COMPETITION OF *STAPHYLOCOCCUS AUREUS* WITH OTHER ORGANISMS

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Sixty-six cultures of food microorganisms were screened by spot-plate tests on their ability to influence the growth of four strains of *Staphylococcus aureus*, two enterotoxigenic and two not. The six test media were selected for differences in complexity and to simulate natural foods. The most consistently inhibitory cultures for *S. aureus* were: *Streptococcus faecium*, *Streptococcus faecalis*, *S. faecalis* var. *liquefaciens*, a nisin-producing *Streptococcus lactis*, and various meat lactobacilli. Other cultures were less consistently inhibitory, and many were not inhibitory or were even stimulatory.

Growth of two enterotoxigenic strains of *S. aureus* in meat infusion broth at 15°, 30° and 44°C. was only moderately reduced by simultaneous growth of *Escherichia coli* strain Gratia, but was markedly reduced by growth of *E. coli* H52, especially at 15°C. and 44°C.

The frequency of outbreaks of staphylococcal food poisoning has stimulated interest in the factors that influence the growth of staphylococci in foods and the production of enterotoxin. One factor that has received comparatively little attention is the effect of simultaneous growth of other competing microorganisms.

Regnier and Lambin (7) reported antagonism of *Escherichia coli* toward *Staphylococcus aureus* in nutrient broth at 37°C., although the coccus attained 319 million cells per ml. in eight hours according to a direct microscopic count. Decreasing inocula of *E. coli* lessened the inhibition of the staphylococcus. Heinemann (5) found that three strains of S. *aureus* added to raw milk grew poorly at 80°F. or lower, and at 110°F., where the staphylococcus grew best, it still was quickly overgrown by the natural flora of the milk. Takahashi and Johns (8) observed a negative correlation between the initial standard plate count of milk and the extent of multiplication of staphylococci. Gibson and Abd-El-Malek (3) found that of all the organisms present in raw milk held between 10° and 20°C., the staphylococci showed the smallest increase in numbers. Mattick, Neave and Chapman (6) reported that in more acid cheeses made with more starter culture, added S. *aureus* died out more rapidly than in sweeter cheeses with less starter.

An attempt has been made to explore by rough screening tests the effects of a number of food microorganisms upon the growth of S. *aureus*. Also a study has been made of the effect of one of these organisms, the commonly occurring *Escherichia coli*, upon the growth of enterotoxigenic cultures of S. *aureus* at different temperatures. *E. coli* was selected because it caused no evident inhibition of S. *aureus* on spot plates and it therefore probably competed without production of appreciable amounts of antibiotic products.

Methods

Sixty-six cultures of important food microorganisms were utilized in agar plate screening tests for their effects on strains of S. *aureus* on different culture media. Four strains of S. *aureus* were employed, two

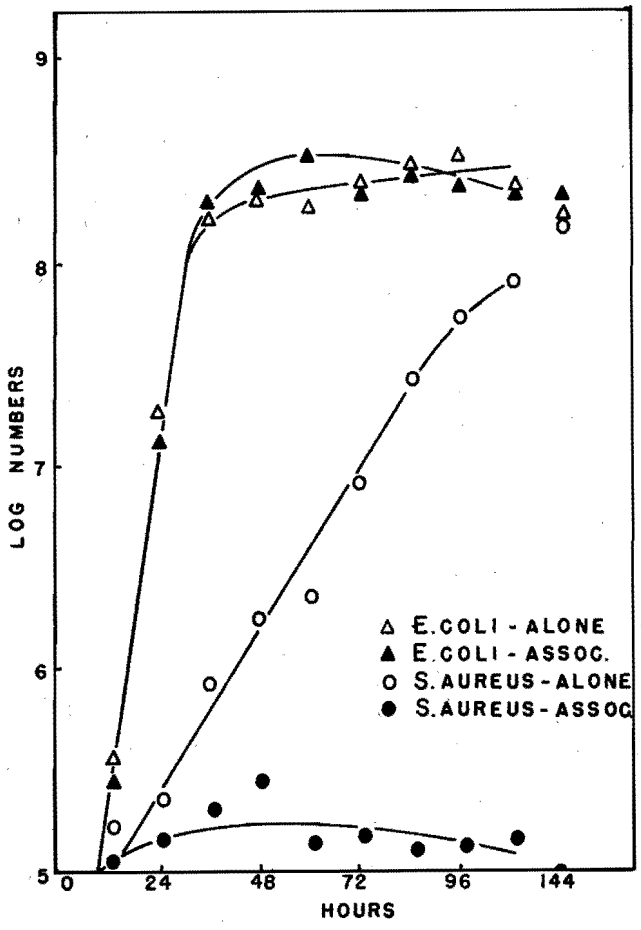

Figure 1. Growth and maximum numbers of *E. coli* H-52 and S. *aureus* 255 in pure culture and in association at 15°C. in meat infusion broth. Direct microscopic counts through 108 hours; plate counts at 144 hours.

enterotoxigenic ones (strains 255 and 261), and two which were not enterotoxigenic (strains W1 and 54B). For studies on competitive growth in broth cultures the two enterotoxigenic strains of *S. aureus* were grown with two strains of *E. coli*, a typically active strain, H-52, and an *Aerobacter*-like strain, Gratia.

The culture media used for screening tests were: (*a*) a vegetable medium, V-8 agar (*1*); (*b*) a meat medium, meat infusion agar (*1*); (*c*) a simple medium, nutrient agar; (*d*) dextrose-tryptone agar (*1*); (*e*) trypticase-soy agar; and (*f*) Evans' and Niven's APT agar (*4*). The medium for growth of *S. aureus* and *E. coli* alone and together was meat infusion broth, which supports good growth of each.

The method of screening was a modification of the "simultaneous antagonism" technique of Gratia (*4*). Agar for plates was seeded with an 18-hr. staphylococcus culture. The inoculum of staphylococci was such as to obtain a semi-confluent lawn of these cocci on the poured plate. After solidification of the agar two spots of an 18-hr. culture of each effector organism were pipetted onto the surface of each agar medium. Four organisms were used per duplicate plate and each experiment was performed thrice. All plates, except those containing high temperature lactic acid bacteria, which were grown at 37°C., were incubated 24 hr. at 30°C.

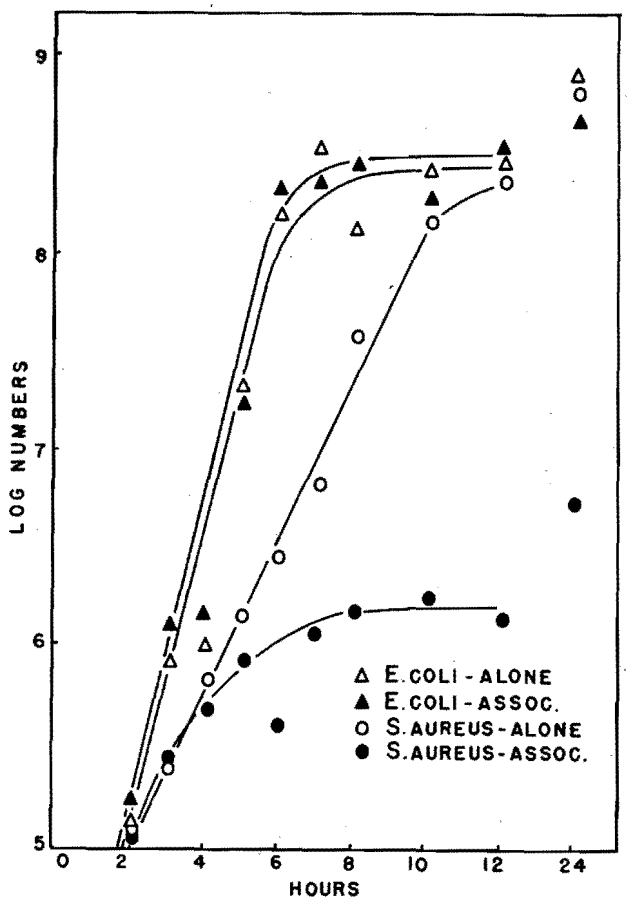

Figure 2. Growth and maximum numbers of *E. coli* H-52 and *S. aureus* 255 in pure culture and in association at 30°C. in meat infusion broth. Direct microscopic counts through 12 hours; plate counts at 24 hours.

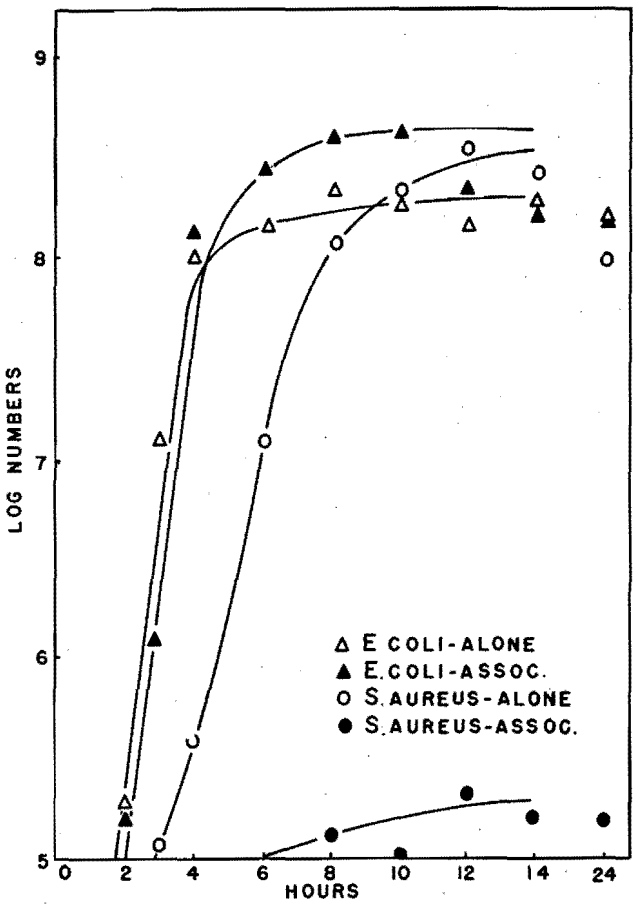

Figure 3. Growth and maximum numbers of *E. coli* H-52 and *S. aureus* 255 in pure culture and in association at 44°C in meat infusion broth. Direct microscopic counts through 14 hours; plate counts at 24 hours.

For the studies of competitive growth in broth, *S. aureus* strains 255 and 261 and *E. coli* strains Gratia and H-52 were first grown in broth at 30°C. for three successive days, the third transfer being incubated for only 18 hr. Numbers of bacteria in this culture were counted by the direct microscopic method and enough of the culture was used as an inoculum to give 100,000 cells per ml. in the inoculated test broth. The final volume in each inoculated broth tube, whether one or two organisms were added, was made up to 10 ml. Incubation temperatures for the experiment were 15, 30 and 44°C. Samples were taken at intervals for direct microscopic counts during the period of active growth and for a plate count at the termination of the incubation. For the plate counts mannitol salt agar was used for numbers of viable staphylococci

and violet red bile agar for *E. coli.* Previous tests had indicated that these media gave practically as high counts as less selective media.

RESULTS

Screening Tests

The results of screening tests with spot plates showed that most consistent in their inhibition of growth of the staphylococci on all of the media employed were *Streptococcus faecium* (4 strains), *Streptococcus faecalis, S. faecalis* var. *liquefaciens, Streptococcus lactis* strain X-13 (nisin-producing), and eight cultures of lactobacilli isolated from meat. Somewhat less consistent inhibitors were *Lactobacillus lactis* strain 39a, *Pseudomonas fluorescens* (2 strains) *Micrococcus freudenreichii* (2 strains) *Pediococcus cerevisiae* (2 strains) and three species of *Leuconostoc.* The streptococci and lactobacilli, especially on meat infusion and nutrient agars, were stimulatory outside the perimeter of the zone of inhibition, indicating that the products of the effector organisms were inhibitory in higher concentrations and stimulatory in lower concentrations.

It was observed, too, that the two enterotoxigenic strains of *S. aureus* were inhibited more often than the two non-enterotoxigenic strains, especially by the lactic acid bacteria, including *Leuconostoc* and *Pediococcus.*

Organisms with little or no apparent effect on the growth of *S. aureus* included: *Streptococcus cremoris, S. lactis, S. thermophilus, Lactobacillus bulgaricus, Pseudomonas aeruginosa, P. fragi, Pseudomonas* cultures from chicken and meat, *Micrococcus flavus, M. ureae, M. varians, Escherichia coli, Aerobacter aerogenes, Alcaligenes viscolactis, Proteus vulgaris, Salmonella gallinarum, Serratia marcescens, Brevibacterium linens, Bacillus subtilis, B. cereus, B. polymyxa, B. coagulans, Microbacterium lacticum* and six yeasts. Some of the above cultures at times were stimulatory to *S. aureus.*

The above results indicated that the effect of the effector organism on *S. aureus* depended upon: (*a*) the strain of *S. aureus* tested; (*b*) the strain or species of the effector organism employed; and (*c*) the culture medium on which the test was conducted.

Competitive Growth of S. aureus and E. coli

When *S. aureus* strain 255 and *E. coli* strain Gratia were grown together in broth at 15°, 30° and 44°C the coliform organism had comparatively little influence on the growth of the staphylococcus although maximum numbers of cocci were reduced about tenfold in the presence of *E. coli.*

However, when enterotoxin-producing *S. aureus* strains 255 and 261 were grown with the more typical *E. coli* strain H-52, the latter was definitely inhibitory to the staphylococci. When *S. aureus* strain 255 was grown with *E. coli* strain H-52 the staphylococcus was strongly suppressed when incubation was at 15°C. (Figure 1) or 44°C. (Figure 3), multiplying less than two generations and attaining numbers insufficient for the production of appreciable amounts of enterotoxin. At 30°C. (Figure 2) the staphylococcus was considerably suppressed but did attain about 2.3 million cells per ml. in 12 hours and about five million in 24 hours as compared to about 720,000,000 per ml. in the culture where *S. aureus* grew alone.

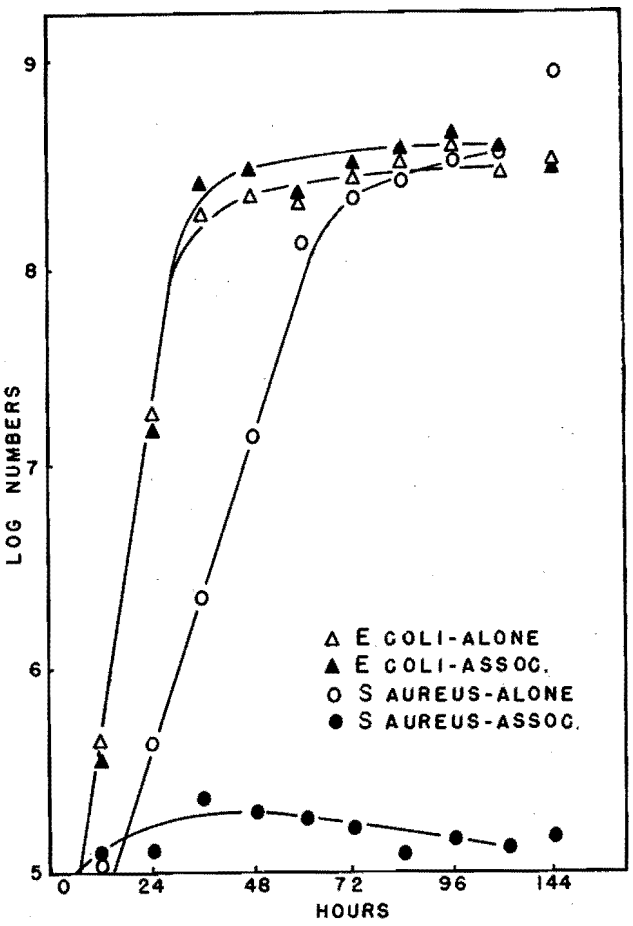

Figure 4. Growth and maximum numbers of *E. coli* H-52 and *S. aureus* 261 in pure culture and in association at 15°C. in meat infusion broth. Direct microscopic counts through 108 hours; plate counts at 144 hours.

Similar results were obtained when *S. aureus* strain 261 was grown with *E. coli* strain H-52. Again at 15°C. (Figure 4) and at 44°C. (Figure 6) *S. aureus* went through less than two generations during the duration of the experiment and the staphylococcus was considerably suppressed at 30°C. (Figure 5).

The results demonstrated that: (*a*) the staphylococci had little apparent effect on the growth of *E.*

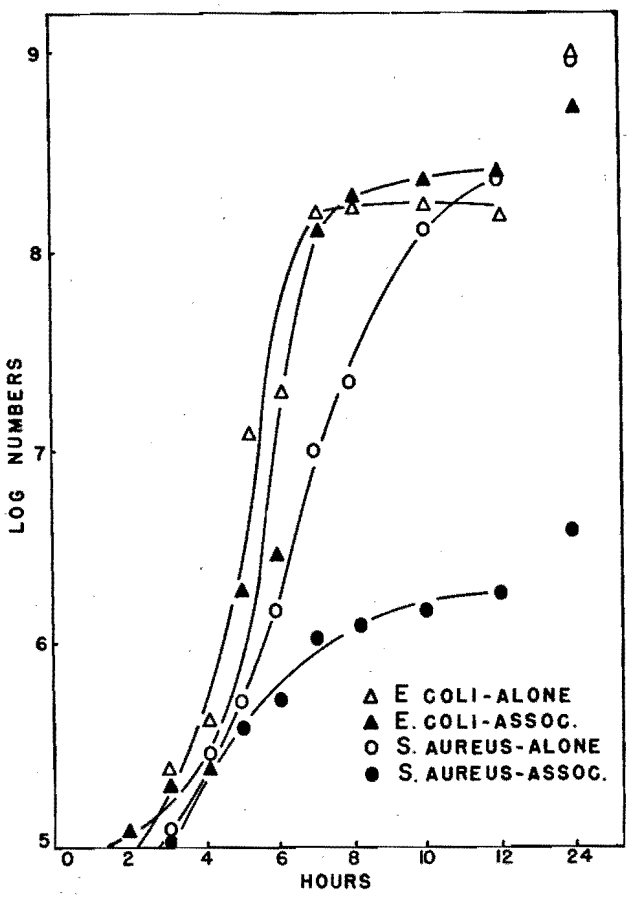

Figure 5. Growth and maximum numbers of *E. coli* H-52 and *S. aureus* 261 in pure culture and in association at 30°C. in meat infusion broth. Direct microscopic counts through 12 hours; plate counts at 24 hours.

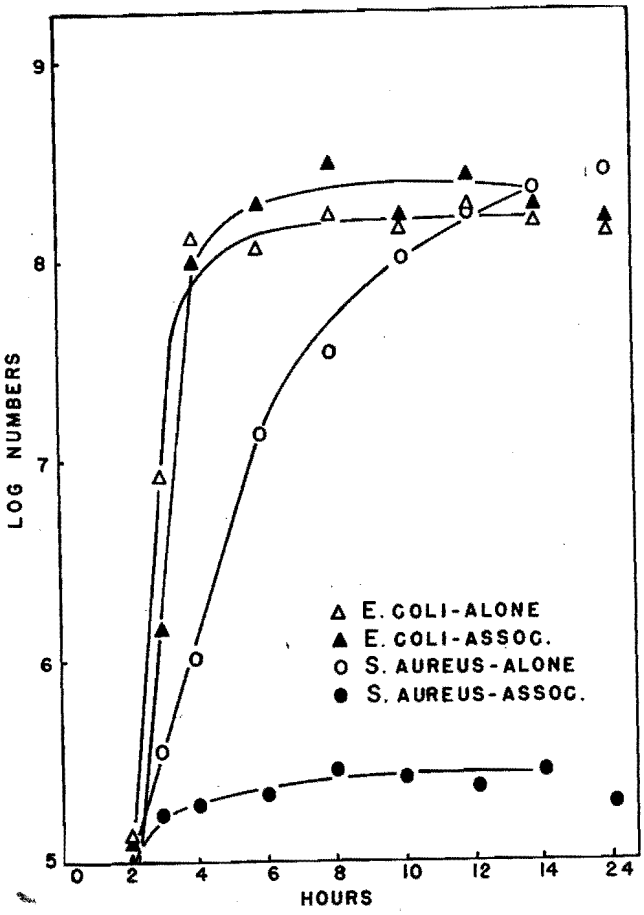

Figure 6. Growth and maximum numbers of *E. coli* H-52 and *S. aureus* 261 in pure culture and in association at 44°C in meat infusion broth. Direct microscopic counts through 14 hours; plate counts at 24 hours.

coli; (*b*) a typical *E. coli* inhibited markedly the growth of two enterotoxigenic strains *of S. aureus;* and (*c*) the inhibitory effect of *E. coli* varied with the strain used.

DISCUSSION

The effect of competing microorganisms on growth of *S. aureus* in foods often must be important in determining whether appreciable enterotoxin formation will take place. The present work has indicated that some species of food bacteria are inhibitory to *S. aureus* according to spot-plate tests. It also has been shown, however, that *E. coli*, which gave no indication of inhibition of the staphylococci, could strongly suppress the growth of *S. aureus*, especially at a low or a high incubation temperature, and that the inhibition was evident from the start of growth of the two organisms. More organisms, both those inhibitory to *S. aureus* on the spot plates and those not, are being tested as competitors for the staphylococcus.

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