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Effect of Variations in Conditions of Incubation upon Inhibition of *Staphylococcus aureus* by *Pediococcus cerevisiae* and *Streptococcus lactis*[1]

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The effects of pH, temperature, proportion of *Staphylococcus aureus* in the inoculum, various strains of effector organism, and various strains of *S. aureus* were examined for their influence on interactions between staphylococci and effector organisms in associative culture. In general, small changes in pH had little effect upon either growth of *S. aureus* or production of enterotoxin in associative culture. Inhibition of growth of *S. aureus* caused by effector organisms was much greater at 25 than at 30 C. Proportion of *S. aureus* in the inoculum greatly affected both growth of the staphylococci and production of enterotoxin. Only slight differences were found between strains of either effector organism or *S. aureus* which affected the interactions in associative culture.

Previous work in this laboratory has shown that several species of lactic acid bacteria, particularly *Streptococcus lactis* and *Pediococcus cerevisiae*, were inhibitory to growth and enterotoxin production by *Staphylococcus aureus* when grown in association with *S. aureus* at temperatures favorable to the organisms concerned.

Several reports (2-8) have indicated that certain conditions of incubation may significantly affect the influence of the competing organisms upon growth of *S. aureus*, but none report any effect on enterotoxin production. The investigation reported herein was conducted to determine the effect of variations in some conditions upon the ability of *S. lactis* and *P. cerevisiae* to inhibit growth and production of enterotoxin by *S. aureus*.

MATERIALS AND METHODS

Cultures. *S. lactis* strains A62, A64, A254, and G18 and *P. cerevisiae* strains FBB39 and FBB63 used as effector organisms were from the stock cultures in the Food Microbiology Laboratory at Michigan State University. *S. lactis* W was obtained from E. H. Marth of the University of Wisconsin. *P. cerevisiae* 10791 was obtained from the American Type Culture Collection, Rockville, Md. *P. cerevisiae* "Accel" is a commercial starter culture from Merck and Co., Rahway, N. J. *S. aureus* strains 265, 243, 137, and 361 were obtained from the late E. P. Casman of the Food and Drug Administration, Washington, D.C.

Procedure. One-liter Erlenmeyer flasks containing 300 ml of all-purpose medium with Tween broth (Difco) were inoculated and incubated 48 hr in a thermostatically controlled gyratory shaker-incubator operated at 175 rev/min. The effect of the initial pH of 6.0, 6.5, and 7.0 on the growth of the cultures of *S. aureus* and the effector organisms was determined by adjusting the pH to these values with 1.0 N HCl or 1.0 N NaOH. The effect of incubation at 25 and 30 C was investigated. In trials involving the influence of temperature, pH, and species of effector organism, the broth in each flask was inoculated with 10^5 cells of *S. aureus* per ml and 10^5 cells of the effector organism per ml. To determine the importance of the relative proportion of *S. aureus* in the inoculum, the numbers of the effector organism in the inoculum were varied, giving initial percentages of *S. aureus* of approximately 10, 50, and 90%. This was accomplished by inoculating the broth in each flask with 10^5 cells of *S. aureus* per ml and then adding 10^4, 10^5, and 10^6 cells of effector organisms per ml to appropriate flasks. Several strains each of *S. lactis*, *P. cerevisiae*, and *S. aureus* were used to determine the extent of variations between strains of the organisms with regard to interactions in associative culture.

Enumeration of S. Aureus. Staphylococcal populations were determined on prepoured mannitol salt agar spread plates incubated 48 hr at 37 C.

Assay for enterotoxin. The microslide double-gel diffusion procedure of Casman and Bennett (1) was used for enterotoxin assays. The assays were performed on samples taken at 3- to 4-hr intervals

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during the first 24 hr of incubation. Reference enterotoxins A, C, and D, and corresponding antisera were obtained from the Food and Drug Administration, Washington, D.C. Reference enterotoxin B and anti-B were obtained from Makor Chemicals Ltd., Jerusalem, Israel.

RESULTS AND DISCUSSION

This investigation involved incubation of cultures over a period of 48 hr and examination of samples taken at intervals of 3 or 4 hr. To conserve space, only maximum populations of

TABLE 1. *Effect of initial pH upon growth and production of enterotoxin by S. aureus 243 grown alone and in association with S. lactis A64 and P. cerevisiae 10791 in APT broth at 30 C*[a]

Effector organism	Approximate initial pH	Maximum population of *S. aureus* observed (cells/ml)	Minimum pH observed	Time required for production of measurable toxin (hr)	Enterotoxin (μg/ml)
None	6.0	7.3×10^9	5.29	18	8
S. lactis	6.0	1.7×10^6	4.21		ND[b]
P. cerevisiae	6.0	4.0×10^7	4.24		ND
None	6.5	1.1×10^{10}	5.85	14	16
S. lactis	6.5	3.7×10^6	4.47		ND
P. cerevisiae	6.5	1.7×10^8	4.34	24	1
None	7.0	1.3×10^{10}	6.42	12	32
S. lactis	7.0	6.6×10^6	4.56		ND
P. cerevisiae	7.0	7.3×10^8	4.55	24	1

[a] APT broth is all-purpose medium with Tween (Difco).
[b] None detected in direct assay or in sample concentrated 10-fold by lyophilization and rehydration.

TABLE 2. *Comparison of growth and production of enterotoxin by S. aureus 243 at 25 C and 30 C when grown alone and in association with S. lactis A64 and P. cerevisiae 10791 in APT broth at pH 6.5*

Effector organism	Incubation temperature	Maximum population of *S. aureus* observed (cells/ml)	Minimum pH observed	Time required for production of measurable toxin (hr)	Enterotoxin (μg/ml)
None	25	1.1×10^{10}	5.33	18	8
S. lactis	25	4.1×10^5	4.31		ND[a]
P. cerevisiae	25	6.0×10^5	4.32		ND
None	30	1.1×10^{10}	5.85	14	16
S. lactis	30	3.7×10^6	4.47		ND
P. cerevisiae	30	1.7×10^8	4.34	24	1

[a] None detected either in direct assay or in sample concentrated 10-fold by lyophilization and rehydration.

TABLE 3. *Effect of ratio of staphylococci to effector organism upon growth and production of enterotoxin by S. aureus 243 grown in association with S. lactis A64 and P. cerevisiae 10791 in APT broth at 30 C and pH 6.5*

Effector organism	% Effector in inoculum	Maximum population of *S. aureus* observed (cells/ml)	Minimum pH observed	Time required for production of measurable toxin (hr)	Enterotoxin (μg/ml)
None	0	1.1×10^{10}	5.85	14	16
S. lactis	10	1.0×10^{10}	5.19	24	8
S. lactis	50	3.7×10^6	4.47		ND[a]
S. lactis	90	2.3×10^5	4.51		ND
P. cerevisiae	10	4.7×10^8	4.50	24	2
P. cerevisiae	50	1.7×10^8	4.44	24	1
P. cerevisiae	0	1.6×10^7	4.34		ND

[a] None detected either in direct assay or in sample concentrated 10-fold by lyophilization and rehydration.

S. aureus and minimum pH are included. These data do not represent terminal populations or pH.

Table 1 illustrates the effects of pH 6.0, 6.5, and 7.0 upon growth of *S. aureus* and production of enterotoxin in association with *S. lactis* and *P. cerevisiae*. At pH values of 6.0, 6.5, and 7.0, there is a trend toward greater inhibition of growth of *S. aureus* strain 243 as the pH decreases, and the decrease in enterotoxin production associated with the decrease in pH is particularly evident in the absence of the effector organisms. Growth of *S. aureus* in the presence of effector organisms was inhibited to a much greater degree at 25 C than at 30 C (Table 2). Also there was more inhibition of toxin production at 25 C.

TABLE 4. *Comparison of the effects of several strains of S. lactis and P. cerevisiae upon growth and production of enterotoxin by S. aureus 243 when grown in association in APT broth at 30 C and pH 6.5*

Effector organism	Maximum *S. aureus* population observed (cells/ml)	Minimum pH observed	Time required for production of measurable toxin (hr)	Enterotoxin (μg/ml)
S. lactis A62	2.9×10^6	4.38		ND[a]
S. lactis A64	3.7×10^6	4.47		ND
S. lactis A254	5.7×10^6	4.85		ND
S. lactis G18	3.8×10^6	4.23		ND
S. lactis W	4.1×10^6	4.39		ND
P. cerevisiae 10791	1.7×10^8	4.34	24	1
P. cerevisiae FBB63	8.1×10^7	4.43	24	1
P. cerevisiae FBB39	3.2×10^7	4.30		ND
P. cerevisiae "Accel"	9.2×10^7	4.24	24	1
None	1.1×10^{10}	5.85	18	16

[a] None detected in direct assay or in sample concentrated 10-fold by lyophilization and rehydration.

Troller and Frazier (8) reported that maximum inhibition of staphylococci by food bacteria occurred in the pH range of 7.4 to 6.2. They also indicated that maximum inhibition of growth of *S. aureus* in association with other organisms occurred at temperatures of 20 to 25 C. Peterson et al. (7) reported similar findings regarding the inhibition of *S. aureus* by psychrophilic saprophytes. Other previous reports (3, 4) also suggest that growth of *S. aureus* is generally inhibited to a greater degree at temperatures lower than 30 C when in association with other organisms.

Table 3 includes data illustrating the importance of the proportion of *S. aureus* in the inoculum upon growth and production of enterotoxin. Growth of *S. aureus* and production of enterotoxin were most inhibited when the proportion of *S. lactis* or *P. cerevisiae* were greatest. The data indicate that the ratio of inoculum was more important when *S. lactis* was used as the effector organism than when *P. cerevisiae* was used. Similar findings regarding the influence of the proportion of staphylococci and effector organisms have been reported by

TABLE 5. *Comparison of susceptibility of four enterotoxigenic strains of S. aureus to antagonism by S. lactis A64 and P. cerevisiae 10791 when grown in association in APT broth at 30 C and pH 6.5*

Strain of staphylococcus	Enterotoxin serotype	Effector organism	Maximum *S. aureus* population observed (cells/ml)	Minimum pH observed	Time required for production of measurable toxin (hr)	Enterotoxin (μg/ml)
265-1	A	None	1.5×10^{10}	5.34	12	2
265-1	A	*S. lactis*	3.1×10^6	4.31		ND[a]
265-1	A	*P. cerevisiae*	5.2×10^8	4.55	24	0.1[b]
243	B	None	1.1×10^{10}	5.85	18	16
243	B	*S. lactis*	3.7×10^6	4.47		ND
243	B	*P. cerevisiae*	1.7×10^8	4.34	24	1
137	C	None	7.2×10^9	5.64	18	8
137	C	*S. lactis*	1.7×10^6	4.42		ND
137	C	*P. cerevisiae*	1.6×10^8	4.53	24	0.4[b]
361	D	None	8.8×10^9	5.84	18	1
361	D	*S. lactis*	3.0×10^5	4.39		ND
361	D	*P. cerevisiae*	3.1×10^8	4.56		ND

[a] None detected in direct assay or in sample concentrated 10-fold by lyophilization and rehydration.
[b] Samples concentrated 10-fold by lyophilization and rehydration.

other investigators (2, 3, 6, 8).

Table 4 indicates that there is little difference among strains of either *S. lactis* or *P. cerevisiae* as inhibitors of *S. aureus* when grown in associative culture, but the *S. lactis* species was more inhibitory than the *P. cerevisiae* species. Similarly, all four strains of *S. aureus* were approximately equal in sensitivity to inhibition by the effector organisms (Table 5) as evidenced by the fact that there was approximately a 4-log reduction in maximum population of all strains of *S. aureus* when grown in association with *S. lactis* and a 1- to 2-log reduction when grown in association with *P. cerevisiae*.

Obviously inhibition of staphylococci is enhanced by selecting conditions of incubation which are not conducive to staphylococcal growth, and inhibition of *S. aureus* in mixed culture is greatly enhanced when the proportion of staphylococci in the population is small. It would generally be expected that the lactic acid culture organisms in a cultured food product would greatly outnumber any staphylococci present.

Since only slight differences were observed between strains of the organisms studied, strain differences which might influence interaction in associative culture probably are uncommon. Kao and Frazier (3) reported data which also indicated that strain variations are not great regarding either the ability of a species of lactic acid bacterium to inhibit *S. aureus* or the susceptibility of *S. aureus* to inhibition. McCoy and Faber (4) found 15 strains of *S. aureus* approximately equal in sensitivity to inhibition by various food microorganisms. It is likely, then, that data obtained by use of selected strains to determine interactions of lactic acid organisms and *S. aureus* in associative culture are generally representative.

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