

Isolation and Identification of *Aeromonas* Spp. and It's Public Health Impacts

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Introduction:

The genus *Aeromonas* was first studied by Kluyver and Niel (1936) in their “natural system of classification of bacteria” which was based on both morphological and physiological characteristics. However, the sixth edition of Bergey’s Manual of determinative bacteriology (Breed *et al.*, 1948) did not incorporate *Aeromonas* as a genus and the species was included under the genus *Pseudomonas*. There were a number of studies during 1960s on the nomenclature and taxonomy of *Aeromonas* and the genus was stabilized and redefined in the seventh edition of Bergey’s manual (Breed *et al.*, 1957).

Aeromonas hydrophila and other *Aeromonas* spp. are ubiquitous organisms found in meat, fish, vegetables, drinking water and various other food items. They cause diarrhea and extra-intestinal infections in normal and immunocompromised patients. A number of *Aeromonas* spp. are able to survive and multiply in a variety of food products including raw, cooked and processed foods at low temperature and can produce virulence factors even at low temperature. (Balakrishna *et al.*, 2010)

Aeromonas are gram-negative rod shaped, oxidase positive, catalase positive, facultative anaerobic ubiquitous microorganisms (Monfort and Baleux, 1990; Farmer *et al.*, 1992). Most mesophilic species are motile due to the presence of flagella and grows in the temperature range from 30 to 45° C. with an optimum growth temperature about 35° C. Psychrophilic strains are non-motile, have an optimum growth temperature range of 15 to 20° C, and can even grow in low temperature as low as 0-5°C (Farmer *et al.*, 1992; Aberoumet *et al.*, 2010)

Public Health Effects:

The most important pathogens are: *A. hydrophila*, *A. caviae*, *A. veronii* biovar *sobria*. The organisms are ubiquitous in fresh and brackish water. They group with the gamma subclass of the Proteobacteria

Two major diseases associated with *Aeromonas* are gastroenteritis and wound infections, with or without bacteremia. Gastroenteritis typically occurs after the ingestion of contaminated water or food, whereas wound infections result from exposure to contaminated water. In its most severe form, *Aeromonas* spp. can cause necrotizing fasciitis, which is life-threatening, usually requiring treatment with antibiotics and even amputation.

Although some potential virulence factors (e.g. endotoxins, hemolysins, enterotoxins, adherence factors) have been identified, their precise roles are unknown.

Aeromonas species cause:

1. opportunistic systemic disease in immunocompromised patients
2. diarrheal disease in otherwise healthy individuals
3. wound infections

Isolation of *Aeromonas* spp :

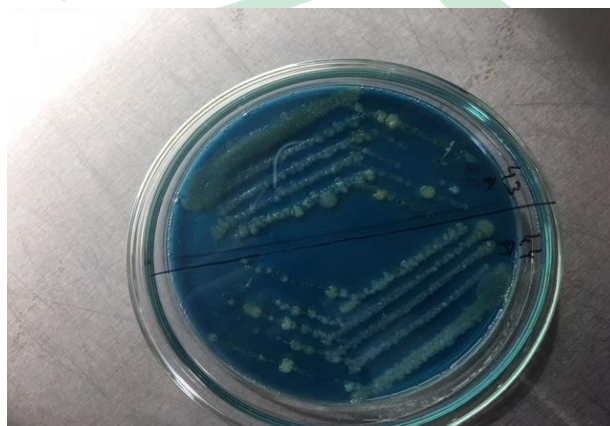
To isolate *Aeromonas* spp. the process prescribed by Balakrishna *et al.*, (2010) with slight modifications is followed.

Enrichment-

Twenty five grams of sample was taken, blended and discharged in 225 ml of Alkaline Peptone water for enrichment and incubated at 37⁰ C for 20-24 hours.

Selective plating-

After proper incubation loopful of enrichment media was streaked on Ampicillin Dextrin agar plate and incubated 37⁰ C for 20-24 hours. The *Aeromonas* colonies appeared as yellow coloured, small and smooth similar to honey drop.



***Aeromonas* isolates showing yellow coloured, small, smooth and honey drop like colonies on Ampicillin dextrin agar (ADA) plate**

Identification by biochemical screening of *Aeromonas* spp:**Catalase test**

In a test tube 2-3 ml of 3% hydrogen peroxide solution was taken and several colonies of the test organism were immersed in the hydrogen peroxide solution using a sterile glass rod. Immediate bubbling was observed in case of positive test.



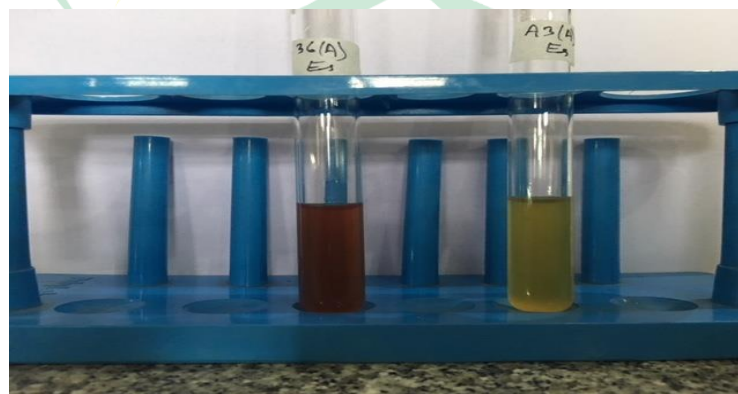
***Aeromonas* isolates showing positive catalase test (immediate bubbling)**

Oxidase test

A piece of filter paper was soaked with 2-3 drops of freshly prepared oxidase reagent. Using a stick or glass rod, remove a colony of the test organism was smeared on the filter paper. Development of a blue purple colour within a few seconds indicated positive test.

Esculin hydrolysis

The test culture was inoculated in esculin broth and incubated at 37°C. The broth was examined daily upto 7 days for blackening in positive test.



***Aeromonas* isolates showing Esculin hydrolysis test A. *Aeromonas* isolates showing blackening of broth (positive test) B. *Aeromonas* isolates showing no change in broth (negative test).**

Conclusion

As *Aeromonas hydrophila* is an ubiquitous bacterial pathogen it might cause serious losses to the intensive culture system across the country. So, it may be concluded that culturists should take proper management practices to avoid such disease of culture fishes especially silver carp and to get rid of the infection by such serious bacterial pathogen like *Aeromonas hydrophila* by improving their culture strategy and culture ecosystem.

