centrifuge, washed twice with buffer, once with distilled water. Twenty gm cell mass was sonicated as described above and stored at - 20 C.

Preparation of Acid Extracts of Arthrobacter tumescens

Acid extracts were prepared by adding 5 vol of 1 N HCl to 1 vol of cell sonicate and heating in a water bath at 100 C for 10 min. The mixture was cooled immediately and adjusted to pH 7.2. The supernatant was collected after centrifugation at 10,000 g for 20 min and concentrated by Aquacide I (Calbiochem) to about 1/45 of the original vol. The extract was dialysed overnight against phosphate buffer at 4 C then centrifuged at 10,000 g to remove any precipitate. The acid extract was stored at - 20 C.

Fractionation of Acid Extract

(A) DEAE cellulose

Diethylaminoethyl (DEAE) cellulose (Sigma, Fine mesh) prepared as described by Wai Kung Lee (30), was washed once in distilled water (DW), twice in 0.5 N NaOH, several times in DW and finally in phosphate buffer. A chromatographic column of 2.5 x 45 cm (Pharmacia) was packed by pouring the degassed DEAE cellulose along a glass rod to the column, and equilibrated by passing through 2 litres phosphate buffer with a pressure of about 120 cm water. Eight ml of the acid extract (20 gm wet cells) was absorbed onto the column, and eluted at 4 C using a continuous linear gradient from 0 up to 1.0 M KCl in the buffer. The flow rate

was about 20 ml/hr and 120 fractions of 8 ml were collected. The protein content of each fraction was determined by UV absorption at 280 nm on a Beckman (Acta III)spectrophotometer. The fractions from each of the absorption peaks were pooled, concentrated by Aquacide, and dialysed against 2 litres of buffer overnight at 4 C.

(B) Sephadex G-200

The dry beads of Sephadex G-200 (Pharmacia) were hydrated in buffer, boiled for 5 hr, allowed to cool and suspended overnight in an excess amt of buffer. The slurry was decanted to remove the smallest particles, resuspended in an excess amt of buffer and degassed. About 20 ml of buffer was pumped up through the bed supporting net at the bottom of a column (2.5 x 45 cm) with a syringe attached to the outlet tubing. The gel, previously swollen in buffer, was poured carefully down a glass rod into an extension tube mounted on top of the column. The flow was started immediately after filling the column in order to obtain even sedimentation. To regulate the operating pressure, the outlet tubing of the column was positioned just below the liquid level in the extension tube, and opened to allow the solvent to flow out slowly. The column outlet tubing was gradually lowered until a pressure of 90 mm was reached, and the column was equilibrated for 2 days with the same buffer solution. Excess buffer above the gel surface was

removed by suction and by opening the column outlet, the remainder was drained away and the outlet was then closed. The fractions (5.5 ml), obtained by DEAE cellulose column chromatography, which gave a precipitin line by the immunodiffusion test (immunoreactive fractions), were carefully layered on top of the bed using a 30 cm long - hollow glass tubing. The outlet was opened and as soon as the sample had passed through, the surface was washed with a small amt of buffer. After the surface had almost dried out, the column was layered with buffer and effluents were collected in 4 ml fractions at a flow rate of 4 ml/hr. The optical densities were determined at 280 nm in a Beckman Spectrophotometer. The fractions from each of the absorption peaks were pooled, dialysed against 2 litre distilled water overnight at 4 C and then lyophilized.

Polyacrylamide Gel Electrophoresis

Polyacrylamide gel electrophoresis was performed as described by Weber and Osborn (46) using sodium dodecyl sulfate (S.D.S.) dissociating system and the Buchler Instruments Disc Electrophoresis Apparatus (Fort Lee, N. J.).

The test sample, obtained by Sephadex G-200 gel filtration which was immunoreactive, was dialysed against phosphate buffer containing 1% SDS and 1% β mercaptoethanol at 37 C for 2 hr and

dialysed against phosphate buffer containing 0.1% S.D.S. and 0.1% β mercaptoethanol overnight at room temp. S.D.S. gel consisted of 15 ml gel buffer (phosphate buffer containing 0.2% S.D.S.), 13.5 ml acrylamide solution, 15 ml of 0.005 % Riboflavin and 50 λ (0.05 ml) of N, N, N', N' tetramethylethylene diamine (TEMED). Pyrex tubes (75 x 8 mm) were filled with DW to flatten the miniscus. When the gel solidified, the water was pipetted out and a small amt of bromophenol blue was layered on gel surface and then with 0.2 ml of the test sample (863.6 µg dry wt). The gel tubes were filled up with buffer and illuminated by fluorescent light for an hour. Electrophoresis was carried out in 50% gel buffer for 5 hr at room temp at 4 mA per gel tube. After completion of the run, the gel columns were removed from the gel tubes, stained for one hour with 0.2% Coomasie blue and destained with 10% acetic acid. Distances of protein and dye, length of gel column before and after destaining were used to determine the molecular weight of the test sample.

Preparation of Antisera

Vaccines were prepared from cell sonicates of C. xerosis, C. fascians, C. diphtheriae, A. globiformis and A. tumescens and standardised to a density equal to McFarland No. 3. Albino rabbits were immunized biweekly according to the procedure of Kwapinsky (26); 0.3 ml subcutaneous, 0.4 ml intramuscular, 0.4 ml foot pad,

0.4 ml another foot pad, 0.4 ml intravenous and 0.5 ml subcutaneous. Two courses of inoculations were usually given. Rabbit inoculated with sonicates of A. tumescens received a third course with vaccines suspended in incomplete Freund's adjuvant and a fourth course without adjuvant.

Absorption of Antisera

One vol of cell sonicates was mixed with 3 vol antisera, incubated at 45 C for 2 hr and placed at 4 C overnight. The absorbed sera were collected after centrifugation at 10,000 g for 30 min and stored at - 20 C.

Immunodiffusion Tests

A modification of Ouchterlony's agar plate method was used (35). The bottom of a smooth clear glass petri dish was coated with a film of 2.0% Noble agar (Difco), and then layered with 20 ml of 0.85 % Noble agar containing 1% sodium chloride and stored at 4 C for 24 hr. A single radial immunodiffusion method was used with a horizontal trough (3 x 50 mm) filled with undiluted antiserum and circular wells (4 mm diam, 5 mm from the trough) containing test samples of antigens. A double radial immunodiffusion was also carried out with the concentrated antiserum placed in a central well (8 mm diam) and antigen samples in 6 - 8 circumferential wells (4 mm diam), cut equal distances apart so that the distance from the edge

of the central well was about 10 mm. The plates were placed in a moist chamber at 37 C overnight and then at 4 C and examined for precipitin lines daily over a period of 7 days.

Chemical Analysis of Purified Antigen

(A) Protein Determination

Protein content of the purified antigen was determined by the Folin method of Lowry et al (32) using crystalline bovine albumin as standard.

(B) Carbohydrate Determination

Carbohydrate content of the purified antigen was determined by a modification of the Anthrone method of Scott and Melvin (40) using dextrose as standard.

(C) Hexosamine Determination

Hexosamine was determined by the modified Elson - Morgan method (15) using glucosamine hydrochloride as standard.

(D) Nucleic Acid Estimation

Nucleic acid content was estimated by the method described by Layne (28).

RESULTS

(A) Type and Distribution of Antigens of Coryneform Bacteria

The antisera produced against cell sonicates of C. xerosis, C. fascians, C. diphtheriae, A. globiformis and A. tumescens, were tested by single and double radial immunodiffusion, using sonicates of homologous and heterologous species.

Single radial immunodiffusion tests of C. xerosis antisera showed 3-4 precipitin lines designated a, b, c and m with cell sonicates of C. xerosis , 1 line b with C. fascians, 1 line designated G with C. diphtheriae, no lines with C. pyogenes, 2 lines G and b with A. globiformis and 3 lines ab, G and e with A. tumescens (Table

Antisera of C. xerosis, which was concentrated to about 1/5 of original volume and examined by double radial immunodiffusion tests gave a maximum of eight precipitin lines with sonicates of C. xerosis (Table 6). The precipitin lines, which were also observed by single radial immunodiffusion test with unconcentrated antisera (Table 1), included one heavy wide band designated ab (Fig. 4,5,6) or 2 separate light lines designated a and b (Fig. 7,8) located close to the antiserum source and 2 bright sharp lines designated c and m.

In addition, faint precipitin lines observed only by double radial immunodiffusion using concentrated antisera were three lines close to the antigen source designated e, n and G and one line d, between c and m, (Fig. 4,5,6,7,8).

In the double radial immunodiffusion test of concentrated C. xerosis antisera with heterologous cell sonicates, the precipitin lines observed with C. fascians were b, G and e, G and a with C. diphtheriae, G with C. pyogenes, G and b with A. globiformis, G, c, ab and (or without) e with A. tumescens (Table 6).

Single radial immunodiffusion tests of C. fascians antisera (Table 2) showed four lines with cell sonicates of C. fascians namely b, e, f and G, 1 line b with C. xerosis, 1 line G with C. diphtheriae, none with C. pyogenes, 1 line b with A. globiformis and 1 line G with A. tumescens.

Single radial immunodiffusion tests of C. diphtheriae antisera (Table 3) showed 4 lines namely a, f, G and h with cell sonicates of C. diphtheriae, 1 line G with C. xerosis, 1 line G with C. fascians, 1 line G with C. pyogenes, 2 lines G and h with A. globiformis and 2 lines G and h with A. tumescens.

Single radial immunodiffusion tests of A. globiformis antisera (Table 4) showed 5 lines b, c, G, h and p with cell sonicates of A. globiformis, 2 lines b and G with C. xerosis, 1 line G with C. diphtheriae, 2 lines b and G with C. fascians, 1 line G with C. pyogenes and 5 lines b, c, G, h and p with A. tumescens.

Double radial immunodiffusion tests of concentrated A. tumescens antisera No. 219 (Table 5) showed one distinct line h and 3 faint lines G, p and e with cell sonicate of A. tumescens, none with C. xerosis, 1 very faint line G with C. fascians, 1 distinct line h and 1 very faint line G with C. diphtheriae, 1 very faint line h with C. pyogenes and none with A. globiformis. Concentrated A. tumescens antisera No. 222 (Table 5) gave one distinct line h and 2 faint lines p and e with sonicates of A. tumescens, none with C. xerosis, none with C. fascians, 1 distinct line h with C. diphtheriae, none with C. pyogenes and 1 very faint line h with A. globiformis.

Immunodiffusion tests of antisera absorbed with heterologous species were tested to determine the type and distribution of antigens among the test species (Table 1, 2, 3 and 4). By analysis of the precipitin lines, obtained with unabsorbed and absorbed antisera, the antigenis pattern of each of the test species was determined (Table 7). The antigen designated the G or group species antigen, showed a line of identity with each of the test species.

(B) Isolation of Group Specific Antigen

The acid extract of cell sonicates of A. tumescens was separated into two absorption peaks by DEAE cellulose column

chromatography (Fig. 1). Concentrated (1:8) pooled fractions of the second absorption peak gave one precipitin line with C. xerosis antiserum which was shown to contain the G antigen. No lines were observed with fractions of the first absorption peak. Fractions from the second absorption peak, eluted on a Sephadex G-200 column were separated into 2 absorption peaks (Fig. 2). Immunodiffusion tests showed that the G antigen was present in the second Sephadex peak. The purity of the G antigen was demonstrated by disc electrophoresis on polyacrylamide gel as one single band of protein. Crude extract of cell sonicates of A. tumescens showed six bands by disc electrophoresis. The purified G antigen gave one precipitin line with A. tumescens antisera No. 219 (Fig. 9) and with C. xerosis antisera (Fig. 4-8) but the precipitin line was not observed with A. tumescens antisera No. 222 (Fig.10). The G line appeared closer to the antigen than the antiserum source and a molecular weight 92,000 was estimated by the disc electrophoresis. The antigen was stable to 1 N HCl at 100 C for 10 min. The biochemical analysis of the antigen showed 46.86% carbohydrate, 44.29% protein, 3.36% hexosamine and 2.52% nucleic acid.

TABLE 1. Single radial immunodiffusion tests of C. xerosis antisera with homologous and heterologous species.

C. xerosis Antiserum No.		Precipitin lines					
		Cell Sonicates					
		C. xer.	C. fas.	C. diph.	C. pyo.	A. glob.	A. tum.
Unabsorbed							
175		ab,c,m	b	G	0	G,b	ab,G,e
153		a,b,c,m	b	G	0	G,b	b,G,e
Absorbing species							
175	C. fas.	c,m	0	a	nd	c	b,c,
153	C. fas.	c,m	0	a	0	c	b,c
175	C. diph.	b,c,m,d	b	0	nd	b,c	b,c
153	C. diph.	b,c,m,d	b	0	0	b	nd
175	C. pyo.	ab,c,m	b	0	0	b	0
153	C. pyo.	b,c,m,	b	nd	0	b	0
175	A. glob.	d	c	a	nd	0	a
153	A. glob.	d	c	a	nd	0	a
175	A. tum.	0	0	0	0	0	0
153	A. tum.	0	0	0	0	0	0

nd = test not done