A SEROLOGICAL STUDY OF THE CELL WALLS OF LACTIC ACID BACTERIA

by

KWOK LEUNG CHUNG, B.Sc.

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ABSTRACT

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ABSTRACT

Cell walls of three species of Lactobacillus and three of Streptococcus, cultured in a synthetic medium, were isolated by mechanical disintegration and purified by washing. Suspensions of cell walls, and also of intact cells, were injected into rabbits--three replicates with cell walls and three with intact cells of each species. Antigens from these species were demonstrated by agglutination, by hemagglutination, and by precipitation tests. A greater number of antigens was detected by cell-wall antisera than by intact-cell antisera. Certain antigens combined with antibodies even though they did not induce them.

A large proportion of the antigens was shared by the different species in the two genera. S. faecalis proved to be an exception.

A new approach for detecting protein-type antigens by the hemagglutination technique was attempted. Cell-wall suspensions were used to sensitize tannin-treated erythrocytes.

The evidence presented shows the possibility of using antisera from species-specific cell-wall antigens for the identification of the six species. The close relationship of these species is discussed.

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INTRODUCTION

INTRODUCTION

Intensive studies on the physical and chemical properties of bacterial cell walls were initiated by Salton and Horne (1951 a, b). Electronmicrographs showed cell walls of Gram-positive bacteria to be thicker than the walls of the Gram-negative bacteria, the former 15 to 20 mμ, and the latter 10 to 15 mμ in thickness (Dawson, 1949; Salton and Horne, 1951 a, b; Birch-Anderson, Maale and Sjostrand, 1953). Two layers were recognized in the cell walls of Spirillum sp, the inner layer composed of spherical macromolecules in a hexagonal pattern (Houwink, 1953).

Chemical studies on cell walls of bacteria have been carried out. Walls of Gram-positive bacteria contained a low concentration of lipids, and a limited range of amino acids but no proline, histidine, arginine, aromatic, or S-containing amino acids. Salton (1952 a, 1953 a) reported lysine, glutamic acid, alanine, glucose, rhamnose and galactose to be the chief components of a strain of Streptococcus faecalis. Cummins and Harris (1956), working with two strains of this species, reported similar findings with two exceptions. Mannose was present, but galactose was not. Species of lactobacilli contained essentially the same components, with only minor differences. L. plantarum lacked alanine and galactose but contained diaminopimelic

acid. L. casei contained aspartic acid and mannose (Cummins and Harris, 1956). L. lactis contained aspartic acid, but neither rhamnose nor galactose (Ikawa and Snell, 1960).

Cummins and Harris (1956) noted a correlation between cell wall composition and taxonomic patterns. The amino acid components appeared to differentiate genera, whereas the sugars and amino sugars to differentiate species within the genera. They expressed the opinion that the chemical constituents and the serological characteristics of the bacterial cell wall are closely related.

Little information concerning the serological properties of the cell wall has been published. The study reported herein involves a comparison of the antigenic components of cell walls and intact cells in certain species of Lactobacillus and of Streptococcus, the two genera being chosen because of their close relationship.

HISTORICAL

HISTORICAL

In the isolation of bacterial cell walls, Vincenzi (1887) treated whole cells of Bacillus subtilis with 0.5 per cent NaOH, and obtained residues similar in outline to those of the original cells. However, alkaline extraction may have altered the chemical properties of the cell walls. Salton and Horne (1951 b), using electron microscopy, demonstrated that cell wall residues prepared by the alkaline method differed from those prepared by other methods. Degradation of walls was thought to have taken place (Salton, 1952 a).

Mechanical disintegration of microorganisms was reported by Bucher (1897). MacFadyen, Harris-Morris and Rowland (1900) used silver sand to rupture yeast cells. MacFadyen and Rowland (1901) employed fine sand to grind cells of typhoid bacilli. Barnard and Hewlett (1901) ruptured yeast cells by grinding in a self-constructed disintegrating device of five steel balls confined in a rotary phosphor-bronze vessel. Thick cell walls were observed when a smear of the cell debris was examined microscopically.

Curran and Evans (1942) disintegrated bacterial spores by violent agitation with small inert particles. King and Alexander (1948) reported that mechanical

destruction was obtained by subjecting bacterial cells to violent shaking with minute round glass beads. Dawson (1949) ruptured intact cells of Staphylococcus aureus with glass beads in the Mickle tissue disintegrator. Cell walls were separated from intact cells and other cytoplasmic components by centrifugation at 8000 r.p.m.. Electron microscopic examination made it clear that intracellular cytoplasmic material had been separated from the cell walls. However, Cooper, Rowley and Dawson (1949) estimated that approximately seven per cent of the cells subjected to the disintegrator remained intact. King and Alexander (1948) observed that a small but definite proportion of organisms was not disintegrated by mechanical treatment, and that the resistant cells did not seem to differ from normal cells in any of the other characteristics investigated.

Bacterial cells ruptured in the Mickle tissue disintegrator (Dawson, 1949) were subjected to differential centrifugation. Salton and Horne (1951 b) separated unbroken cells of Streptococcus faecalis and Salmonella pullorum at 3000 r.p.m.; and separated cell walls by centrifuging the supernatant at 10,000 r.p.m.. Ribi, Milner and Perrine (1958) centrifuged suspensions of ruptured cells of Salmonella enteritidis at 2500 x G. The sediment consisted of a translucent upper layer of cell walls, and an opaque under layer of unbroken cells. The upper layer was removed, and freed from intact cells by repeated washings and

centrifugations. Munoz, Ribi and Larson (1959) centrifuged a suspension of disintegrated cells of Bordetella pertussis at 10,000 r.p.m.. These workers recognized an upper layer of cell walls, a middle layer of a mixture of intact cells and cell walls, and a lower layer of intact cells. The cell wall layer was removed, resuspended and centrifuged repeatedly, discarding in each case the heavier intact cell layer and collecting only the upper layer, until a pure preparation of cell walls was obtained.

The application of sonic energy in the disintegration of bacterial cells was investigated by Mudd, Polevitzky, Anderson and Chamber (1941). These authors subjected intact cells of Bacillus megaterium, B. subtilis and B. anthracis to a sonic oscillator, and examined the disrupted cells by electron microscopy. They demonstrated that cell walls have definite, solid morphological structure.

Of the 16 organisms exposed to ultrasonic disintegration by Stump, Green and Smith (1946), 12 were ruptured easily, and the remaining four, particularly the Gram-positive cocci, were refractory. Citing evidence that some cells in every batch resisted the disruptive force of the sonic oscillator, they postulated that complete disintegration even of the non-refractory cells was impossible. These authors also stressed that the viscosity of the cell suspension was a factor of importance. They recognized that ultrasonic vibration was largely converted

to heat when the thickness of the cell suspension exceeded a certain limit, and that, as a result, the disruptive power was lost.

Salton (1953 a), in his work on the isolation of cell wall by a Raytheon sonic oscillator, showed that Escherichia coli and Bacillus megaterium were ruptured in 10 minutes, whereas micrococci presented no evidence of disruption after treatment for an hour.

Marr and Cota-Robles (1957), in a study of the sonic disintegration of Azotobacter spp., demonstrated that 40 per cent of the surface of the intact cells was fragmented to submicroscopic particles. These workers, as did Slade and Vetter (1956), further demonstrated that when continuous oscillation was maintained the cell walls lost structural features and finally were reduced to a soluble state.

Using a different approach, Salton and Horne (1951 b) ruptured bacteria by heating cell suspensions at 75 degree C and 100 degree C, and then separated cell walls from coagulated protoplasts by shaking with glass beads. This method was considered to have little value due to the possible destruction of surface features of cell walls by heat and to the low yield.

The crude cell wall material, obtained after disintegration by differential centrifugation, has been shown to contain contaminating cytoplasmic substances such as nucleic acids, denatured proteins, and cellular particles

(Salton and Horne, 1951 b; Barkulis and Jones, 1957). Salton (1956) reported that the adherent cytoplasmic material was removed more easily from the walls of Gram-positive than from those of Gram-negative bacteria. Salton and Horne (1951 b), working with cell walls of Streptococcus faecalis and Salmonella pullorum claimed that cell walls, prepared by disintegrating in distilled water and washing a few times in 0.1 M phosphate buffer at pH 7.0, were free of cytoplasmic contaminants. Barkulis and Jones (1957) purified cell walls of type 14 hemolytic streptococci by the same method. Vennes and Gerhardt (1959) reported that washing with saline facilitated the removal of adherent small particles. McCarty (1952 a, b), Salton (1956), and Cummins (1956) obtained cell walls free of cytoplasmic material by digesting crude walls with ribonuclease, pepsin or trypsin. Salton (1956) objected to the unrestricted use of proteolytic enzymes for this purpose. He expressed the opinion that enzymes like trypsin might contaminate wall preparations with insoluble plasteins, and that crude enzymes probably contained small amounts of wall-degrading enzymes. Cummins (1954) showed that prolonged treatment of cell wall material with enzymes like pepsin eventually destroyed certain specific antigens.

The immunological properties of bacterial cell walls have been investigated by McCarty (1952 a, b) and Salton (1952 c, 1953 a), each of whom produced evidence that the group-specific antigen for group A streptococci was located

in the cell walls. Jones and Shattock (1960) reported that for group D streptococci the opposite was true. The group-specific antigen was detected in the cell content after the cell walls had been removed. McCarty (1952 a, b) dissolved the isolated cell walls of several strains of group A streptococci with enzymes extracted from Streptomyces albus. The extract on analysis was found to consist of approximately two-thirds carbohydrate (mainly N-acetyl-glucosamine and rhamnose), and one-third protein. He pointed out that the carbohydrate portion contained the group-specific C carbohydrate.

Salton (1953 a) and Barkulis and Jones (1957) observed the presence of type-specific M-protein in cell walls of group A streptococci prepared by mechanical disintegration. This protein was isolated from the cell walls and partially purified. In the presence of trypsin, it was destroyed.

Cummins (1954) made a serological study on cell walls of a mitis strain of Corynebacterium diphtheriae, using suspensions of intact cells and cell walls prepared by mechanical disintegration. He demonstrated the presence of two antigens by agglutination and agglutinin absorption techniques. One was a superficial heat-labile specific antigen and the other, a deeper heat-stable group antigen. The former, a protein, was considered to be responsible for the agglutination of intact cells, and the latter for that of cell walls. The author suggested that the deeper group

antigen, which was detected in all cultures of Corynebacterium diphtheriae studied and was also present in one strain of C. ovis, was probably polysaccharide in nature, since it was attacked rapidly by periodate. Cummins considered that these cell wall antigens might be valuable in classification.

Yoshida, Nagayuki, Fukuya, Kakutani, Tanaka, Tegawa and Tadayo (1954) studied cell walls of Bacillus subtilis stained with 0.1 per cent phosphotungstic acid. Electron micrographs revealed the rough appearance of the outer surface of the cell wall, compared to the smooth inner surface. However, the rough structure of the outer surface disappeared when cell walls were treated with pepsin and trypsin. They suggested that these electron microscopical observations supported the assumption that the protein component was situated in the superficial outer layer and the polysaccharide components in the inner layer.

Tomcsik and Guex-Holzer (1954) examined the immunological properties of surface layers of bacterial cells. These workers provided evidence that protoplast and the intact cell, or the cell wall, of Bacillus megaterium were antigenically distinct. Vennes and Gerhardt (1956, 1959), working with B. megaterium also, reported no cross-reaction between intact cells (or cell walls) and antiserum to protoplast (or protoplast membrane). With the agglutinin absorption technique, these authors demonstrated that intact

cells absorbed only a portion of the cell-wall antibodies.

Sharpe (1955) investigated the group-specific antigens of lactobacilli. This author tested crude HCl extracts with group-specific antisera by the precipitin test, and classified 312 of the 442 strains studied into six groups and one subgroup.

The presence of a surface protective antigen in the cell wall was reported by Yoshida and his associates (1955). They demonstrated that cell walls of Bordetella pertussis were as effective as intact cells for protection against experimental infections. Munoz, Ribi, and Larson (1959) confirmed this finding, and in addition found that the protective antigen and the histamine sensitizing factor were predominantly located in the cell wall, whereas the heat-labile toxin was in the protoplasm.

Shafa (1958) detected a surface antigen in the cell walls of Salmonella gallinarum and Vibrio metchnikovi. This antigen was identical with the O-suspensions of these organisms. Ribi, Milner and Perrine (1958) reported on the antigens in cell walls of Salmonella enteritidis, noting that the toxicity, the ability to produce antibody, and the reactivity with O-antibody were associated principally with the cell wall. Other authors who have provided evidence that cell walls contain antigens which participate in immunologic reactions: Salton (1952 c) and Barkulis and Jones (1957), with Streptococcus pyogenes;

Shepard, Ribi and Larson (1955), with Histoplasma capsulatum; Carey and Baron (1958), with Salmonella typhosa; and Ribi, Larson, List and Wicht (1958), with Mycobacterium sp.

The direct adsorption of bacterial extracts on erythrocytes and subsequent hemagglutination by antisera to these extracts was reported by Landsteiner (1945), Keogh, North and Warburton (1948), Middlebrook and Dubos (1948), Norden (1949), and Boyden (1950, 1951). Keogh, North and Warburton (1947, 1948) suggested that polysaccharides from bacterial extracts caused the direct sensitizing of erythrocytes; and that hemagglutination by immune sera was due to a serological reaction between specific antibody and adsorbed polysaccharides. Middlebrook and Dubos (1948), with Mycobacterium tuberculosis, and Alexander, Wright and Baldwin (1950), with Pasteurella tularensis, reported that polysaccharides from these organisms also sensitized erythrocytes; and that the subsequent hemagglutination reaction was highly specific.

Purified protein, likewise, has been adsorbed to erythrocytes. Boyden (1950) noted that treatment of sheep erythrocytes with a suitable concentration of tannic acid rendered them capable of adsorbing protein molecules such as Tuberculin P.P.D., ovalbumin, chicken serum albumin, horse serum albumin, horse serum globulin, and protein preparation from Streptococcus sp. The sensitized erythrocytes

subsequently were hemagglutinated in the presence of the corresponding antiprotein sera. Several factors in the test were of critical importance. A suitable dilution (1:250) of normal rabbit serum for washing the tannin-treated and sensitized erythrocytes eliminated excessive hemolysis. A saline diluent containing 1:100 dilution of normal rabbit serum avoided autoagglutination of the tannin-treated and sensitized erythrocytes. An optimum pH and the proper dilution of tannic acid provided maximum adsorption of antigens by tannin-treated erythrocytes.

The agglutination of erythrocytes treated with tannic acid had been known for many years before Boyden applied the principle to adsorption of proteins and hemagglutination. Reiner and Fischer (1929) suggested that the tannic acid treatment altered the surface properties, and changed the erythrocytes from the hydrophilic to the hydrophobic state. Freud (1929, 1931) proposed that the tannic acid which altered surface potential probably caused aggregation of the erythrocytes in the presence of electrolytes. Boyden (1950) did not consider these tannic acid-erythrocyte factors to be important in the adsorption of protein by the tannin-treated erythrocytes. He proposed that the adsorption procedure took place in two steps. In the first step, some components of the system acted on the surface properties of the erythrocytes. In the second, other components were adsorbed by the erythrocytes as a result of their newly acquired

surface properties.

MATERIALS AND METHODS

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Cultures

The following species were used:

Lactobacillus plantarum (Laboratory culture)

Lactobacillus lactis (NCIB 11983, Strain 8011, 1954)

Lactobacillus casei (ATCC 4691)

Streptococcus cremoris (ATCC 9265)

Streptococcus lactis (ATCC 7962)

Streptococcus faecalis (Laboratory culture)

Culture Medium

The synthetic medium of Morgan, Campbell and Morton was used with certain modifications. Nine stock solutions designated A, B, C, D, E, F, G, H and J were prepared as follows:

Solution A

Double strength Hank's balanced salt solution containing neither glucose nor sodium bicarbonate	1000.0	ml.
l-Arginine monohydrochloride	800.0	mg.

l-Histidine	800.0	mg.
l-Lysine monohydrochloride	400.0	mg.
d-l-Phenylalanine	400.0	mg.
d-l-Methionine	400.0	mg.
d-l-Serine	400.0	mg.
d-l-Threonine	400.0	mg.
d-l-Leucine	400.0	mg.
d-l-Isoleucine	400.0	mg.
d-l-Valine	400.0	mg.
d-l-Glutamic acid monohydrate	400.0	mg.
d-l-Aspartic acid	400.0	mg.
d-l-alpha-Alanine	400.0	mg.
Norleucine	400.0	mg.
Glutamine	100.0	mg.

Solution B

d-l-Tyrosine	800.0	mg.
l-Cystine hydrochloride	400.0	mg.
0.075 N HCl	1000.0	ml.

Dissolved with heat.

Solution C

Niacin	4.0	mg.
Pyridoxine HCl	8.0	mg.
Pyridoxal HCl	8.0	mg.
Thiamine HCl	4.0	mg.
p-Aminobenzoic acid	0.8	mg.

Ion-exchange water	1000.0	ml.

Solution D

Folic acid	0.8	mg.
Ion-exchange water (with the inclusion of just sufficient N/100 NaOH to effect solution)	1000.0	ml.

Solution E

d-Biotin	0.008	mg.
N HCl	10.0	ml.
Ion-exchange water	990.0	ml.

Solution F

Adenine sulphate	400.0	mg.
Ion-exchange water	1000.0	ml.
NH_4OH (conc.)	6.0	ml.

Solution G

Guanine HCl	200.0	mg.
Uracil	200.0	mg.
NH_4OH (conc.)	5.0	ml.
Ion-exchange water	1000.0	ml.
Dissolved with heat.		

Solution H

K_2HPO_4	6.0	g.
Sodium acetate	72.0	g.
NH_4Cl	30.0	g.

$(NH_4)_2SO_4$	30.0	g.
KH_2PO_4	6.0	g.
$FeSO_4$	60.0	mg.
NaCl	60.0	mg.
$MnSO_4$	60.0	mg.
Ion-exchange water	1200.0	ml.

Solution J

Glucose	240.0	g.
Ion-exchange water	1200.0	ml.

These stock solutions were mixed in the following proportions with ion-exchange water:

Solution A	250.0	ml.
Solution B	250.0	ml.
Solution C	50.0	ml.
Solution D	25.0	ml.
Solution E	25.0	ml.
Solution F	25.0	ml.
Solution G	50.0	ml.
Solution H	100.0	ml.
Solution J	50.0	ml.
Ion-exchange water	175.0	ml.

The medium was sterilized by passing through membrane filters[i] with a maximum pore size of 0.65 μ. Each species

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[i]Millipore Filter Corp., Bedford, Mass., U. S. A.

was cultured at it's optimum temperature for 48 hours. Cells were harvested by centrifugation at 3000-4000 r.p.m. for 15 minutes, and washed three times in saline.

Reagents

Saline: (a) for washing; physiological isotonic saline. (b) as a diluent for agglutination tests; physiological isotonic saline plus 0.1 per cent sodium azide.

Buffered saline: (a) for washing and suspending erythrocytes; one volume of physiological isotonic saline and one volume of buffer prepared by mixing 0.15 M KH_2PO_4 and 0.15 M Na_2HPO_4 in the proper proportions to give the desired pH for the purpose used. (b) as a diluent for hemagglutination tests; as in (a) but with one per cent normal rabbit serum.

Tannic acid: 1:20,000 in buffered saline, pH 7.2, freshly prepared each time of use.

Alsever's solution: dextrose 2.05 g., sodium citrate 0.8 g., sodium chloride 0.42 g., distilled water 100 ml., adjusted to pH 6.1 with 10 per cent citric acid and sterilized at 110 degree C for 15 minutes.

Preparation of Intact-Cell Antigens

The bacteria were suspended in saline to give a density of 10 mg. of cells (dry weight) per ml.. This

suspension was heated at 56 degree C for one hour and tested for sterility. Clumps (indicating spontaneous agglutination) were removed by filtering through cotton. The cell suspension was stored at 4 degree C, and used for vaccinations, for agglutination, and for agglutinin absorption tests.

Preparation of Cell-Wall Antigens

Ten grams (wet weight) bacterial cells were suspended in 10 ml. distilled water, placed in a plastic centrifuge tube immersed in ice, and disintegrated for 20 minutes by the MSE-Mullard Ultrasonic Disintegrator with a 1/4 inch (9:1 ratio) stainless steel vibrator probe (133 mm. overall length) connected to the magnetostrictive transducer and the generator. The disintegrated cell suspension was centrifuged at 10,000 r.p.m. for 20 minutes in a Servall SS-1 high speed angle centrifuge and the supernatant discarded. The cell walls formed a distinct layer on top of the intact cells. By adding distilled water and gentle agitation, the surface layer, containing mainly cell walls, was resuspended. This suspension was decanted and centrifuged. The above procedure was repeated many times--until all intact cells were removed.

Removal of impurities from cell walls was accomplished by washing twice with distilled water, twice with saline, four times with 0.05 M phosphate buffer (pH

7.6), and seven times with distilled water. The purity of cell wall preparations was checked by phase contrast microscopy.

Cell-wall antigens were prepared by suspending sufficient cell walls in saline to give a density of 2 mg. per ml. (dry weight). These antigens were stored at -20 degree C.

Preparation of Antisera

Twelve groups of adult albino rabbits were immunized with intact cells or cell walls of each of the six species of lactic acid bacteria. The method of immunization was that of Kabat and Mayer (1948) with minor modifications, using three rabbits for each group. Each rabbit received a total of 3.0 ml. of antigen in 12 doses administered at a rate of three per week as follows: 0.1 ml. the first week, 0.2 ml. the second week, 0.3 ml. the third week, and 0.4 ml. the fourth week. Each week the first injection was given intraperitoneally, and the second and third intravenously. Serum samples were obtained from each rabbit prior to immunization, and again two days after the final injection. Cell-free sera were obtained by centrifugation at 2,200 r.p.m. for 10 minutes; and stored at -20 degree C until serological tests were performed.

Agglutination Tests

The antisera were set up in a series of halving dilutions of 0.5 ml. volumes, ranging from 1:10 to 1:20, 480. The intact-cell, or cell-wall, antigens were added in 0.5 ml. volumes to each dilution. A negative control with saline and intact-cell, or cell-wall, antigen was included with each test. Incubation was carried out in a water-bath at 37 degree C for four hours followed by refrigeration at 10 degree C for 48 hours. Readings were made with the aid of a Fisher Kahn Viewer and graded into four groups: 4 +, 3 +, 2 +, 1 +. The activity of an antiserum in terms of titer was expressed as the reciprocal of the highest dilution of serum showing agglutination of 2 + or higher. A titer of 1:80 or less was considered to be not significant.

Agglutinin Absorption Technique

One volume packed bacterial cells was added to 20 volumes undiluted antiserum, agitated at 37 degree C for two hours and refrigerated overnight. Without removing the sedimented cells, the process of absorption was repeated twice, each time by the addition of another volume of packed cells. The absorbed serum was then separated by centrifugation, and tested for completeness of absorption by the agglutination test with its homologous antigen. If

absorption was incomplete, the absorption procedure was repeated. When absorption was complete, the serum was stored at -20 degree C.

Hemagglutination Tests

One part sheep's blood was collected in 1.2 parts Alsever's solution, stored at 6 degree C and used from the fourth to the twenty-first day after bleeding.

(a) Preparation of washed, packed sheep's erythrocytes

The erythrocytes were centrifuged at 2,200 r.p.m. for 10 minutes, washed three times with six volumes of saline (pH 7.2), suspended in the saline at a 2.5 per cent concentration, stored at 10 degree C, and used the same day.

(b) Treatment of erythrocytes with tannic acid

Equal volumes of a 2.5 per cent suspension of erythrocytes and of a 1:20,000 tannic acid were mixed and allowed to react at room temperature for 10 minutes. The erythrocytes were separated by centrifuging at 2,200 r.p.m. for 10 minutes, washed with saline (pH 7.2), and resuspended in saline (pH 6.4) at a concentration of 0.5 per cent. These tannin-treated erythrocytes were stored at 10 degree C and used the same day.

(c) Sensitization of erythrocytes with cell-wall antigens

One-tenth milliliter packed tannin-treated erythrocytes was added to 12 ml. cell-wall antigen; incubated at room temperature for 90 minutes, with agitation at 15-minute intervals; separated by centrifuging at 1800 r.p.m. for eight minutes; washed with saline (pH 6.4) containing one per cent normal rabbit serum (hereafter designated as N.R.S.); and suspended in the saline with N.R.S. at a 2.5 per cent concentration. The sensitized erythrocytes were stored at 10 degree C and used the same day.

(d) Hemagglutination of tannin-treated sensitized erythrocytes

The antisera were diluted with saline (pH 6.4) containing one per cent N.R.S. in a series of halving dilutions ranging from 1:32 to 1:4096. One drop of sensitized erythrocytes was added to each dilution of serum. Six negative controls were included: three contained antiserum (1:32), the first with untreated erythrocytes, the second with tannin-treated erythrocytes, and the third with saline (pH 6.4) containing one per cent N.R.S.; and three contained the saline, the first with untreated erythrocytes, the second with tannin-treated erythrocytes, and the third with tannin-treated sensitized erythrocytes. Incubation was at room temperature for 12 hours. Readings were made with the aid of a Fisher Kahn Viewer, and the

degree of clumping graded as follows: 4 + compact granular agglutinate; 3 + smooth mat on the bottom of the tube with folded edges; 2 + smooth mat on the bottom of the tube with edges somewhat ragged; 1 + narrow red ring around the edge of smooth mat; ± smaller area of the bottom of the tube covered than one plus and heavier ring around the edge; - a discrete red button clearly visible in the centre at the bottom.

The activity of an antiserum in terms of titer was expressed as the reciprocal of the highest dilution of serum showing hemagglutination of 2 + or higher. A titer of 1:64 or less was considered to be not significant.

Precipitin Tests--Lancefield Grouping

Group-specific Antigens and Antibodies:

(a) Preparation of extracts

Lancefield's (1933) hot-acid method for the preparation of group-specific antigens from intact cells and cell walls was used. The extracts were stored in stoppered tubes at -20 degree C.

(b) Precipitin tests

Antisera to cell walls of the bacteria were layered on the group-specific antigens, mentioned above, in 0.7 to 1 mm. diameter capillary tubes. These were incubated at

room temperature, and examined for ring formation at the junction at 30 minutes and again at 12 hours.

Commercial group D and group N antisera[i] were used to test each hot-acid extract.

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[i]Bacto-Streptococcal Antisera

RESULTS

RESULTS

A. Agglutination

The results of agglutination tests of antisera to cell walls with intact-cell antigens are presented in Table 1 A, and those of absorbed antisera in Table 1 B.

Antiserum to each of the six species agglutinated its homologous antigen. Antiserum from each species cross-agglutinated with each of the other species, with one exception. Antiserum to Lactobacillus lactis did not agglutinate any other species.

Antiserum to Lactobacillus plantarum agglutinated L. lactis, S. cremoris and S. lactis. After absorption with S. cremoris, the antiserum agglutinated its homologous antigens, L. lactis and S. lactis. After absorption with L. lactis, it agglutinated its homologous antigen and S. lactis. After absorption with S. lactis, it agglutinated its homologous antigen and L. lactis.

Antiserum to Lactobacillus casei agglutinated L. lactis, L. plantarum and S. lactis. After absorption with L. lactis, the antiserum agglutinated its homologous antigen, L. plantarum and S. lactis. After absorption with L. plantarum, it agglutinated its homologous antigen and S. lactis. After absorption with S. lactis, it agglutinated its homologous antigen and L. plantarum.

Antiserum to Streptococcus cremoris agglutinated L. lactis and L. plantarum. After absorption with L. lactis, the antiserum agglutinated only its homologous antigen. After absorption with L. plantarum, it agglutinated only its homologous antigen.

Antiserum to Streptococcus faecalis agglutinated L. lactis. After absorption with L. lactis, the antiserum agglutinated only its homologous antigen.

Antiserum to Streptococcus lactis agglutinated L. lactis, L. casei, L. plantarum and S. cremoris. After absorption with L. plantarum, the antiserum agglutinated its homologous antigen, L. casei and S. cremoris. After absorption with L. lactis, it agglutinated its homologous antigen, and S. cremoris, L. casei and L. plantarum. After absorption with S. cremoris, it agglutinated its homologous antigen, and L. casei and L. plantarum. After absorption with L. casei, it agglutinated its homologous antigen, L. lactis, L. plantarum and S. cremoris.

On the basis of reciprocal testing, L. lactis was agglutinated by the antiserum of each of the other five species; L. plantarum was agglutinated by the antisera of S. lactis, S. cremoris and L. casei; S. cremoris was agglutinated by the antisera of L. plantarum and S. lactis; S. lactis was agglutinated by the antisera of L. casei and L. plantarum; L. casei was agglutinated by the antiserum of S. lactis; but S. faecalis was not agglutinated by the

antiserum of each of the other species.

On the basis of reciprocal agglutination tests of unabsorbed and absorbed cell-wall antisera, antigenic components present in each of the species were ascribed. These antigens are designated by small letters.

The antigenic components of L. lactis are a, b, c, d, f and g; of L. plantarum are b, c, e, f, g, k and p; of L. casei are c, k, l, n and p; of S. cremoris are b, f, h and n; of S. faecalis are d and m; and of S. lactis are c, e, f, l, n and p (Table 3 A). Antigen a was found in L. lactis; antigen b in L. lactis, L. plantarum and S. cremoris; antigen c in L. lactis, L. plantarum, L. casei and S. lactis; antigen d in L. lactis and S. faecalis; antigen e in L. plantarum and S. lactis; antigen f in L. lactis, L. plantarum, S. cremoris and S. lactis; antigen g in L. lactis and L. plantarum; antigen h in S. cremoris; antigen k in L. plantarum and L. casei; antigen l in L. casei and S. lactis; antigen m in S. faecalis; antigen n in L. casei, S. cremoris and S. lactis; and antigen p in L. plantarum, L. casei and S. lactis.

The antigens shared by one species with another are presented in Table 3 B. L. lactis and L. plantarum share four antigens: b, c, f and g; L. plantarum and S. lactis share four antigens: c, e, f and p; L. plantarum and L. casei share three antigens: c, k and p; L. casei and S. lactis share three antigens: l, n and p; L. lactis and

S. cremoris share two antigens: b and f; L. lactis and S. lactis share two antigens: c and f; L. plantarum and S. cremoris share two antigens: b and f; S. cremoris and S. lactis share two antigens: f and n; L. lactis and L. casei share one antigen: c; L. lactis and S. faecalis share one antigen: d; and L. casei and S. cremoris share one antigen: n.

The antigens shared by lactobacilli, by streptococci, and by both are presented in Table 3 C. The lactobacilli share six antigens: b, c, f, g, k and p; the streptococci share two antigens: f and n; and the lactobacilli and streptococci share eight antigens: b, c, d, e, f, l, n and p. The antigens shared by two species in one genus were not necessarily shared by another combination of species in the genus.

The results of agglutination tests of antisera to intact cells with intact-cell antigens are presented in Table 2 A, and those of absorbed antisera in Table 2 B.

Antiserum to each of the six species agglutinated its homologous antigen. Antiserum from each species cross-agglutinated with each of the other species, with one exception. Antiserum to Lactobacillus lactis did not agglutinate any other species.

Antiserum to Lactobacillus plantarum agglutinated L. lactis, L. casei and S. cremoris. After absorption with L. lactis, the antiserum agglutinated its homologous antigen

and S. cremoris. After absorption with L. casei, it agglutinated its homologous antigen and S. cremoris. After absorption with S. cremoris, it agglutinated only its homologous antigen.

Antiserum to Lactobacillus casei agglutinated L. lactis. After absorption with L. lactis, the antiserum did not agglutinate either its homologous antigen or any other species.

Antiserum to Streptococcus cremoris agglutinated L. lactis and L. plantarum. After absorption with L. lactis, the antiserum agglutinated its homologous antigen and L. plantarum. After absorption with L. plantarum, it agglutinated only its homologous antigen.

Antiserum to Streptococcus faecalis agglutinated L. lactis, L. plantarum and S. cremoris. After absorption with L. lactis, the antiserum agglutinated its homologous antigen, L. plantarum and S. cremoris. After absorption with S. cremoris, it agglutinated its homologous antigen, L. lactis and L. plantarum. After absorption with L. plantarum, it did not agglutinate either its homologous antigen or any other species.

Antiserum to Streptococcus lactis agglutinated L. lactis. After absorption with L. lactis, the antiserum did not agglutinate its homologous antigen or any other species.

On the basis of reciprocal testing, L. lactis was agglutinated by the antiserum of each of the other five

species, L. plantarum by the antisera of S. cremoris and S. faecalis, L. casei by the antiserum of L. plantarum, and S. cremoris by the antisera of S. faecalis and L. plantarum. Neither S. faecalis nor S. lactis was agglutinated by the antiserum of any of the other species.

On the basis of reciprocal agglutination tests of unabsorbed and absorbed intact-cell antisera, antigenic components present in each of the species were ascribed. These antigens are designated by capital letters.

The antigenic components of L. lactis are A, C, E, F, M and P; of L. plantarum are B, D, E, G, M and P; of L. casei are C and E; of S. cremoris are B, D, E, H and P; of S. faecalis are D and M; and of S. lactis are F and N (Table 4 A). Antigen A was found in L. lactis; antigen B in L. plantarum and S. cremoris; antigen C in L. lactis and L. casei; antigen D in L. plantarum, S. cremoris and S. faecalis; antigen E in L. lactis, L. plantarum, L. casei and S. cremoris; antigen F in L. lactis and S. lactis; antigen G in L. plantarum; antigen H in S. cremoris; antigen M in L. lactis, L. plantarum and S. faecalis; antigen N in S. lactis; and antigen P in L. lactis, L. plantarum and S. cremoris.

The antigens shared by one species with another are presented in Table 4 B. L. plantarum and S. cremoris share four antigens: B, D, E and P; L. lactis and L. casei share

two antigens: C and E; L. lactis and S. cremoris share two antigens: E and P; L. plantarum and S. faecalis share two antigens: D and M; L. lactis and L. plantarum share one antigen: E; L. lactis and S. faecalis share one antigen: M; L. lactis and S. lactis share one antigen: F; L. plantarum and L. casei share one antigen: E; L. casei and S. cremoris share one antigen: E; and S. cremoris and S. faecalis share one antigen: D.

The antigens shared by lactobacilli, by streptococci, and by both are presented in Table 4 C. The lactobacilli share two antigens: C and E; the streptococci share one antigen: D; and the lactobacilli and streptococci share six antigens: B, D, E, F, M and P. The antigens shared by two species in one genus were not necessarily shared by another combination of species in the genus.

B. Hemagglutination

The results of hemagglutination tests with cell walls adsorbed to tannin-treated erythrocytes and cell-wall antisera are presented in Table 7, and illustrated in Plate I.

Hemagglutination occurred with the cell-wall antigen of each species and its homologous antiserum. In addition, it occurred with L. lactis and the antisera of S. lactis, S. cremoris, L. casei and L. plantarum; with L. plantarum and the antiserum of S. lactis; with L. casei and the anti-

sera of L. lactis and S. cremoris; and with S. lactis and the antisera of S. cremoris, L. casei and L. lactis.

On the basis of hemagglutination tests of cell-wall antisera, antigenic components present in each of the species were ascribed. These antigens are designated by Roman numerals.

The antigenic components of L. lactis are I, II and VI; of L. plantarum are II and V; of L. casei are I, III and VII; of S. cremoris are VI and VII; of S. lactis are I, V and VI; and of S. faecalis is IV (Table 6 A). Antigen I was found in L. lactis, L. casei and S. lactis; antigen II was in L. lactis and L. plantarum; antigen III in L. casei; antigen IV in S. faecalis; antigen V in L. plantarum and S. lactis; antigen VI in L. lactis, S. cremoris and S. lactis; and antigen VII in L. casei and S. cremoris.

The antigens shared by one species with another are presented in Table 6 B. L. lactis and S. lactis share two antigens: I and VI; L. lactis and L. plantarum share one antigen: II; L. lactis and L. casei share one antigen: I; L. lactis and S. cremoris share one antigen: VI; L. plantarum and S. lactis share one antigen: V; L. casei and S. cremoris share one antigen: VII; L. casei and S. lactis share one antigen: I; and S. cremoris and S. lactis share one antigen: VI.

The antigens shared by lactobacilli, by streptococci, and by both are presented in Table 6 C. The lactobacilli

share two antigens: I and II; the streptococci share one antigen: VI; and the lactobacilli and streptococci share four antigens: I, V, VI and VII. The antigens shared by two species in one genus were not necessarily shared by another combination of species in the genus.

C. Precipitation

The precipitation tests of cell-wall extracts with antisera to cell walls are presented in Table 7.

Extracts of each species precipitated its homologous antiserum, with two exceptions--S. cremoris and S. faecalis. Only extracts from S. faecalis were precipitated by antisera of other species, namely the three species of lactobacilli.

The results of precipitation tests with cell-wall and intact-cell extracts and commercial group N and group D antisera are presented in Table 8. Precipitation occurred with cell-wall, and also intact-cell, extracts and group N antiserum in each species, with one exception. Neither cell-wall nor intact-cell extracts of S. faecalis precipitated group N antiserum. The intact-cell but not the cell-wall extracts precipitated group D antiserum.

DISCUSSION

DISCUSSION

In this work, as in other biological investigations, there was a difference in the degree of serological response of individual rabbits to cell walls and likewise to intact cells. In each group, certain rabbits produced more antibodies than others. For this reason, the interpretation of results was based on the antisera which gave the highest titers.

It appears that cell walls are more effective in stimulating the production of antibodies than are intact cells, since a greater number of antigens was detected by cell-wall antisera than by intact-cell antisera. This is in agreement with findings reported by Vennes and Gerhardt (1956). It may be that the location of antigens in the cell wall determines antibody production. The antigens on the inner surface of the wall may be bound to the cytoplasm in such a way that they are unable to stimulate antibody-forming cells in the animal, whereas the antigens in the isolated cell wall may be free.

A considerable number of antigens failed to produce antibodies, although they combined with antibodies produced by heterologous species. For example, L. lactis produced only one antibody, but was agglutinated by antisera of the other five species. Antigens of this kind may be complex

haptenes (Carpenter, 1956); or it may be that the antigens are present in amounts insufficient to stimulate the production of antibodies, although they unite with them (Fabiyi, Lief and Henle, 1958).

On the basis of common cell-wall antigens, the six species of lactic acid bacteria in this study may be grouped as follows. Group 1 includes those species that share three or more antigens; group 2, those that share two antigens; and group 3, those that do not share antigens.

This grouping suggests that the possessing of common cell-wall antigens might be considered in establishing taxonomic relationships. Thus, S. lactis on this basis is more closely related to the lactobacilli than it is to S. cremoris, or to S. faecalis. Likewise, this grouping provides further evidence of the difference between S. faecalis and the other two streptococci.

As may be observed in Tables 3 C, 4 C and 6 C, a considerably greater number of antigens was shared by the lactobacilli and streptococci than by the species of either genus. This provides additional evidence of the close relationship between the rods and the cocci in Lactobacillaceae.

The significance of determining the antigens of cell walls of lactic acid bacteria lies in the possibility that they may be used to identify large numbers of lactic acid bacteria more accurately than methods presently used. In

this study, certain antigens were found to be highly species-specific. Antisera prepared from these specific cell-wall antigens may be used to identify the six species. Thus serum from antigen "a" identifies L. lactis; from "h", S. cremoris; and from "m", S. faecalis. Since L. casei contains "l" and "k", S. lactis contains "l" but not "k", and L. plantarum contains "k" but not "l", it follows that positive reactions with serum prepared from "l" and with serum from "k" identifies L. casei; with serum from "l" but not from "k", S. lactis; and with serum from "k" but not from "l", L. plantarum.

The ability of a cell-wall suspension to sensitize erythrocytes treated with tannic acid provides a new approach to the hemagglutination technique for the study of cell-wall antigens. Boyden (1950) sensitized tannin-treated erythrocytes with purified protein extracts. This author suggested that some components of protein extracts acted on the surface properties of the erythrocytes. The reaction eventually led to the adsorption of other components by the erythrocytes as a result of their newly acquired surface properties. It is probable that in the present study tannin-treated erythrocytes were sensitized by the protein components of the cell wall in the same manner. This method was used to detect the protein-type antigens in cell walls of the six species studied. The finding of fewer of these

antigens suggests a higher degree of specificity. Cell-wall suspensions applied to hemagglutination technique could serve as a useful tool for serological studies of other bacteria.

Preliminary precipitation tests with cell-wall antisera showed the presence of polysaccharide-type antigens in cell walls. The specific group N antigen was demonstrated in the cell walls of each species with one exception, S. faecalis. Previously McCarty (1952 a, b) had shown that the group antigen of group A streptococci was located in the cell walls. The group antigen of S. faecalis was detected in intact cells, but not in cell walls. This is in agreement with the report by Jones and Shattock (1960) that the group antigen of group D streptococci was present in the cell contents after the cell walls had been removed.

On the basis of agglutination and hemagglutination results in this report, even though cell-wall and intact-cell antigens are designated by different types of letters and numbers, it is probable that at least certain antigens in one group might be the same as those in another group. Confirmation of this theory was beyond the scope of this investigation.

TABLES

TABLE 1 A

AGGLUTININ TITERS OF UNABSORBED CELL-WALL ANTISERA WITH INTACT-CELL ANTIGENS

Antisera	Antigens					
	L. lactis	*L. plantarum*	*L. casei*	*S. cremoris*	*S. faecalis*	*S. lactis*
L. lactis	1280	40	0	0	0	10
L. plantarum	2560	640	0	160	0	160
L. casei	1280	160	160	0	0	160
S. cremoris	640	160	10	160	0	0
S. faecalis	320	10	20	40	160	80
S. lactis	1280	320	640	160	10	320

TABLE 1 B

AGGLUTININ TITERS OF ABSORBED CELL-WALL ANTISERA WITH INTACT-CELL ANTIGENS

Antisera	Absorbed with	Antigens					
		L. lactis	*L. plant.*	*L. casei*	*S. cremor.*	*S. faecal.*	*S. lactis*
L. lactis	Unabsorb.	1280	40	0	0	0	10
	L. lactis	0	40	-	-	-	-
L. plantarum	Unabsorb.	2560	640	0	160	0	160
	L. plant.	0	0	-	40	-	0
	L. lactis	0	320	-	80	-	160
	S. cremor.	1280	320	-	0	-	160
	S. lactis	640	160	-	0	-	0
L. casei	Unabsorb.	1280	160	160	0	0	160
	L. casei	0	0	10	-	-	20
	L. lactis	0	160	160	-	-	160
	L. plant.	10	0	320	-	-	160
	S. lactis	40	320	160	80	-	0
S. cremoris	Unabsorb.	640	160	10	160	0	0
	S. cremor.	0	0	-	0	-	-
	L. lactis	0	80	-	160	-	-
	L. plant.	0	10	-	160	-	-
S. faecalis	Unabsorb.	320	10	20	40	160	80
	S. faecal.	0	0	0	10	0	40
	L. lactis	0	-	-	-	160	80
S. lactis	Unabsorb.	1280	320	640	160	10	320
	S. lactis	10	0	40	0	0	0
	L. lactis	0	160	640	320	-	640
	L. plant.	10	0	320	320	-	640
	L. casei	160	320	0	160	-	160
	S. cremor.	0	320	640	0	-	160

TABLE 2 A

AGGLUTININ TITERS OF UNABSORBED INTACT-CELL ANTISERA WITH INTACT-CELL ANTIGENS

Antisera	Antigens					
	L. lactis	*L. plantarum*	*L. casei*	*S. cremoris*	*S. faecalis*	*S. lactis*
L. lactis	2560	40	80	0	80	10
L. plantarum	1280	2560	320	160	80	10
L. casei	1280	40	1280	20	0	20
S. cremoris	1280	640	40	320	0	40
S. faecalis	1280	1280	80	1280	640	40
S. lactis	640	80	80	20	0	640

TABLE 2 B

AGGLUTININ TITERS OF ABSORBED INTACT-CELL ANTISERA WITH INTACT-CELL ANTIGENS

Antisera	Absorbed with	Antigens					
		L. lactis	*L. plant.*	*L. casei*	*S. cremor.*	*S. faecal.*	*S. lactis*
L. lactis	Unabsorb.	2560	40	80	0	80	10
	L. lactis	0	-	20	-	40	-
L. plantarum	Unabsorb.	1280	2560	320	160	80	10
	L. plant.	40	0	20	40	80	80
	L. lactis	0	160	40	640	-	40
	L. casei	20	640	0	320	0	0
	S. cremor.	80	640	10	0	0	40
L. casei	Unabsorb.	1280	40	1280	20	0	20
	L. casei	0	40	40	-	-	40
	L. lactis	0	80	0	-	-	-
S. cremoris	Unabsorb.	1280	640	40	320	0	40
	S. cremor.	80	80	-	0	-	-
	L. lactis	0	320	-	160	-	-
	L. plant.	0	0	-	320	-	-
S. faecalis	Unabsorb.	1280	1280	80	1280	640	40
	S. faecal.	10	20	0	40	10	0
	L. lactis	0	1280	80	640	320	80
	L. plant.	0	0	80	80	80	40
	S. cremor.	640	160	0	0	160	80
S. lactis	Unabsorb.	640	80	80	20	0	640
	S. lactis	0	40	10	-	-	0
	L. lactis	0	80	-	-	-	80

TABLE 3 A

ANTIGENS IN CELL WALLS OF CERTAIN LACTIC ACID BACTERIA

Bacteria	Antigens[i]
L. lactis	a, b', c', f', g'
L. plantarum	b, e, g, c', f', k', p'
L. casei	c, k, l, n', p'
S. cremoris	b, h, f', n'
S. faecalis	d, m
S. lactis	f, n, p, c', e', l'

[i]Small letters without the prime designate antigens producing antibodies and combining with them. Those with the prime do not produce antibodies, but do combine with them.

TABLE 3 B

CELL-WALL ANTIGENS SHARED BY CERTAIN LACTIC ACID BACTERIA

Common Antigens	Number	Shared by
b, c, f, g	4	L. lactis, L. plantarum
c	1	L. lactis, L. casei
b, f	2	L. lactis, S. cremoris
d	1	L. lactis, S. faecalis
c, f	2	L. lactis, S. lactis
c, k, p	3	L. plantarum, L. casei
b, f	2	L. plantarum, S. cremoris
-	0	L. plantarum, S. faecalis
c, e, f, p	4	L. plantarum, S. lactis
n	1	L. casei, S. cremoris
-	0	L. casei, S. faecalis
l, n, p	3	L. casei, S. lactis
-	0	S. cremoris, S. faecalis
f, n	2	S. cremoris, S. lactis
-	0	S. faecalis, S. lactis

TABLE 3 C

CELL-WALL ANTIGENS COMMON TO *LACTOBACILLUS* SPP., TO *STREPTOCOCCUS* SPP., AND TO CERTAIN SPECIES OF BOTH

Common Antigens	Number	Species of
b, c, f, g, k, p	6	*Lactobacillus*
f, n	2	*Streptococcus*
b, c, d, e, f, l, n, p	8	*Lactobacillus* and *Streptococcus*

TABLE 4 A

ANTIGENS IN INTACT CELLS OF CERTAIN LACTIC ACID BACTERIA

Bacteria	Antigens[i]
L. lactis	A, C', E', F', M', P'
L. plantarum	B, E, G, D', M', P'
L. casei	C, E'
S. cremoris	B, H, P, D', E'
S. faecalis	D, M
S. lactis	F, N

[i]Capital letters without the prime designate antigens producing antibodies and combining with them. Those with the prime do not produce antibodies, but do combine with them.

TABLE 4 B

INTACT-CELL ANTIGENS SHARED BY CERTAIN LACTIS ACID BACTERIA

Common Antigens	Number	Shared by
E	1	L. lactis, L. plantarum
C, E	2	L. lactis, L. casei
E, P	2	L. lactis, S. cremoris
M	1	L. lactis, S. faecalis
F	1	L. lactis, S. lactis
E	1	L. plantarum, L. casei
B, D, E, P	4	L. plantarum, S. cremoris
D, M,	2	L. plantarum, S. faecalis
-	0	L. plantarum, S. lactis
E	1	L. casei, S. cremoris
-	0	L. casei, S. faecalis
-	0	L. casei, S. lactis
D	1	S. cremoris, S. faecalis
-	0	S. cremoris, S. lactis
-	0	S. faecalis, S. lactis

TABLE 4 C

INTACT-CELL ANTIGENS COMMON TO *LACTOBACILLUS* SPP., TO *STREPTOCOCCUS* SPP., AND TO CERTAIN SPECIES OF BOTH

Common Antigens	Number	Species of
C, E	2	*Lactobacillus*
D	1	*Streptococcus*
B, D, E, F, M, P	6	*Lactobacillus* and *Streptococcus*

TABLE 5

HEMAGGLUTINATION TITERS OF CELL-WALL ANTISERA WITH TANNIN-TREATED ERYTHROCYTES SENSITIZED WITH CELL WALLS

Antisera	Erythrocytes sensitized with cell walls					
	L. lactis	*L. plantarum*	*L. casei*	*S. cremoris*	*S. faecalis*	*S. lactis*
L. lactis	2048	0	128	0	32	512
L. plantarum	4096	1024	0	32	0	0
L. casei	1024	64	1024	0	0	512
S. cremoris	1024	0	256	256	0	1024
S. faecalis	0	0	32	64	512	32
S. lactis	32	256	0	64	0	4096

TABLE 6 A

ANTIGENS IN CELL WALLS--

HEMAGGLUTINATION DATA

Bacteria	Antigens[i]
L. lactis	I, II, VI'
L. plantarum	II, V'
L. casei	I, III, VII'
S. cremoris	VI, VII
S. faecalis	IV
S. lactis	V, I', VI'

[i]Roman numerals without the prime designate antigens producing antibodies and combining with them. Those with the prime do not produce antibodies, but do combine with them.

TABLE 6 B

CELL-WALL ANTIGENS SHARED BY CERTAIN LACTIC ACID BACTERIA--
HEMAGGLUTINATION DATA

Common Antigens	Number	Shared by
II	2	*L. lactis*, *L. plantarum*
I	1	*L. lactis*, *L. casei*
VI	1	*L. lactis*, *S. cremoris*
-	0	*L. lactis*, *S. faecalis*
I, VI	2	*L. lactis*, *S. lactis*
-	0	*L. plantarum*, *L. casei*
-	0	*L. plantarum*, *S. cremoris*
-	0	*L. plantarum*, *S. faecalis*
V	1	*L. plantarum*, *S. lactis*
VII	1	*L. casei*, *S. cremoris*
-	0	*L. casei*, *S. faecalis*
I	1	*L. casei*, *S. lactis*
-	0	*S. cremoris*, *S. faecalis*
VI	1	*S. cremoris*, *S. lactis*
-	0	*S. faecalis*, *S. lactis*

TABLE 6 C

CELL-WALL ANTIGENS COMMON TO *LACTOBACILLUS* SPP., TO *STREPTOCOCCUS* SPP., AND TO CERTAIN SPECIES OF BOTH--HEMAGGLUTINATION DATA

Common Antigens	Number	Species of
I, II	2	*Lactobacillus*
VI	1	*Streptococcus*
I, V, VI, VII	4	*Lactobacillus* and *Streptococcus*

TABLE 7

PRECIPITIN TESTS WITH CELL-WALL EXTRACTS

Antiserum to cell walls	Hot-acid Extract					
	L. lactis	*L. plantarum*	*L. casei*	*S. cremoris*	*S. faecalis*	*S. lactis*
L. lactis	+++	±	-	+	++	±
L. plantarum	+	++	±	±	++	±
L. casei	±	±	++	+++	++	-
S. cremoris	±	-	±	±	-	-
S. faecalis	-	-	-	-	±	±
S. lactis	±	±	±	+	-	+++

TABLE 8

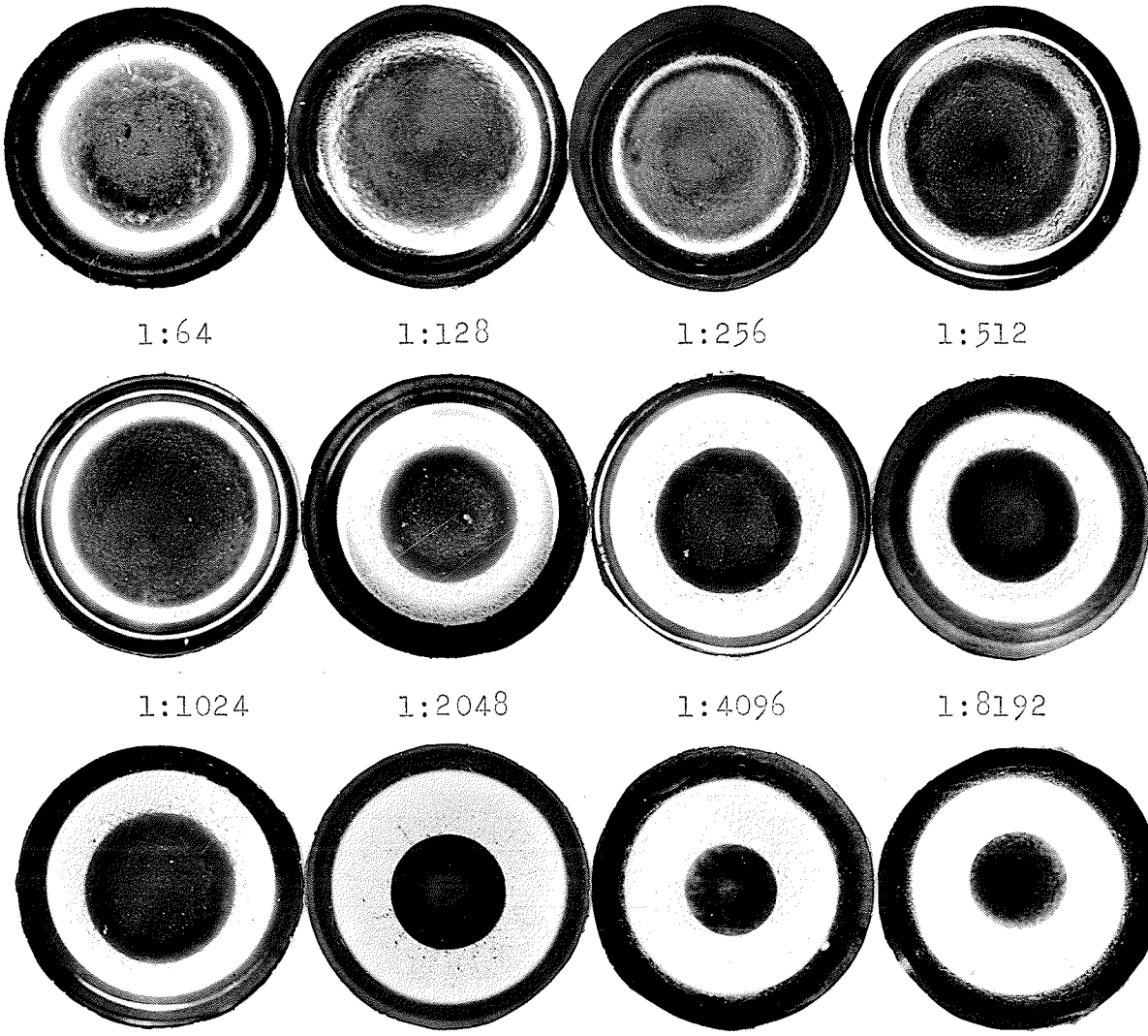
PRECIPITIN TESTS WITH GROUP SPECIFIC ANTISERA

Extract--Lancefield's hot HCl method		Antiserum Group N[i]	Antiserum Group D[i]
Lactobacillus lactis	Intact cells	+++	-
	Cell walls	+++	-
Lactobacillus plantarum	Intact cells	+++	-
	Cell walls	+++	-
Lactobacillus casei	Intact cells	+++	-
	Cell walls	+++	-
Streptococcus cremoris	Intact cells	+++	-
	Cell walls	+++	-
Streptococcus faecalis	Intact cells	-	++
	Cell walls	-	-
Streptococcus lactis	Intact cells	+++	-
	Cell walls	+++	-

[i]Bacto-Streptococcal Antisera

PLATE I

HEMAGGLUTINATION OF TANNIN-TREATED ERYTHROCYTES SENSITIZED WITH CELL WALL



1:64 1:128 1:256 1:512

1:1024 1:2048 1:4096 1:8192

1:16384 1:32768 1:65536 Control

Serum dilution 1:64 to 1:512 ++++ ; 1:1024 +++ ;
1:2048 to 1:16384 + ; 1:32768 to 1:65536 -

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