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VARIATION ANALYSIS OF PROTEIN DENSITOMETRY PROFILES OF *AEROMONAS HYDROPHILA* ISOLATED FROM OREOCHROMIS NILOTICUS FISH CAUGHT AT CAIRO, GHARBIA, MANZALA AND EDQUO AREAS

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SUMMARY

Aeromonas hydrophila was isolated from water, sediment soil and pathogenic fish lesions in four different areas at Cairo, Gharbia, Manzala and Edquo. Six optic density major bands 36kDa, 40kDa, 52kDa, 60kDa, 71kDa, and 80kDa, were observed in commassie stained separated crude protein profile of pathogenic form organism in sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE). The saprophytic forms isolated from water and sediments showed increase in bands located at 40kDa and 180 kDa, which may be related to virulence factor.

INTRODUCTION

Bacteria of genus Aeromonas are motile gram negative, short flagellated rods, cytochrome oxidase positive. Many authors showed that the organism is a constant component of microbiota of fresh reservoirs where, it together with other microorganisms, plays an important role in fish diseases syndromes (Companets et al 1992). Aeromonas were isolated from fish cases suffering from tail rot, wounds, ulcers, inflamed spots on skin and from septicaemic cases .These bacteria were isolated from wounds affecting trout as well as frogs causing the so-called "red leg" disease (Reed and Toner, 1942). Ulcerative disease syndrome is an epizootic fish disease caused mainly by Aeromonas hydrophila and characterized by the presence of severe, open dermal ulcers on the head, mid-body and dorsal regions of the fish (McGarey, et al., 1991). Motile aeromonads are ubiquitous aquatic bacteria that can cause motile aeromonads septicaemia and produce significant economic loss (Ford and Thune, 1991).

The aim of this study was to evaluate the etiologic pathogenic form proteins of Aeromonads which *infect Oreochromis* niloticus popular fish as well as saprophytic forms in different localities in Egypt.

MATERIALS AND METHODS

1- Isolation of *Aeromonas hydrophila* From caught fish

Sixty swabs were taken from hypraemic skin, wounds or ulcer lesions of *Oreochromis neloticus* fish at 4 localities: Cairo, Gharbia, Manzala, and Edquo areas representing water, bottom sediment of water environment surrounding fish and from the pathogenic lesions of diseased fish. The isolated bacteria from diseased fish as well as water and sediment were grown under identical conditions (24 hours at 25 °C) in 3% (wt/vol) tryptone soya broth medium supplemented with vitamins and inorganic ions according to Fyfe et al., (1987).

2- Separation and staining of Aeromonas hydrophila proteins by SDS-PAGE.

The isolated strain was mixed with antiprotease buffer (50 mM tris pH 8, 5 mM EDTA, 5mM iodo acetamide, 0.1mM TLCK, 44mM PMSF and 10mM leupeptin) then cold sonicated at 4°C. The solubilized protein was spectrophotometrically determined according to Bradford, (1976) and denatured in sample buffer containing 0.025mMTris-HCL (pH6.8), 2% (wt./vol.) SDS, 15%glycerol, 2.5% (vol./vol.) 2-mercaptoethanol and 0.02% bromophenol blue), then loaded as 75 ug protein per lane and separated in 7.5-17.5 gradient SDS-PAGE polyacrylamide gel electrophoresis at constant electrical current 40 mA for 3 hours according to Takacs, (1979). Labeled standard marker (10ul) was used for molecular weight comparison (Amersham, Arlington Heights, III ranging from 200-14.3 kDa.). The gels were stained with commassie brilliant blue stain R-250 (Baker USA) according to Frederick et al. (1994). The commassie stained protein bands profile in the gels were densitometrically analyzed to estimate the polypeptide profile fingerprint of loaded antigenic bacterial protein in comparison with standard molecular weight rainbow markers ranging from 14.3 kDa to 200 kDa (Amersham life science Co.).

RESULTS

Protein profile of natural and pathogenic aeromonads:

The densitometrical analysis of commassie stained SDS-PAGE containing separated crude proteins of *Aeromonas hydrophila* organism isolated from four different localities showed major common bands located nearly at 36kDa, 40kDa, 52kDa, 60kd, 71kDa and 80kDa (Fig.1). The relation between fractionated bands in intensity and

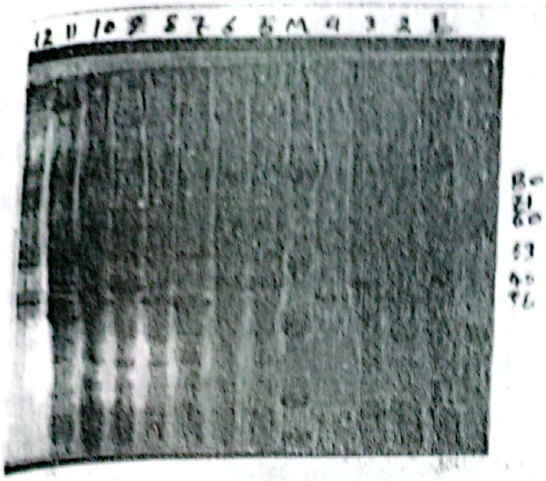

Fig. (1) SDS-PAGE - differential densitometry analysis of *Aeromonas hydrophila* strains isolated from four localities (M= Amersham molecular weight marker 200, 97.4, 69, 46, 30, 21.3, 14.3kDa. & 1,2,3 = Cairo , 4,5,6 = Gharbia , 7,8,9 = Manzala 10,11,12 = Edquo).

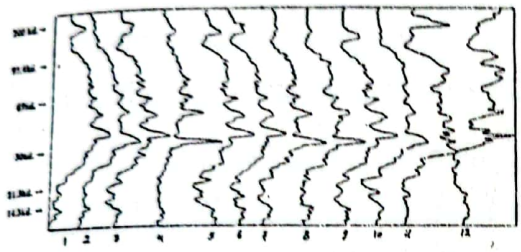

Fig. (2) Differential densitometry analysis of *Aeromonas hydrophila* strains isolated from four localities (1,2,3 = Cairo , 4,5,6 = Tanta , 7,8,9 = Manzala 10,11,12 = Edquo).

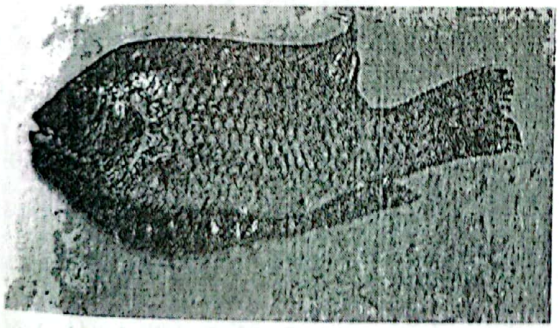

Fig. (3): *Oreochromis niloticus* fish affected with fin rot.

locality verification was varied. The fractionated protein patterns of isolated *Aeromonas hydrophila* from pathogenic lesions was characterized by major aggregated protein clusters band at 36kDa, 52kDa, 60kDa, 71kDa, 80kDa, while the Aeromonas isolated from water and sediment have increased major protein aggregation at 40kDa.and 180kDa. On the other hand, the saprophytic isolates at Cairo governorate showed intensities 3.7%, 5.2% & 10.6 %, 21.1% & 7.6%, 10.8% & 6.8%, 6.1% & 5.4%, 4.2% & 4.1%, 2.2% & 7.2%, 5.1% of total loaded sample optic density at 36kDa, 40kDa, 52kDa, 60kDa, 71kDa, 80kDa, 180kDa, respectively, while the isolated bacterial antigen at the same locality from pathologic lesions showed 15.8%, 3.9%, 12.1%, 8%, 4%, 6.2%, 0.6% optic density at the same molecular weight, respectively. The densitometry of isolated wound bacterial *A. hydrophila* protein from fish caught at Gharbia governorate revealed that 7.8%, 1.8%, 8.3%, 7.8%, 3.7%, 4.9%, 1.5% as optic density of total loaded sample at 36kDa, 40kDa, 52kDa, 60kDa, 71kDa, 80kDa, 180kDa, respectively, while the analytic optic densities of the same bacteria isolated from water and sediment were 3.4%,2.8% & 11%, 11.1% & 9.5%, 12.3% & 6.5%, 6% & 3%, 2.3% & 1.9%, 4.7% & 4.4%, 2.9 of total loaded sample at the same molecular weights. The water of lake Manzala showed presence of aeromonads isolated from fin rot pathogenic lesions (Fig.3). The separated protein of pathogenic organism recorded optic density ranged 14.3%, 3.6%, 12.6%, 8.5%, 3.5%, 2.7%,

0.9%, while natural living aeromonad bacteria isolated from water and sediment at the same locality showed 12.1%, 6.2% & 3.5%, 15.1% & 10.3%, 8.9% & 6.3%, 8.8% & 5.4%, 3.5% & 3.8%, 3.8% & 1.4%, 2.6% of total loaded sample at previously defined molecular weight masses. Lastly, the isolated pathogenic profile of *Aeromonas hydrophila* separated protein at lake Edquo showed 14.2%, 3.9%, 8.5%, 3.4%, 5.7%, 8.5%, 0.9% optic density of total loaded sample, while the naturally habitated ones were 3.2%,4.7% & 6.1%, 11.5% & 4.1%, 6.3% & 2.6%, 1.8% & 1.6%, 1.2% & 4.7%, 4.1% & 11.6%, 4.1% of total loaded sample percent at 36kDa, 40kDa, 52kDa, 60kDa, 71kDa, 80kDa and 180kDa. respectively (Table 1).

DISCUSSION

The principal virulence factor of Aeromonas is its A-layer which is composed of tetragonally arrayed approximately 50,000 protein subunit tethered to the cell surface. Physically, this layer protects the microorganism against bacteriophage, proteases as well as immune and non-immune complement. Specific mutants containing a disorganized A-layer are virulent and provide significant protection to bacteria (Kay and Trust 1991). Ascencio et al., (1991) showed that the *A. hydrophila* strain cell bind to the various proteins greater than other aeromonide types due to binding of collagen fibronectin and laminin to aeromonas cells, while the bacteria incubated with proteolyt-

Table (1) Densitometry analysis of aeromonads isolated from fish at different localities.

M.W.	Cairo			Ghatbia			Manzala			Edquo		
Optic density	% lesion	% soil	% water	% lesion	% soil	% water	% lesion	% soil	% water	% lesion	% soil	% water
36kDa.	15.8	3.7	5.2	7.8	3.4	2.8	14.3	12.1	6.2	14.2	3.2	4.7
40kDa.	3.9	10.6	21.1	1.8	11	11.1	3.6	3.5	15.1	3.9	6.1	11.5
52Da.	12.1	7.6	10.8	8.3	9.5	12.3	12.6	10.3	8.9	8.5	4.1	6.3
60kDa.	8	6.8	6.1	7.8	6.5	6	8.5	6.3	8.8	3.4	2.6	1.8
71kDa.	4	5.4	4.2	3.7	3	2.3	3.5	5.4	3.5	5.7	1.6	1.2
80kDa.	6.2	4.1	2.2	4.9	1.9	4.7	2.7	3.8	3.8	8.5	4.7	4.1
180kDa.	0.6	7.2	5.1	1.5	4.4	2.9	0.9	1.4	2.6	0.9	11.6	4.1

ic enzymes lose the ability of binding to protein. This finding agrees with the present results, where the pathogenic forms recorded more percentage of total loaded microbial protein sample than saprophytic ones. Mateos et al., (1992) observed two surface characters, agglutinating and non-agglutinating acriflavine *A. hydrophila* phenotypes significantly associated with water and sediment strains which agrees with present result, where the saprophytic isolated organisms showed increase of bands at 40kDa., 180kDa. percentage of total loaded sample than pathogenic ones. Tu and Lin (1992) isolated *A. hydrophila* bacteria from affected septicemic carp fish and separated a heat stable extracellular toxin with molecular weight 52.5kDa. on SDS-PAGE as a single polypeptide. The toxine named "hec" and had haemolytic and cytotoxic activities and recorded LD50 for carp 4.44 micrograms. This finding agrees with the present results in that the separated *A.hydrophila* profile from pathogenic lesion showed increase in band 52 kDa. optic density percentage of total separated protein sample specially from Cairo and Manzala fish samples. which may contain the same toxin, and also support the finding of Ford and Thune, (1991) who showed that the aeromonads produce a wide variety of hydrolytic enzymes and expressed cell surface characteristics linked to virulence, where as the other bacterial species rarely produced the same enzymes or cell surface characteristics and play an important role in this degenerative disease. This explains why the crude protein of pathogenic aeromonad has more percentage of separated bands contents than saprophytic ones.

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