EFFECT OF PSEUDOMONADS AND ACHROMOBACTERACEAE ON GROWTH OF STAPHYLOCOCCUS AUREUS[1, 2]

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SUMMARY

Pseudomonas and *Achromobacteraceae* cultures, mostly from foods, were tested for their effect on the growth of *Staphylococcus aureus* 196E in Trypticase Soy Broth at different temperatures (10 to 30 C) and with different ratios of effectors to staphylococci in inocula. Most cultures inhibited the staphylococcus, with inhibition becoming greater with decreasing proportions of *S. aureus* in the inoculum and decreasing temperatures of incubation, but inhibition usually was not as great as had been found with most coliform and lactic acid bacteria. Only a few of the *Pseudomonas* cultures could keep numbers of *S. aureus* below 5 x 10^6 cells per ml, even with an initial ratio of effectors to staphylococci of 100 : 1 and a low incubation temperature, although most cultures of *Pseudomonas* and *Achromobacteraceae* delayed the attainment of these numbers. Especially effective in inhibition of S. aureus were strains of *Pseudomonas striata* and *P. mildenbergii* or *convexa*, and a culture of *Alcaligenes viscolactis*.

At 15 C *Pseudomonas fluorescens*, *Alcaligenes faecalis*, and *Achromobacter xerosis* stimulated *S. aureus* enough during early growth to hasten the attainment of hazardous numbers of staphylococci by several hours. At 15, 25 and 30 C most cultures, however, delayed the growth of *S. aureus*, and all kept maximal numbers of staphylococci below those reached by the coccus growing alone, although numbers usually were less by only about one- to two-thirds.

Most strains of two *Pseudomonas* species affected *S. aureus* similarly, and the effects of eight species of effectors on two strains of *S. aureus* were, for the most part, similar.

Previous work by DiGiacinto and Frazier *(1)* had indicated that most of the coliform bacteria tested and lactic acid bacteria as reported by Kao and Frazier *(3)*, inhibited the growth of *Staphylococcus aureus*, with greater inhibition as the incubation temperature was lowered toward 10-15 C and as the proportion of staphylococci in the inoculum was decreased. Some of the lactic acid bacteria, however, stimulated growth of *S. aureus* during early hours of incubation. Present work was with some of the gram-negative, nonsporeforming, nongasforming rods commonly found in foods.

Graves and Frazier *(2)* reported that most cultures of the *Pseudomonas-Achromobacter* group stimulated *S. aureus*, according to spot-plate tests, but some were inhibitory, *Flavobacterium* and *Alcaligenes* being most inhibitory. On spot plates Oberhofer and Frazier *(5)* found that *Pseudomonas* cultures from chicken and meat, together with *P. aeruginosa*, *P. fragi*, and *Alcaligenes viscolactis*, had no apparent effect on growth of *S. aureus*, whereas two strains of *Pseudomonas fluorescens* were somewhat inhibitory. Troller and Frazier *(7)* concluded that a *Pseudomonas* culture inhibited *S. aureus* by outcompeting it for nutrients. Peterson, Black, and Gunderson *(6)* reported that naturally occurring mixed populations of mesophiles and psychrophiles in precooked frozen foods repressed added staphylococci during thawing at different temperatures. Presumably *Pseudomonas-Achromobacter* bacteria were involved.

MATERIALS AND METHODS

A few of the 28 effector cultures tested were from stock culture collections, but most were isolated from foods. The 19 *Pseudomonas* cultures represented species tentatively identified as *P. fluorescens*, *P. aeruginosa*, *P. fairmontensis* (strain R35), *P. effusa* (S82), *P. striata* (F2, R39), *P. mildenbergii* or *P. convexa* (A24, B63, F72), and *P. incognita* or *P. rugosa* (A47). Strain F11 was not identified. The *S. aureus* cultures were strain 196E, an enterotoxigenic strain from G. M. Dack, and strain W-1, a nonenterotoxigenic mastitis strain from J. B. Wilson. All effector cultures were tested for their effect on *S. aureus* when grown with it on Trypticase Soy Agar spot plates at 15, 30, and 37 C. Also effector cultures were grown with *S. aureus* in Trypticase Soy Broth at different temperatures and with different initial ratios of effectors to staphylococci, and growth of the staphylococci was followed. A third transfer, grown in the broth for 12 hr at 30 C, was used as inoculum. In all experiments the inoculum of staphylococci was about 2 x 10^4 cells per ml, and inocula of effectors were 2 x 10^2, 2 x 10^4, or 2 x 10^6 cells per ml. Trypticase Soy Broth was chosen to represent a complete medium, favorable to growth of both staphylococci and effectors, and in this way resembling most raw foods.

Counts of *S. aureus* during the course of growth were made by means of Mannitol Salt Agar spread plates, with incubation for 48 hr at 30 C. All dilutions were in sterile 0.1% peptone solution.

RESULTS

Spot plates. None of the *Pseudomonas*, *Alcaligenes*, *Achromobacter*, or *Flavobacterium* cultures

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TABLE 1. INFLUENCE OF EFFECTOR BACTERIA ON GROWTH OF *S. aureus* 196E IN TRYPTICASE SOY BROTH AT 25 AND 30 C AFTER 8 HR, AND AT 18 C AFTER 24 HR, WITH INITIAL EFFECTOR TO COCCUS RATIO OF 1 : 1

Cultures	Source	No. of *S. aureus*[a]/ml x 10^6 30 C	25 C	18 C
S. aureus 196E (alone)	—[b]	53.0	13.0	14.0
Pseudomonas aeruginosa	—	39.0	5.9	14.0
Pseudomonas fluorescens	—	25.0	3.5	8.3
Pseudomonas A8	beef	45.0	4.2	4.9
Pseudomonas A24	beef	2.7	2.0	8.3
Pseudomonas B63	beef	23.0	1.9	11.0
Pseudomonas F2	fish	19.0	5.8	8.2
Pseudomonas F11	fish	23.0	6.5	16.0
Pseudomonas F72	fish	41.0	5.9	8.3
Pseudomonas R35	milk	42.0	2.8	3.9
Pseudomonas R39	milk	58.0	3.9	6.4
Pseudomonas S82	chicken pie	12.0	4.6	5.4
Alcaligenes viscolactis	—	10.0	0.9	1.6
Alcaligenes faecalis	—	20.0	1.2	16.0
Alcaligenes P142	peas	31.0	2.9	6.7
Achromobacter xerosis	—	58.0	0.4	7.0
Achromobacter M80	milk	43.0	3.0	2.9
Flavobacterium capsulatum	—	56.0	0.6	7.7

[a]Initial number for *S. aureus* 196E (alone) and for *S. aureus* and each effector was 2 x 10^4 per ml.

[b]— indicates previously identified stock cultures.

tested affected the growth of *S. aureus* 196E on spot plates at 15, 30, or 37 C.

Growth in broth. Table 1 shows the influence of effector cultures on the growth of *S. aureus* in broth after 8 hr at 25 and 30 C, and after 24 hr at 18 C, with equal initial numbers of effector and staphylococcus. Of 19 *Pseudomonas* cultures (11 are shown in the table), only one, strain A24 (which resembles *P. convexa*), markedly repressed the growth of *S. aureus* at 30 C. At 25 C all were inhibitory, and at 18 C all were moderately inhibitory except *P. aeruginosa* which had no effect, and an unidentified strain (F11) which was stimulatory. The four *Alcaligenes* cultures (3 in table) inhibited at 30, 25, and 18 C, except that *A. faecalis* stimulated somewhat at 18 C. *Achromobacter xerosis* and *Flavobacterium capsulatum* stimulated at 30 C, but otherwise all four *Achromobacter* cultures (2 in Table) and one *Flavobacterium* inhibited *S. aureus.*

By the 72nd hr at 18 C and the 24th hr at 25 and 30 C, all 19 *Pseudomonas* cultures had inhibited *S. aureus,* but in most instances numbers were reduced to only about one-third to two-thirds of numbers of *S. aureus* growing alone. At 18 C, by the 72nd hr, *P. fluorescens* had caused no decrease in final numbers, and only two pseudomonads, A24 and R35, had markedly inhibited the staphylococcus.

Of cultures in the other genera, only *Alcaligenes viscolactis* had held down numbers of staphylococci appreciably (85%) after 24 hr at 30 C. This culture and the others held down numbers of cocci most effectively at 25 C, where numbers of cocci were lower than in the control by about 99.5% with *A. viscolactis,* by about 85% with *Alcaligenes faecalis, Achromobacter xerosis,* and *Flavobacterium capsulatum,* and by about 56 to 77% with the others. The exception was *Achromobacter* P15, which was most effective at 18 C. At 18 C by the 72nd hr most of the cultures had repressed *S. aureus* to only about one-third to two-thirds of its population when growing alone.

Eight cultures, representing the four genera, were studied for their effect on growth of *S. aureus* by means of detailed growth curves at 10, 15, and 22 C and with ratios of effectors to staphylococci in inocula of 1 : 100, 1 : 1, and 100 : 1. In general, inhibition increased with increasing proportions of effector bacteria in the inocula and with decreasing temperature, but inhibition was not as great as with the other bacteria studied.

Again special attention was paid to the time required to reach 5 x 10^6 staphylococci per ml, a number approximating the minimal number of *S. aureus* organisms assumed by DiGiacinto and Frazier *(1)* to be necessary for appreciable enterotoxin production. Only one of the eight test organisms, *Pseudomonas* A24, kept numbers of staphylococci below 5 x 10^6 cells per ml with inoculum ratios of 100 : 1 and 1 : 1, as shown in Table 2. These numbers were exceeded eventually in the presence of the other seven test organisms, regardless of temperature or inoculum ratio. Similar results also were obtained in other tests at 18, 25 and 30 C. Table 2 shows the delay or hastening of the attainment of 5 x 10^6 staphylococci per ml with the eight cultures. Except for *Pseudomonas* A24, delay was for 1.3 to 5.5 hr at 22 C. At 15 C, however, *P. fluorescens* and *Alcaligenes faecalis* caused *S. aureus* to reach 5 x 10^6 cells per ml 2.2 to 4.4 hr sooner than when growing alone, when inoculum ratios were 1 : 100 and 1 : 1, and *Achromobacter xerosis* shortened the time 4.4 hr at a 1 : 100 ratio. Otherwise at 15 C delays were, for the most part, considerable, some for as long as 9 to 23 hr. There was no stimulation at 10 C, and

TABLE 2. TIME IN HOURS FOR *Staphylococcus aureus* TO REACH 5 x 10^6 CELLS PER ML WHEN GROWN WITH EFFECTOR ORGANISMS IN TRYPTICASE SOY BROTH AT 22, 15, AND 10 C, AND WITH INITIAL RATIO OF EFFECTOR TO COCCUS OF 1 : 100, 1 : 1, OR 100 : 1

Effector organism	22 C			15 C			10 C		
	1 : 100	1 : 1	100 : 1	1 : 100	1 : 1	100 : 1	1 : 100	1 : 1	100 : 1
P. aeruginosa	11.5	11.5	14.5	45.8	52.4	64.4	93.6	102.0	172.8
P. fluorescens	11.3	11.3	12.0	37.1	37.1	45.2	93.6	96.0	100.8
Pseudomonas A24	11.5	16.3	—[a]	61.1	—	—	175.2	—	—
Pseudomonas R39	12.5	12.5	15.5	43.6	51.8	57.8	93.6	96.0	109.2
A. viscolactis	13.0	13.0	14.0	50.2	53.5	56.1	91.2	100.8	121.0
A. faecalis	12.0	12.0	12.0	39.3	37.1	43.1	81.6	92.4	115.2
F. capsulatum	13.5	13.5	13.5	41.5	41.5	50.2	87.6	87.6	103.2
A. xerosis	12.0	12.0	13.0	37.1	42.5	49.1	96.0	96.0	112.8
S. aureus		10.0			41.5			81.6	

[a]— = a population of 5 x 10^6 *S. aureus* per ml was not attained.

S. aureus usually was delayed from reaching hazardous numbers for a number of hours, especially when ratio of effectors to staphylococci was 1 : 1 or 100 : 1.

A comparison of the eight selected cultures at 10, 15, 18, 22, 25, and 30 C, with an initial ratio of effector to coccus of 1 : 1, showed that most inhibition of the coccus was obtained at 15 C with the pseudomonads, except for *P. fluorescens* which was best at 25 C. *Alcaligenes* cultures had the greatest effect at 18 to 22 C; *Flavobacterium capsulatum* was best at 22 C, and *Achromobacter xerosis* at 10 or 15 C.

A comparison of strains of *Pseudomonas* species showed that three of five other strains of *P. mildenbergii* or *P. convexa* resembled strain A24 in being markedly inhibitory toward the staphylococcus at 18 and 25 C; however, they were not inhibitory at 30 C. All five strains of *P. striata* were markedly inhibitory at 18 and 25 C, but not at 30 C.

A comparison of the effect of the eight selected cultures on the enterotoxigenic *S. aureus* 196E and the nonenterotoxigenic *S. aureus* W-1, made at 30 C with an effector to staphylococcus ratio in the inoculum of 1 : 1, showed mostly general agreement with the two strains, except with *A. xerosis* and *F. capsulatum* which stimulated strain W-1 more than strain 196E.

DISCUSSION

The representatives of the genus *Pseudomonas* and the family *Achromobacteraceae* tested were, in general, less inhibitory toward *S. aureus* than the coliform bacteria were shown to be by DiGiacinto and Frazier *(1)*, and than most of the lactic acid bacteria as reported by Kao and Frazier *(3)*. This is contrary to expectations, because the psychrophiles, and in particular low-temperature pseudomonads, are believed to play an important role in the repression of *S. aureus* at temperatures below those of the room, and to cause changes in foods that make them unfit to eat before numbers of staphylococci become significant. Of the 17 *Pseudomonas* cultures isolated from beef, fish, milk, and chicken pot pie, 14 came from these foods incubated at 15 C or below, and 10 from foods held at 5 C. All 19 of the pseudomonads in 1 : 1 ratio inhibited *S. aureus* within 8 hr at 18 and 25 C, but only 12 kept numbers below 5 x 10^6 cocci per ml at 25 C, and only 5 within 24 hr at 18 C. Only one repressed *S. aureus* that much in 8 hr at 30 C. The commonly found *P. fluorescens* was not an effective inhibitor, nor were the *Achromobacteraceae* tested. Of course if the foods are held at near the minimal temperature for growth of *S. aureus*, the psychrophilic pseudomonads usually will be able to repress the staphylococcus.

Tests should be made on the special types of *Pseudomonas* and *Achromobacter* reported by Lerke, Adams, and Farber *(4)* to be concerned with the spoilage of fish.

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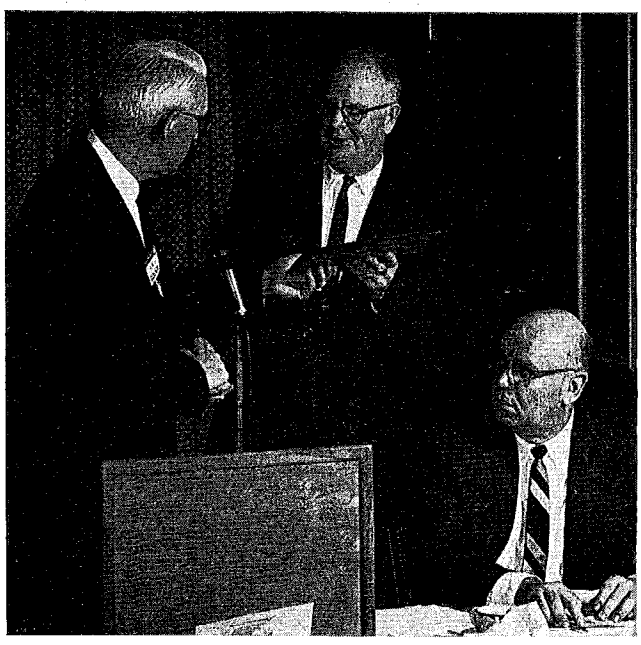
ASSOCIATION AFFAIRS

RAY B. WATTS HONORED BY OHIO STATE UNIVERSITY

Ray B. Watts, Chief, Division of Sanitation, Ohio Department of Health, has been presented the Merit Award In Milk Sanitation by the Ohio State University Department of Dairy Technology.

Ray Watts was honored in recognition of "his outstanding contributions to Public Health and to Milk Sanitation in Ohio, for engendering the spirit of cooperation and understanding between all facets of the Dairy Industry in respect to milk control needs, and for providing leadership in educational programs designed to improve the professional status of the Sanitarian." The Award was presented by Dr. I. A. Gould, Chairman of the Department of Dairy Technology, at the 33rd Annual Dairy Industry Conference Banquet, February 9.

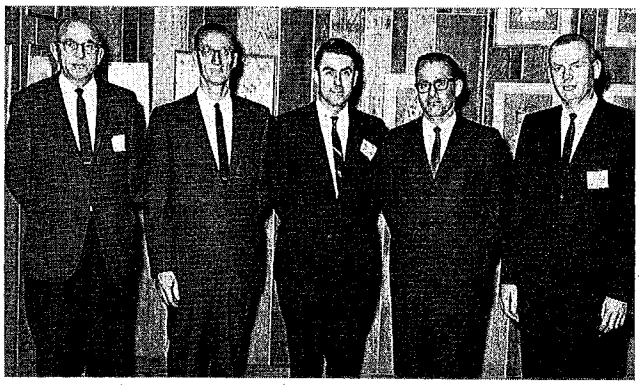


Ray B. Watts is presented the Merit Award in Milk Sanitation by Dr. I. A. Gould.

A native of Connecticut, Ray Watts has been engaged in milk and food sanitation work in Ohio since 1939. In 1949, he became associated with the Ohio Department of Health and he was named Sanitarian in Charge of Milk and Food Protection in 1951. He assumed his present position with the Department in 1964.

DALE R. COOPER RECEIVES MERLE BAKER AWARD

Left to Right: Dr. Merle P. Baker; Dale R. Cooper; E. G. Haupt, President Iowa Milk Sanitarians Association.



1966 Officers of the Iowa Association of Milk Sanitarians, left to right: H. E. Hansen, Sec'y.-Treas.; E. H. Wegerman, Retiring President; E. G. Haupt, President; C. W. Yeager, Jr.; Vice-President; D. E. Hagedon, 2nd Vice President.