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Review

Aeromonas Species in Foods

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ABSTRACT

Aeromonas species have been recognized as potential or emerging foodborne pathogens for more than 20 years. Aeromonads are estuarine bacteria and are ubiquitous in fresh water, fish and shellfish, meats, and fresh vegetables. Actual sourced foodborne outbreaks are few, but epidemiological evidence suggests that the bacterium can cause self-limiting diarrhea, with children being the most susceptible population. Most aeromonads are psychrotrophic and can grow in foods during cold storage. Aeromonads are not resistant to food processing regimes and are readily killed by heat treatment. A host of virulence factors are present, but the exact role of each in human disease has not been fully elucidated.

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The bacterial genus *Aeromonas* is now officially classified within the family *Aeromonadaceae* and consists of 14 different confirmed species *(51)* (an asterisk indicates that a species has been isolated from clinical specimens): *A. allosaccharophila, A. hydrophila,* A. bestiarum,* A. caviae,* A. encheleia, A. eucrinophila, A. jandaei,* A. popoffii, A. media,* A. salmonicida, A. schubertii,* A. sobria, A. veronii,** and *A. trota.** The genus was formerly classified within *Vibrionaceae,* and misidentification of *Aeromonas* spp. as *Vibrio* spp. still poses a problem in clinical settings *(2, 8).* Originally, four *Aeromonas* species were identified: *A. hydrophila, A. sobria, A. caviae,* and the fish pathogen *A. salmonicida (82).* Subsequent work with these four species indicated distinct biochemical groupings (designated phenospecies) and distinct DNA hybridization groupings (designated genospecies, sometimes referred to as hybridization groups [HGs]), leading to variability in species designation and classification *(1, 8, 13, 18, 38, 54, 58).* These gram-negative facultative organisms are ubiquitous in water and in many foods. Their role in foodborne illness is still not firmly established. The purpose of this review is to provide a general overview of *Aeromonas* spp. in foods and the role they may play in foodborne illness.

GENERAL CHARACTERISTICS

Aeromonads are gram-negative, oxidase-positive, glucose-fermenting, facultatively anaerobic rods, and most are motile by polar flagella *(82).* The word *Aeromonas* was derived from the Greek words *aer,* meaning air or gas, and *monas,* meaning unit or monad. Cells are typically 1.0 to 4.4 μm in length and occasionally form filaments of up to 8.0 μm. Aeromonads can ferment the sugars glucose, fructose, maltose, and trehalose to acid or to acid and gas. They can also hydrolyze starch, dextrin, and glycerol *(55).* Recently, pectinolytic activity has been reported in a newly identified strain *(80).* Most *Aeromonas* spp. can grow at a pH of 5.5 to 9.0. Colonies formed after 24 h on nutrient agar are 1 to 3 mm in diameter, convex, smooth, whitish, and translucent. Colonies turn a light beige color after prolonged incubation *(55).*

Similar to *Vibrio* species, aeromonads have been reported to have a viable but nonculturable state *(92).* Certain strains display a rapid decline following 12 to 18 h of growth in media containing glucose. This phenomenon is referred to as the suicide phenomenon *(70).* The optimal growth temperature is generally believed to be 28°C *(58, 82),* but a wide growth range and variability in the optimum temperature have been observed *(54, 57, 58, 65, 80).* Many strains can grow at <5°C, making aeromonads particularly significant in refrigerated foods *(24, 27, 77, 78). A. hydrophila* has been reported to have an optimal growth temperature of 28°C, although it can grow at temperatures ranging from 1 to 42°C *(65, 74).*

Aeromonads are considered opportunistic pathogens of both aquatic and terrestrial animals *(25, 67).* Since 1960, it has been speculated that these bacteria may be pathogenic to humans *(67, 72).* In 1984, the Food and Drug Administration introduced *A. hydrophila* as a ''new'' foodborne pathogen *(89).* Aeromonads have been considered controversial human pathogens, since results from human volunteer studies have been inconclusive *(57)*; however, epidemiological evidence continues to indicate that these organisms are capable of causing gastroenteritis *(7, 26, 62, 69)* and other complications, including wound infections, septicemia, and endocarditis *(2, 14, 48).* Gastroenteritis involves inflammation of the gastrointestinal tract due to invasion of bacteria into the intestinal mucosa. Septicemia or blood poisoning involves the growth of bacteria in the blood *(9).* Studies indicate that *Aeromonas* spp. can act as both infectious and enterotoxigenic pathogens *(56).* Non-

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motile aeromonads, namely, *A. salmonicida,* are thought to be obligate fish parasites *(25).* The motile mesophilic aeromonads, consisting of *A. hydrophila, A. sobria,* and *A. caviae,* are considered causative agents of human gastroenteritis, wound infections, and septicemia *(34).*

The taxonomy of the genus *Aeromonas* is still developing. Molecular genetic evidence now indicates that *Aeromonas* spp., formerly members of the family *Vibrionaceae,* are quite different from each other and from the vibrios *(58).* Early studies of aeromonads classified the mesophilic, pathogenic, and nonpathogenic strains as *A. hydrophila* and the psychrophilic strains that were fish pathogens as *A. salmonicida (58, 72).* In the early 1980s, Popoff *(82)* used DNA-DNA hybridization to show that at least nine distinct HGs existed among the mesophilic species *A. hydrophila.* Among the HGs, three separate phenotypic groups were found by using 8 to 18 biochemical tests. These three "phenospecies" were named *A. hydrophila, A. sobriae,* and *A. caviae.* Most clinical laboratories have accepted phenotypic classification of the mesophilic aeromonads. *A. hydrophila* is now limited to HG1, HG2, and HG3 *(58).* A decade ago, investigators used DNA-DNA hybridization studies to apply the classification schemes of genomospecies, genospecies, or hybridization groups, which were defined as having at least 70% DNA homology with the designated type strain *(72).* These hybridization groups are geographically dispersed. Moreover, no evidence has suggested a significant correlation between these hybridization groups and virulence *(67).* While DNA homology analysis is the traditional tool for species confirmation, 16S rRNA sequencing, amplified fragment length polymorphism, and fatty acid methyl ester analysis have all been proposed to characterize new isolates *(51).*

Aeromonas spp. have properties that are shared by bacteria in the families *Enterobacteriaceae* and *Vibrionaceae (55). Aeromonas* spp. are not particularly fastidious and can grow well on many common laboratory media, such as MacConkey agar, Hektoen enteric agar, and xylose lysine desoxycholate agar *(47).* In efforts to isolate *Aeromonas* spp., selective media are used that take advantage of unique characteristics of amylase activity and ampicillin resistance. Selective media such as glutamate starch penicillin agar *(52),* ampicillin dextrin agar *(40),* and starch ampicillin agar *(77)* are typically used for the isolation of aeromonads from foods. Gavriel and Lamb *(33)* studied the abilities of all three media to grow different species of *Aeromonas* and reported wide disparity in the recovery of certain *Aeromonas* species, namely, *A. veronii, A. schubertii,* and *A. sobria,* indicating that not all species are equally recovered with these selective media.

Aeromonads do not exhibit unusual resistance to food processing procedures. These organisms are not heat- or acid-resistant and do not grow at a pH below 5 or at a sodium chloride concentration above 3.5% *(58, 78, 79).* Combinations of sodium chloride and polyphosphates are also inhibitory *(76),* as are sorbates and smoke *(37).* The chlorine resistance of aeromonads is also similar to that of other gram-negative bacteria *(58).* One interesting phenomenon of *Aeromonas* species is their tendency for biphasic thermal survivor curves. Palumbo et al. *(76, 79)* reported tailing or two-phase thermal survivor curves with three clinical and two food isolates of *A. hydrophila.* Isonhood *(45)* also reported biphasic thermal survivor curves for two strains of *A. hydrophila.*

FOODBORNE OUTBREAKS

Currently, reports of foodborne *Aeromonas* outbreaks lack hard evidence that indisputably establishes the organism as a causative agent of foodborne illness. Kirov *(57)* reviewed incidences of *Aeromonas*-related foodborne gastroenteritis that occurred between 1977 and 1991. Most outbreaks involved seafood, such as oysters, sashimi, prawns, and shrimp. Although *Aeromonas* was implicated, viruses were not ruled out as a possible cause of the illness. The only report presenting good evidence leading to *Aeromonas* as the cause of gastroenteritis involved a 38-year-old man who consumed a ready-to-eat shrimp cocktail. Through ribotyping, the patient's stool and the shrimp were found to contain identical *Aeromonas* spp. This report offers the best evidence known from a study in which molecular techniques were used to identify *Aeromonas* spp. as the causative agent in foodborne gastrointestinal infection *(47).* Saad et al. *(84)* reported an increased rate of *Aeromonas* spp. isolated from human stools during the summer months and hypothesized an association with the incidence of motile aeromonads in fresh vegetables. The increased frequency of human stool–associated aeromonad isolation in the summer months was not linked with microorganisms isolated from nearby river and tap water, but it did correlate with large numbers of *Aeromonas* from food samples.

VIRULENCE FACTORS

The role of aeromonads and the toxins they produce and their relationship to virulence in cases of human gastroenteritis have not been fully elucidated. There is no animal model for *Aeromonas* gastroenteritis. Most cases of gastroenteritis associated with *Aeromonas* occur in children, elderly people, and immunocompromised people. A number of putative virulence factors have been identified, including hemolysins, invasins, adhesins, endotoxin (lipopolysaccharide [LPS]), proteases, fimbriae, pili, capsules, S-layers, siderophores, and a variety of extracellular enzymes *(47, 58, 66).* Cytotoxic activity on Vero cells has occasionally been reported and has been associated with hemolysin activity. Several hemolysins may be produced, but the most well characterized of them is aerolysin. Aerolysin is a lytic channel-forming exotoxin encoded by *aerA (11, 42)* that was first characterized >25 years ago. Other cytotoxic enterotoxins similar to aerolysin and loosely grouped as aerolysins have since been isolated and characterized *(15, 30).* Heat-labile and heat-stable cytotoxic enterotoxins have also been reported *(6, 20, 58).* In addition, two *A. hydrophila* phospholipases were recently demonstrated to play a role in virulence in fish and mice *(66).*

The formation of S-layers has been considered by some to be an indicator of pathogenicity in gram-negative and gram-positive microorganisms. An S-layer is an arrangement of protein or glycoprotein subunits on the cell wall

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(95). Other than *A. salmonicida,* the mesophilic *Aeromonas* spp. of serotype O:11, *A. hydrophila* and *A. sobria,* are the only ones thus far to show the presence of S-layers *(58, 68).* S-layers increase the ability of the cells to adhere to and colonize the intestinal epithelium. Presumably, S-layers of *Aeromonas* spp. of serotype O:11 contribute directly to diarrheal symptoms. The S-layer also allows the bacteria to be less susceptible to opsonophagocytosis. Opsonophagocytosis is phagocytosis of a cell facilitated by an opsonin, which is a substance that binds to bacteria, erythrocytes, or other particles, making them more susceptible to phagocytosis *(23). Aeromonas* spp. of serotype O:11 are also frequently isolated in cases of septicemia *(68).*

The endotoxin LPS produced by *Aeromonas* is similar to those produced by other gram-negative bacteria. Similarities in the LPS are related to a T-independent antigen that activates polyclonal B-cell activation and produces a predominantly immunoglobulin M response. The injection of LPS into animals tends to cause pyrogenicity, leukopenia leading to leukocytosis, shock, hemorrhagic necrosis of tumors, diarrhea, and death *(68).* These reactions are caused by lipid A, a common LPS. Excretion of some exotoxins has been shown to be dependent on the presence of the O-antigen LPS. The significance of this finding is that those strains lacking the O-antigen LPS (rough strains) excrete less toxin than those strains that have ample O-antigen LPS (smooth strains). It is important to note that O-antigen LPS is one of the key factors involved in bacterial colonization of the gut mucosa. Research has revealed that these strains, which are characterized by O-antigen LPS, are more virulent when grown at low temperatures. Some reports of *Enterobacteriaceae* are related to these findings *(68).*

Fimbriae (filamentous and nonfilamentous) adhesins have been described in studies of mesophilic *Aeromonas* spp. These fimbriae enable *Aeromonas* to adhere to different cell lines. Two types of fimbriae have been characterized. Fimbriae of the first type are short and rigid (S/R) and occur in large numbers in bacteria cells. *A. hydrophila* and *A. sobria* are the most adherent to various surfaces, a finding that has been attributed to the nonfilamentous (outer membrane) adhesins *(68).* Fimbriae of the second type are long and flexible (L/W) and occur in smaller numbers in bacterial cells. These two types of fimbriae have been found on both clinical and environmental isolates of mesophilic *Aeromonas* spp. Isolates from different *Aeromonas* spp. share a high degree of homology with the N-terminal amino acid sequences with respect to L/W fimbriae. Growth at low temperatures in a liquid medium helps to promote fimbriae expression among most isolates *(68).*

A. salmonicida, a fish pathogen, has been shown to produce a capsular polysaccharide when grown in vivo or in a glucose-rich medium. This capsular polysaccharide has been an important surface structure and a pathogenicity determinant in virulent strains. *A. hydrophila* and *A. sobria* were shown to have the same ability to produce a capsular polysaccharide. Studies have shown that the ability to produce a capsular polysaccharide improved the adhesion of the bacteria to various surfaces *(68).* While a capsular polysaccharide certainly enhances the virulence of these bacterial strains, its effects on enteropathogenicity are still unclear *(68).*

Abeyta et al. *(4)* studied *A. hydrophila* involved in 472 cases of gastroenteritis occurring in November 1982 associated with the consumption of oysters harvested from Louisiana. Samples obtained from the oysters all tested negative for *Salmonella,* pathogenic *Vibrio parahaemolyticus,* and diarrhetic shellfish poison. Twenty-three of 28 *A. hydrophila* strains tested positive in at least one of the virulence assays, which included the suckling mouse test, the adrenal Y-1 mouse cell test, and hemolysin assays. While these tests did not prove that *A. hydrophila* was the sole cause of the outbreak, they did indicate that *A. hydrophila* should be considered when foodborne outbreaks involving oysters are investigated *(4).* Krovacek et al. *(61)* found few differences in virulence factors between human diarrheal and marine environmental isolates of *A. hydrophila.* Kuhn et al. *(62)* reported that hemolysin and cytotoxin production were more frequent for *Aeromonas* spp. isolated from individuals with diarrhea than for those isolated from healthy individuals. However, the number of environmental isolates that exhibited hemolysin and cytotoxin production was larger than the number of human isolates that did. Albert et al. *(6)* compared *A. hydrophila* isolated from children with diarrhea and with those isolated from healthy children and from surface water in Bangladesh. The three toxins studied were a heat-labile cytotonic enterotoxin (ALT), a heat-stabile cytotonic enterotoxin (AST), and a cytotoxic enterotoxin (ACT). The quantities of *Aeromonas* isolates that tested positive for the presence of the *alt* gene were similar for all three sources. The *ast* gene was significantly more commonly encountered in isolates from environmental samples than in those from children with diarrhea. Isolates positive for both the *alt* and the *ast* genes were significantly more numerous for children with diarrhea than for the other sources. This led to the conclusion that *alt* and *ast* may have a synergistic effect in inducing diarrhea. For a study in which *Aeromonas* spp. were the only pathogens isolated from the stools of 26 patients, clinical data for 11 of these patients also suggested that watery diarrhea occurred when both the *alt* and the *ast* genes were present, while loose stools occurred when only the *alt* gene was present *(6).* Schiavano et al. *(87)* also reported mixed results from a study of *Aeromonas* isolates from healthy individuals and from those suffering from diarrhea. Cytotoxic activity as well as adhesin and invasin production was found in four of eight diarrheal isolates but was also found in isolates from healthy individuals. Of 141 food isolates, 66% tested positive for the presence of a cytolytic enterotoxin gene, as did 67% of clinical isolates and 58% of environmental isolates *(56).* Ninety-four percent of 767 shellfish isolates were hemolysin positive *(1).* Of the hemolysin-positive isolates, 59% exhibited cytotoxic activity. Wang and Silva *(93)* also detected hemolytic activity in 86% of catfish *Aeromonas* isolates.

VIRULENCE IN FOOD

The infectious dose for *Aeromonas* spp. in foods is unknown, as is the exact mechanism of how they cause

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gastroenteritis. *A. hydrophila* and *A. caviae* are the species most commonly associated with diarrhea. Mateos et al. *(65)* studied the expression of selected virulence factors in *A. hydrophila* isolates as a function of temperature. Environmental and human isolates were studied; environmental isolates were inhibited at 37°C, while human isolates were not. The hemolytic and cytotoxic activity of environmental isolates decreased at 37°C, while this activity was stimulated for some human isolates (4 of 9). All environmental isolates were pathogenic for trout (100% mortality), while only 44% (4 of 9) of the human isolates were. Okrend et al. *(73)* and Tsai and Chen *(89)* determined the toxigenicity of aeromonads isolated from meats and seafood, respectively. Cytotoxins were detected in more than 90% of *A. hydrophila* isolates. In a study involving an *A. hydrophila* strain isolated from the oyster, Tsai et al. *(90)* investigated the production of hemolysin and cytotoxin under different environmental conditions. *A. hydrophila* was found to produce both hemolysin and cytotoxin at 37, 28, and 5°C. Toxins were more stable and were produced faster at 28°C than at 37°C. In the presence of 1 to 5% (wt/vol) NaCl in brain heart infusion broth, the production of hemolysin and cytotoxin was decreased. Toxin production also decreased when the pH was raised or lowered from 7.2. Toxin production increased with an increase in dissolved oxygen during the stationary growth phase *(90). Aeromonas* spp. generally grow at refrigeration temperatures, and toxin production is not necessarily inhibited *(28, 64).* Enterotoxin and hemolysin were produced in meat extracts stored at 5 or 12°C in an experiment that revealed not only that motile *Aeromonas* spp. grew well in food products at refrigeration temperatures, but also that production of enterotoxin and hemolysin by these bacteria was not limited by refrigeration of the food *(64).*

A study by Kirov et al. *(59)* addressed the virulence of strains isolated from milk. Isolates recovered from raw and pasteurized milk were tested for exotoxin production. Most strains were not enterotoxigenic, and some produced small amounts of hemolysin. Piliation was observed in most strains. *A. sobria* from pasteurized milk produced all of the exotoxins (hemolysins, cytotoxin, and enterotoxin) measured. This strain grew well at 43°C. Almost half of the strains showed some ability to bind to Hep-2 cells (≥5 bacteria per cell). This test was run with control adherent strains isolated from human and chicken feces (12 to 20 bacteria per cell). Strains grown at low temperatures exhibited increased adhesive ability and piliation. These strains were able to grow to large numbers in refrigerated milk without spoilage. Evaluation of this study suggests that consumption of pasteurized milk with preformed toxins may be of little concern, since exotoxin production was significantly lower in milk than in bacteriological medium. In addition, bacteria with the capability to form such toxins appear rarely in milk isolates *(59).*

SOURCES OF CONTAMINATION

The ubiquity of aeromonads in foods suggests that foods are vehicles for illness caused by *Aeromonas* spp. Merino et al. *(68)* speculated that occurrence of foodborne illness with *Aeromonas* spp. as the cause could increase in the future because of a higher consumer demand for less processed, more "natural" foods. *Aeromonas* spp. are commonly isolated from aquatic environments such as rivers, lakes, sewage effluents, marine waters, and chlorinated drinking water *(4, 6, 34, 39, 60, 62, 80)* and from retail fresh vegetables *(84)* and foods of animal origin such as seafood *(4, 5, 39),* red meat *(27, 51, 77),* ground pork, poultry *(77),* raw milk *(47, 77),* and cheese made from raw milk *(86).* Recovery of *Aeromonas* spp. from estuarine environments has been reported to be seasonal *(34, 83).* Aeromonads appear to be ubiquitous on freshwater fish (both wild and pond-raised) *(35, 36, 71, 93).* Refrigeration of these foods has not proven adequate for control, as researchers have reported growth at 4 and 5°C *(16, 24, 27, 31).* Estimates show that *Aeromonas* may cause up to 13% of the gastroenteritis cases in the United States *(56).*

As waterborne and environmental bacteria, *Aeromonas* spp. encounter a range of environmental changes that include acid and temperature stresses. The ability to adapt to a wide variety of environments accounts for its threat as a human pathogen and its frequent isolation from various habitats *(53). Aeromonas* spp. are considered a potential cause of human gastroenteritis, and water supplies, particularly drinking water, have been investigated as sources of these bacteria. A recent study in northern Scotland focused on a drinking water distribution system *(34).* The various factors within the system, such as chlorine concentration, pH, temperature, and rainfall, were evaluated. The results of the study revealed that although some reservoirs with low chlorine concentrations were shown to contain low numbers of *Aeromonas,* the organism was frequently isolated from highly chlorinated reservoirs in significant numbers. Gavriel et al. *(34)* suggested a relationship between rainfall and the densities of *Aeromonas* isolated. Extended periods of rainfall increased the incidence of the recovery of *Aeromonas* from the reservoirs. This increase is thought to have occurred because an increased organic load caused the formation of chloramines, which are less effective in killing microorganisms.

Cotton and Marshall *(22)* evaluated microflora on processing equipment in two different catfish processing plants. *Aeromonas* spp. (37.5%) and *Pseudomonas* spp. (37.5%) were the most common gram-negative bacteria isolated. The deheaders, conveyors, and cutting boards were the principal areas in the plant from which *Aeromonas* spp. were isolated. Automated filleting machines were not shown to harbor aeromonads. An interesting observation was that *Aeromonas* spp. were isolated at a higher frequency from the smaller, less automated plant than they were from the larger, automated plant. The researchers concluded that these differences may have resulted from the differences in the frequencies with which equipment was sanitized in the two plants. The smaller plant cleaned and sanitized equipment at the end of the processing day, while at the larger, automated plant, sanitizer was sprayed on equipment surfaces during employee break periods, and cleaning and sanitation operations were carried out after processing runs. Furthermore, the results of this study may implicate

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Aeromonas spp. as possible indicators of processing equipment sanitation *(22).*

The control of biofilms on food processing equipment is essential to minimize the risk of contamination of a product with pathogens. *Aeromonas* can form biofilms, which contribute to increased resistance to normal bactericidal treatments. Bal'a et al. *(10)* examined the effects of heat and chlorine on the inactivation of *Aeromonas* biofilms on stainless steel surfaces. These researchers found that older biofilms were more heat resistant than were less established biofilms. An 8-h biofilm was destroyed within 1 min at 50°C or by exposure to 25 ppm chlorine for 1 min. Eight-day-old biofilms were effectively destroyed by either 60°C or 75 ppm chlorine for 1 min.

Some studies have shown that fresh vegetables can also harbor *Aeromonas* spp. *(84).* Since vegetables are normally consumed raw, the presence of aeromonads on these foods can present a significant health risk. A study conducted in Brazil *(84)* isolated motile aeromonads from 43 of 90 vegetable samples tested. Most isolates were found on watercress, with lettuce having fewer positive samples and escaroles having slightly fewer positive samples than lettuce. Positive samples were reported to range from $<10^2$ to $>10^6$ CFU/g. Sixteen positive samples had counts of $\geq 10^4$ CFU/g *(84).* Jacxsens et al. *(46)* reported that the growth of *Aeromonas* spp. at 7°C on fresh-cut vegetables was influenced more by vegetable type than by atmosphere. Growth occurred on shredded lettuce and chicory but not on carrots or brussels sprouts. Velazquez et al. *(91)* demonstrated that *A. hydrophila* survived and grew on fresh tomato surfaces and in fresh chopped tomatoes at refrigeration temperatures.

Pin et al. *(81)* conducted a survey of the frequencies of mesophilic *Aeromonas* spp. isolated from various foods. A total of 87 different foods (meats, water, dairy products) were sampled for the presence of *A. hydrophila, A. sobria,* and *A. caviae. A. hydrophila* was isolated from 33 of the 87 samples, while *A. sobria* was isolated from 17 samples and *A. caviae* was isolated from 6 samples. Isolation frequencies were highest for meat products such as fish, seafood, and poultry, with 82.7% of the strains being isolated from these products. The other 17.3% were isolated from water, cheese, and milk *(81).* In a different study *(97),* poultry meat and eggs were investigated as potential sources of enterotoxigenic *Aeromonas* spp. All eggs sampled were free of *Aeromonas.* From 130 broiler meat samples, 17 *Aeromonas* strains were isolated. Of these strains, 8 were positive by the vasopermeability test and 7 were positive by the mouse foot pad test *(97).* Milk has also been investigated as a potential vehicle of *Aeromonas*-related gastroenteritis. In a study by Kirov et al. *(59),* 72 samples of raw milk and 183 samples of pasteurized milk were collected in Australia. *Aeromonas* spp. were recovered from 60% of raw samples and from only 3.8% of pasteurized samples. Of the species recovered from raw milk, 74% were *A. hydrophila,* while *A. sobria* was the species most recovered from pasteurized milk *(59).* Santos et al. *(85)* reported the inhibition *of A. hydrophila* in the presence of *Lactococcus lactis* subsp. *lactis.* Although aeromonads were isolated from raw sheep's milk cheese *(86),* this was high-pH (>6.4) cheese without an added lactic starter. Thus, although *Aeromonas* is found in raw milk, it is unlikely to be found in fermented dairy products or pasteurized milk.

Doherty et al. *(27)* evaluated the growth of *A. hydrophila* on refrigerated lamb meat stored in vacuum and modified-atmosphere packages at high and neutral pHs. These researchers found that numbers of aeromonads decreased in all types of packages tested at 5°C. However, in high-pH lamb meat, aeromonads significantly increased or maintained their numbers in all packages except those containing 100% CO_2, in which the bacteria slowly died. At 0°C, *A. hydrophila* was recovered from all samples with high pHs. In high-pH minced lamb meat stored at 5°C in air or vacuum packages, there was a significant increase in cell numbers *(27). A. hydrophila* grew slowly at 2°C on cooked crayfish tails with air or vacuum storage, but modified-atmosphere storage inhibited growth *(43).* The ability of *Aeromonas* spp. to survive and grow at refrigeration temperatures is a cause for concern and results in a need for other means to control this psychrotrophic foodborne pathogen *(64).*

STRESS RESPONSE OF MICROORGANISMS

The use of mild processing techniques in the production of food products to meet consumer demand for higher quality products is increasing in popularity. Foods are processed with mild heat treatments, are formulated with less acid, salt, and sugar, and are not as dependent on preservatives such as sulfite and nitrite. For these minimally processed foods, psychrotrophic and mesophilic microorganisms are a concern because of their ability to grow in a wide range of temperatures and their tolerance to low levels of heat *(3).* In the environment and in foods, microorganisms encounter changing conditions and stresses that they must survive and overcome in order to reproduce *(12).* Some of these stresses include starvation, cold shock, heat shock, weak acids, high osmolarity, and high hydrostatic pressure *(3).* The physiological, biochemical, and genetic mechanisms that different bacteria undergo during certain stresses have not been elucidated for many microorganisms. However, it is well documented that when microorganisms are exposed to a stress, they will adapt and may become resistant to stronger doses of the stress (homologous stress hardening).

Another adaptive response that has been observed is the cross-protective effect of stress (heterologous stress hardening). Cross-protection occurs when the adaptation of a microorganism to one stress carries over to the increased survival of another stress. As an example of cross-protection, *Listeria monocytogenes* grown under low-nutrient conditions was shown to have an increased tolerance to chlorine sanitizers *(12).* The well-characterized foodborne pathogens *L. monocytogenes, Escherichia coli,* and *Salmonella enterica* all have documented stress responses that can lead to homologous and heterologous stress hardening, and this finding has implications for food processing techniques *(16, 17, 29, 41, 49, 50, 63, 88).*

Adaptation to host defenses within the gastrointestinal

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tract is crucial to enable a gastrointestinal pathogen to reach the site of the infection. The acid barrier in the stomach, the physical barrier of the epithelial cells lining the intestinal walls, and nonspecific immune responses of the body such as macrophages are the main barriers that the microorganism encounters on its path to the gastrointestinal tract in healthy individuals *(32).* Response to certain stress conditions such as the ones implicated in foods can induce stress tolerance and thereby enhance virulence *(3, 12).* From a food safety standpoint, it is important to understand the adaptation, survival, and growth of microorganisms in foods under such stresses. For acid stress, a low-pH environment may be used in a food to inhibit the growth of microorganisms. In effect, what may actually happen is that the microorganism may adapt to this imposed stress in the stored food, allowing it to adapt and survive the acid barrier of the stomach better.

STRESS IN *AEROMONAS*

Acid stress, also called acid tolerance response, involves a complex process in which many changes in the levels of different proteins and other events occur through gene regulation *(32). Aeromonas* spp. are not acid-resistant organisms, but they do demonstrate an adaptive stress response to acid. Karem et al. *(53)* studied the effects of sublethal acid adaptation followed by a severe acid environment. The death of *A. hydrophila* was rapid at a pH of 3.5, but when the bacteria were adapted at a pH of 5.0 for 20 min before they were challenged at a pH of 3.5, a >5-log increase in survival relative to that with no acid adaptation was observed within 1.5 h after the acid challenge. Karem et al. *(53)* hypothesized that *A. hydrophila* was able to adapt and survive in the acid environments by producing "protective" proteins. Chloramphenicol-treated cells (no protein synthesis) did not show an acid tolerance response. On the basis of this research, it seemed clear that proteins were synthesized during the adaptation step, allowing the bacteria to survive the acid environment at pH 3.5. Iron was not required for acid tolerance response in *Aeromonas (53).*

Condon et al. *(21)* evaluated the effects of culture age, incubation at low temperatures, and pH on the thermal resistance of *A. hydrophila.* In their study, bacteria in the late log phase were twice as resistant as those cells in the early log stage. The maximum *D*-value was obtained after 72 h of incubation. Incubation at low temperatures had no effect on *D*-values. At a pH of 4.0, the *D*-value was reduced by a factor of 5 when compared with thermal resistance at a pH of 6.0 *(21).* For various food models, Palumbo and Buchanan *(75)* showed that *A. hydrophila* did not grow when both a pH of 6.1 and a salt content of >3% were used. However, the bacteria did not die at these pH and salt levels *(75).*

To date, few studies have investigated the ability of *A. hydrophila* to adapt to a stress and then survive that same stress at a level that would normally be lethal to the cell. Cross-protective effects for *Aeromonas* following exposure to a stress were observed in only one previous study *(21).* Isonhood *(45)* studied the heat and freeze-thaw tolerances of two *A. hydrophila* strains following starvation or cold stress. Following stress, bacteria were not more heat or freeze-thaw resistant. Exposure to moderate acidic conditions did enhance subsequent acid resistance to more extreme pH conditions, findings that are similar to results reported by Karem et al. *(53).*

The alternative sigma factor RpoS orchestrates the stress responses of many of the *Enterobacteriaceae,* including *E. coli (19), Salmonella (96),* and *Shigella (94).* Strains of *E. coli* or *Shigella flexneri* without functional *rpoS* show a decreased stress response to acid and starvation, and the cross-protective effects of these stresses are also decreased. *Salmonella* virulence is decreased, *(96),* as is that of *Yersinia enterocolitica (44),* in the absence of RpoS. Yildiz and Schoolnik *(98)* isolated and characterized an *rpoS* homolog in *Vibrio cholerae.* Mutants of the *Vibrio rpoS* exhibited a decreased ability to survive environmental stress. *rpoS* did not enhance acid resistance for *V. cholerae* as it did for *Enterobactericeae.* An *rpoS* gene has not been reported for *Aeromonas.* Further studies on the genotypic and phenotypic characterization of the stress response of *Aeromonas* will further elucidate its role in foodborne disease and its pathogenicity.

CONCLUSIONS

Aeromonas is an estuarine bacterium implicated in occasional gastroenteritis outbreaks. *Aeromonas* is not unusually resistant to traditional food processing techniques but is ubiquitous on seafoods, meats, and fresh produce. Additional research is necessary to elucidate the stress response of this organism and to expand knowledge of virulence and pathogenicity and their relation to foods and food processing.

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