Agglutinating Antigens of *Lactobacillus jugurti* ATCC 521

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SUMMARY

Serological studies were conducted on *Lactobacillus jugurti* (ATCC 521) in an attempt to identify its agglutinating antigens.

Glycerol teichoic acid was isolated from the cell wall of ATCC 521. Isolated wall teichoic acid removed I21 and I22 antibodies from ATCC 521 antiserum, thereby demonstrating that it is an antigen involved in agglutination. I27 antibodies were not removed from antisera by the wall teichoic acid. Isolated ATCC 521 cell walls did not agglutinate in anti-I12 antiserum, nor did wall teichoic acid from the same organism react with this serum.

Intracellular teichoic acid of ATCC 521 also removed agglutinating antibody from whole antisera. Gel double-diffusion experiments indicated that cell wall and intracellular teichoic acid are immunologically identical.

Lactobacillus jugurti (ATCC 521) mucopeptide was prepared from the cell walls, and this component did not remove agglutinating antibody from antisera.

INTRODUCTION

Williams (1948), the first to study the agglutinating antigens of *Lactobacillus casei* and *Lactobacillus plantarum* by agglutinin adsorption analysis, detected five agglutinating antigens, which were lettered from A to E. Additional agglutinating antigens of *L. casei* and *L. plantarum*, designated as F, G, H and I, were detected by Orland (1950). Miller (1957) detected a K and an L antigen in strains of *L. lactis*. The antigenic structure of *L. bulgaricus*, *L. helveticus* and *L. jugurti* was investigated by Mageau (1966) by agglutinin adsorption analysis; he found that *L. helveticus* ATCC 10797 contained an I20 and an I21 antigen. Mageau (1966) also detected in *L. bulgaricus* ATCC 11842, 15062 and 15065 an I21 antigen and a new agglutinin, I22. The I12 antigen, one previously detected by Efthymiou & Hansen (1962) in *L. acidophilus*, was also observed in ATCC 11842, 15062 and 15065. The I20 and I21 antigens (see footnote to Table 1) have also been observed in an additional strain of *L. helveticus* (ATCC 12046) (personal observation). The I12, I21, I22 and a newly discovered agglutinin, the I27 antigen, have been observed in *L. jugurti* ATCC 521 (personal observation, see Table 1). In the published investigations mentioned above, no attempts were made to identify the agglutinating antigens.

Baddiley & Davison (1961) found that *Lactobacillus jugurti* NCIB 2889 (ATCC 521) possessed both wall and intracellular teichoic acid. The intracellular teichoic acid has been described as the *L. helveticus-jugurti* serological group A antigen by Sharpe, Davison & Baddiley (1964), but they found that cell-wall teichoic acid from these species did not react with group A antiserum. They did not, however, study the role of the latter acid in the agglutination reaction. Juergens, Sanderson & Strominger (1963) and Nathenson & Strominger (1962) found, however, that the precipitinogen

ribitol teichoic acid, when added to homologous antiserum, inhibited cell-wall agglutinin of *Staphylococcus aureus*. This evidence indicates that wall teichoic acid may play a role in the agglutination reaction.

Another component of the bacterial cell wall, the mucopeptide, has been studied extensively. Much of the research performed on bacterial mucopeptide has dealt with the elucidation of its structure (Strominger, Izaki, Mutsuhashi & Tipper, 1967; Plapp, Schleifer & Kandler, 1967). Karakawa & Krause (1966) found streptococcal mucopeptide to be immunogenic, but no studies were conducted to determine the role of the mucopeptide in the agglutination reaction. Morse (1962) however found that mucopeptide of *Staphylococcus aureus* did not absorb agglutinating antibody from antiserum.

Table 1. *Antigenic composition of lactobacilli determined by agglutinin-adsorption analysis*

Antisera	Antigens*				
	I 12	I 20	I 21	I 22	I 27
<i>L. acidophilus</i> , ATCC 4356‡	+	—	—	—	—
<i>L. bulgaricus</i> , ATCC 11842†	+	—	+	+	—
<i>L. bulgaricus</i> , ATCC 15062†	+	—	+	+	—
<i>L. bulgaricus</i> , ATCC 15065†	+	—	+	+	—
<i>L. helveticus</i> , ATCC 10797†	—	+	+	—	—
<i>L. helveticus</i> , ATCC 12046§	—	+	+	—	—
<i>L. jugurti</i> , ATCC 521§	+	—	+	+	+

* Williams (1948) used letters to designate the antigens in lactobacilli. Williams *et al.* (1953) later suggested the use of a system involving numbers to replace the letters, i.e. A = I 1, B = I 2, etc., since the alphabet may not contain a sufficient number of letters to accommodate all of the antigens.

† Mageau (1966). ‡ Efthymiou & Hansen (1962). § Personal observation.

Foley & Wheeler (1945) detected four types of agglutinating antigens within the group D streptococci and found that this was in agreement with the number detected by the precipitin reaction. Sharpe & Shattock (1952) found in typing group D streptococci that there appeared to be some correlation between the precipitin and the agglutination reactions. Lancefield (1943) observed that the M antigen in group A streptococci could be detected by agglutination and precipitin tests. No studies were performed, in the cases cited, to determine whether agglutination and precipitin reactions are caused by identical antigens.

In view of the findings of others, studies were conducted to determine the roles of the cell-wall teichoic acid and the mucopeptide of *Lactobacillus jugurti* ATCC 521 in the agglutination reaction and the composition of the I 12, I 21, I 22 and I 27 antigens. Studies were also made to ascertain whether the agglutinogens for *L. jugurti* (ATCC 521) are also precipitinogens and whether there is an immunological similarity between the wall and intracellular teichoic acids.

METHODS

Organisms and conditions of growth. The strains used in these studies were *Lactobacillus bulgaricus* ATCC 11842, ATCC 15060, ATCC 15062, ATCC 15064, ATCC 15065, L 933 and L 934; *L. helveticus* ATCC 10797, ATCC 10386 and ATCC 12046; *L. jugurti* ATCC 521, ATCC 15063, L 801 and L 803; and *L. acidophilus* ATCC 4356. The L strains

were obtained from Dr P. A. Hansen of the University of Maryland, College Park, Maryland. MRS medium (DeMan, Rogosa & Sharpe, 1960) was used for growing the organisms, which were harvested after incubation at 37° for 18 to 24 hr.

Chemical analysis. For identification of monosaccharides, materials were hydrolyzed for 2 hr at 100° in 2N-H₂SO₄. The residue was dissolved in water and chromatographed with ethyl acetate+methanol+acetic acid+water (80+10+10+15 v/v) by thin-layer chromatography with the Eastman Chromagram System (supplied by Fisher Scientific, Inc.). In the determination of amino acids, materials were hydrolysed for 20 hr at 100° in 6N-HCl with evaporation to dryness. The residue was taken up in an ethanolic solution of 0.5M-HCl and chromatographed in butanol+acetic acid+water (80+20+20 v/v) or propanal+water (70+30 v/v). After the chromatography, amino acids were detected with the ninhydrin reagent, carbohydrates with aniline hydrogen phthalate and phosphoric esters by the method of Wade & Morgan (1953). Protein determinations were performed by the method of Lowry, Rosebrough, Farr & Randall (1951).

Preparation of teichoic acid. Cell-wall teichoic acid was prepared by the method of Sharpe *et al.* (1964) by extractions of cell walls with 10% trichloroacetic acid (TCA). Intracellular teichoic acid was prepared by the extraction of freeze-dried cytoplasmic material with 10% TCA; the cytoplasmic material was obtained by centrifuging down the cell walls and combining the supernatant with the cell-wall washings. The intracellular teichoic acid was further purified by ethanol precipitation.

The mucopeptide portion of the cell wall was prepared by the method of Plapp & Kandler (1967).

Serological methods. Immune sera were prepared by injecting rabbits 12 times over a period of 3 weeks with bacterial suspensions. Five days after the last injections the rabbits were bled.

The tube-agglutination test was performed by the double-dilution method of Williams, Norris & Gyorgy (1953). The final dilution range was between 1/20 and 1/10,240 and the final volume in any one tube was 0.4 ml., 0.2 ml. being diluted serum and 0.2 ml. the antigen suspension. In those cases in which only small amounts of antisera were available, the above procedure was utilized, but the total volume was 0.04 ml., using equal volumes of antigen and antisera.

The antigen+antiserum system was incubated at 37° for 2 hr and refrigerated at 4° overnight. The tubes were read the following day, and the titre recorded as the highest dilution showing agglutination. The control was normal rabbit serum and antigen.

Adsorption of agglutinins with intact organisms was performed by the method of Efthymiou & Hansen (1962). Adsorption of antibody with soluble antigen was accomplished by diluting the antiserum with the latter until the required dilution was obtained. Following this procedure, the system was incubated at 37° and 4°, as described above, after which it was centrifuged to remove all precipitins.

Ring precipitin test. The method of Jones & Shattock (1960) was used. The concentration of teichoic acid in this test was 1 mg./ml. The concentration of teichoic acid in the agar-diffusion test of Ouchterlony (1953) was 1 mg./ml.

Preparation of cell walls. Bacteria previously freeze dried were broken in the French pressure cell at 12,560 lbs/sq. in., after which the material was centrifuged at 480 × G.R.C.F. in a Sorvall Super Speed RC-2 refrigerated centrifuge. The unbroken bacteria were deposited, and the walls were recovered in the supernatant and washed nine

times with M-phosphate buffer, pH 7, followed by an equal number of washings with distilled water. During the washing the walls were exposed to ultrasonic vibrations for 20 sec. at a setting of 2 W. on a Sonifier Cell Disrupter (Heat Systems Co., Melville, Long Island, New York). This procedure was carried out in the presence of glass beads, 1/2 mm. diam., in order to break cell-wall masses. After each washing the protein content of the supernatant was determined by the method of Lowry *et al.* (1951). When little or no protein was found in the washings of the cell walls, the preparation was judged clean. The purity of the cell-wall suspensions was further confirmed by phase microscopy.

Table 2. *Agglutination of Lactobacillus jugurti* ATCC 521 and walls with whole and adsorbed antisera

Antisera	Freeze dried			ATCC 521 cells in antisera adsorbed with 521 cells	ATCC 521 cells in antisera adsorbed with 521 cell walls	Homologous cells in untreated antisera
	ATCC 521 cells in unadsorbed antisera	ATCC 521 cells in unadsorbed antisera	ATCC 521 cell walls in unadsorbed antisera			
<i>L. acidophilus</i> , ATCC 4356	160	160	—	—	—	1280
<i>L. bulgaricus</i> , L 933	80	80	40	—	—	640
<i>L. bulgaricus</i> , L 934	160	320	80	—	—	1280
<i>L. bulgaricus</i> , ATCC 11842	320	320	320	—	—	640
<i>L. bulgaricus</i> , ATCC 15060	160	160	160	—	—	640
<i>L. bulgaricus</i> , ATCC 15062	1280	640	320	—	—	1280
<i>L. bulgaricus</i> , ATCC 15064	320	160	160	—	—	5120
<i>L. bulgaricus</i> , ATCC 15065	320	40	160	—	—	80
<i>L. helveticus</i> , ATCC 10386	640	320	320	—	—	2560
<i>L. helveticus</i> , ATCC 10797	320	320	320	—	—	1280
<i>L. helveticus</i> , ATCC 12046	320	160	160	—	—	640
<i>L. jugurti</i> , ATCC 521	1280	1280	1280	—	—	1280
<i>L. jugurti</i> , L 801	1280	1280	1280	—	—	1280
<i>L. jugurti</i> , L 803	5120	1280	5120	—	—	5120
<i>L. jugurti</i> , ATCC 15063	40	40	40	—	—	640

See footnote to Table 3

RESULTS

Location of the bacterial agglutinating antigens. In order to determine the role of the cell wall in the agglutination reaction, the following experiment was performed. Agglutination tests were conducted with antisera prepared against strains of *Lactobacillus bulgaricus* (ATCC 11842, ATCC 15060, ATCC 15062, ATCC 15064, ATCC 15065, L 933 and L 934), *L. jugurti* (ATCC 521, ATCC 15063, L 801 and L 803), *L. helveticus* (ATCC 10386, ATCC 10797 and ATCC 12046) and *L. acidophilus* (ATCC 4356), all known to agglutinate *L. jugurti* ATCC 521 (see Table 2). Simultaneous agglutination tests were performed

with freeze-dried organisms from which the cell walls were prepared in order to determine whether the antigenic properties of the cells had been impaired during the freeze-drying process. Agglutination tests were also performed with the previously mentioned antisera adsorbed with ATCC 521 or with its corresponding cell walls. These tests indicated that the bacterial cell wall contained the agglutinating antigens.

Role of the cell-wall teichoic acid in the agglutination reaction and its immunologic similarity to intracellular teichoic acid

Having demonstrated that the cell wall contained the antigens that were responsible for agglutination, experiments were made to determine the role of wall and intracellular teichoic acid in the reaction. Chemical analysis of the isolated acids revealed that the wall acid contained glycerol phosphate, alanine and variable traces of glucose. On the other hand the intracellular teichoic acid contained, in addition to these same components, a trace of ribose.

Two mg. of wall teichoic acid and the same amount of intracellular teichoic acid of ATCC 521 were added to separate 0.05 ml. quantities of homologous, whole antiserum to remove the corresponding antibody. The adsorbed serum was then used to perform agglutination tests with various species of lactobacilli (Table 3). *Lactobacillus helveticus* 10797 and 12046, shown in Table 3, contain the I21 antigen and are antigenically identical. *Lactobacillus bulgaricus* 11842, 15062 and 15065 contain the I12, I21 and I22 antigens. An examination of the table indicates that both wall and intracellular teichoic acid absorbed agglutinating antibody from antisera, thereby suggesting that these two substances may be immunologically identical. To investigate this possibility an Ouchterlony double-diffusion test was performed. The line of identification connecting the intracellular and wall teichoic acids indicated that these substances were antigenically identical.

Table 3. Agglutination of *Lactobacillus bulgaricus*, *L. helveticus*, and *L. jugurti* with *Lactobacillus jugurti* ATCC 521 antiserum adsorbed with wall and intracellular teichoic acid

Antigen	ATCC 521 antiserum	ATCC 521 antiserum adsorbed with wall teichoic acid	ATCC 521 antiserum adsorbed with intracellular teichoic acid
<i>L. bulgaricus</i> , ATCC 11842	320*	160	160
<i>L. bulgaricus</i> , ATCC 15062	1280	320	320
<i>L. bulgaricus</i> , ATCC 15065	320	80	160
<i>L. helveticus</i> , ATCC 10797	320	—	40
<i>L. helveticus</i> , ATCC 12046	320	—	—
<i>L. jugurti</i> , ATCC 521	1280	320	640

* Numerical values indicate the denominator of the reciprocal agglutination titre; — no agglutination.

To substantiate these findings further, ATCC 521 antiserum was adsorbed with homologous cell walls after which a gel-diffusion plate was prepared (Fig. 1). This adsorbed serum no longer reacted with wall and intracellular teichoic acid. Furthermore, it no longer agglutinated intact homologous cells.

Because absorption of whole ATCC 521 antiserum with intracellular and wall teichoic

acid reduced the agglutination titre in six strains of lactobacilli tested (Table 3), the indication was that these strains contained immunologically similar or identical teichoic acids. To explore this possibility an Ouchterlony double-diffusion test was performed with antisera to ATCC 11842, 15062, 15065, 10797, 12046 and 521 and with wall teichoic acid from the latter organism (Fig. 2). Examination of the Ouchterlony plate revealed that five of the antisera tested reacted with ATCC 521 wall teichoic acid, although the reaction with anti-15062 was very faint. A different sample of ATCC 11842 antiserum, with a low titre of 1/80, was used in the gel-diffusion plate from that utilized in the tests reported in Table 2. It did not react with wall teichoic acid from ATCC 521 in the Ouchterlony plate; however this antiserum did react with the teichoic acid in the ring precipitin test.

Fig. 1

Fig. 2

Fig. 1. Ouchterlony double-diffusion plate. Well 1: normal rabbit serum, well 2: ATCC 521 antiserum; well 3: wall teichoic acid; well 4: ATCC 521 antiserum adsorbed with homologous cell walls; centre well: intracellular teichoic acid.

Fig. 2. Ouchterlony double-diffusion plate. Well 1: 11842 antiserum; well 2: 15062 antiserum; well 3: 15065 antiserum; well 4: 10797 antiserum; well 5: 12046 antiserum; well 6: 521 antiserum; centre well: wall teichoic acid.

The previous experiments indicated that wall teichoic acid is an important antigen in agglutination. Therefore an experiment was designed to determine whether this component was the only or the major antigen responsible for agglutination. Increments of wall teichoic acid of ATCC 521 were added to whole homologous antiserum until no more precipitate formed. Completeness of precipitation was substantiated by finding the antigen in the supernatant. When all of the teichoic acid antibody was removed from the antiserum, the antiserum still exhibited a titre of 1/40, indicating that other factors are involved in agglutination. This absorbed antiserum no longer reacted with ATCC 10797 and 12046, which contain the I21 antigen. Neither did it react with ATCC 11842, 15062 and 15065, which contained the I21 and I22 antigens. The serum, however, agglutinated the homologous organism at a very low titre apparently because of the I27 antigen.

Role of mucopeptide in agglutination. ATCC 521 antiserum was absorbed with the corresponding mucopeptide, after which the former was used for an agglutination test. The titre of the adsorbed serum was identical with that of the unadsorbed serum. Antiserum prepared against mucopeptide also did not agglutinate intact, untreated cells of ATCC 521; however, agglutination was observed with homologous anti-cell-wall serum.

Nature of the I12 antigen. It was noted (Table 2) that anti-I12 serum prepared from

ATCC 4356 did not agglutinate cell walls of ATCC 521. Since evidence from previous experiments indicated that some of the precipitinogens found in the lactobacilli studied were also agglutinogens, experiments were undertaken to determine whether or not this was true of the I12 antigen. Utilization of the precipitin and double-diffusion gel tests revealed that the teichoic acid of ATCC 521 did not react with anti-4356 serum. It was also found that extracts of ATCC 4356 did not react with 521 serum. It was further observed that 521 antiserum did not agglutinate ATCC 4356.

DISCUSSION

As previously mentioned, Morse (1962) found that the mucopeptide of *Staphylococcus aureus* did not adsorb agglutinating antibody from antiserum. This also was found to be true with the mucopeptide of *Lactobacillus jugurti* ATCC 521. However, when working with bacterial mucopeptide prepared by formamide extraction it should be remembered that alteration may occur during the isolation process. Such alteration would result in a component dissimilar to and possessing different immunological properties from those of the native mucopeptide. Perkins (1965) found that formylation of the free amino groups by hot formamide occurred during preparation of the mucopeptide from cell walls.

Evidence presented here indicates that cell-wall and intracellular teichoic acid are immunologically identical, thereby disagreeing with the findings of Sharpe *et al.* (1964). These authors found that wall teichoic acid of ATCC 521 (NCIB 2889) did not react with group antisera. Perhaps the differences are due to the methods by which the antisera were prepared. Antisera used in the present studies were obtained after immunizing each animal for 3 weeks with approximately 12 injections and a total of 14.1 ml. of antigen. A schedule of this nature produces an antiserum with a very high titre. There is the possibility that the antiserum utilized by Sharpe *et al.* (1964) was of a much lower titre, the reaction with wall teichoic acid thereby not being discernible. There is also the possibility that the medium in which the organisms were grown might be responsible for the differences in wall teichoic acid as detected in these studies and as reported by Sharpe *et al.* (1964). This possibility should be considered in light of the findings of Ellwood & Tempest (1967) and Tempest, Dick & Ellwood (1968), who found that the teichoic acid prepared from *Bacillus subtilis* grown in a magnesium-limited medium contained glycerol, glycerol phosphate and glucose. When this organism was grown in a phosphate-limited environment, the wall teichoic acid, upon hydrolysis, showed the absence of glycerol, glycerol phosphate and glucose but the presence of uronic acid and an amino sugar, indicating a teichuronic acid-type compound. Ellwood & Tempest (1968) also found that the cell walls of *Bacillus subtilis* w 23, which normally contain ribitol teichoic acid, manifested a teichuronic acid polymer when the organism was grown in a phosphate-limited medium. Baddiley (1968) also reported that the number of glucosyl substituents on the ribitol teichoic acid from the walls of *Lactobacillus arabinosus* 17-5 depended partly upon the concentration of glucose in the growth medium.

Sharpe *et al.* (1964) observed that *Lactobacillus helveticus* and *L. jugurti* contained wall glycerol teichoic acid. If this is true of all representatives of these species, then it can be assumed that ATCC 10797 contains the same kind of polymer. *Lactobacillus helveticus* ATCC 10797 and *L. jugurti* ATCC 521, although containing wall glycerol teichoic acid, manifest differences in agglutinating antigens. *Lactobacillus helveticus* 10797

contains the I₂₀ and I₂₁ antigens (Mageau, 1966), and *L. jugurti* 521 the I₁₂, I₂₁, I₂₂ and I₂₇ antigens. Since it appears that the main agglutinating antigen in the species discussed is wall glycerol teichoic acid, these antigenic dissimilarities are probably due to differences in this substance.

ATCC 10797 and 521 antisera exhibited a reaction of identity when challenged with *L. jugurti* ATCC 521 wall teichoic acid. Because teichoic acid removes the anti-I₂₁ and anti-I₂₂ components from homologous antiserum, one is led to suspect that two separate and different acids exist in the wall of 521, one the I₂₁ and the other the I₂₂ antigen. In reference to this last speculation, *Staphylococcus aureus* H was found by Baddiley, Buchanan, Martin & Rajbhandary (1962) to contain a wall ribitol teichoic acid substituted with alanine and *N*-acetyl-glucosamine in α - and β -glycosyl linkages. It was further found that the α and β anomers occurred on different chains. Although the possibility of 521 walls containing two types of teichoic acid exists, it was not borne out by gel-diffusion tests. If two different types of teichoic acid were present in the walls of ATCC 521, one would expect to observe two lines in a gel-diffusion test when challenged with homologous serum, but this was not seen. A phenomenon similar to this was observed by Torii, Kabat & Bezer (1964): they demonstrated that *S. aureus* walls contained two types of ribitol teichoic acid, one with α -*N*-acetyl-glucosaminyl and the second with β -*N*-acetyl-glucosaminyl linkages. They were able to separate these two polymers and to demonstrate that they were different immunologically. Attempts to demonstrate two lines in a gel-diffusion system with homologous antiserum resulted in failure.

Although the wall and intracellular teichoic acid appear to be immunologically identical, it is doubtful whether the latter plays a role in the agglutination reaction. The fact that the agglutinating antibody can be removed from antisera with cell walls substantiates the conclusion that the intracellular teichoic acid plays no part in bacterial agglutination. Furthermore, agglutination of intact cells via intracellular teichoic acid would be unlikely because this involves passage of antibody across the cell wall.

The nature of the I₁₂ antigen detected in *Lactobacillus jugurti* ATCC 521 could not be determined. An examination of Table 2 reveals that isolated cell walls of ATCC 521 did not agglutinate in I₁₂ antiserum. There is a distinct possibility that the antigen involved was destroyed during the isolation of the walls. Walls of strains of *L. acidophilus* investigated by Ikawa & Snell (1960) were found to contain glycerol phosphate, a component of teichoic acid. Dr O. Kandler (personal communication) found teichoic acid in the cell walls of strains of *L. acidophilus* investigated. Morse (1963) observed that dilute acid extracts of *Staphylococcus albus* contained wall teichoic acid. In the present study it was found that acid extracts of *L. acidophilus* ATCC 4356, which should contain wall teichoic acid, did not react with ATCC 521 antiserum. This preliminary experiment, along with the fact that ATCC 4356 (I₁₂) antiserum did not react with ATCC 521 teichoic acid, indicates that immunologically dissimilar acids are found in the cell walls of these two species. It also demonstrates that ATCC 521 wall teichoic acid is probably not the I₁₂ antigen.

Experiments revealed that wall teichoic acid did not remove all of the agglutinating antibody from homologous antiserum. This might indicate either that another component might be responsible for a portion of bacterial agglutination or that the teichoic acid or mucopeptide was altered to such an extent during the isolation process that its antigenicity was impaired.

Some mention should be made of the serological cross reactions between strains of *Lactobacillus helveticus*, *L. jugurti* and *L. bulgaricus*. Tables 1 and 2 indicate a close immunological relationship between the species investigated. *L. jugurti* does not seem to be any closer to *L. helveticus* antigenically than to *L. bulgaricus*. The close serological relationships between *L. helveticus* and *L. jugurti* correlates with the similarity in their physiological characterization established by Orla-Jensen (1919). These organisms are so similar physiologically that it is hardly justified to recognize two species (Hansen, 1965). The serological studies performed demonstrate that *L. bulgaricus* is quite close to *L. helveticus* and *L. jugurti* antigenically, but it is well known that the former is quite distinct physiologically from the latter two (Hansen, 1965).

It has been known for some time that serological relationships exist between some lactobacilli which are physiologically dissimilar. Miller (1957) observed that various strains of *L. lactis* contained I1, I2 and I3 and other agglutinating antigens formerly detected only in *L. casei* and *L. plantarum*. Efthymiou & Hansen (1962) found agglutinating antigens previously observed in *L. casei*, *L. plantarum* and *L. lactis* present in strains of *L. acidophilus*. Mageau (1966) found agglutinating antigens previously observed in *L. casei*, *L. plantarum*, *L. lactis* and *L. acidophilus* present in *L. jugurti* and *L. bulgaricus*. To complicate the situation further Chung & Hawirko (1962) found that *L. casei* and *L. lactis* shared agglutinating antigens with *Streptococcus lactis* and *Streptococcus cremoris*. These facts substantiate the observation made in this study that the sharing of agglutinating antigens among different species of lactobacilli observed in this study is not an unusual phenomenon.

One important aspect of the studies conducted is the fact that the agglutinating antigens can be detected via the precipitin test with pure wall teichoic acid. This should prove useful for the identification of species of the genus *Lactobacillus* and should be of value in the elucidation of various taxonomic difficulties.

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