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SHORT COMMUNICATION

DETECTED OF *AERO* GENE IN *AEROMONAS HYDROPHILA* ISOLATES FROM SHRIMP AND PEELED SHRIMP SAMPLES IN LOCAL MARKETS

*Alaa Kareem Niamah**

Address: Food Science Dep., Agriculture College, Basrah University, Basrah City, Iraq

*Corresponding author. Tel.:+9647709042069; E-mail: alaakareem2002@yahoo.com

ABSTRACT

A 62 isolates were isolated from 33 samples of shrimp and peeled shrimp in three local markets in Basrah city. A 36 isolates were identified to be *Aeromonas hydrophila* bacteria depending on morphological, microscopic examinations and ten biochemical tests. 83.33% (30 isolates) from isolates produced ß-haemolysis on tryptone soy agar with 5% sheep blood. *Aero* gene detection by polymerase chain reaction technique, Theband appearance in the amplified gene of bacteria shows the molecular weight of aerolysin (424 bp). A 100% (36 isolates) of *Aeromonas hydrophila* isolates were contained *Aero* gene.

Keywords: *Aeromonas hydrophila*, ß-haemolysis**,** *Aero* gene

INTRODUCTION

Aeromonas organisms are straight gram-negative rods with occasional filaments. The majority are motile with polar flagellae, however, some strain are non-motile. *Aeromonas* species are indigenous to aquatic environments world-wide. The main virulence factors are 3 toxins that are enterotoxins, aerolysin and hemolysin in addition to other factors such as adhesions and mucinase production **(Rabaan *et al*., 2001).**

A. hydrophila are Gram-negative, facultative anaerobic bacteria that can be isolated from many sources, such as food, drinking water, sewage, environmental water and human clinical samples with a world-wide distribution. These bacteria can develop in refrigeration temperatures and are responsible for food and water-borne diseases, that can cause a range of

human diseases that vary in severity from a self-limiting gastroenteritis to potentially fatal septicemia **(Tsai *et al.*, 2006)**.

Aerolysin and hemolysin genes are reported to be the putative virulence genes of *A.hydrophila.* Aerolysin, produced by some strains of *A. hydrophila,* is an extracellular, soluble, hydrophilic protein exhibiting both hemolytic and cytolytic properties. Polymerase chain reaction (PCR) technique was used to assay for the detection of *aero* and *hlyA* genes in *Aeromonas* spp. isolated from environmental and shellfish sources **(Yousr *et al.*, 2007)**.

Isolation six types of *Aeromonas* spp. were diagnosed from tap water in Basrah City which including: *A. hydrophila* (35 isolates) dominant species isolated from 9 districts, 32 isolates of *A. caviae*, 26 isolates of *A. schubertii* , and 21 isolates of *A. eucrenophila* while the total number of isolates of *A. encheleia* and *A. veronii* bv. *veronii* was 17 and 15 respectively **(Vartan, 2009)**.

The aim of this study was isolated *A. hydrophila* bacteria from samples of shrimp and peeled shrimp taken from the markets of Basra city, Iraq. And estimated the *Aero* gene by PCR.

MATERIALS AND METHODS

Samples collection and bacteria isolation

A 21 samples from shrimp and 12 samples peeled shrimp was collected from three local markets (A, B and C) in Basrah City. Twenty five grams of samples were weighed aseptically and homogenized 2 min with 225ml of alkaline peptone water (pH 8.5). After 24h of incubation at 37°C. A 1ml of the enrichment was inoculated in Starch Ampicillin Agar (SAA) and incubated for 18-24 h at 37°C **(Palumbo *et al.*, 1985)**.

The plates were flooded with approximately 5 ml of iodine solution. Amylase and oxidase positive colonies were isolated.

Identification of bacteria

Aeromonas hydrophila were identified by Microscopic test (Gram staining) and Biochemical tests to species level. Ten tests were using such as motility, Kovac's oxidase, glucose fermentation, catalase production, indole test, methyl red test, mannitol fermentation test, voges proskauer test, H_2S production and growth on eosin methylene blue agar **(Barrow and Feltham, 2003)**.

Haemolysis assay

Haemolysis was assayed on tryptone soy agar (Mast laboratories Ltd., U.K.) plates with 5% whole sheep blood **(Lye and Dufour , 1991)**. Plates were incubated at 30°C and were checked for the type (α or ß) of haemolytic activity after 24 h.

Detection of *Aero* gene

The polymerase chain reaction (PCR) was used to detect the presence of the aerolysin in all isolates. The primers used (Aero 2F: 5'-AGC GGC AGA GCC CGT CTA TCC A-3' and Aero 2R: 5'-AGT TGG TGG CGG TGT CGT AGC G-3') PCR was carried out on a cycler using the following cycle: preheating at 95°C for 5 min followed by 30 cycles at 95°C for 2 min, 55°C for 1 min and 72°C for 1 min, followed by 7 min final extension at 72°C. PCR products were examined by electrophoresis in 1.5% agarose gel in TBE buffer. The gel was stained with EtBr and saw under UV light **(Yogananth *et al*., 2009)**.

RESULTS AND DISCUSSION

A 62 isolates were obtained from total samples (33 samples). These were distributed among 40 from shrimp and 22 from peeled shrimp. As isolated 24 from A market, 18 from B market and 20 from C market (Tab 1).

Table 1 Number of isolates from Local markets in Basrah city

Samples / Markets	Shrimp	Peeled shrimp	Total
A	18	6	24
B	10	8	18
C	12	8	20

A 36 isolates (58% from total isolates) were G^-, short rod, oxidase and catalase production, acid production from glucose and mannitol, indole test, methyl red test, voges proskauer test and H_2S production positive, the colonies were red mucosa when growth on eosin methylene blue agar (Fig.1). These results were consistent with **(Holt *et al*., 1994).** Therefore, 36 isolates belonging to *A. hydrophila* bacteria.

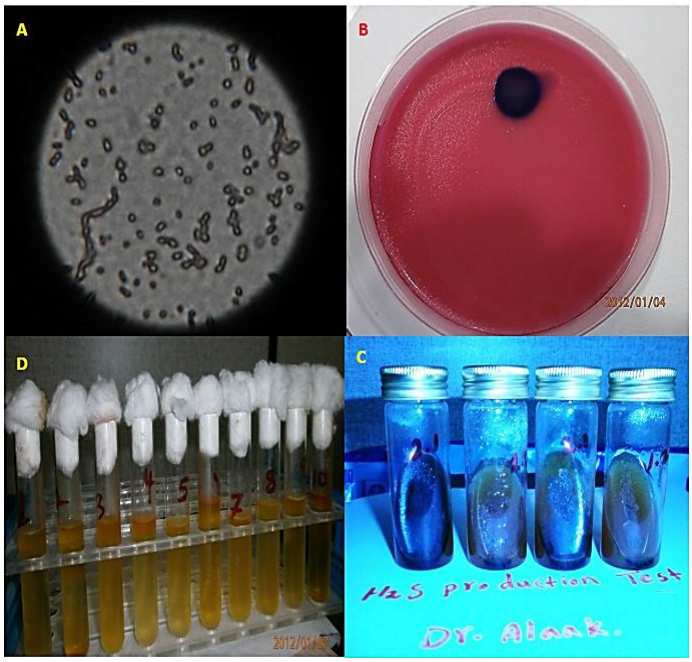

Figure 1 A result some of the tests of *A. hydrophila* bacteria, (A) The bacteria cells after Gram staining were under light microscope, (B) oxidase production, (C) H_2S production, (D) fermentation of mannitol

The majority (30 of 36) of isolates 83.33% of the *A. hydrophila* produced ß-haemolysis on tryptone soy agar with 5% sheep blood. The β-hemolysis pattern results in the media displaying clear halos around bacterial colonies (Fig. 2) The haemolysins produced by *A. hydrophila* are divided into two major groups, such as extracellular haemolysin and aerolysin based on immunological studies **(Kozaki *et al.*, 1989)**.

Figure 2 ß-haemolysis production by *A. hydrophila* on tryptone soy agar with 5% sheep blood

The bands appearance in the amplified *Aero* gene of screened shrimp and peeled shrimp shows the molecular weight of aerolysin (*Aero* gene 424 bp). Amplification result obtained through the present study also shows the same molecular weight of aerolysin gene (Fig. 3).

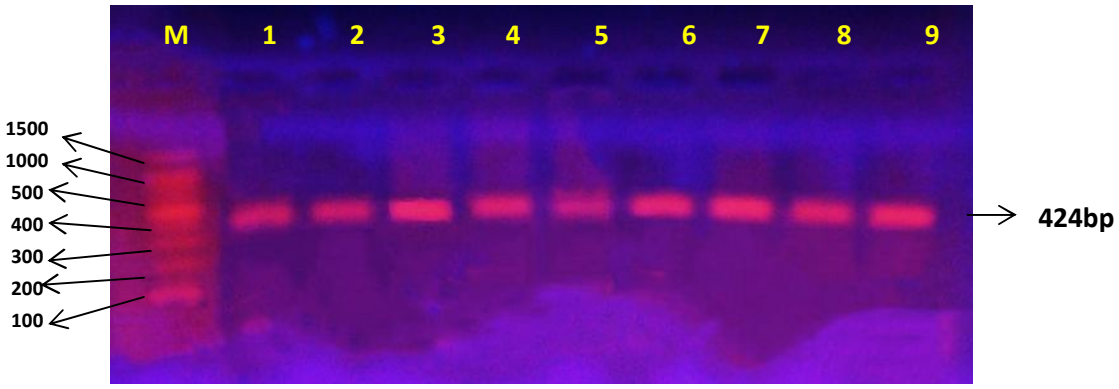

Figure 3 Electrophoresis of PCR production on 2% agarose gel, Lane 1 to Lane 9 *Aero* gene, Lane M marker DNA standard (100-1500) bp

All isolates of *A. hydrophila* (n=36) have the *Aero* gene. The PCR results matched with **(Yogananth *et al*., 2009).** As indicated Possibility to detect *Aero* gene in *A. hydrophila* bacteria Isolated from infected fish in the markets of India. This study proved presence *A. hydrophila* in shrimp and peeled shrimp samples .The ice and water used in cooling an important role in the presence of these bacteria in shrimp and peeled shrimp. The bacterium has been recognized as an active spoiler of fish under refrigeration **(Pansare *et al.*, 1986)**.

CONCLUSION

This study isolated *Aeromonas hydrophila* bacteria from shrimp and peeled shrimp samples in three local markets in Basrah city of Iraq. The majority isolates were ß-haemolysis production and. All isolates were contained *Aero* gene charge of aerolysin.

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