Food Microorganisms Influencing the Growth of *Staphylococcus aureus*[1]

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ABSTRACT

GRAVES, R. R. (University of Wisconsin, Madison), AND W. C. FRAZIER. Food microorganisms influencing the growth of *Staphylococcus aureus*. Appl. Microbiol. 11:513–516. 1963.—Some 870 cultures of predominating microorganisms were isolated from market samples of hamburger, fresh pork sausage, fresh fish fillets, stewing beef, frozen chicken pot pie, frozen corn, frozen peas, and pasteurized and raw milk, before and after storage at different temperatures. The isolates were screened for their ability to influence the growth of *Staphylococcus aureus* strain 196E by means of spot-plate tests on APT and nutrient agars at 25 C. The 438 cultures that influenced the growth of *S. aureus* were retested on spot plates at 15, 30, and 42 C. After elimination of replicates, the 143 remaining cultures were classified into species, genera, or groups, and 14 different cultures were tested for their influence on the growth of *S. aureus* in APT broth at 25 C. Over half of the effective cultures inhibited *S. aureus* and less than half were stimulatory. Pork sausage had the highest proportion of inhibitory cultures, and stewing beef had the lowest. APT agar was better than nutrient agar for screening, and incubation at 15 C gave more effector organisms than at 30 and 42 C. Most of the lactic acid bacteria were inhibitory, but other groups of bacteria contained more stimulatory cultures than inhibitory ones. The three *Escherichia coli* cultures were stimulatory, but most other *Escherichia* cultures were inhibitory. *Aerobacter* and *Paracolobactrum* isolates were mostly stimulatory. Cultures of other kinds of bacteria were more or less evenly distributed between inhibitory ones and stimulatory ones. Genera containing mostly inhibitory bacteria were *Streptococcus*, *Leuconostoc*, and *Lactobacillus*. Inhibitory species were *E. freundii* and *E. intermedia*. Tests with *S. aureus* in broth indicated that all cultures inhibitory according to spot plates were inhibitory in broth, but stimulation on spot plates did not always indicate the same phenomenon in broth.

A number of workers have reported on the inhibition of growth of *Staphylococcus aureus* by other microorganisms, some of which were food organisms, but there is little information on the incidence of these effector organisms in foods. Stimulation of the staphylococcus by species of *Streptococcus* and *Lactobacillus* was noted by Judge (1958), and by species of *Candida* by Emmanouilidou-Arseni and Soultani (1960). Ability to inhibit the staphylococcus was reported by Régnier and Lambin (1934), Heatley and Florey (1946), and Oberhofer and Frazier (1961) for *Escherichia coli*; by Blackford, Parr, and Robbins (1951), Lockhart and Powelson (1953), Wynne (1947), and Higginbottom (1959) for coliform bacteria; by Hettche (1932) for *Serratia marcescens*; by Thompson and Johnson (1951) for salivary streptococci; by Hirsch and Wheater (1951) for *Streptococcus lactis*; by Hirsch, McClintock, and Macquot (1952) and Vincent, Veomett, and Riley (1959) for lactobacilli; by Rosebury, Gale, and Taylor (1954) for *S. faecalis* and *S. mitis*; by Oxford (1944) for lactic streptococci; by Su (1948) and Loeb, Moyer, and Murray (1950) for micrococci; by Dubos (1939) and Lockhart and Powelson (1953) for *Bacillus* species; by Garré (1887), Lewek (1890), Lode (1903), Lewis (1929), and others for *Pseudomonas* species; and by Grecz, Dack, and Hedrick (1961) for *Brevibacterium linens*.

Oberhofer and Frazier (1961) tested 66 cultures of food bacteria from various culture collections and found that *E. coli*, various fecal streptococci, a nisin-producing strain of *S. lactis*, and unidentified meat lactobacilli were inhibitory toward *S. aureus* and that this inhibitory effect varied with the medium used and the temperature. Troller and Frazier (1963*a*, *b*) studied the effect of seven inhibitory cultures of food bacteria on *S. aureus* and found that the medium and the proportions of bacteria in the inocula were important in determining the amount of inhibition. Five of the organisms apparently inhibited by means of antibiotic substances and two out-competed the staphylococcus. Peterson, Black, and Gunderson (1962) found depression of added staphylococci by mixed populations of microorganisms in thawed frozen chicken pot pies.

MATERIALS AND METHODS

Some 870 cultures of microorganisms which predominated in market samples of hamburger, fresh pork sausage, fresh fish fillets (perch), stewing beef, frozen chicken pot pie, frozen corn, frozen peas, and pasteurized and raw

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milk, before and after storage at different temperatures, were isolated, checked for purity, and screened for their ability to influence the growth of *S. aureus* strain 196E, by means of spot-plate tests on APT (Difco) and nutrient agars at 25 C. Solid food items were incubated at 5, 15, 25, 37, and 45 C; milks were incubated at 5, 10, 30, 37, and 45 C. Samples were taken at 1-, 2-, or 3-day intervals, and oftener at the higher temperatures of storage. Foods at 5 C were held for 6 to 12 days, others only 3 days. The 444 cultures that influenced the growth of *S. aureus* were purified and retested on *S. aureus* 196E on nutrient and APT agar plates at 15, 30, and 42 C. On the basis of a study of morphological, cultural, and physiological characteristics, enough replicates were eliminated to reduce the number of cultures to 143, and these were classified into genera as far as feasible and into species in some instances according to descriptions given in *Bergey's Manual of Determinative Bacteriology* (Breed, Murray, and Smith, 1957). Preliminary tests had shown that strains 196E and 255 (both enterotoxigenic) of *S. aureus* and Wi and Wis. 523 (both nonenterotoxigenic) reacted similarly when grown in broth with an inhibiting strain (P-111) of *Leuconostoc*.

A total of 14 cultures, including a yeast and a representative from each of 13 different genera of bacteria, were inoculated into APT broth to which was added an equal number of cells of *S. aureus* 196 E. Plate counts of viable staphylococci were made in mannitol-salt-agar at the time of inoculation and after 8 and 16 hr of incubation at 25 C, and comparison was made with numbers in a pure culture of *S. aureus* growing in the same medium at 25 C.

RESULTS

General results with all cultures isolated. Screening tests showed that 438 (50.4%) of the 870 cultures isolated from foods influenced the growth of *S. aureus* on the agar media. Over half (56.6%) of the effective cultures inhibited the staphylococcus and less than half (43.4%) were stimulatory, the effects ranging from slight to marked. About one-fourth of the inhibitory and stimulatory cultures were only slightly so. Table 1 shows the total numbers of isolates from each food and the number and percentage of these cultures that were inhibitory, stimulatory, or without effect. A few cultures showed both inhibition and stimulation on the plates. The ratio of inhibitory cultures to stimulatory ones was highest in isolates from pork sausage and lowest in isolates from stewing beef. Hamburger had the highest percentage of ineffective cultures.

In the screening tests, the inhibitory or stimulatory effect of food organisms on the staphylococcus usually was more apparent with APT agar than with nutrient agar, and, for the most part, was greatest at 15 C, less at 30 C, and least or absent at 42 C. Table 2 shows the results of screening tests at 15, 30, and 42 C with different groups of effector organisms. The inhibitory streptococci were effective almost as often at 30 C as at 15 C, and some were active at 42 C. The gram-negative rods were effective about three times as often at 15 C as at 30 C, and only one had any effect at 42 C. The lactobacilli did little at 30 or 42 C. The bacilli, on the other hand, were effectively inhibitory or stimulatory at the higher temperatures. Most of the stimulation by all other groups of organisms was demonstrated best at 15 C.

The isolates from the foods were grouped as shown in Table 2 on the basis of morphological, cultural, and physiological tests. The streptococcus group, which included some leuconostocs, contained mostly inhibitory cultures. Grouped as coliforms were gram-negative rods that produced acid and gas from lactose in 48 hr at 37 C. These included *E. coli*, *E. freundii*, *E. intermedia*, and *Aerobacter aerogenes* and *A. cloacae*, but not *Paracolobactrum* isolates which fell into the group of "other gram-negative gas-formers." Organisms in this group produced gas from glucose, but not from lactose, within 48 hr. Some of the bacteria in this group were in the genus *Proteus*. The nongas-forming, gram-negative rods were primarily *Pseudomonas-Achromobacter* organisms, although a few

TABLE 1. *Effect of all cultures from various foods on Staphylococcus aureus 196 E in spot-plate tests*

Food	Total no.	Cultures isolated					
		Inhibitory		Stimulatory		No effect	
		No.	Per cent	No.	Per cent	No.	Per cent
Pork sausage	86	33	38.37	4	4.65	49	56.98
Hamburger	78	8	10.26	6	7.69	64	82.05
Beef	81	4	4.94	33	40.74	44	54.32
Chicken pie	88	27	30.68	27	30.68	33	38.64
Fish	87	10	11.49	24	27.59	53	60.92
Frozen corn	117	37	31.62	25	21.37	55	47.01
Frozen peas	150	51	34.00	35	23.33	64	42.07
Raw milk	83	34	40.96	18	21.69	31	37.35
Pasteurized milk	100	44	44.00	18	18.00	38	38.00
Total	870	248	28.50	190	21.84	432	49.66

TABLE 2. *Effect of different groups of effector organisms on Staphylococcus aureus 196 E in spot-plate tests at 15, 30, and 42 C*

Organism	No. of inhibitory actions				No. of stimulatory actions			
	Total	15 C	30 C	42 C	Total	15 C	30 C	42 C
Cocci								
Streptococci	85	85	71	14	3	3	0	0
Micrococci	32	20	15	5	35	33	6	1
Gram-negative rods								
Coliforms*	14	13	5	1	28	22	9	4
Other gas-formers†	35	35	11	0	39	34	16	6
Nongas-forming	34	32	14	0	44	43	8	1
Gram-positive rods								
Lactobacilli	18	18	1	0	3	3	1	0
Bacilli	10	5	6	6	16	8	2	9
Others	5	5	2	0	10	10	0	0
Yeasts	5	4	4	0	8	7	1	0

* Acid and gas from lactose in 48 hr at 37 C.

† Gas from glucose, but not from lactose, in 48 hr at 37 C.

Flavobacterium and *Alcaligenes* cultures were found. The few gram-positive rods other than the bacilli and lactobacilli probably belonged to the genus *Brevibacterium*.

The incubation of the various foods at different temperatures before plating resulted in most streptococci and gram-negative gas-forming rods being isolated after incubation at 37 C, most micrococci and lactobacilli after incubation at 15 to 25 C, and most nongas-forming, gram-negative rods after incubation at 5 C. The *Bacillus* cultures came mostly from foods held at 37 and 45 C.

As is shown in Table 3, more of the coliform bacteria that were identified as such were stimulatory toward *S. aureus* than were inhibitory. The three *E. coli* cultures were stimulatory, but most other *Escherichia* cultures were inhibitory. The *Aerobacter* and *Paracolobactrum* isolates, on the other hand, were, for the most part, stimulatory.

Table 4 shows numbers of cultures of different groups of organisms obtained from the kinds of foods yielding at least three cultures in a group. Streptococci, mostly inhibitory, were obtained from all of the foods, but especially from milk, peas, corn, and pork sausage. Four of these cultures were inhibitory leuconostocs. Micrococci also were found in all foods examined. Most of the inhibitory micrococci came from frozen peas and pork sausage, whereas stimulatory ones were obtained primarily from chicken pie, fish, and peas. The few coliform cultures from meats and fish were mostly stimulatory. About two-thirds of those from corn, peas, and milk were stimulatory, and the rest were inhibitory. The other gram-negative gas-forming bacteria from beef and peas were all stimulatory, whereas most such organisms from fish, corn, and milk were inhibitory. The gram-negative rods that did not form gas from glucose (mostly *Pseudomonas-Achromobacter* cultures plus a few *Flavobacterium* and *Alcaligenes* isolates) from chicken pie were, for the most part, inhibitory. Most cultures from fish, beef, and milks, however, were stimulatory. Most of the lactobacilli were inhibitory. The bacilli from beef, peas, and milk were stimulatory, but chicken pie yielded more inhibitory cultures than stimulatory ones. About two-thirds of the yeasts, mostly from corn and peas, stimulated *S. aureus*, and one-third were inhibitory.

Previously all 870 cultures isolated have been considered. After obvious replicates of effector organisms had been eliminated, the species, genera, or groups of organisms shown in Table 5 remained. The table shows from what

TABLE 3. *Effect of coliform bacteria from various foods on Staphylococcus aureus 196 E in spot-plate tests*

Kind of coliform	IMViC reactions				H_2S production	No. of cultures affecting *S. aureus*	
	Indole	Methyl red	Voges-Proskauer	Use of citrate		Inhibitory	Stimulatory
Escherichia coli	+	+	−	−	−	0	3
E. freundii	−	+	−	+	+	5	1
E. intermedia	−	+	−	+	−	4	1
Aerobacter cloacae	+	−	+	+	−	0	1
Aerobacter aerogenes	−	−	+	+	−	4	17
Paracolobactrum aerogenoides	−	−	+	+	−	2	7
Unidentified	−	+	+	+	−	1	0
Total						16	30

TABLE 4. *Foods as sources of effector organisms*

Organism	No. of cultures affecting *S. aureus*		Foods yielding 3 or more effectors (no. of effectors in parentheses)
	Inhibitory	Stimulatory	
Streptococci	86	3	Milks (45), peas (17), corn (16), sausage (5), chicken (3)
Micrococci	35	35	Peas (28), sausage (14), chicken (10), milks (5), fish (4)
Coliforms	14	28	Milks (21), corn (10)
Other gas-formers	35	39	Milks (25), corn (20), chicken (11), beef (7), fish (6), peas (5)
No gas, gram-negative	34	44	Peas (23), fish (16), chicken (12), beef (11), milks (9), corn (4)
Lactobacilli	18	4	Sausage (10), milks (4), hamburger (4), beef (3)
Bacilli	11	16	Chicken (14), beef (5), milks (3), peas (3)
Other gram-positive rods	10	12	Sausage (6), fish (6), beef (5)
Yeasts	5	9	Corn (9), peas (3)
Total	248	190	

TABLE 5. *Effect of cultures selected for different determinative characteristics on growth of Staphylococcus aureus 196 E on spot plates*

Species, genus, or group	Effect on *S. aureus**		Sources of cultures
	I	S	
Streptococcus	21	1	All foods
Leuconostoc	4	0	Chicken, corn, peas, raw milk
Lactobacillus	9	2	All meats, milks
Micrococcus	13	12	All foods
Pseudomonas-Achromobacter	6	15	All foods except sausage and corn
Flavobacterium	2	1	Sausage, fish, peas
Alcaligenes	2	0	Peas
Escherichia coli	0	2	Hamburger, raw milk
E. freundii	5	0	Chicken, peas, milks
E. intermedia	2	0	Milks
Aerobacter	1†	12	Corn, peas, chicken, milks
Paracolobactrum aerogenoides	2	7	All except sausage and fish
Proteus morganii	1	1	Fish, beef
P. vulgaris	1	0	Fish
Bacillus	5	5	Chicken, hamburger, peas, milk
Yeast	0	6	Sausage, corn, raw milk

* I = inhibitory; S = stimulatory.
† IMViC reaction = + − ++.

foods these cultures came and the numbers of cultures that were inhibitory or stimulatory. In agreement with results shown in Table 4, most organisms in the genera *Streptococcus* and *Lactobacillus* were inhibitory. Table 5 indicates that the tested isolates in the genera *Lenconostoc* and *Alcaligenes* and in the species *E. freundii*, *E. intermedia*, and *Proteus vulgaris* were inhibitory, although the samples were small. On the other hand, *Pseudomonas-Achromobacter*, *E. coli*, *Aerobacter*, *Paracolobactrum aerogenoides*, and yeast cultures were mostly stimulatory.

Growth of S. aureus with effectors in APT broth. When equal inocula of *S. aureus* and effector were added to APT broth and incubation was at 25 C, plate counts of the staphylococcus after 8 and 16 hr showed inhibition by all seven cultures that had been inhibitory according to spot-plate tests. Of the seven cultures previously found stimulatory, two were stimulatory in the broth, two were slightly inhibitory, and three were definitely inhibitory. It had been observed that the best stimulation of these cultures on spot plates was at 15 C. It is apparent that an indication of inhibition on the plates is more reliable than an indication of stimulation.

Discussion

The cultures selected for further study will be tested on other strains of *S. aureus* at different incubation temperatures, in different culture media including human foods, and with variations in size and proportions of inocula. Also, attempts will be made to identify the more active cultures.

Most of the cultures isolated were predominant in the foods after storage at different temperatures and, hence, were the ones that would compete with *S. aureus* if it were present. Apparently there were inhibitory cultures growing at all temperatures, but stimulatory ones also grew. It is possible that inhibition may be balanced by stimulation in some instances. Indications are, however, that inhibition is more common than stimulation, despite the large proportion of stimulating cultures found, for the limited number of tests in broth showed that some of the cultures that were stimulatory to the staphylococcus according to spot-plate tests really were inhibitory in broth. By the same token, some of the cultures discarded as ineffective might have exhibited an effect on the staphylococcus in a liquid medium.

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