

AN ABSTRACT OF THE THESIS OF

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Microbial Spoilage of Shrimp

Abstract approved: \_\_\_\_\_  
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Pacific shrimp (Pandalus jordani) was washed repeatedly and the eluted bacteria were enumerated and identified. Selected isolates were tested for their adhesive properties.

Washing reduced the microbial load by 3.84 to 42.04%. The bacteria which most resisted wash-off were Staphylococcus and Pseudomonas spp. The easiest to wash off was Flavobacterium spp. In higher-count samples, Moraxella and/or Lactobacillus spp. washed off readily, but they still constituted large proportions of the residual bacteria on shrimp.

Adhesiveness, measured by hydrophobic interaction with octane, showed 43.3% change in absorbance by Staphylococcus spp., followed by 21.5% by Moraxella spp., and Arthrobacter spp. at 13.5%. Pseudomonas spp. showed only 5.7% change in absorption.

Attachment, measured by hanging glass cover slips in broth, however, showed Pseudomonas and Staphylococcus spp. to have the greatest ability to adhere, with 0.47 and 0.46% attachment, respectively. Moraxella spp. showed the least ability to adhere to glass (.02%),

followed by Lactobacillus spp. at 0.11%. Arthrobacter and Flavobacterium spp. adhered at 0.30 and 0.37% levels, respectively.

Attachment of Pseudomonas spp. to glass was the least affected by media composition, temperature, or presence of a surface-active agent (sodium hexametaphosphate).

Staphylococcus spp., on the other hand, attached most strongly under optimum growth conditions but were most affected by varying growth conditions, temperature, and presence of a metabolic inhibitor (sodium hexametaphosphate).

This indicates that the adhesive ability of Staphylococcus spp. is directly related to its metabolic activity, while Pseudomonas spp. is less sensitive to changes in metabolism and may depend on motility for adhesion.

Bacteria that could adhere strongly on solid surfaces (Pseudomonas and Staphylococcus spp.) tend to be found in greater proportions and, hence, contribute more to the spoilage of shrimp.

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Its Relationship to Microbial Spoilage  
of Shrimp

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HETEROTROPHIC BACTERIA ASSOCIATED WITH  
A FEED ALGAE FOR OYSTER LARVAE

LITERATURE REVIEW

Microbiology of Pacific Shrimp

The Pacific shrimp (Pandalus jordani) is one of the major seafood products of the Pacific Northwest. Shrimp, like most fresh seafoods, are highly susceptible to microbial attack. The vast majority of seafood spoilage is due to the growth and metabolic activity of psychotrophic bacteria (Herbert et al., 1971). The obvious conclusion which can be drawn from this statement is that reducing the initial bacterial load and inhibiting subsequent microbial multiplication would lead to slower seafood deterioration.

Lee and Pfeifer (1977) studied the microbial population of Pacific shrimp (Pandalus jordani) at each phase of processing: from landing to shipment to market. They noted that at the completion of processing the microbial level was  $3.3 \times 10^4$ /g. The predominant microorganisms at landing were Moraxella, Pseudomonas, Acinetobacter, Arthrobacter, and Flavobacterium-Cytophaga spp. The blanching and peeling process tended to selectively eliminate Moraxella spp.

Analyses of the growth characteristics and heat sensitivity of the bacteria associated with shrimp supported the conclusion that the presence of Arthrobacter and Acinetobacter spp. in peeled shrimp might indicate inadequate cleaning of raw shrimp or a shorter

blanching time. The presence of Moraxella and Flavobacterium spp. would indicate the degree of secondary contamination, while the presence of Pseudomonas spp. would indicate the shelf age of shrimp.

These experiments demonstrated several factors that influence the composition of shrimp microflora. Another factor that would determine the microbial composition of food is the ability of certain bacteria to associate themselves more firmly to a food surface. The attachment of microorganisms to a surface is termed bacterial adhesion (Costerton et al., 1978). A better understanding of the forces involved in the attachment on and detachment from the surfaces of a food product would be useful in designing techniques to reduce numbers of bacteria on food surfaces. To maintain them at reduced levels could improve shelf life and, possibly, reduce public health hazards (Butler et al., 1979).

#### Nature of Bacterial Adhesion

The attachment of bacteria to both solid and inanimate surfaces is known to be a widespread phenomenon in nature (Butler et al., 1979). In fact, the adhesion of bacteria to animal cells is believed to be the first step in microbial infection. Microbial attachment to food surfaces is also believed to be one of the initial events in degradation of foods (Firstenberg-Eden, 1981).

Ofek and Beachey (1980) stated that if bacteria failed to adhere to the surface they would be simply swept away by the fluids in the surrounding media, whether the environment be body fluids in a mammalian system or the aqueous environment of a marine ecosystem.

The mechanisms involved in bacterial adhesion are exceedingly complex. For example, Jones (1981) demonstrated that Vibrio cholera infections of the gastro-intestinal tract involved bacterial motility, chemotactic attraction, penetration of the mucous gel on the intestinal pili, adhesion to receptors in the mucous gel, chemotaxis into deeper intervillous spaces, adhesion to the epithelial surface and ultimate toxin production.

Bacterial adherence is known to be a three-stage process: the first stage is the reversible attachment of the bacteria to the surface, at which time the bacteria are susceptible to wash-off; while, after a period of several hours, a more firm attachment occurs (Zobell, 1943). Marshall et al. (1971) termed the first two phases "reversible" and "irreversible" sorption, respectively. The third phase is in actuality an outgrowth of the irreversible sorption phase, during which time the attached cells multiply and are joined by additional attaching cells, which then leads to micro-colony formation (Fletcher, 1981).

Reversible sorption to the surface was found by Marshall et al. (1971) to be an instantaneous interaction between the bacteria and the surface to which they were adsorbed. This event was partly dependent on the electrolyte concentration in the media. They suggested that this could be explained in terms of the Derjaguin-Landau and Verwey-Overbeek theory (DLVO). This theory involves an estimation of the magnitude, and variation with interparticle distance, of the London-Van der Waals attractive forces between surfaces and electrical repulsive energies resulting from the

overlapping ionic atmospheres around the surfaces. Tadros (1980) summarized three forces responsible for particle adhesion. These three forces are strong interactions (covalent and ionic), the relatively weak interactions (Van der Waals forces) and those of intermediate energy (hydrogen bonds, electronic charge transfer bonds and electrostatic double-layer forces).

Thus, the reversible sorption of bacteria to a solid surface can be thought of as a complex group of interactions between the bacterial cell wall components which involve hydrogen bonding, Van der Waals forces, hydrophobicity, and electrostatic interactions.

The reversible phase is followed by an irreversible sorption which is time-dependent (Marshall et al., 1971). This is thought to be mediated by the formation of polymers which act as a biological cement gluing the bacterial cell to the surface (Costerton et al., 1978). Electron-microscopy has provided the necessary proof that these long polymers are a principal mechanism in bacterial attachment (Marshall et al., 1971; Fletcher and Floodgate, 1973; Costerton et al., 1978; Schwach and Zottola, 1982).

Costerton and Irwin (1981) call this polymer cement the bacterial glycocalyx, which can be defined as any polymer that is formed outside the cell wall. Sutherland (1980) studied the chemical composition of the glycocalyx and have shown that it may be either homopolymers or complex heteropolymers, made up of varying monosaccharides. However, neutral hexoses, 6-deoxyhexoses, polyols, uronic acids and amino sugars predominate. Sutherland (1980) noted that the chemical composition of exopolysaccharides were

genus- or strain-dependent. Different strains of Streptococcus pneumoniae produced differently-structured polymers while others, such as all strains of Pseudomonas aeruginosa, produced polysaccharides of similar chemical composition.

Glycocalyx formation mediates the formation of microcolonies. A bacteria, once adherent to a surface, surrounds itself with additional exopolysaccharide and then replicates within this environment. This protective polysaccharide shell around the bacterial colony offers significantly greater protection from the effects of heat, antibiotics, antibodies and various cleaning and bactericidal agents (Costerton and Irwin, 1981; Firstenberg-Eden, 1981). Much of the research done on bacterial adhesion has been to elucidate the relationship of adhesiveness and pathogenicity. One area of particular interest has been the relationship between bacterial adhesion and production of dental caries by bacteria indigenous to the oral cavity. Hamada and Slade (1980) state that Streptococcus mutans of human and animal origin produced dental caries in various experimental animals, if the animals were fed a high sucrose diet. The two virulence factors involved are the ability of the organism to adhere to the dental surface and subsequent acid production when fermentable sugars were available. The adherence of S. mutans to a surface depends mainly on its ability to utilize sucrose for the synthesis of water-soluble glucans. Germaine and Schachtele (1976) and Staat et al. (1980) showed the polysaccharide adhesions produced by S. mutans to be alpha 1,3 and alpha 1,6 branched glucans produced by a group of glucosyltransferases.

Hamada and Slade (1980) conducted a series of studies involving bacterial adherence to glass slides to quantify the in vitro adherence of S. mutans and other species of oral bacteria. Fletcher and Floodgate (1973), Firstenberg-Eden et al. (1979), and Schwach and Zottola (1982), have demonstrated that the glycocalyx production was not limited to oral bacteria. Fletcher and Floodgate (1973) showed that marine organisms commonly produce an acidic polysaccharide, which was used in the firm adhesion of these organisms to surfaces in the aquatic environment. Firstenberg-Eden et al. (1979) showed that Staphylococcus aureus produced a polymeric substance which aided in the adhesion of this bacteria to cow teats.

Schwach and Zottola (1982) used scanning electron microscopy (SEM) to examine the surface of stew meat. They were able to visualize the attachment fibrils produced by various bacteria found on the meat surface. They also showed the adhesion process of Pseudomonas fragi when it came in contact with the beef stew meat. Thirdly, they demonstrated that, when the meat surface containing the adherent bacteria was placed in contact with an inert surface (in this case stainless steel), some of the bacteria were transferred to the inert surface. Transfer of microorganisms from the beef surface to the contacting stainless steel occurred in all cases tested. In some, microorganisms were observed to attach to the stainless steel surface with their polymer fibrils in less than 4 h.

### Impact of Fimbriae and Motility on Adherence

Pearce and Buchanan (1980) stated that fimbriae, which are smaller and more numerous than flagella, are known to occur in many gram-negative genera but are not found in gram-positive bacteria, with the exception of Corynebacteria spp., played a significant role in both specific and non-specific aspects of bacterial attachment. Wadstrom et al. (1978) evaluated the hydrophobic nature of the K88 and K99 antigens (fimbriae) of Escherichia coli and concluded that this provided evidence for the suspected role of hydrophobic interaction in the adhesive properties of certain strains of E. coli associated with diarrheal disease.

Fimbriae are also known to participate in specific binding of bacteria to a substrate, a topic which will be discussed later in more detail. While it appears that the fimbriae play a direct role in adsorption of microbes to surfaces, bacterial motility seems to have a more subtle role in the association of bacteria to surfaces.

Iijima et al. (1981) found flagella in Vibrio parahaemolyticus to be an accessory factor in adhesion, in that the adherence of the non-flagellated species was weak or non-existent; but some flagellated cells did not adhere. This observation supports Fletcher's (1980) conclusion that flagellation and the resultant motility act in guiding the bacteria to the surface so that other adhesive factors may come into play. She stated that this was simply a result of increasing the statistical chance of a bacterium encountering a surface. Motility may also be important in

overcoming the electrostatic repulsion between the surface and the bacterium, increasing the force with which the bacterium encounters the surface.

### Specific and Non-Specific Adhesion

The specificity of the adhesive nature of a particular bacteria is of great interest, especially in the study of microbial pathogenicity. This selectiveness has been postulated by Costerton et al. (1978) to be a result of the interactions between the bacterial glycocalyx and the animal cellular structure, whose specificity comes from the varying chemical makeup of the bacterial exopolysaccharide itself. Walker and Nagy (1980) have identified a number of specific adhesions associated with the bacterial cell wall. These were K or capsular antigens and were proteinaceous or polysaccharide in composition. In proteinaceous attachment, the fimbriae (pili) attached to specific receptors on the host cell.

In specific polysaccharide adhesion, the glycocalyx of the bacteria interacts with similar structures from the animal cell. The sugar units at the ends of the fibres can interact directly through polar attraction, negatively charged sugars being linked by divalent cations or, alternatively, a bridge may be supplied by a lectin, a protein which carries specific receptor sites for which the fibrous sugar units have an affinity. Such interactions may be very specific in nature (Costerton et al., 1978; Walker and Nagy, 1980).



The specificity of attachment has been studied by numerous researchers. Aronson et al. (1979) successfully blocked the binding of Escherichia coli to the urinary tract of mice with methyl alpha-d-mannopyranoside (A-MM). This gave support to the theory that the binding of E. coli to the epithelial cells of the mucosa is mediated by a mannose-specific, lectin-like substance present on the bacterial surface, which binds to a mannose-like residue site on the mucosal cell (Fein, 1981). The A-MM either specifically inhibited bacterial adherence or displaced adherent bacteria from the epithelial cell surface, most probably blocking the mannose binding of the bacteria. To further illustrate the specificity of the reaction, Aronson et al. (1979) found no inhibition of the attachment of Proteus mirabilis when tested under the same experimental conditions. Chitnis et al. (1982) demonstrated that, in Vibrio cholerae, the specificity for the epithelial lining of the gut was derived from a relationship between the bacterial somatic antigen and the intestinal mucous membrane.

While specific adhesion of bacteria is of great importance in the pathogenicity of bacteria, no study was apparently conducted which relates the effect of the specific type of adhesion to adherence to food products. Since most food products are composed of cellular substances, it seems likely that there must be some type of specific receptors for some of the indigenous bacteria associated with food.

Much work has been done in the area of the non-specific adhesion of bacteria to varying surfaces. Marshall (1976) defined

non-specific interactions as those associations that did not involve any specific bonding between complementary molecular structures on the microorganism and the interface. He stated that most associations between microorganisms and interfaces in aquatic ecosystems are of the non-specific type.

Fletcher (1980) suggested that marine organisms first become associated with the surface to which they are to become attached by the reversible sorption process--a mechanism previously stated to be mediated by Van der Waals forces, electrical repulsive energies and hydrophobic interactions. This is followed, in most instances, by the production of fibrous polysaccharides by the bacteria. These react with the electrostatic structure of the surface to glue the bacteria irreversibly to the surface. The fact of critical importance here is that the glycocalyx does not react with any specific receptor site on the surface but reacts in a more non-specific manner with the surface in general.

#### Effects of Environment on Adherence

Marshall et al. (1971) stated that the concentration of dissolved electrolytes had a direct effect on the attachment of marine Pseudomonads. For example, he inhibited their attachment by omitting the divalent cations  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  from the growth media. This electrolyte deficiency also had an inhibitory effect on bacterial growth.

The effects of temperature on bacterial physiology are complex, making it impossible to make a general prediction about the effect

of temperature on the production of polymeric adhesives (Fletcher, 1980). Experimental work with marine bacteria (Fletcher, 1977) has shown an increase in attachment with an increase in temperature. However, Sutherland (1979) indicated that low temperatures could favor polysaccharide synthesis. The specific effect of temperature on the mechanism of bacterial adherence is, therefore, not clearly defined at this time. However, temperature does indeed have a definite effect on adhesion.

Polyphosphates are currently used widely in the shrimp processing industry to improve product yield. Therefore, the effect of added phosphate on bacterial adhesion is of interest. Numerous researchers have studied the effect of phosphates on microbiological growth, but the effect of polyphosphates on bacterial adhesion is apparently unknown.

Chen et al. (1973) reported that chicken soaked in 3.0% polyphosphate resulted in controlled growth of gram-positive cocci but did not reduce the growth of gram-negative rods. Petchsing (1982) found that the inhibition of bacterial growth by phosphates depended on the initial number and type of bacteria. This was found to be particularly important in the case of Pseudomonas, Moraxella, Lactobacillus, and Corynebacter spp. She found that, if present in phosphate-treated seafoods, Pseudomonas spp. and Moraxella spp. quickly became predominant. Lactobacillus spp. and Corynebacter spp. became the more predominant when Pseudomonas and Moraxella spp. were absent.

## MATERIALS AND METHODS

### Sampling Procedure

The shrimp (Pandalus jordani) used in this investigation were purchased from five grocery stores in Corvallis, Oregon. These samples were to represent different stages of microbial contamination and/or spoilage.

### Microbial Enumeration and Isolation

A 10 g. portion from each sample was aseptically weighed and placed in a sterile Buchner funnel. The shrimp were placed as a mono-layer on the surface of the Buchner funnel; then 10 ml of sterile distilled water was poured evenly over the shrimp, and the effluent was collected in a sterile 20 mm culture tube. This process was repeated five times. Following the fifth wash, the shrimp were removed and blended in an osterizer jar with 20 ml of sterile 0.067 M Butterfields phosphate buffer. Microbial counts of the five washings and the blended shrimp sample were made by spread-plating proper dilutions on tryptone peptone extract (TPE) agar and counting the viable colonies after 48 H. incubation in air at 25° C. These plates also served as the source of microbial isolates.

### Identification of Isolates

The replica plating technique of Lee and Pfeifer (1975) was used to identify the majority of the isolates to the genus level. This scheme involved the use of a series of selective and differential media. Gram reaction and cell morphology were also utilized in identification. Figure 1 schematically describes the identification scheme.

### Affinity to Hydrocarbons

Nine predominant bacterial types isolated from the shrimp were selected. These were Pseudomonas spp. (types I, II and III), Moraxella spp., Flavobacterium spp., Lactobacillus spp., Arthrobacter spp., Corynebacterium spp. and Staphylococcus spp.

Cells were grown in TPE broth for two days at 25° C. Five different isolates from each genera were tested. After this period, the cells were centrifuged at 10,000 x G for 15 min. The resultant pellet was washed by resuspending and centrifuging in 20 ml of 0.067 M Butterfields phosphate buffer. This was repeated twice. The final cell pellet was resuspended in phosphate buffer to give an absorbance reading of 0.3 at 400 nm (Bausch and Lomb, Spectronic 20). One ml of octane (J. T. Baker Co., reagent grade) was added to this suspension. After incubating for 10 min. at 25° C, the cell suspension with octane was mixed in a vortex mixer (Scientific Products) at the maximum speed for two min. The tubes were then allowed to stand at room temperature for 30 min. to permit the

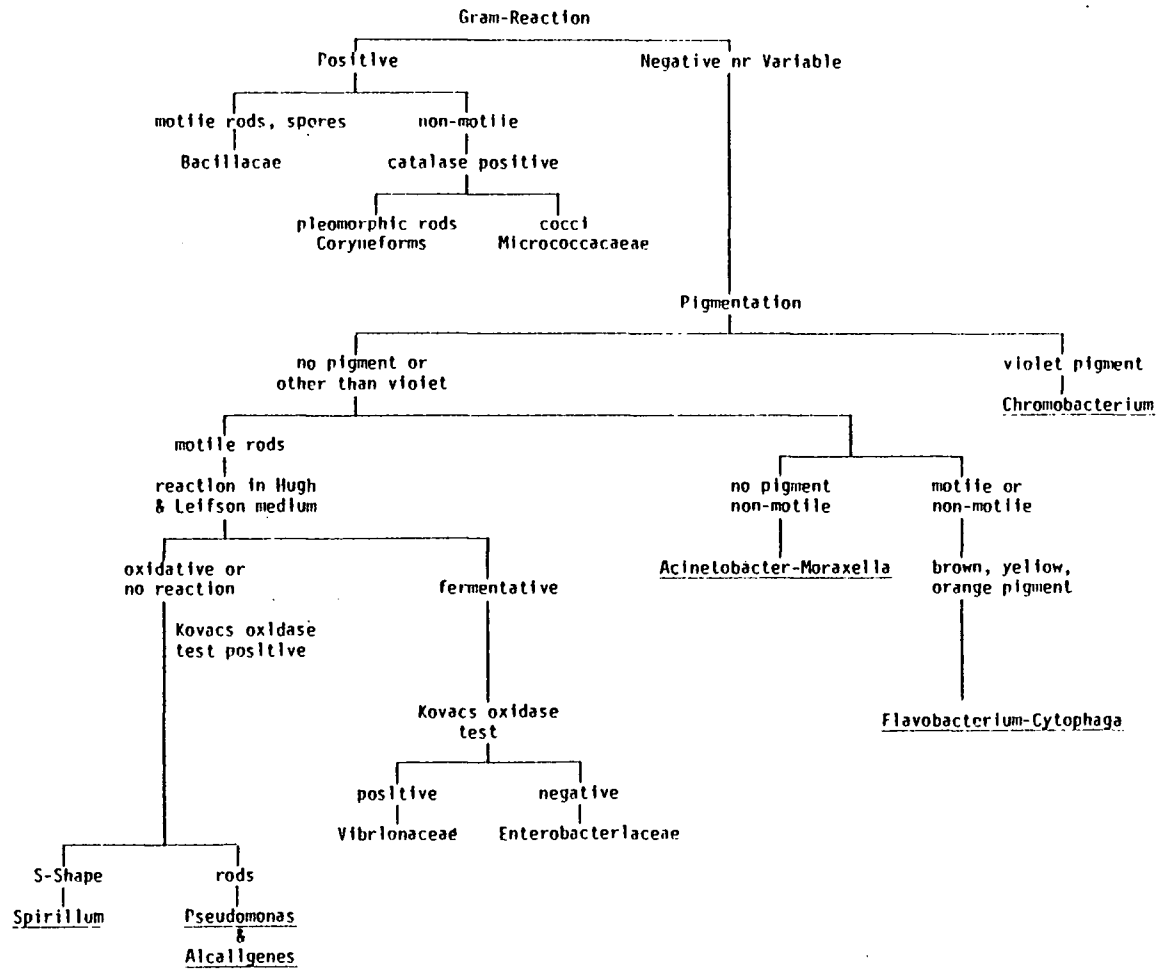


Figure 1. Identification Scheme for Bacteria of Seafood Origin.

aqueous phase to separate completely from the hydrocarbon phase. Complete separation was verified by the absorbance reading of the phosphate control which returned to a zero reading at 400 nm. Once the phases had completely separated, a 2-3 ml aliquot of the aqueous phase was carefully removed with a Pasteur pipette and the absorbance at 400 nm was recorded. This measured the density of cells not absorbed into the hydrocarbon phase.

#### Adherence of Bacteria to Glass Surface

The same isolates studied for affinity to hydrocarbon were examined for their ability to adhere to glass cover slips. An acid-cleaned 18 mm glass cover slip was placed at the bottom of a 20 mm tube. A 10 ml solution of Difco proteose peptone broth plus 0.025 g per liter of Difco yeast extract was added. Five such tubes were prepared for each isolate and autoclaved. Microorganisms were initially grown for 48 h in TPE broth at 25° C, and 0.1 ml of bacterial suspension was added to each of five tubes. At various intervals, for up to 48 h, one of the test tubes for each bacterial type was removed from the incubator, the media decanted, and the cover slip carefully removed with sterile forceps and gently washed in sterile 0.067 M Butterfields phosphate buffer to remove loosely adhered bacteria. The cover slips were then mounted on clean glass slides and the top of each cover slip was carefully wiped to remove bacteria attached to the top side of the cover slip. The number of bacteria adhering per cm<sup>2</sup> of each slide was calculated by multiplying the average number adhering in 10 randomly-selected

fields by a conversion factor which gave results in number per  $\text{cm}^2$ . The conversion factor was derived as follows: radius of one phase contrast field equals .125mm (as measured by an American Optical stage micrometer) divided by two. The product was then squared and multiplied by pi or  $(0.125/2)^2 \times \pi/100 = \text{area of a field expressed in } \text{cm}^2$ . The reciprocal of this value equaled the conversion factor or the number of fields per  $\text{cm}^2$ .

#### Adhesion to a Glass Surface During Growth

The same basic procedure as described for adhesion study was followed. Rather than using proteose peptone with added yeast extract, TPE broth was used to stimulate the growth of cells. Additionally, initial cell concentration was standardized using the standard plate count (SPC) and direct count procedures, as described by Gilliland et al. (1976) and by Pusch et al. (1976). The SPC and direct counts were correlated with the optical density of culture suspension at 420 nm. This experiment, then, measured the ability of each organism to adhere to the glass surface during growth.

#### Effect of Temperature on Bacterial Adhesion

The basic procedure followed was the same as described above, except that in this instance the cultures were incubated at 15, 25 and 37° C, respectively, in TPE over a 24 h period. The cover slips were removed at various intervals and adhering bacteria were counted. Six bacterial strains selected for this experiment were:



Moraxella spp., Lactobacillus spp., Pseudomonas spp. (types I and II), Flavobacterium spp., and Arthrobacter spp.

#### Effect of Polyphosphates on Bacterial Adhesion

Each microorganism was grown in TPE at 25° C for 2 days, following which equal concentrations of each organism were transferred to culture tubes containing glass cover slips and TPE with 6% sodium hexametaphosphate. As before, the tubes were incubated at 25° C, and adhesion was evaluated at various times over a 24 h period. Four bacterial groups included in this study were: Lactobacillus spp., Staphylococcus spp., Pseudomonas spp. (type III), and Arthrobacter spp.

## RESULTS AND DISCUSSION

Bacteria Associated with Pacific Shrimp

The total numbers of viable, aerobic bacteria associated with the five shrimp samples are presented in Table 1, which is shown by count of each of the five washings and the count remaining on the blended shrimp. The total bacterial count, which is the sum of the six counts, is also shown for each sample.

The total bacterial counts ranged from  $5.12 \times 10^3$  to  $9.28 \times 10^7$  per g, giving an average of  $3.66 \times 10^7$  per g with a sample standard deviation of  $4.70 \times 10^7$  per g. These counts, with the exception of sample E, were significantly (1,000 ~ 10,000 times) greater than the counts reported by Lee and Pfeifer (1977) for Pacific shrimp obtained at the dock side. The higher count could have resulted from either contamination during processing or might have been the result of microbial growth during storage. There is also the possibility that the high-count samples were improperly handled in the retail market. Based on this analysis, sample E is probably the freshest sample and/or the one most carefully handled during processing and subsequent marketing.

Table 2 shows the percent of the total bacterial load which was washed off at each washing. The cumulative percentage of the population which washed off is also presented, as well as the percent remaining on the shrimp after five washings.

Table 1. Microbial load of shrimp wash water.

Treatment	Count per ml or g				
	Sample				
	A	B	C	D	E
First wash	$2.00 \times 10^6$	$3.58 \times 10^5$	$1.82 \times 10^5$	$1.13 \times 10^4$	$1.60 \times 10^3$
Second wash	$2.76 \times 10^6$	$1.20 \times 10^6$	$6.40 \times 10^5$	$1.63 \times 10^4$	$2.15 \times 10^2$
Third wash	$3.30 \times 10^6$	$1.53 \times 10^6$	$4.81 \times 10^5$	$1.28 \times 10^4$	$1.30 \times 10^2$
Fourth wash	$2.54 \times 10^6$	$1.02 \times 10^6$	$3.94 \times 10^5$	$1.14 \times 10^4$	$1.00 \times 10^2$
Fifth wash	$2.50 \times 10^6$	$1.65 \times 10^6$	$5.10 \times 10^5$	$8.90 \times 10^3$	$1.05 \times 10^2$
Washed shrimp	$6.99 \times 10^7$	$8.70 \times 10^7$	$3.60 \times 10^6$	$1.52 \times 10^6$	$2.97 \times 10^3$
Total	$8.30 \times 10^7$	$9.28 \times 10^7$	$5.81 \times 10^6$	$1.58 \times 10^6$	$5.12 \times 10^3$

Table 2. Percent microbial load removed at each washing.

Treatment	% Microorganism Washed Off				
	Sample				
	A	B	C	D	E
1st Wash	2.41	0.39	3.13	0.72	31.25
2nd Wash	3.32	1.29	11.02	1.03	4.20
3rd Wash	3.98	1.65	8.28	0.81	2.54
4th Wash	3.06	1.10	6.78	0.72	2.00
5th Wash	3.01	1.78	8.78	0.56	2.05
% Total Washed Off	15.78	6.21	37.99	3.84	42.04
% Remaining in Shrimp	84.22	93.79	62.01	96.16	57.96

It is of note that the lower-count samples (C and E) had the greatest proportion of bacteria being washed off, with totals of 37.99 and 42.04 percent, respectively. This is consistent with the notion (Fletcher, 1980) that bacteria adhere more strongly when allowed to grow over time, where they form microcolonies which provide a protective shell over the underlying organisms, thereby aiding the bacteria to resist wash-off. It might have been that many of the organisms found in low-count samples represented the recent contaminants which had insufficient time to firmly adhere to the shrimp surface (Firstenburg-Eden, 1981) and were, therefore, unable to withstand the relatively gentle washing process employed in this experiment.

#### Identities of Bacteria Associated with Shrimp

Bacteria isolated from the first wash, last wash, and the residual on the blended shrimp from each of the five samples were isolated and identified in accordance with the replica plating technique and proposed classification scheme (Lee and Pfeifer, 1975). Less than 10% of the bacterial population could not be classified by this scheme; therefore, no attempt was made to further classify them. Tables 3 through 7 show the microbial population distribution expressed as a percent of each genus found in the sample. Twelve different bacterial groups were isolated from the five samples. They were: Pseudomonas spp. (types I, II, and III), Moraxella spp., Flavobacterium-Cytophaga spp., Micrococcus spp., Staphylococcus spp., Arthrobacter spp., Lactobacillus spp.,

Table 3. Distribution of bacterial types in shrimp sample A.

Microorganisms	Percent		
	First Wash	Third Wash	Shrimp <sup>(1)</sup>
<u>Pseudomonas</u> spp. (2)	2.13		12.68
Type I			
Type II			
Type III	2.13		12.68
<u>Moraxella</u> spp.	80.85	76.36	46.48
<u>Acinetobacter</u> spp.			2.82
<u>Flavobacterium</u> spp.	4.26	16.36	2.82
Unidentifiable Gram Neg. Rods			2.82
<u>Arthrobacter</u> spp.	12.77	5.45	9.86
<u>Staphylococcus</u> spp.			22.54
<u>Micrococcus</u> spp.	1.82		

(1) Residual left on shrimp after 5 washes.

(2) Total Pseudomonas spp.

Table 4. Distribution of bacterial types in shrimp sample B.

Microorganisms	Percent		
	First Wash	Fifth Wash	Shrimp <sup>(1)</sup>
<u>Pseudomonas</u> spp. <sup>(2)</sup>	4.24	8.24	7.93
Type I		1.03	
Type II	3.40	3.09	6.93
Type III	0.84	4.12	1.00
<u>Moraxella</u> spp.	1.70		7.92
Unidentifiable Gram Neg. Rods	6.78	1.03	7.92
<u>Lactobacillus</u> spp.	68.64	70.10	73.26
<u>Arthrobacter</u> spp.		2.06	1.00
<u>Corynebacterium</u> spp.	17.74	14.43	11.88
<u>Staphylococcus</u> spp.		1.03	1.00
Unidentifiable Gram Pos.		2.06	
Other unidentifiabes	0.84		1.00

(1) Residual left on shrimp after 5 washes.

(2) Total Pseudomonas spp.

Table 5. Distribution of bacterial types in shrimp sample C.

Microorganisms	Percent		
	First Wash	Fifth Wash	Shrimp <sup>(1)</sup>
<u>Pseudomonas</u> spp. <sup>(2)</sup>	13.64	8.49	15.12
Type I	3.03	8.49	5.81
Type II	6.06		2.33
Type III	4.55		6.98
<u>Moraxella</u> spp.	9.09	8.49	13.95
<u>Acinetobacter</u> spp.	6.06		4.65
<u>Flavobacterium</u> spp.	4.55	0.94	
Unidentifiable Gram Neg. Rods	9.09	5.66	5.81
<u>Bacillus</u> spp.			1.16
<u>Lactobacillus</u> spp.	1.52	7.55	3.49
<u>Arthrobacter</u> spp.	33.33	14.15	17.44
<u>Corynebacterium</u> spp.	22.73	52.83	34.88
<u>Staphylococcus</u> spp.		0.94	3.49
<u>Micrococcus</u> spp.		0.94	

(1) Residual left on shrimp after 5 washes.

(2) Total Pseudomonas spp.



Table 6. Distribution of bacterial types in shrimp sample D.

Microorganisms	Percent		
	First Wash	Fifth Wash	Shrimp <sup>(1)</sup>
<u>Pseudomonas</u> spp. <sup>(2)</sup>	26.13	20.69	20.69
Type I	12.50	12.64	12.64
Type II	6.94	1.15	3.45
Type III	6.94	6.90	4.60
<u>Moraxella</u> spp.	63.89	62.07	62.07
<u>Acinetobacter</u> spp.	1.39	1.15	1.15
<u>Flavobacterium</u> spp.	6.94	2.30	4.60
Unidentifiable Gram Neg. Rods	1.39		
<u>Lactobacillus</u> spp.		1.15	
<u>Arthrobacter</u> spp.		10.34	10.34
<u>Corynebacterium</u> spp.	5.56		
<u>Staphylococcus</u> spp.		1.15	
<u>Micrococcus</u> spp.	1.39		1.15
<u>Yeast</u>		1.15	

(1) Residual left on shrimp after 5 washes.

(2) Total Pseudomonas spp.

Table 7. Distribution of bacterial types in shrimp sample E.

Microorganisms	Percent		
	First Wash	Fifth Wash	Shrimp <sup>(1)</sup>
<u>Pseudomonas</u> spp. <sup>(2)</sup>	13.69	18.18	18.89
Type II	3.16		2.22
Type III	10.53	18.18	16.67
<u>Moraxella</u> spp.	23.16		7.78
<u>Acinetobacter</u> spp.	6.32	9.09	11.11
<u>Flavobacterium</u> spp.	3.16	4.55	14.14
Unidentifiable Gram Neg. Rods	6.32	9.09	1.11
<u>Lactobacillus</u> spp.	1.05		
<u>Arthrobacter</u> spp.	42.11	13.64	32.22
<u>Corynebacterium</u> spp.	1.05		1.11
<u>Staphylococcus</u> spp.	3.16	33.36	13.33
<u>Micrococcus</u> spp.		9.09	

(1) Residual left on shrimp after 5 washes.

(2) Total Pseudomonas spp.

Corynebacterium spp., Acinetobacter spp., and Bacillus spp. Sample D yielded 1.15 percent yeast.

#### Bacteria Isolated from Shrimp Wash

The identity of bacteria found in wash water were quite different among the five samples. In sample A, Moraxella and Arthrobacter spp. appeared to have washed off more readily. These two genera were also the predominant species. Staphylococcus and Pseudomonas spp. were found primarily in shrimp after washings and could represent the microbial groups that had adhered to the shrimp more strongly than the other bacteria.

Sample B yielded Lactobacillus spp., which were found both in washes and the blended shrimp. Corynebacterium spp. also was found in significant proportions. Petchsing (1982) found greater proportions of Lactobacillus and Corynebacterium spp. in phosphate-treated shrimp, where Moraxella and Pseudomonas spp. were selectively removed.

The microbial flora of sample C were dominated by Arthrobacter spp. and Corynebacterium spp. The Arthrobacter spp. did not appear to have been firmly attached, as they were washed off more readily.

While sample E had the lowest total microbial counts among the five, a significant proportion of its flora consisted of Staphylococcus spp., which were not easily removed by the washing process. Moraxella spp. clearly dominated the microbial flora of sample D, with approximately equal percentages of them found in both washes and in the final blended shrimp.

Numerous factors could have contributed to which bacteria would wash off more readily than others (Marshall, 1976). Some of these factors are: how long the microorganisms have been in contact with the seafood, their relative concentrations, how well each bacteria initially adsorbed to the shrimp surface and, ultimately, how well they irreversibly sorbed to the shrimp (Firstenberg-Eden, 1981).

In sample A, the predominant bacteria that washed off was Moraxella spp., followed by Flavobacterium and Arthrobacter spp. However, a significant proportion of Moraxella spp. still remained on the shrimp after washing. This would seem to indicate that Moraxella spp. would grow readily on shrimp and are a major contributor to seafood spoilage. Staphylococcus spp. and Pseudomonas spp., on the other hand, did not wash off readily at all but represented major proportions of the microbial load of the blended shrimp, i.e., 22.5% and 12.68%, respectively. This would indicate that these two groups had formed some type of adherent relationship with the shrimp flesh which resisted wash-off.

The bacterial flora of sample B showed a divergence from that of sample A. In this sample, Lactobacillus and Corynebacterium spp. showed clear predominance and were isolated in approximately equal proportions from two washes and from the shrimp.

The percentage distribution of each bacterial genus in each wash cannot be viewed as an independent entity but must be viewed as the percentage of the total population which washed off. For example, sample B contained 68.64% Lactobacillus spp., but only

6.21% of the total population came off in this wash. Therefore, despite high proportions of Lactobacillus spp. found in the wash water, Lactobacillus spp. seemed to have adhered fairly firmly to the shrimp. On the other hand, in sample E, where 42.04% of the total microbial population had washed off, the bacterial genera which remained attached to the shrimp can be considered to have adhered well, while those which washed off would have had adhered poorly. This is particularly true of the bacteria which came off in the first wash. In the instances where the total percentage which washed off was low and a particular group had dropped significantly, it can be said that this microorganism probably had a lesser affinity for the shrimp surface. This was the case for Moraxella spp. in sample A, where the percentage of Moraxella spp. dropped from 78% in the washes to only 46% on the residual shrimp meat. In sample D, where only 3.84% of the population washed off and the Moraxella spp. concentration remained the same, the apparent anomaly can be explained as, possibly, that the Moraxella spp. had been associated with the shrimp for a longer period of time and were able to form adherent colonies.

In summary, Staphylococcus and Pseudomonas spp. appeared to adhere most strongly to the shrimp, regardless of their initial concentrations. Lactobacillus and Arthrobacter spp. seem to adhere less strongly than Staphylococcus or Pseudomonas spp. but seem to adhere more strongly than Moraxella spp.

The purpose of this experiment was to rank bacteria isolated from shrimp according to their abilities to adhere to the shrimp

flesh. Although crude, the data provided us with sufficient guidance in selecting groups of bacteria to be tested in the following, more specific, experiments.

#### Affinity of Bacteria to Hydrocarbon

Ability of a bacterium to attach to a solid surface can be measured by its hydrophobicity (Rosenberg et al., 1980). This measures the ability of the organism to become initially associated with the surface to which it may ultimately become irreversibly adherent (Marshall et al., 1971). Nine genera isolated from shrimp were tested for their ability to adhere to the hydrocarbon octane; the results are shown in Table 8. The values expressed in this table are the percent change in absorbance, at 400 nm, from before addition to after addition of octane. Therefore, the greater the affinity the bacteria had for octane, the greater the percentage adsorbance change.

Octane was selected as the test hydrocarbon, based on the experiments of Johnson (1982) and Rosenberg et al. (1982), who tested various hydrocarbons such as P-xylene, pentane, hexane, and octane, and concluded that the most consistent results were obtained using octane. Staphylococcus spp. showed the strongest affinity for octane, with an average percentage change of 43.30%. This finding coincided with that of Rosenberg et al. (1980), where they found that Staphylococcus spp. strongly adhered to the hydrocarbon phase, even though it lacked the ability to degrade this substance. This result also explains the difficulty in washing

Table 8. Hydrocarbon affinity of shrimp isolates (adherence to octane measured by % absorbance change at 400 nm).

Microorganism	Average % Change	Standard Deviation
<u>Moraxella</u> spp.	21.50	7.40
<u>Lactobacillus</u> spp.	1.75	0.96
<u>Flavobacterium</u> spp.	0.40	0.89
<u>Arthrobacter</u> spp.	13.50	1.29
<u>Staphylococcus</u> spp.	43.30	4.20
<u>Pseudomonas</u> spp., Type I	6.00	2.60
<u>Pseudomonas</u> spp., Type II	7.20	3.60
<u>Pseudomonas</u> spp., Type III	3.80	2.80
<u>Corynebacterium</u> spp.	13.00	6.80

Staphylococcus spp. from the shrimp. The Staphylococcal cell wall is probably very hydrophobic in nature and would, therefore, have a greater tendency to remain associated with the relatively more polar shrimp surface than to be displaced by the very polar water molecules. Of the other bacterial isolates, none had showed as strong a hydrophobic tendency as did Staphylococcus spp.; but Moraxella spp. (at 21.5%) and Arthrobacter spp. (at 13.5%) were more strongly adsorbed to octane than the others. Flavobacterium spp. had virtually no affinity for octane at all. The hydrocarbon affinity data (Table 8) would aid in explaining why Flavobacterium spp. and Moraxella spp. were readily washed off, while Staphylococcus spp. remained strongly associated with the shrimp.

The data do not, however, explain the ability of the Pseudomonas spp. to remain associated with shrimp after washing. It seems that the adhesive ability of Pseudomonas spp. is not related to its hydrophobicity. Marshall et al. (1971) stated that no single factor is solely responsible for the adhesiveness of a microorganism to a solid surface. While hydrophobicity is a convenient measure of the adhesiveness of bacteria, it cannot measure all other mechanisms by which bacteria become associated with a surface.

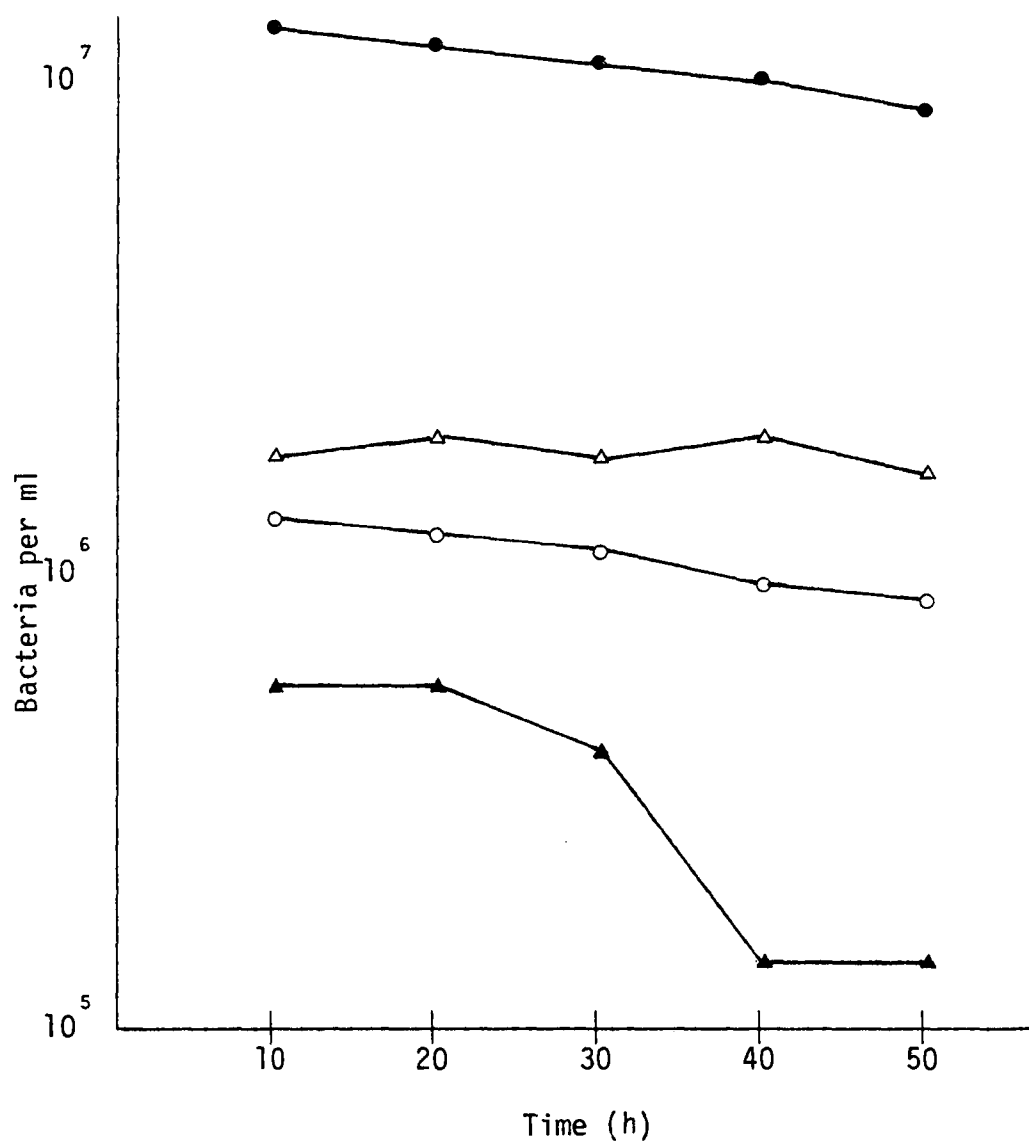
#### Adherence of Bacteria to Glass Surface

The hydrophobicity of an organism seems to reveal its ability to initiate adhesion. Other physical, chemical and metabolic factors also seem to play significant roles in adhesion. Another method utilized to study the ability of microorganisms to colonize



solid surfaces is the measurement of their capability in adhering to glass (Shiba and Taga, 1981). Glass is hydrophobic by nature; therefore, hydrophobic attraction would play a role in the initial association of a non-polar bacterium with the glass surface. However, using pure cultures and incubation times up to 24 h, some of these other factors involved in bacterial adhesion can be measured.

Marshall et al. (1971) stated that bacterial adhesion was a two-stage process with an initial adsorption to the surface followed, over time, by an irreversible sorption of the organism to the surface. Use of glass cover slips allows the study of the combined result of these two processes, presenting an ultimate test to measure the ability of a particular organism to adhere to a solid surface. This, however, has to be measured in the absence of microbial growth, as some bacteria tend to form cementing compounds during growth (Costerton et al., 1978). The medium selected for this study was proteose peptone-yeast extract, which maintained viability but not growth of the four test bacteria groups for up to 24 h (Figure 2). Table 9 shows the ratio of bacteria adhered versus those remaining in the medium, measured during a 48-h period. Fletcher (1980) reported that in order for bacteria to produce adhesive polysaccharides, they have to be actively growing. The adhesion of Lactobacillus spp., Staphylococcus spp., Pseudomonas spp., and Arthrobacter spp., presented in Table 9, therefore seem to reveal the ability of the organisms to adhere through factors such as hydrophobicity, Van der Waals forces, and electrostatic attraction (Firstenberg-Eden, 1981).



- (○) Lactobacillus spp.
- (●) Staphylococcus spp.
- (△) Pseudomonas, type I
- (▲) Arthrobacter spp.

Figure 2. Viable Count of Bacteria in Proteose Peptone Yeast Extract Test Medium.

Table 9. Ratio of bacteria adhered to glass surface vs. remaining in proteose peptone yeast extract medium.

Microorganism	Adhered/Unadhered x 100						
	Hour						
	7	12	24	28	48	$\bar{x}$	s.d.
<u>Lactobacillus</u> spp.	0.025	0.08	0.06	0.108	0.105	0.075	0.03
<u>Staphylococcus</u> spp.	0.24	0.06	0.07	0.05	0.05	0.094	0.08
<u>Pseudomonas</u> spp. Type I	0.30	0.18	0.15	0.25	0.35	0.246	0.083
<u>Arthrobacter</u> spp.	0.10	0.07	0.03	0.09	0.08	0.074	0.03

The experiments in the poor-growth medium may indicate the value of motility to adhesion. Since none of the organisms tested grew well in the medium, it can be assumed that their metabolic processes were functioning at a reduced level. Phase contrast microscopy, however, showed that the Pseudomonas spp. retained their characteristic motility. It is likely, therefore, that the relatively good adhesion of the Pseudomonas spp. was made possible by their motility, by which they were able to attain closer proximity to the glass surface, overcoming the electrostatic repulsive forces (Fletcher, 1980). Staphylococcus spp., on the other hand, was probably not able to produce the polysaccharides necessary for its firm adherence to the surface due to its slowed metabolic processes, and was only held to the surface through hydrophobic interactions which were not sufficient to withstand rinsing with phosphate buffer.

The same experiment was conducted in TPE broth. This medium supported the growth of all test organisms well and, therefore, it could allow the production of extracellular adhesives. Table 10 shows the ratio of adhesive bacteria per square centimeter of the glass cover slip divided by the concentration of the bacteria per ml at varying incubation times, using an incubation temperature of 25° C.

It can easily be seen that Pseudomonas spp. (type I) had the greatest over-all ability to adhere to glass, followed by Staphylococcus spp., Pseudomonas spp. (type II), Pseudomonas spp. (type III), and Arthrobacter spp. Lactobacillus and Moraxella spp. showed

Table 10. Ratio of bacteria adhered to glass surface vs. remaining in tryptone peptone extract medium (TPE).

Microorganism	Adhered/unadhered x 100					
	Hour					
	5	9	12	24	$\bar{x}$	s.d.
<u>Moraxella</u> spp.	0.004	0.007	0.03	0.04	0.02	0.02
<u>Staphylococcus</u> spp.	0.30	0.40	0.49	0.65	0.46	0.15
<u>Pseudomonas</u> spp. Type I	0.60	0.66	0.77	0.80	0.58	0.20
<u>Pseudomonas</u> spp. Type II	0.33	0.35	0.47	0.58	0.43	0.10
<u>Pseudomonas</u> spp. Type III	0.18	0.33	0.49	0.60	0.40	0.18
<u>Arthrobacter</u> spp.	0.25	0.20	0.32	0.45	0.30	0.11
<u>Flavobacter</u> spp.	0.23	0.48	0.40	0.40	0.37	0.11
<u>Lactobacillus</u> spp.	0.03	0.08	0.11	0.20	0.11	0.07

little propensity to adhere themselves to the glass surface. Some of the variation of the data, as seen in Table 10, can be explained by the fact that microorganisms both adsorb and desorb from surfaces over time, and do so at varying rates (Fletcher, 1980).

#### Effect of Temperature on Bacterial Adhesion to Glass

Temperature has a great impact on the growth of microorganisms. As with any food product, maintenance of a constant chilled condition in shrimp is a vital element in reducing microbial growth. This experiment evaluated Pseudomonas spp. (type III and type II), two Staphylococcus and an Arthrobacter spp. for their ability to adhere to glass cover slips at 15, 25 and 37° C (Table 11).

Pseudomonas spp. of marine origin tend to be psychotropic and, as suspected, showed poor growth at 37° C (Buchanan and Gibbons, 1974), but grew quite well at 15 and 25° C. Both Pseudomonas types demonstrated better adhesion at 15 and 25° C than that observed at 37° C but not significantly so (Table 12).

This conclusion is further substantiated by a statistical analysis of the data. Using analysis of variance (ANOVA) techniques, it is shown that there is a significant ( $\alpha = 0.01$ ) difference between the means of the Staphylococcus spp. adhesion to concentration ratios at the three test temperatures, while no such statistical variation can be derived from the observations of the Pseudomonas spp. Arthrobacter spp. also showed significant variation in average adhesion ratio between the three temperatures (Table 12).

Table 11. Effect of temperature on adhesion of bacteria to glass surface.

Microorganism	Adhered/Unadhered x 100					
	Temperature °C					
	15		25		37	
	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.
<u>Pseudomonas</u> spp. Type II	.23	.05	.35	.11	.19	.06
<u>Pseudomonas</u> spp. Type III	.31	.10	.34	.05	.18	.09
<u>Staphylococcus</u> spp.	.13	.02	.60	.48	1.26	.21
<u>Staphylococcus</u> spp.	.17	.07	.42	.34	1.10	.62
<u>Arthrobacter</u> spp.	.23	.04	.27	.04	.14	.03

Table 12. Analysis of variance of the effect of temperature on adhesion to glass surface.

Microorganism	Test Statistic (F)
<u>Pseudomonas</u> spp. Type II	4.60
<u>Pseudomonas</u> spp. Type III	4.28
<u>Staphylococcus</u> spp.	13.90
<u>Staphylococcus</u> spp.	28.90
<u>Arthrobacter</u> spp.	26.22

Null hypothesis  $H_0 : U_{15} = U_{25} = U_{37}$

Alternate hypothesis  $H_a : U_{15} \neq U_{25} \neq U_{37}$

Critical value of F (d.f. 2,9)(alpha = .01) = 8.02

If test statistic > critical value of F, then reject null hypothesis.

If test statistic < critical value of F, then accept null hypothesis.



Staphylococcus spp., on the other hand, grew extremely well at 37° C. This would be expected, since Staphylococcus spp. is a commensal organism on human skin and would be anticipated to grow well at body temperature (Buchanan and Gibbons, 1974). Staphylococcus spp., however, grew very poorly at 15° C and, as also expected, showed little adhesiveness at this temperature.

Staphylococcus spp. had a significantly greater ability to adhere at its optimal growth temperature (37° C) than at any other temperature tested, but did show adhesion at 25° C. Fletcher (1980) showed that temperature, which influenced metabolic activity, also influenced attachment. She did not, however, explain the cause-and-effect relationship. The present data is also in concurrence with the earlier observation that an active metabolism is important in the ability of Staphylococcus spp. to adhere, while it is not as critical in the adherence of Pseudomonas spp. It may be that, at the lower temperatures, Staphylococcus spp. loses some of its ability to produce extracellular polysaccharides, while Pseudomonas spp. is not dependent on metabolic activity for adhesion but to its motility. Pseudomonas spp. retained their characteristic motility at all growth temperatures. Arthrobacter spp. did not grow well at 37° C, but did grow at 15° C. There was little variation between the adhesiveness of this organism at the two lower temperatures, but it had much less adherence at 37° C.

### Effect of Polyphosphate on Bacterial Adhesion to Glass

Current shrimp processing procedures almost inevitably involve a phosphate dip of fresh shrimp prior to peeling. This process has been shown to increase the total yield of peeled shrimp by increasing the water-holding capacity of the shrimp protein. The effect of added phosphate on shrimp microbial flora was studied by Petchsing (1982) and, as discussed previously, has an impact on the over-all bacteriological makeup of shrimp.

When TPE broth with 6% sodium hexametaphosphate was used as a test medium for adhesion study, different bacteria reacted differently to the presence of phosphate. Table 13 shows that phosphate effectively inhibited the growth of Staphylococcus spp., with a resultant reduction in adhesion. Growth of Pseudomonas spp. was likewise reduced, but the ability to adhere was not appreciably affected. Lactobacillus spp. growth was the least affected by the phosphate, even though its rate of growth was considerably lower than that in the control. This was also reflected by the reduced ability to adhere to the glass. Phosphate also retarded the growth of Arthrobacter spp., which exhibited poor adhesive tendency in this medium.

These data add more credence to the hypothesis that the mechanism of adhesion between genera are not the same, particularly in the case of Staphylococcus spp. versus Pseudomonas spp. It can be postulated that at least part of this difference can be attributed to the activity of Pseudomonas spp. and the lack thereof by

Table 13. Ratio of adhered bacteria vs. remaining  
in TPE plus 6% sodium hexametaphosphate.

Microorganism	Adhered/Unadhered x 100					
	Hour					
	6	9	18	24	$\bar{x}$	s. d.
<u>Staphylococcus</u> spp.	0.09	0.19	0.27	0.30	0.21	0.10
<u>Pseudomonas</u> spp. Type III	0.18	0.24	0.30	0.26	0.25	0.05
<u>Lactobacillus</u> spp.	0.05	0.06	0.07	0.19	0.09	0.06
<u>Arthrobacter</u> spp.	0.09	0.15	0.18	0.20	0.16	0.05

Staphylococcus spp. Pseudomonas spp. retained its characteristic motility in the phosphate medium.

## SUMMARY

The purpose of this study was to examine the role of bacterial adhesion on spoilage of seafood. The initial contaminants of the shrimp flesh, following processing, play a large part in determining the ultimate bacterial flora of the shrimp. Pseudomonas spp. tend to become predominant, if present on the shrimp following processing. Lactobacillus spp. will dominate in the absence of Pseudomonas spp. while, when neither of these microorganisms are present, Moraxella spp. will usually become predominant. The first organism to disappear from the shrimp flora, with age, is Flavobacterium spp.

While bacterial attachment cannot alone explain these changes in microbial population, study of the relative adhesiveness of the microorganisms associated with the shrimp can shed some light on the varying microbial flora.

We found that Pseudomonas spp. and Staphylococcus spp. had the greatest ability to adhere. This was observed both in vivo and in vitro. The data indicated that Staphylococcus spp. are very hydrophobic, which explained their strong association with the shrimp meat. Since they do not possess any other attachment features (pili or flagella), their ability to form adherent colonies is, possibly, a result of their metabolizing cementing polysaccharides. Pseudomonas spp., while lacking the high degree of hydrophobicity of the Staphylococcus spp., are also able to form close relationships with the shrimp flesh. Their initial adsorption to the surface is

probably related to their motility, which allows them to overcome electrostatic repulsive energies near the surface by striking the surface with greater frequency, at which times they may be held by Van der Waals forces. Adhesive substances may then be produced by the organism.

The relative degree by which varying bacteria adhere to shrimp is likely a combination of the bacterial ability to: (1) initially adhere to the surface or, (2) form a glycocalyx and/or interact with receptors on the shrimp surface to firmly attach the bacteria to the surface.

The data show that, if given time, both Moraxella spp. and Lactobacillus spp. firmly attach to shrimp. This occurred only in those instances where these organisms were not in competition with Pseudomonas spp. or Staphylococcus spp.

Staphylococcus spp., while not typically indigenous to shrimp, is a common contaminant from food handlers. We demonstrated that, given proper growth conditions, this organism has a significant ability to adhere to the surface and can become a dominant microorganism. Clearly, all measures should be made throughout the processing chain to reduce contamination and outgrowth of this microorganism. The best means to reduce Staphylococcal hazard is to store foods at lower temperature conditions, as they lose their adhesive ability at chill temperatures.

Pseudomonas spp., on the other hand, is more difficult to control. It is part of the normal flora of the shrimp and retains good adhesiveness in a multitude of growth conditions. Here again,

good processing techniques which reduce the initial bacterial counts, followed by storage temperatures which are low enough to retard out-growth, are the critical features.

## BIBLIOGRAPHY

- Aronson, M., O. Medalia, L. Schori, D. Mirelman, N. Sharon, and I. Ofek. 1979. Prevention of colonization of the urinary tract of mice with Escherichia coli by blocking of bacterial adherence with methyl  $\alpha$ -D-Mannopyranoside. *J. Inf. Dis.* 139:329-332.
- Bell, C. R. and L. J. Albright. 1982. Attached and free-floating bacteria in a diverse selection of water bodies. *Appl. Environ. Microbiol.* 43:1227-1237.
- Brown, C. M., D. C. Ellwood, and J. R. Hunter. 1979. Growth of bacteria at surfaces: Influence of nutrient limitation. *FEMS Microbiol. Lett.* 1:163-166.
- Buchanan, R. E. and N. E. Gibbons (eds.) 1974. Bergey's Manual of Determinative Bacteriology, 8th ed. Baltimore, Md.: Waverly Press.
- Butler, J. L., J. C. Stewart, C. Vanderzant, Z. I. Carpenter, and G. C. Smith. 1979. Attachment of microorganisms to pork skin and the surfaces of beef and lamb carcasses. *J. Food Protect.* 42:401-406.
- Chen, H-C. and T-J. Chai. 1982. Microflora of drainage from ice in fishing vessel fishholds. *Appl. Environ. Microbiol.* 43:1360-1365.
- Chen, T. C., J. R. Culotta, and W. S. Wang. 1973. Effect of water and microwave energy precooking on microbiological quality of chicken parts. *J. Food Sci.* 38(1):155-157.
- Chitnis, D. S., K. D. Sharma, and R. S. Kamat. 1982. Role of somatic antigen of Vibrio cholerae in adhesion to intestinal mucosa. *J. Med. Microbiol.* 15:53-61.
- \_\_\_\_\_. 1982. Role of bacterial adhesion in the pathogenesis of cholera. *J. Med. Microbiol.* 15:43-51.
- Corlett, D. L. Jr., J. S. Lee, and R. O. Sinnhuber. 1965. Application of replica plating and computer analysis for rapid identification of bacteria in foods. I. Identification scheme. *Appl. Microbiol.* 13:808-817.
- Costerton, J. W., G. G. Greesly, and K. J. Cheng. 1978. How bacteria stick. *Sci. Amer.* 238:86-95.



- Costerton, J. W., and R. T. Irwin. 1981. The bacterial glycocalyx in nature and disease. *Ann. Rev. Microbiol.* 32:299-324.
- Duguid, J. P. 1959. Adhesive properties in Klebsiella strains. *J. Gen. Microbiol.* 21:271-286.
- Faris, A., T. Wadstrom, and J. H. Freer. 1981. Hydrophobic adsorptive and hemagglutinating properties of Escherichia coli possessing colonization factor antigens (CFA/I or CFA/II), type 1 pili or other pili. *C. Microbiol.* 5:67-72.
- Fein, J. E. 1981. Screening of uropathogenic Escherichia coli for expression of mannose-sensitive adhesions: Importance of culture conditions. *J. Clin. Microbiol.* 13(6):1088-1095.
- Firstenberg-Eden, R. 1981. Attachment of bacteria to meat surfaces: A review. *J. Food. Protect.* 44(8):86-95.
- Firstenberg-Eden, R., S. Notermans, F. Thiel, S. Henstra, and E. H. Kempelmacher. 1979. Scanning electron microscopic investigation into attachment of bacteria to teats of cows. *J. Food Prot.* 42:305-309.
- Fletcher, M. 1977. The effects of culture concentration and age, time and temperature on bacterial attachment to polystyrene. *Can. J. Microbiol.* 23:1-6.
- Fletcher, M. 1980. Adherence of marine microorganisms to smooth surfaces. In: E. H. Beachey (ed.), Bacterial Adherence. New York: Chapman and Hall, pp. 345-374.
- \_\_\_\_\_. 1980. The question of passive versus active attachment mechanisms in non-specific bacterial adhesion. In: R. C. W. Berkeley et al. (eds.), Microbial Adhesion to Surfaces. Chichester, England: Ellis Horwood, pp. 197-210.
- Fletcher, M., and G. P. Floodgate. 1973. An electron-microscopic demonstration of an acidic polysaccharide involved in the adhesion of marine bacterium to solid surfaces. *J. Gen. Microbiol.* 74:325-334.
- Fletcher, M., and G. I. Loed. 1979. The influence of substratum characteristics on the attachment of a marine Pseudomonad spp. to solid surfaces. *J. Gen. Microbiol.* 74:325-334.
- Fletcher, M., and K. C. Marshall. 1982. Bubble contact angle method for evaluating substratum interfacial characteristics and its relevance to bacterial attachment. *Appl. Environ. Microbiol.* 44:184-192.

- Germaine, G. R. and C. F. Schachtele. 1976. S. mutans dextran-sucrase: Mode of interaction with high molecular weight dextran and role in cellular aggregation. Infect. and Immun. 13:365-372.
- Gibbons, R. J. 1980. Adhesion of bacteria to the surfaces of the mouth. In: R. C. W. Berkeley et al. (eds.), Adhesion of Bacteria to Surfaces. Chichester, England: Ellis Horwood, pp. 351-388.
- \_\_\_\_\_. 1980. Bacterial adherence and the formation of dental plaques. In: E. H. Beachey (ed.), Bacterial Adherence. New York: Chapman and Hall, pp. 61-104.
- Gibbons, R. J. and J. van Houte. 1975. Bacterial adherence in oral microbial ecology. Annual Rev. of Microbiol. 29:14-44.
- Gilliland, S. E., F. F. Busta, J. J. Brinda, and J. E. Campbell. 1976. Aerobic plate count. In: M. L. Speck (ed.), Compendium of Methods for the Microbiological Examination of Foods. Washington, D.C.: American Public Health Association, pp. 107-131.
- Hamada, S. and H. D. Slade. 1980. Mechanisms of adherence of Streptococcus mutans to smooth surfaces in vitro. In: E. H. Beachey (ed.), Bacterial Adherence. New York: Chapman and Hall, pp. 105-135.
- Harrison, J. M. and J. S. Lee. 1968. Microbiological evaluation of pacific shrimp processing. Appl. Microbiol. 18:188-192.
- Herbert, R. A., M. S. Hendrie, D. M. Gibbson, and J. M. Shewen. 1971. Bacteria active in the spoilage of certain seafoods. J. Appl. Bacteriol. 34:41-50.
- Hugh, R. and E. Leifson. 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram negative bacteria. J. Bact. 66:24-26.
- Iijima, Y., H. Yamada, and S. Shinoda. 1981. Adherence of Vibrio parahaemolyticus and its relation to pathogenicity. Can. J. Microbiol. 27:1252-1259.
- Johnson, L. I. 1982. Heterotrophic Bacteria Associated with a Feed Algae for Oyster Larvae. M.S. Thesis. Corvallis, Oregon: Oregon State University.
- Jones, G. W. 1980. The adhesive properties of Vibrio cholerae and other vibrio species. In: E. H. Beachey (ed.), Bacterial Adherence. New York: Chapman and Hall, pp. 221-249.

- Kelly, C. A. and J. F. Dempster. 1981. The effect of temperature, pressure and chlorine concentration of spray washing water on numbers of bacteria on lamb carcasses. *J. Appl. Bacteriol.* 51: 415-424.
- Kennedy, R. S., W. R. Finnerty, K. Sudarsanan, and R. A. Young. 1975. Microbial assimilation of hydrocarbons. *Arch. Mikrobiol.* 102:75-83.
- Lee, J. S. and E. Kolbe. 1982. Microbiological profile of pacific shrimp, Pandalus jordani, stowed under refrigerated seawater spray. *Mar. Fish. Rev.* 44(3):12-17.
- Lee, J. S. and D. K. Pfeifer. 1975. Microbiological characteristics of dungeness crab (Cancer magister). *Appl. Microbiol.* 30:72-78.
- \_\_\_\_\_. 1977. Microbiological characteristics of pacific shrimp (Pandalus jordani). *Appl. Environ. Microbiol.* 33:853-859.
- Lee, J. S. and G. C. Wolfe. 1967. Rapid identification of bacteria in foods: Replicapating and computer method. *Food Tech.* 21 (6):24-26.
- Lupton, F. S. and K. C. Marshall. 1981. Specific adhesion of bacteria to heterocysts of Anabaena spp. and its ecological significance. *Appl. Environ. Microbiol.* 42:1085-1092.
- Marshall, K. C. 1976. Interfaces in Microbial Ecology. Cambridge, Mass.: University Press.
- \_\_\_\_\_. 1980. Bacterial adhesion in natural environments. In: R. C. W. Berkeley et al. (eds.), Microbial Adhesion to Surfaces. Chichester, England: Ellis Horwood, pp. 187-196.
- Marshall, K. C. and R. H. Cruickshank. 1973. Cell surface hydrophobicity and the orientation of certain bacteria at interfaces. *Arch. Mikrobiol.* 91:29-40.
- Marshall, K. C., R. Stout, and R. Mitchell. 1971. Mechanism of the initial events of the sorption of marine bacteria to surfaces. *J. Gen. Microbiol.* 68:337-348.
- Meadows, P. S. 1971. The attachment of bacteria to solid surfaces. *Arch. Mikrobiol.* 75:374-381.
- Moore, R. L. and K. C. Marshall. 1981. Attachment and rosette formation by Hyphomicrobia. *Appl. Environ. Microbiol.* 42:751-757.

- Mukasa, H. and H. D. Slade. 1973. Mechanism of adherence of Streptococcus mutans to smooth surfaces. I. Roles of insoluble dextran-levan synthetase enzymes and cell wall polysaccharide antigen in plaque formation. *Infect. and Immun.* 8:555-562.
- Ofek, I. and E. H. Beachey. 1980. General concepts and principles of bacterial adherence in animals and man. In: E. H. Beachey (ed.), Bacterial Adherence. New York: Chapman and Hall, pp. 3-29.
- Pearce, W. A. and T. M. Buchanan. 1980. Structure and cell membrane-binding properties of bacterial fimbriae. In: E. H. Beachey (ed.), Bacterial Adherence. New York: Chapman and Hall, pp. 289-344.
- Petchsing, U. 1982. Effect of Polyphosphate on Microbial Characteristics of Seafood. M.S. Thesis. Corvallis, Oregon: Oregon State University.
- Post, F. J., G. B. Krishnamurty, and M. D. Flanagan. 1963. Influence of sodium hexametaphosphate on selected bacteria. *Appl. Microbiol.* 11:430-435.
- Pusch, D. J., F. F. Busta, W. A. Moats, and A. E. Schulze. 1976. Direct microscopic count. In: M. L. Speck (ed.), Compendium of Methods for the Microbiological Examination of Foods. Washington, D.C.: American Public Health Association, pp. 132-151.
- Rosenberg, M. 1981. Bacterial adherence to polystyrene: A replica method of screening for bacterial hydrophobicity. *Appl. Environ. Microbiol.* 42:375-377.
- Rosenberg, M., E. Rosenberg, and D. Gutnick. 1980. Bacterial adherence to hydrocarbons. In: R. C. W. Berkeley et al. (eds.), Microbial Adhesion to Surfaces. Chichester, England: Ellis Horwood, pp. 541-542.
- Rosenberg, M., S. Rottem, and E. Rosenberg. 1982. Cell surface hydrophobicity of smooth and rough Proteus mirabilis strains as determined by adherence to hydrocarbons. *FEMS Microbiol. Lett.* 13:167-169.
- Schwach, T. S. and E. A. Zottola. 1982. Use of scanning electron microscopy to demonstrate microbial attachment to beef and beef contact surfaces. *J. Food Sci.* 47:1401-1405.
- Shiba, R. and N. Taga. 1981. Attachment properties of Flavobacterium cytophaga. *Japan. Soc. Sci. Fish.* 4(9):1199-1203.

- Staat, R. H., S. D. Langley, and R. J. Doyle. 1980. Streptococcus mutans adherence: Presumptive evidence for protein mediated attachment followed by glucan-dependent cellular accumulation. Infect. and Immun. 27:675-681.
- Steinhauer, J. E. and G. J. Banwart. 1964. The effect of food grade polyphosphates on microbial population of chicken meat. Poultry Sci. 43(3):618-625.
- Sugarman, B. and S. T. Donta. 1979. Specificity of attachment of certain enterobacteriaceae to mammalian cells. J. Gen. Microbiol. 115:509-512.
- Sutherland, I. W. 1980. Polysaccharides in the adhesion of marine and freshwater bacteria. In: R. C. W. Berkeley et al. (eds.), Microbial Adhesion to Surfaces. Chichester, England: Ellis Horwood, pp. 329-338.
- Tadros, R. F. 1980. Particle-surface adhesion. In: R. C. W. Berkeley et al. (eds.), Microbial Adhesion to Surfaces. Chichester, England: Ellis Horwood, pp. 93-116.
- Wadstrom, T., C. J. Smyth, A. Fairs, P. Jonsson, and J. H. Freer. 1978. Proceedings Second International Symposium on Neonatal Diarrhea. Canada: University of Saskatchewan, pp. 29-55.
- Walker, P. D. and L. K. Nagey. 1980. Adhesion of organisms to animal tissues. In: R. C. W. Berkeley et al. (eds.), Microbial Adhesion to Surfaces. Chichester, England: Ellis Horwood, pp. 329-338.
- Zobell, C. E. 1943. The effect of solid surfaces upon bacterial activity. J. Bacteriol. 46:39-59.
- Zvyagintsev, D. G. 1959. Adsorption of microorganisms by glass surfaces. Microbiol. (USSR)(English Trans.) 28:104-108.