Serological Characters of the *Pediococci*

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SUMMARY

Rabbit antisera were prepared against 12 *pediococcus* isolates and tested by precipitin, precipitin-absorption and agglutination tests. Common precipitins for the three species *Pediococcus cerevisiae*, *P. parvulus* n.sp. and *P. damnosus* were demonstrated. An antiserum prepared against *Aerococcus viridans* (NCTC 7599) reacted with extracts of the homologous strain and 7 out of 10 heterologous *Aerococcus viridans*.

INTRODUCTION

Renewed interest in the genus *Pediococcus* has led in recent years to extensive studies of the cultural and physiological characters of its members (Pederson, 1949; Felton & Niven, 1953; Pederson, Albury & Breed, 1954; Jensen & Seeley, 1954) but, so far, no study has been reported of the serological properties of the organisms. In view of the successful application of precipitin and agglutination tests in the characterization of other lactic acid bacteria it was decided to explore the possibility of using serological methods in the classification of *pediococci*. Some preliminary results of this investigation are reported in this paper.

METHODS

The cultures and methods of cultivation are described in our previous paper (Günther & White, 1961).

Organisms. Twelve strains representative of the physiological groups described in the same previous publication were selected for antisera production.

Pediococcus cerevisiae (group I) was represented by strains 8081, F-166, 2-170, N-91, A-1, FP-1; *P. parvulus* (group II) by strains M-31, A-181, S-190, SS-69; *P. damnosus* (possible group IV) by strain M-1, and *P. halophilus* (possible group V) by strain Tc.1.

We intended to prepare an antiserum for strain L-148, which belongs to the unnamed group (group III), but this proved unsatisfactory and no further attempts have as yet been made to prepare antisera against strains belonging to this group.

One antiserum against *Aerococcus viridans* H-9 (NCTC 7599) was prepared.

Preparation of antisera. Intravenous injections of (a) whole living organisms, or (b) disintegrated suspensions, were given to rabbits which had been tested previously for the absence of naturally occurring antibodies. For method (a) 40 ml. glucose Lemco (GL) broth (Shattock & Hirsch, 1947) were inoculated with 0.5 ml. of a 24 hr. 'Oxoid' tomato juice (TJ) broth culture and incubated for 24 hr. at 30°. The culture was centrifuged, washed twice in 0.9 % (w/v) sodium chloride solution

resuspended in 0.9% sodium chloride solution to give an opacity equivalent to Brown's tube No. 7 and then stored at 4° for use in one course of animal injections. In method (b) fresh suspensions of organisms were prepared for each injection. Organisms from 20 ml. of a culture in GL broth were washed and adjusted to the same optical density as in method (a). They were then disrupted for 30 min. at room temperature in a tissue disintegrator (Mickle, 1948) in a vessel containing 5 g. sterile Ballotini beads grade No. 12. After centrifugation the supernatant fluid was found to be sterile and was used for animal injection.

When the two methods of antibody production were compared it was noted that the disintegration of the organism before inoculation did not result in a speedier production of antibodies, nor was the potency of the resulting antiserum increased. Suspensions of whole living organisms were therefore used for the routine preparation of antisera; the results recorded below refer in all instances to sera prepared by this method.

The experimental animals received 1 ml. volumes of suspensions of organisms prepared as above at 3-4 days intervals. One course of injections consisted of five such inoculations. When a test bleeding at this stage indicated that the antibody produced was of insufficient potency, a second course was given. Occasionally, a third course had to be administered before satisfactory antibody was obtained. The animals were bled 3 or 4 days after the last injection. Sera were stored at 4° without preservative.

The presence of non-specific antibodies was excluded by precipitin tests with each serum against extracts of streptococci (representative of Lancefield's groups A to N) and against extracts of 7 staphylococcus and 3 leuconostoc strains.

Preparation of extracts. (a) *Crude extracts:* Lancefield's (1933) HCl extraction method was used as modified by Sharpe (1955). The cultures were incubated for 48 hr. at 30° in tomato juice broth. The reactions of at least two extracts, prepared at different times, were tested to ensure reproducibility of results. (b) *Ethanol precipitation.* The difficulty in reading the results with some of the sera and crude extracts prepared as above made it desirable to concentrate the antigenically active material in the extracts by ethanol precipitation as suggested by Shattock (1949) for group D streptococci. A slight increase in activity in the precipitate was noted with most of the extracts, but in a few this treatment resulted in the complete loss of activity in the precipitate as well as in the supernatant fluid. Ethanol precipitation was not used, therefore, for the routine preparation of extracts.

Precipitin tests. Ring tests were carried out as described by Sharpe (1955). Observations were continued for 30 min., or up to 60 min. when a loss of potency in the antiserum was noted after prolonged storage. Results obtained with antisera which had been stored for a long time were always regarded as unreliable but were useful in preliminary work.

Precipitin-absorption tests. The organisms were cultivated in tomato juice broth for 24 hr. at 30°. The technique outlined by Sharpe (1955) was followed.

Agglutination tests. Tube agglutination, employing suspensions of organisms from 16 hr. tomato juice broth cultures in 0.9% (w/v) sodium chloride solution, was used. The tubes were read after overnight incubation at 50°.

RESULTS

The selected *pediococcus* isolates differed in their capacity to induce antibody response in the rabbit and the choice of suitable isolates for antiserum production was of great importance. The difficulty experienced in obtaining active extracts with some isolates merits further investigation. In the future, modifications may

Table 1. *Precipitin tests with antisera of pediococci and Aerococcus viridans*

	Antisera												
	<i>P. cerevisiae</i>						<i>P. parvulus</i>				<i>P. dam-</i> <i>nosus</i>	<i>P. halo-</i> <i>philus</i>	<i>A. viri-</i> <i>dans</i>
	F-166	2-170	8081	N-91	A-1	FP-1	M-31	A-181	S-190	SS-69	M-1	Tc.1	H-9
<i>P. cerevisiae</i> (group I)													
F-166	++	+	+	+	+	+	+	+	+	+	±	—	—
2-170	+	++	+	±	±	+	+	+	+	±	—	—	—
8081	+	+	++	+	+	+	+	+	+	+	+	—	—
N-91	+	+	+	++	+	+	+	±	—	+	—	—	—
A-1	+	+	+	—	++	+	—	—	—	—	—	—	—
FP-1	+	+	+	—	—	++	+	—	—	+	—	—	—
Others	$+\frac{30}{31}$	$+\frac{29}{31}$	$+\frac{27}{31}$	$+\frac{24}{30}$	$+\frac{26}{30}$	$+\frac{29}{31}$	$+\frac{24}{30}$	$+\frac{14}{16}$	$+\frac{18}{30}$	$+\frac{28}{30}$	$+\frac{21}{28}$	$-\frac{28}{28}$	$-\frac{30}{30}$
<i>P. parvulus</i> (group II)													
M-31	+	+	+	+	.	+	++	+	—	+	+	—	—
A-181	+	+	±	—	+	±	+	++	+	+	+	—	—
S-190	+	+	+	+	+	+	+	.	++	—	—	—	—
SS-69	+	+	+	+	±	±	+	.	—	++	+	—	—
Others	$+\frac{20}{24}$	$+\frac{21}{24}$	$-\frac{17}{24}$	$-\frac{17}{24}$	$-\frac{14}{18}$	$+\frac{10}{24}$	$+\frac{22}{24}$	$-\frac{9}{13}$	$-\frac{18}{23}$	$+\frac{18}{24}$	$+\frac{18}{24}$	$-\frac{24}{24}$	$-\frac{24}{24}$
<i>P. damnosus</i> (? group IV)													
M-1	+	±	±	—	—	—	+	.	—	+	++	—	—
Others	$+\frac{2}{2}$	$+\frac{2}{2}$	$+\frac{1}{2}$	$+\frac{1}{2}$	.	$-\frac{2}{2}$	$+\frac{2}{2}$	.	$-\frac{2}{2}$	$+\frac{1}{2}$	.	$-\frac{2}{2}$	$-\frac{2}{2}$
<i>P. halophilus</i> Tc.1 (? group V)	—	—	—	—	.	—	—	.	—	—	—	++	±
<i>A. viridans</i> H-9	—	—	—	—	—	—	—	—	—	—	—	—	++
Others	$-\frac{8}{10}$	$-\frac{10}{10}$	$-\frac{9}{10}$	$-\frac{10}{10}$	$-\frac{3}{3}$	$-\frac{9}{9}$	$-\frac{9}{9}$	$-\frac{1}{1}$	$-\frac{8}{10}$	$-\frac{9}{10}$	.	$-\frac{10}{10}$	$+\frac{7}{10}$
Leuconostocs	$-\frac{3}{3}$	$-\frac{2}{3}$	$-\frac{3}{3}$	.	$-\frac{3}{3}$	$-\frac{3}{3}$	.	$-\frac{3}{3}$	.	.	.	.	.
Streptococci	$-\frac{24}{24}$	$-\frac{24}{24}$	$-\frac{24}{24}$	$-\frac{12}{24}$	$-\frac{12}{24}$	$-\frac{24}{24}$	$-\frac{12}{24}$	$-\frac{24}{24}$	$-\frac{12}{24}$	$-\frac{12}{24}$	$-\frac{12}{24}$	$-\frac{12}{24}$	$-\frac{24}{24}$
Staphylococci													
<i>Staph. aureus</i> 7447	—	—	—	—	.	—	—	.	—	—	—	—	+
<i>Staph. albus</i> 7292	—	—	—	—	.	—	—	.	—	—	—	+	+
Others	$-\frac{5}{5}$	$-\frac{5}{5}$	$-\frac{5}{5}$	$-\frac{5}{5}$	.	$-\frac{5}{5}$	$-\frac{5}{5}$	.	$-\frac{5}{5}$	$-\frac{5}{5}$	$-\frac{4}{5}$	$-\frac{3}{5}$	$-\frac{4}{5}$

++ = strong precipitate, + = precipitate, ± = weak reaction, — = no reaction, . = not tested.

The figures are $\frac{\text{no. of strains giving reaction}}{\text{no. of strains tested}}$.

have to be made in the method of preparation in order to obtain satisfactory extracts of consistently high activity.

Precipitin tests. These results are summarized in Table 1. Positive reactions were obtained with extracts of the homologous and with the majority of heterologous strains of the same species. Antisera against *Pediococcus cerevisiae* strains F-166, 2-170, and FP-1 also precipitated consistently with extracts of *P. parvulus*. Antisera against *P. cerevisiae* strains 8081, N-91, and A-1 were less consistent in their reactions with extracts of *P. parvulus*, but they too gave positive reactions with a large number of extracts of that species.

Antisera against *Pediococcus parvulus* and *P. damnosus* reacted with the homologous and with the majority of the heterologous extracts of the same species as well as with extracts of *P. cerevisiae*. Cross-reactions occurred also between *P. parvulus* and *P. damnosus*.

Reactions could not be observed between sera against *Pediococcus cerevisiae*, *P. parvulus* or *P. damnosus* on the one hand, and between 'group III' isolates or *P. halophilus* on the other. The serum against *P. halophilus* was not precipitated by extracts of any of the other pediococci.

Table 2. *Absorption of antisera of* *Pediococcus cerevisiae* *strains F-166 and 2-170 with cells of homologous and heterologous* *P. cerevisiae* *strains*

Antigen (extracts)	Antiserum									
	F-166					2-170				
	Unab- sorbed	Absorbed with strains			Unab- sorbed	Absorbed with strains				
		F-166	D-32	PUE		2-170	F-166	8081	FP-1	A-1
<i>P. cerevisiae</i> (group I)										
F-166	++	—	—	+	+	—	—	—	—	—
2-170	+	—	.	—	++	—	—	.	.	.
PUE	+	—	.	—	+	.	.	.	.	.
Others	+	—	—	—	+	—	—	—	—	—
<i>P. parvulus</i> (group II)	+	—	—	—	+	.	.	—	—	—

Symbols as Table 1.

Six of the pediococcus antisera gave positive reactions with one or the other aerococcus extract, but in these instances ring formation was markedly delayed and considerably less intense than that observed with the pediococci.

The *Aerococcus viridans* H-9 serum reacted with extracts of the homologous and with 7 of the 10 other strains of *A. viridans* tested. A weak cross-reaction was obtained also with extracts of *Pediococcus halophilus* and with strain L-171 of the unclassified group III.

Cross-reactions were not found between the pediococcus antisera used and the extracts of streptococci, staphylococci and leuconostoc strains, with the exception of one reaction between a strain of *Staphylococcus albus* and *Pediococcus halophilus* antiserum.

Precipitin-absorption tests. Four antisera were selected for absorption tests. The results are shown in Tables 2, 3, 4. These results indicate that certain antisera contained more than one antibody, one of which was absorbed by heterologous

Table 3. *Absorption of antiserum Pediococcus cerevisiae strain F-166 with cells of P. parvulus and strain L-148 (group III)*

Antigen	Antiserum F-166 absorbed with strains <i>P. parvulus</i>					
	Un-absorbed	A-181	S-190	SS-69	S-182	L-148
<i>P. cerevisiae</i> (group I)						
F-166	++	+	—	+	—	+
2-170	+	—	.	—	.	+
PUE	+	.	—	.	.	.
Others	+	—	—	—	—	.
<i>P. parvulus</i> (group II)	+	—	—	—	—	+

Symbols as Table 1.

Table 4. *Absorption of antisera Pediococcus parvulus strains S-190 and SS-69 with cells of P. cerevisiae and P. parvulus*

Antigen	Antiserum							
	S-190				SS-69			
	Unab-sorbed	Absorbed with <i>P. parvulus</i>		<i>P. cerevisiae</i> F-166	Unab-sorbed	Absorbed with <i>P. cerevisiae</i>		
		S-190	SS-69			<i>P. parvulus</i> SS-69	F-166	PUE
<i>P. parvulus</i> (group II)								
S-190	+	—	+	+	+	—	—	—
SS-69	+	.	.	.	+	—	+	+
Others	+	—	—	—	+	—	—	—
<i>P. cerevisiae</i> (group I)	+	—	—	—	+	—	—	—

Symbols as Table 1.

Table 5. *Agglutination tests with pediococcus and aerococcus antisera*

Antigen	Antisera							
	<i>P. cerevisiae</i>					<i>P. parvulus</i>		<i>A. viridans</i> H-9
	F-166	2-170	8081	N-91	FP-1	M-31	SS-69	
<i>P. cerevisiae</i> (group I)								
F-166	1280 +	—	—	—	—	—	—	—
2-170	80	1280 +	—	40	80	—	—	—
8081	20	40	1280 +	40	40	40	40	20
N-91	—	—	—	640	—	—	—	—
FP-1	40	80	—	40	1280 +	—	160	—
<i>P. parvulus</i> (group II)								
M-31	—	—	—	—	—	1280 +	—	—
SS-69	320	40	40	40	160	80	1280	40
<i>A. viridans</i> H-9	—	—	—	—	—	—	320	1280 +

Figures are the reciprocals of the highest titres read. — = > 20.

strains and the other absorbed only by the homologous strain. Antibodies in *Pediococcus cerevisiae* antiserum were absorbed by *P. parvulus* strains and vice versa, indicating that these two species share at least one common antigen. Absorption with strain L-148 organisms, with strains of *Aerococcus viridans*, streptococci and staphylococci did not result in loss of antibodies from the antisera of *P. cerevisiae* or *P. parvulus*.

Agglutination tests. The results are shown in Table 5. With homologous strains titres of more than 1/1280 were obtained generally but with heterologous strains, when any reaction at all was obtained, the titres seldom exceeded 1/80. A number of cross-reactions occurred between antisera of *Pediococcus cerevisiae* and *P. parvulus*. In one instance cross-reactions occurred between *P. parvulus* strain SS-69 and *Aerococcus viridans* strain H-9.

DISCUSSION

The present investigation indicates that serological techniques may be applied successfully to the separation and classification of the pediococci. Antisera prepared against pediococci did not react with extracts of organisms from the closely related genera *Streptococcus* and *Leuconostoc* and generally not with extracts from aerococci. It becomes evident that pediococci possess precipitins which are common to members of more than one species. Such antigens were demonstrated in *Pediococcus cerevisiae*, *P. parvulus* and *P. damnosus*, but not in *P. halophilus* and in members of the as yet unclassified subgroup (III). As these antigens were shared by more than one species they may be referred to as 'group' antigens by analogy to the group antigens of streptococci.

Some pediococcus strains showed an additional antigen which appeared to be more strain specific and may therefore represent a 'type' antigen. The presence of more than one antigen was also demonstrated in agglutination tests. These tests revealed relationships apparently much narrower than were demonstrated in precipitin tests. It is probable, then, that pediococci possess a 'group' antigen demonstrable by precipitin tests and a 'type' antigen which can be shown by precipitin and agglutination tests, as with streptococci and lactobacilli. No study has as yet been made to determine whether the 'type' antigens demonstrable in the two tests were identical. The possibility that the organisms possess more than one 'type' antigen cannot be excluded.

Some indication that the 'group' antigen may be protein in nature was obtained from ethanol precipitation of some of the extracts since the antigenically active material had been found in the precipitate. However, the antigen might be carbohydrate carried down with the protein.

The serological position of *Pediococcus halophilus* and of the unnamed group III could not be established satisfactorily. Absorption of *P. cerevisiae* antiserum with one strain of group III did not remove the 'group' antigen from the serum. This indicates that members of group III are not related antigenically to *P. cerevisiae* (group I) and *P. parvulus* (group II). They may possibly represent a separate serological group.

The reaction of strain PUE is of interest. Pederson *et al.* (1954) excluded it from the pediococci but the strain was classified by us (Günther & White, 1961) as *Pediococcus cerevisiae*. This organism gave strong precipitin reactions with antisera

of *P. cerevisiae*, *P. parvulus* and *P. damnosus*. When PUE was used for absorption of *P. cerevisiae* antiserum the 'group' antigen was absorbed. This is further evidence that the correct classification of this strain is with *P. cerevisiae*.

The serological work also confirms the classification of strain A-2 isolated by Dacre and classified by him tentatively as *P. cerevisiae* (Dacre, 1958) and of the old '*Leuconostoc mesenteroides* P-60' reclassified by Garvie (1959) as *P. cerevisiae*.

The cross-reactions between the aerococci and pediococci might have been due to non-specific antibodies, but more experimental evidence is required. The work is as yet incomplete but it is being continued and we hope to clarify at least some of the issues raised in this and the preceding paper.

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