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Aeromonas hydrophila: A Challenging Food Borne Pathogen

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ABSTRACT:
Aeromonas hydrophila is one of the most challenging, ubiquitous and opportunistic food borne pathogens. It mainly resides in aquatic environments and is capable of growth within a wide temperature range (-0.1°C to 37°C). Due to its capacity to grow even at low temperatures it has major role in spoilage of packaged foods. It causes various diseases like endophthalmitis, gastroenteritis, cellulitis, meningitis, diarrhoea, etc. It is also resistant to many antibiotics, hence it has become one of the most problematic food borne pathogens to handle. It is required to develop novel approaches for controlling this organism.

Key words: Foodborne pathogen, Gastroenteritis, Aerolysin.

INTRODUCTION

Aeromonas hydrophila is an ubiquitous, heterotrophic food borne pathogen which is widely distributed in aquatic environments like fresh, marine, estuarine, salt, etc. [1-3]. *A. hydrophila* is a gram-negative, gammaproteobacteria, facultatively anaerobic, nonsporeforming straight rod [1]. They are 0.3 to 1 µm wide and 1 to 3.5 µm in length. They exert motility in aquatic environments with polar flagella on their surface. The optimal temperature for the growth of *A. hydrophila* is 28°C but some strains can grow upto 37°C. Few strains can grow even at temperature as low as -0.1°C [4]. Recently, effect of packaging atmosphere on the microbial and biochemical attributes of fresh pearlspot (*Etroplus surantensis* Bloch) stored at 0-2°C was investigated and *A. hydrophila* was found to grow under modified packaging atmosphere [5]. This organism is neither salt (<5%) nor acid (min. pH ~ 6) tolerant [6].

HISTORY, NOMENCLATURE AND CLASSIFICATION

A. hydrophila was first isolated from drinking water by Zimmerman in 1890s and after that Sanarelli isolated it from frogs, but they called them *Bacillus punctata* and *Bacillus hydrophila* respectively till 1930s, when the genus *Aeromonas* was first described. In 1936, Kluyver and Van Niel used the genus name *Aeromonas* in their "natural system of classification of bacteria", but they termed *A. hydrophila* as *A. liquifaciens*. It was again discovered by Hoshina in 1962 while working for finding the causes of eel and fish disease "red fin". MacInnes et al. performed the first DNA hybridization experiments with *Aeromonas* and concluded that the genus *Aeromonas* consisted of two main evolutionary lines: a diverse group of motile aeromonads, and the genetically more homogenous non motile aeromonads, comprising the species *Aeromonas salmonicida* [7].

ISOLATION AND IDENTIFICATION

This organism can be isolated from fresh water, soil, brakish water, marine water and sewage streams. Since they are found in water and sewage they are potential contaminants of foodstuff like vegetables and other foods of animal origin like milk and dairy products. Their presence can be detected in raw, refrigerated or frozen food [8-12]. Isolation of *A. hydrophila* can be achieved on starch ampicillin agar, bile salts Irgasan brilliant green agar, blood ampicillin agar, Ryan *Aeromonas* agar and in alkaline peptone water. Most widely used broths for enrichment of *A. hydrophila* are trypic soy ampicillin broth and alkaline peptone water broth [7]. For identification of *A. hydrophila* various biochemical tests can be performed. *A. hydrophila* shows positive result in various sugar fermentation tests, viz. glucose, sucrose, maltose, sorbitol, trehalose, etc. Its inability to ferment xylose serves an an useful distinguishing feature for identification. It gives positive result in voges-proskauer, indole production, oxidase and catalase tests. It also has the ability to hydrolyse esculin [7,13,14].

GENOME

Complete genome of *A. hydrophila* strain ATCC 7966^T has been sequenced which is made up of single circular chromosome comprising 4,744,448 bp with 61.5% GC content [15-17]. The genome consists of 4288 genes out of which 4129 are protein coding genes and 159 are RNA coding genes: 128 t-RNA genes and 30 r-RNA genes and one gene codes for other RNA. Of the coding sequences 72.3% have been known to have function while 21.5% possess similarity to genes of unknown function and no function has been assigned to 6.2% of the coding sequences [18].

Genomic study of *Aeromonas* explains what makes it a pathogen. *Act* (Aerolysin cytotoxic enterotoxin) gene is the primary gene involved in the pathogenicity and Act

is made up of single polypeptide. Act is secreted via type II secretion apparatus and has cytotoxic, enterotoxic, and haemolytic properties [15]. *Aeromonas* species possesses a large variety of virulence factors but how they are responsible for causing diarrhoea is not yet clear. But it was found that a cytotoxic enterotoxin (Act), a heat labile cytotonic enterotoxin (Alt) and a heat stable enterotoxin (Ast) are responsible for diarrhoea. Products of both *Alt* and *Ast* genes may act synergistically to induce severe diarrhoea [19]. Many bacterial pathogens of animals and plants have been shown to inject anti-host virulent determinants into the host via type III secretion system (TTSS). Yu et al. located the TTSS gene cluster in *A. hydrophila* and found that insertional inactivation of two of the TTSS genes (*apoB and apoC*) led to decreased cytotoxicity, increased phagocytosis and reduced virulence [20].

PATHOLOGY

A. hydrophila secretes a wide range of extracellular virulence products like enterotoxins, cytotoxins, lipases, haemolysin, proteases etc. When it enters into the host, it travels through the blood and enters into the available organs, where it produces various enterotoxins most notably aerolysin cytotoxic enterotoxin (Act), which causes tissue damage. *A. hydrophila* has been known to be responsible for causing intestinal infections like gastroenteritis and diarrhoea [19, 21].

Aerolysin is the main cause of pathogenicity of *A. hydrophila* which is found to be associated with diarrhoeal disease and wound infections. It is secreted as protoxin of 52 kDa which is proteolytically cleaved into a 25 residue carboxyterminal peptide and 48 kDa active protein. Further conversion of water soluble form of the toxin into a transmembrane channel (ca 1.5 nm in diameter) is a multistep process which is responsible for destruction of the cells by breaking their permeability barriers [22].

A. hydrophila is also found to be associated with variety of extraintestinal infections like peritonitis, cholangitis, skin and soft tissue infections, pneumonia, meningitis, haemolytic uremic syndrome,. myonecrosis, bacteraemia, septicemia, eczema and occular infections. When *A. hydrophila* crosses the blood–occular barrier to reach the eye via blood stream, it causes a sight-threatening condition known as endogenous endophthalmitis [23, 29]. Out of these diseases some are exclusively found in immunocompromised individuals, where as gastroenteritis and diarrhoea can occur in healthy individuals too.

Ljungh et al. found *A. hydrophila* as causative agent of acute diarrhoeal disease [24]. They proposed that the enterotoxin produced by *Aeromonas* should be classified among the cytotonic enterotoxins rather than cytotoxins. Guera et al. performed studies on patients with gastroenteritis and diarrhoea in Brazil. They found that most of the genes responsible for virulence like *aerA*-aerolysin/haemolysin, *ahpA*-serine protease, *satA*-glycerophospholipid-cholesterol acetyl transferase, *lipA*-lipase and *ahyB* elastase and virulence factors such as haemolytic, proteolytic, lipolytic activities and biofilm formation were present in majority of the *A. hydrophila* isolates. Multiple factors are involved in virulence viz. adhesins, S-layer, lipopolysaccharides, siderophores and a variety of exoenzymes and exotoxins i.e. aerolysin/haemolysin, lipases, proteases. Gene *aerA* was found in *A. hydrophila* isolates from all the patients with toxigenic diarrhoea which supports the fact that aerolysin is associated with watery diarrhoea. All the strains with a*erA* gene also possessed serine protease gene. This serine protease is responsible for activation of aerolysin and other extracellular enzymes which further adds on to the virulence of *A. hydrophila.* The *ahpA* gene was present in only some of the dysenteric sample isolates (23%) whereas *ahyB* gene that codes for metalloprotease (elastase) was present in most of the isolates (88.9%). Most of the isolates were also showing the presence of *lipA* and *satA* genes. They have been found to be associated with leucocyte and intestinal damage. Biofilm formation was also found in most of the isolates which helps in polar flagellar assembly and bacterial adhesion to host tissues [25,26]. *A. hydrophila* causes diarrhoea by producing enterotoxin after initial colonization of the epithelial cells through type IV pili [27,28].

Aerolysin positive *A. hydrophila* strains have been found to be occurring in sea water and in association with marine copepods which could facilitate the transport of the bacteria to other locations. This suggests that this organism should be included as one of the microbial indicator in routine water quality monitoring programs [30].

Molecular characterization of three enterotoxins (Act, Alt, and Ast) produced by *A. hydrophila* helped in defining their individual contribution in diarrhoea. It was found that bloody diarrhoea was associated with the production of cytotoxic enterotoxin - Act, where as nonbloody diarrhoea was due to cytotonic enterotoxins - Alt and Ast. These studies were performed by developing mutants with various combinations of deletions of enterotoxin genes. Apart from various enterotoxins produced by *A. hydrophila*, the two major extracellular proteolytic activities of: (1) 38-kDa thermostable metalloprotease and (2) 68-kDa heat labile serine protease also contributes to its virulence properties. It was found that *AhyB* gene which codes for Ahy B protease contributes significantly to the

elastolytic activity of these bacteria [31-33].

Recently, Erova et al. reported that DNA methyltransferase influences the virulence of *A. hydrophila*. They found that *dam* gene was essential for the viability of the bacterium, and as they overproduced this Dam in *A. hydrophila* SSU strain, using arabinose-inducible, P_{BAD} promoter-based system, the virulence and pathogenicity of the pathogen got reduced by 58%. However, cytotoxic and haemolytic activities associated with Act as well as the protease activity in the culture supernatant of a Dam-overproducing strain were increased by 10-, 3-, and 2.4-fold, respectively, compared to the control [34].

PREVENTION AND TREATMENT

A. hydrophila spreads through water and food. Applying sodium hypochlorite (1%) and calcium hypochlorite (2%) solution can kill this pathogen thus eliminating them and preventing the infection [35]. *A. hydrophila* can infect specially to those fishes, which are in poor environment. Consumption of such infected fishes leads to transmission of disease. This can be prevented by providing good environmental conditions like proper nutrition, enough dissolved oxygen, good water quality, less carbon dioxide and reduced stress conditions. As consumption of infected fishes can lead to diseased condition, certain precautions should be taken while dealing with fishes. To avoid infection and for maintaining proper personal hygiene and sanitation it is suggested to wear mask and gloves, while handling fishes. In order to minimize the chances of occurrence of disease, transfer of fishes from hatchery to hatchery should be avoided or transfer should be with proper sanitation. Fishes should be handled gently and checking for the infection should be done during each operation [36-38].

A. hydrophila shows resistance to antibiotics such as cabenicillin, vancomycin, ampicillin, cephalothin, rifampicin, penicillin, cefoxitin, sulbactam, erythomycincefoxitin, bacitracin, and trimethoprim [39, 35] and is susceptible to cephalosporins, carbapenems, and quinolones. Chemotherapeutic agents such as colistin, amphenicol, kanamycin, tetracycline, gentamicin, netilmicin, amikacin, cefuroxime, norfloxacin, and cefotaxime also show activity against *A. hydrophila* and inhibit the growth of this organism upto certain extent [7, 40]. The inhibitory effect can be increased by combination of cefotaxime and minocycline. By far, *A. hydrophila* is most susceptible to ciprofloxacin than any of above antibiotics [12].

CHALLENGE FOR FOOD INDUSTRY

A. hydrophila at low temperature

Most significant feature with regard to any threat *A. hydrophila* may pose in foods is its ability to grow down to chill temperatures. It has a lag time of >22 days (at 0 to 1°C), 6 to 10 days (at 2 to 3°C) and 3 to 4 days (at 5°C). Generation time at 0-1°C is reported to be in excess of 49 h^{-1}. [6] Bacteria surviving at low temperature have been shown to possess cold shock proteins (Csp). In the family of cold shock proteins Csp A has the highest induction level hence it is termed as major cold shock protein. After cold shock in *A. hydrophila* no Csp A like protein was present but only a 11 kDa protein was weakly and transiently expressed. Bacteria experiencing a cold shock response enter a state called as viable but not culturable (VBNC) state. During this state, the metabolic activity is maintained and the cells become coccoid in shape if they are normally rod shaped. *A. hydrophila* is also known to enter VBNC state after exposure to low temperature. This suggests that the ability to enter VBNC state could be responsible for the organism to be capable of surviving below temperature optimum for their growth with minimal metabolic rate [41]. Studies on growth of *A. hydrophila* at low temperatures would be useful as consumption of frozen foods is on rise throughout the globe.

A. hydrophila under modified atmosphere packaging

Ferial and Kareem performed characterization of ten *Aeromonas* species isolated from local food and *A. hydrophila* ATCC 7965. All strains of *A. hydrophila*, *A. sobria* and one strain of *A. caviae* showed strong haemolytic activity. Except one strain of *A. caviae*, all were protease producers. Some strains of *A. hydrophila* and *A. caviae* fermented lactose, coagulated milk and reduced litmus. When the food was frozen at -16ºC viability of the tested strains greatly decreased. *A. hydrophila* isolated from salt tolerated 6.5% salt and all the strains grew normally in the presence of 1 and 3% salt. In presence of potassium sorbate at pH 5.0, growth of the four strains was prevented while 0.05-0.3% potassium sorbate at pH 7.0 supported the growth of organisms [42]. García-Gimeno et al. evaluated the survival and growth of *A. hydrophila* in commercial mixed vegetable salads composed of lettuce, red cabbage and carrots, packed under modified atmosphere and stored at 4 and 15°C, by determining evolution of carbondioxide, oxygen and pH values. They found that in the first 24 h, *A. hydrophila* was able to grow at levels of 10^8 cfu/g with a subsequent decline in growth in salads stored at 15°C. The reason for decline in growth was low pH along with high CO_2 levels. At 4°C *A. hydrophila* was able to survive but unable to grow [43]. The spoilage of food can be prevented by modified atmosphere packaging (MAP), i.e. packaging under a gas atmosphere different from ambient air and by moderate vaccum packaging (MVP) - which is a special type of MAP that operates at a pressure of 400 mB. *A. hydrophila* is able to survive at low temperature hence

its growth is not inhibited by MAP. Although under moderate vacuum, growth of *A. hydrophila* on chicory endive was prevented when stored at 6.5°C. However there was only limited inhibitory effect in case of mung bean sprouts under same conditions [44]. Papageorgiou et al. inoculated *A. hydrophila* NTCC 8049 and wild isolate (food strain) in rice pudding (rice milk) at levels of ca. 2.5×10^2–4.0×10^2 cfu/g and stored at 4°C and 12°C. They found that the generation time of the type strain NTCC 8049 was 17.75 h and that of food strain was 20.38 h at 4°C. At 12°C generation time of type strain was 4.12 h and it was 4.20 h for the food strain. After 22 days at 4°C and 6-9 days at 12°C, maximum population of *A. hydrophila* was observed which was ranging from 8.00 to 9.23 $\log_{10}$ cfu/g [45].

In a survey [46], fresh vaccum packed commercial pork cuts stored at 5°C for 7 to 28 days and vaccum packed leg roast at 5°C for 21 days were sampled and further holding was done at -18°C for 90 days. It was found that out of 54 samples 20% were showing high pectinolytic colonies of *A. hydrophila* which were also cytotoxic. High frequency (92%) of contamination with the organism was found in chilled water. *A. hydrophila* was isolated from oysters frozen at -72°C for one and half year. Mary *et al.* reported that *A. hydrophila* can show cross-protection, in which cells starved for short (1 day) or prolonged (50 days) periods developed increased resistance to down shift at 4°C and ethanol stress [47]. This indicates that the cross-protection ability of the pathogen enables it to survive in harsh conditions that adds to problems related to this pathogen in food industries.

FINAL COMMENTS

Aforementioned properties and characteristics of *A. hydrophila* justify for considering this pathogen as worthy of prime attention as it is getting infamous for spoilage of various packaged food products and also in causing gastrointestinal infections. Controlling this organism with conventional chemotherapeutic agents is not easy as it is resistant to many of them. Hence it is necessary to design and develop some novel agents to overcome the threat posed by this organism. More investigation is needed to control the growth of this challenging pathogen which will help to avoid spoilage of frozen packaged food products and in precluding various gastrointestinal problems associated with *A. hydrophila* infection.

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