Fishery Technology
1994, Vol. 31(1) pp: 41-47

Antagonistic Actions of *Escherichia coli*, Faecal Streptococci and Natural Bacterial Flora on *Staphylococcus aureus* in Shrimp Homogenate*

T.S. Gopalakrishna Iyer and K.P. Shrivastava**
Central Institute of Fisheries Technology
Cochin - 682 029, India

The antagonistic effect of *Escherichia coli*, faecal streptococci and natural bacterial flora on *Staphylococcus aureus* in shrimp homogenate was studied at 28, 10 and 0°C. It was observed that staphylococci could not grow in competition with *E. coli*, faecal streptococci and natural bacterial flora at any of the temperatures studied.

Antagonistic relations between microorganisms occur frequently in foods. The significance of such antagonism, particularly in the field of public health cannot be ignored or under estimated. The relatively low recorded incidence of staphyloenterotoxicosis must be attributed, atleast in part, to such phenomena. *Staphylococcus aureus* is sensitive to competition and suppression of its growth by the natural microflora of foods including spoilage bacteria (Oberhofer and Frazier, 1961; Peterson *et al.*, 1962; Dack and Lipputz, 1962; Bryan, 1968; Digiacinto and Frazier, 1966; Haines and Harmon, 1973; Kraft *et al.* 1976; Mossel, 1982). The genera and species often exerting their antagonistic influence on *S. aureus* are *Pseudomonaceae, Enterobacteriaceae, Lactobacillaceae, Escherichia coli, Streptococcus faecalis, Bacillus cereus, Micrococci, Lactobacillus, Achromobacter and Pseudomonas* (*Ibid*). However, in cooked foods *S. aureus* is known to compete with other bacteria (Bryan, 1973, Mossel, 1982). Available information regarding the interaction between different species of bacteria pertains only to a few food items. However, knowledge of this important aspect relating to fish and fishery products is lacking. The present study was carried out to find the ability of staphylococci to grow in competition with *E. coli*, faecal streptococci and natural bacterial flora at 28, 10 and 5°C in shrimp homogenate.

Materials and Methods

Different strains of coagulase - positive staphylococci, *E. coli* and faecal streptococci isolated from frozen shrimps in our laboratory were used for the intercompetition studies. Five sets of experiments were carried out employing the following combinations of bacterial cultures and in the case of each set, the influence of temperature (28, 10 and 0°C) and initial bacterial load on the growth and viability of the relevant organisms were also studied: i) Coagulase positive staphylococci, No. 4 and *E. coli* No. II; ii) Coagulase positive staphylococci No. 4 and faecal streptococci A; iii) Coagulase positive staphylococci No. 2 and natural bacterial flora; iv) Coagulase positive staphylococci No. 4 and natural bacterial flora; v) Coagulase positive staphylococci No. 6 and natural bacterial flora

The substrate used in the first two sets of experiments was sterile 20% (w/v) shrimp-homogenate (autoclaved at 121°C for 30 min) whereas in the remaining three sets, a 20% (w/w) homogenate of fresh uncooked shrimps was used.

*Formed part of the Ph.D. thesis of the first author approved by the University of Kerala.
**Export Inspection Agency, World Trade Centre, 14/1B EZM Street, Calcutta - 700 001, India

Twenty four h old cultures of the organisms in brain-heart infusion broth incubated at 37°C were centrifuged for 15 min at 5000 rpm and the supernate was aseptically decanted off. Cells were washed twice by suspending in 10 ml isotonic saline and recovered by centrifugation. Washed cells were resuspended in 10 ml of fresh saline and finally diluted 10^5 times using sterile isotonic saline.

In each set of experiments cited above, 0.1 to 10 ml of the diluted suspensions of the concerned organism were pipetted into the shrimp homogenate contained in conical flasks. After thorough stirring, 100 ml of the homogenate from each set of the experiment was dispensed into three sterile 250 ml conical flasks. One flask from each set of the experiment was incubated at room temperature (28°C), 10°C and 0°C. One ml each of the test homogenate was drawn from each flask just before incubation and thereafter at fixed intervals for bacteriological enumeration. The growth of *S. aureus* when inoculated alone into sterile shrimp homogenate and incubated at different temperatures were also determined. The strain and the substrate used in these studies were identical with those used in the intercompetition studies.

E. coli, faecal streptococci and coagulase positive staphylococci in one ml each of the homogenates were determined using Tergitol 7 agar, KF agar and Baird-Parker medium (BP) respectively. Natural bacterial flora were enumerated using Tryptone glucose extract agar (TGE).

The Tergitol 7 agar medium was incubated at 37°C for 24 h, while all other media were incubated at 37°C for 48 h.

As TGE agar also allows growth of coagulase - positive staphylococci, the endogenous microflora was determined by deducting the number of coagulase positive staphylococci from the total number of bacteria that grew on TGE.

Results and Discussion

The intercompetition between coagulase positive staphylococci and *E. coli* for growth in shrimp homogenate at 28°C, 10°C and 0°C is shown in Tables 1, 2 & 3. From the tables, it is clear that coagulase positive staphylococci cannot grow in shrimp homogenate in competition with *E. coli* at any of the three temperatures. In the experiments where the number of staphylococci was equal or greater than that of *E. coli*, the staphylococci showed an increase in growth upto 24 h, but, the growth rate was significantly lower than that of *E. coli*. After 24 h, staphylococci exhibited a sharp decline in the rate of increase whereas the number of *E. coli* continued to be on the increase throughout the period of study. It was further observed that when greater numbers of *E. coli* than staphylococci were present in the homogenate (Table 1), the growth of staphylococci was suppressed to a greater extent. At 10°C, the maximum growth of staphylococci was attained in 12 to 16 days followed by a steady decline whereas the number of *E. coli* continued to increase throughout the period of study (Table 2). At 0°C, however, the number of both the organisms were found to decrease, the rate of decrease in the case of staphylococci being faster, indicating intercompetition between the two groups of organisms. (Table 3).

Data presented in Tables 4, 5 and 6 clearly indicated that staphylococci could not grow in shrimp homogenate in competition with faecal streptococci at 28, 10, 0°C. Staphylococci could grow well only for a maximum of (Table 4) 24 h and, thereafter, it was outgrown by faecal streptococci. When the initial population of faecal streptococci in the substrate was more, the antagonistic effect of streptococci on staphylococci was evident even at 12 h. At 10°C, the growth of staphylococci was found to be restricted from the 8th day onwards, depending upon the number of streptococci in the substrate. At 0°C, even though the

Table 1. *Intercompetition between Staphylococci and E. coli for growth and viability in shrimp - homogenate at 28°C*

Expt. no	Organism & strain no.	Original number per ml	Growth rate* 2h	6h	12h	24h	2days	4days	6days	10days
1	Staphylococci 4	3200	2	400	2010	8800	4300	2100	980	350
	E. coli II	4800	3	200	6500	12400	18300	35880	61450	75820
2	Staphylococci 4	1.65×10^4	3	320	6400	10000	6400	3300	1200	320
	E. coli II	3800	2	420	12420	18100	25820	44700	60100	87800
3	Staphylococci 4	2400	2	20	420	300	150	110	80	50
	E. coli II	4.62×10^4	2	25	920	12100	29800	35400	62100	74200

*The number of times of increase in the orginal number of the organisms

Table 2. *Intercompetition between Staphylococci and E. coli for growth and viability in shrimp - homogenate at 10°C*

Expt. no	Organism& strain no.	Original number per ml	Growth rate* 1day	4days	8days	12days	16days	20days	24days
1	Staphylococci 4	3200	2	175	450	500	400	95	40
	E.coli II	4800	2	2352	22610	23530	24450	25220	27250
2	Staphylococci 4	1.65×10^4	3	210	450	680	810	520	210
	E.coli II	3800	2	410	12450	15640	18310	23810	30150
3	Staphylococci 4	2400	3	30	200	160	110	90	70
	E. coli II	4.62×10^4	4	300	1250	2850	3580	3750	3810

*The number of times of increase in the orginal number of the organisms

Table. 3 *Intercompetition between Staphylococci and E. coli for growth and viability in shrimp - homogenate at 0°C*

Expt. No.	Organism & Strain no.	Original number per ml	Death rate* 1day	4days	8days	12days	16days	20days	24days
1	Staphylococci 4	3200	1.1	1.6	2.2	10.4	50	120	208
	E.coli II	4800	1.3	2.5	14	18	22	50	70
2	Staphylococci 4	1.65×10^4	0.5	4	25	120	250	340	440
	E.coli II	3800	1.6	8	14	23	31	38	42
3	Staphylococci 4	2400	1.5	4	25	80	150	250	810
	E. coli II	4.6×10^4	2	5.5	6	25	30	35	45

*The number of times of decrease in the orginal number of the organisms

number of both the organisms decreased, the rate of decrease in respect of staphylococci was comparatively more rapid.

The results further indicated that coagulase - positive staphylococci were unable to grow along with the natural bacterial flora present in shrimp-homogenate at any of the three temperatures studied (Tables 7, 8 and 9). At 28°C, the growth of staphylococci was inhibited in 15-18 h by the growth of natural bacterial flora, while at 10°C, staphylococci could grow for 16 days without being suppressed. In both the cases, the ratio between the number of staphylococci and the natural bacterial flora was also a controlling factor as in the case of *E. coli* and faecal streptococci. At 0°C

Table 4. *Intercompetition between staphylococci and faecal streptococci for growth and viability in shrimp - homogenate at 28°C*

Expt. No.	Organism& Strain no.	Original number per ml	Growth rate* 2h	6h	12h	24h	2days	4days	6days	10days
1	Staphylococci 4	3.62×10^4	3	660	1570	1240	200	180	150	110
	Streptcocci A	1.21×10^4	2	30	1400	14210	48000	50000	51500	53000
2	Staphylococci 4	7200	3	20	400	1850	1100	800	500	375
	Streptococci A	560	2	150	820	1800	4400	75000	112100	125800
3	Straphylococci 4	740	3	70	1880	1400	1800	1000	950	780
	Streptococci A	1.05×10^4	2	20	910	5400	91000	121000	164000	181500

* The number of times of increase in the orginal number of the organisms

Table 5. *Intercompetition between staphylococci and faecal streptococci for growth and viability in shrimp-homogenate at 10°C*

Expt No.	Organisms strain No.	Original number per ml	Growth rate* 1 d	4 d	8 d	12 d	16 d	20 d	24 d
1	Staphylococci 4	3.62×10^4	1.2	10	120	220	150	120	100
	Streptococci A	1.21×10^4	2.2	345	6500	10200	15080	20200	25100
2	Staphylococci 4	7200	2	15	210	450	300	210	190
	Streptococci A	650	3	450	7300	10200	14300	18500	21200
3	Staphylococci 4	740	2.6	25	200	180	140	110	75
	Streptococci A	1.05×10^4	1.5	750	9500	15650	18200	20400	22700

*The number of times of increase in the original number of the organisms

Table 6. *Intercompetition between staphylococci and faecal streptococci for growth and viability in shrimp-homogenate at 0°C*

Expt No.	Organisms strain No.	Original number per ml	Death rate* 1 d	4 d	8 d	12 d	16 d	20 d	24 d
1	Staphylococci 4	3.62×10^4	IS	2	3.5	6.5	10.2	15.1	25.0
	Streptococci A	1.21×10^4	IS	1.5	2.0	3.1	3.6	3.8	4.5
2	Staphylococci 4	7200	IS	1.8	3.5	9.2	15.5	22.0	29.0
	Streptococci A	650	IS	1.5	1.9	2.5	3.2	6.2	7.1
3	Staphylococci 4	740	IS	1.5	6.5	8.5	12.0	18.0	22.0
	Streptococci A	1.05×10^4	IS	2.0	2.5	3.5	4.4	6.8	7.5

IS : insignificant decrease in the original number

*The number of times of decrease in the original number of the organisms

(Table 9) while staphylococci gradually reduced in numbers, fairly rapid multiplication of the natural bacterial flora was recorded. However, *S. aureus* had an uninterrupted growth alone in shrimp homogenate (Tables 10, 11 and 12).

Apparently, no similar work seems to have been done in fish or shrimps and, therefore, the results of this work cannot be compared with other studies. The studies of McCoy and Farber (1966) on meat, Bryan (1968) on beef pies and Mossel (1982) on foods in general indicate the inability of *S. aureus* to grow in competition with the natural bacterial flora which is in total agreement with the observations of the present work. Oberhofer & Frazier (1961) found that

Table 7. *Intercompetition between staphylococci and natural bacterial flora for growth and viability in shrimp-homogenate at 28°C*

Expt No.	Organism strain no.	Original number per ml	Growth rate* 2 h	6 h	12 h	15 h	18 h	24 h	2 days	6 days
1	Staphylococci 2	3.62×10^4	10	60	120	185	160	30	15	10
	Natural flora	6.21×10^4	35	350	1500	1800	2300	3200	4200	5800
2	Staphylococci 4	8300	5	25	90	75	60	50	35	20
	Natural flora	1.05×10^5	25	340	1650	2100	2800	3800	5200	6100
3	Staphylococci 6	2.61×10^5	10	28	32	40	16	10	8	6
	Natural flora	1.81×10^4	14	210	2110	2500	3300	4050	4150	5200

*The number of times of increase in the original number of the organisms

Table 8. *Intercompetition between staphylococci and natural bacterial flora for growth and viability in shrimp-homogenate at 10°C*

Expt No.	Organism strain no.	number per ml	Growth rate* 1 d	4 d	8 d	12 d	16 d	20 d	24 d
1	Staphylococci 2	1.62×10^4	3	18	35	65	150	105	70
	Natural flora	6.21×10^4	10	220	540	1210	2900	3050	4200
2	Staphylococci 4	8300	2	16	45	160	120	85	35
	Natural flora	1.05×10^5	25	110	540	1100	2500	3600	4400
3	Staphylococci 6	2.61×10^5	3	40	120	450	520	370	220
	Natural flora	1.81×10^4	20	65	450	1600	1720	2800	3400

*The number of times of increase in the original number of the organisms

Table 9. *Intercompetition between staphylococci and natural bacterial flora for growth and viability in shrimp-homogenate at 0°C*

Expt No.	Organism strain no.	Original number per ml	Growth rate*/death rate** 1 d	4 d	8 d	12 d	16 d	20 d	24 d
1	Staphylococci 2	3.62×10^4	0	1	4	5	6	8	12
	Natural flora	6.21×10^4	4	6	13	185	1300	3200	4500
2	Staphylococci 4	8300	0	2	4	6	10	15	20
	Natural flora	1.05×10^5	4	6	12	250	780	1050	2100
3	Staphylococci 6	2.61×10^5	0	3	5	10	40	85	110
	Natural flora	1.81×10^4	2	3	6	150	750	1820	2700

*In the case of natural bacterial flora; **in the case of staphylococci. As number of times increase/decrease in original number of organisms

streptococci were inhibitory to the growth of *S. aureus*. The study of Graves & Frazier (1963) on the microorganisms isolated from different food products indicated that *E. coli* and faecal streptococci were inhibitory to *S. aureus*. According to these authors, the temperature of incubation also markedly influenced the extent of such inhibition in the growth of staphylococci. The results obtained in the present study support these findings.

S. aureus requires a good complement of a number of amino acids like cystine, valine, glycine, proline, arginine and aspartic acid (Troller & Frazier, 1963). Niacin and biotin too are vital nutrients for the growth

Table 10. *Growth of S. aureus 4 in shrimp homogenate at 37°C*

	Count ml^{-1}											
	0 h	2 h	4 h	6 h	8 h	10 h	12 h	24 h	2 d	4 d	6 d	8 d	12 d
Numbers	1.4×10^4	2.4×10^4	2.0×10^5	5.2×10^5	6.0×10^7	2.4×10^8	4.6×10^8	5.2×10^8	6.1×10^8	8.2×10^8	8.5×10^8	8.8×10^8	7.0×10^8
Fold increase over initial numbers		1.7	14	371	4285	17142	32857	38571	43571	58571	60714	62857	50000

Table 11. *Growth rate of S. aureus 4 in shrimp homogenate at 10°C*

	Count ml^{-1}									
	0 d	1 d	2 d	3 d	4 d	5 d	8 d	12 d	18 d	20 d
Numbers	3.8×10^4	4.5×10^4	1.5×10^5	7.5×10^5	2.1×10^7	8.9×10^7	1.9×10^8	2.1×10^8	3.5×10^8	3.0×10^8
Fold increase over initial numbers		1.18	3.9	20	553	2342	5000	5526	9210	7894

Table 12. *Death rate of S. aureus 4 in shrimp-homogenate at 0°C*

	Count/ml								
	0 day	1 day	4 days	6 days	10 days	16 days	24 days	32 days	42 days
Numbers	2.8×10^4	2.18×10^4	2.0×10^4	1.1×10^4	9200	1800	460	160	75
Fold increase over initial numbers		1.3	1.4	2.55	3.04	15.55	60.86	175	373

of *S. aureus* (*Ibid.*). As the antagonistic microorganisms exhaust these essential nutrients during their competitive growth, staphylococci lose its chance for growth. Many metabolites like trimethylamine, hypoxanthine, indole, hydrogen sulphide, dimethyl sulphide, furfural, isovaleraldehyde, isobutyraldehyde etc., are formed during the growth of saprophytic bacterial flora on fish. In the present study, the toxic effect of any one or more of these metabolites on the growth of *S. aureus* may also be suspected.

One of the main aims of the present work was to investigate whether under normal commercial handling practices, coagulase positive staphylococci present in fishery products, can multiply to the extent which may result in the outbreak of food poisoning. On the basis of the results obtained in the present study, it can be concluded that the food poisoning outbreak due to consumption of raw frozen shrimps contaminated with coagulase positive staphylococci is seldom possible as the natural bacterial flora prevent staphylococci from attaining a dangerous level. However, in the case of cooked frozen shrimps, where the competing microorganisms are much less, staphylococci, if present, can multiply to high levels, thereby increasing the hazard of food poisoning.

The authors are thankful to Dr. K. Gopakumar, Director, Central Institute of Fisheries Technology, Cochin for his kind permission to publish this paper.

References

Bryan, F.L. (1968) *J. Milk Food Technol.* **31**, 110

Bryan, F.L. (1973) in *Microbial Safety of Fishery Products* (Chicester, C.O. & Graham, H.D., Eds.), p.273, Academic Press, New York

Digiacinto, J.V. & Frazier, W.C. (1966) *Appl. Microbiol.* **14**, 124

Graves, R.R. & Frazier W.C. (1963) *Appl. Microbiol.* **11**. 513

Haines, W.C. & Harmon, L.G. (1973) *Appl. Microbiol.* **25**, 436

Kraft, A.A., Oblinger, J.L., Walker. H.W., Kawal M.C., Moon, N.J. & Reinhold, M.C. (1976) in *Microbiology in Agriculture, Fisheries and Food* (Skinner, F.A. & Carr, J.G., Eds.), p.141, Academic Press, London

McCoy, D.W. & Farber, J.E. (1966) *Appl. Microbiol.* **14**, 372

Mossel, D.A.A. (1982) *Microbiology of Foods*, 3rd edn., The University of Utrecht, Netherlands

Oberhofer, T.R. & Frazier, W.C. (1961) *J. Milk Food Technol.*. **24**, 72

Peterson, A.C., Black, J.J. & Gunderson, M.F. (1962) *Appl. Microbiol.* **10**, 16

Troller, J.A. Frazier, W.C. (1963) *Appl. Microbiol.* **11**, 11