Study on Outer Membrane Protein (OMP) Profile of *Aeromonas* Strains using SDS-PAGE

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Abstract

Mesophilic aeromonads are being increasingly reported pathogen of humans and lower vertebrates. Water and foods are considered to be the chief source of *Aeromonas* spp. At present there are several techniques available for the detection of *Aeromonas* spp. from water and foods. However, there is still need to develop immunodiagnostics for rapid detection of *Aeromonas* spp. irrespective of their species or serotype. To meet out this requirement present study was undertaken to identify the common protein moiety in their OMPs by SDS-PAGE so, that immunoassays can be developed for efficient and rapid detection of *Aeromonas* spp. from foods.
Key words: *Aeromonas*, OMP, SDS-PAGE, immunoassay, foods.

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Introduction

Emergence of *Aeromonas* spp. as an important human pathogen has led to a considerable interest in the organism in last two decades. Aeromonads are ubiquitous in nature being isolated from wide variety of sources. They are normal flora of aquatic and terrestrial animals as well as etiologic agents of disease in numerous cold- blooded and warm blooded animals (Cahill 1990; Joseph and Carnahan, 1994). The aquatic environment is considered to be the principal reservoir of *Aeromonas* spp. (Von Graevenitz and Mensch, 1968, Davis *et al.*, 1978; Burke *et al*., 1984a, b' Wadstrom and Ljungh, 1991) and organism is isolated from different waters including chlorinated drinking water (Lechevallier *et al*., 1982; Alvarez and Bitton, 1983; Boussaid *et al*., 1991; Kersters *et al*., 1995).

These bacteria are classified into; a psychrophilic, non motile group and a mesophilic, motile group. Complexity of mesophilic *Aeromonas* is further aggravated by various serotypes which they contain. Ewing *et al.*, (1961) is credited with preliminary work on serology of *A. hydrophila*. The first isolation of *Aeromonas* was made in 1890 from tap water in Chimnitz and named *Bacillus punctatus* (Zimmerman, 1890). A variety of foods and food products including seafood, chicken and red meat, vegetables, raw milk and its products have been shown to harbour *Aeromonas* spp. (Hunter and Burke, 1987; Majeed *et al*., 1989; Palumbo *et al.*,1989; Kirov *et al*., 1990). Cases of *Aeromonas* associated gastroenteritis have been reported from all over the world including Australia (Burke *et al*., 1983), Bangladesh (Sack *et al.*, 1987), Brazil (Schorling *et al*., 1990), Ethiopia (Wadstrom *et al*., 1976).

In India, Chatterjee and Neogy (1972) reported 8% isolation of *Aeromonas* spp. from the cases of choleric diarrhoea in Calcutta. Since then some reports of *Aeromonas* associated gastroenteritis have appeared from different parts of the country (Bhat *et al*., 1974; Sanyal *et al.*, 1983; De and Pal, 1984). Aeromonads are also known to cause extra intestinal human infections such as meningitis, endocarditis, peritonitis, septic arthritis, eye and urinary tract infections (Khardori and Fainstein, 1988; Krovacek *et al*., 1983). Since *Aeromonas* spp. are resistant to the antibiotics commonly used for treatment of wound infections, wounds exposed to water are

likely to be infected with *Aeromonas* spp. (Skiendzielewski and O'Keefe, 1990).

Materials and Methods

Aeromonas strains were isolated from different sources such as chicken meat, fish, milk, egg etc. using Buffered Cephalothin Dextrin Broth -10 (BCDB-10) as selective enrichment broth. Isolates were serotyped by the courtesy of Dr. T. Shimada, Chief, Laboratory of Enteric Infection 1, National Institute of Health, Tokyo, 162, Japan (Table 1).

Table-1: OMP strains and their concentrations

Strain no. (OMP)	Serotype	Protein concentration (mg/ml)
VPH 5	Rough	11.3
10	Rough	12.4
Buff 1	Unknown	14.4
C19	Unknown	16.2
69	Rough	12.2
F2	O73	12.6
F6	O6	11.5
AC5	O60	13.2
G3	O38	12.9
F17	O38	11.8
F7	O38	12.2
F14	O16	13.4
Floor 2	O16	14.4
C5	O16	11.8
C11	O16	13.5
C23	O16	13.2

Separation of Outer membrane protein (OMP): OMP of 16 *Aeromonas* strains was separated as per the method of Crosa and Hodges (1981) as modified by Santos *et al*. (1996). Briefly, *Aeromonas* grown overnight in 100 ml of tryptic soya broth (TSB, Difco) were recovered by centrifugation at 5000 rpm for 30 min. Cells were resuspended in 3 ml of 10 m mol/l tris buffer containing 0.3% (w/v) (pH 8.0) and sonicated with a sonifier (MSE, Ultrasonicator) (10 amplitude, 45 seconds, 4-5 times). After centrifugation at 10,000 g for 2 min. the supernatant fluids were transferred to new tubes and centrifuged for 1 hr at 17,000 g at 4^0C. Cell envelop suspensions were incubated with 3% sodium lauroyl sarcosinate (sarcosyl) (w/v) in 10 m mol/l tris buffer at room temperature for 20 min. Outer membrane protein was obtained by centrifugation at 17000 g for 1 hr and washed twice with distilled water. The OMP was stored at -20°C.

Protein estimation: The protein content was determined by the method of Schaterle and Pollack (1973) with little modifications. A small amount (25 µl) of OMP was diluted with 400 µl of distilled water. Different concentrations (25, 50, 75, 100, and 125 µg) of bovine serum albumin (BSA) were prepared in distilled water to a total volume of 400 µl. To all the tubes, 400 µl of 2x lowry concentrate was added and incubated at room temperature for a minimum of 10 min. Thereafter, 200 µl of the 0.2 N Folin reagents was added and incubated (55°C for 5 min). The Absorbances were read at 650 nm using polystyrene cuvettes. A standard graph was plotted against optical density (OD) of BSA in different concentrations. Protein concentration of OMP was estimated using standard graph.

SDS-PAGE analysis of OMP: OMP fractions were analysed by SDS-PAGE as per the method of Laemmli (1970) employing 12.5% (w/v) acryl amide in the resolving gel and 5% (w/v) acryl amide in the stacking gel. The samples were diluted with sample buffer in a ratio of 4:1 and heated at 95ºC for 5 min. Approximately, 30µlof the sample containing 100µg protein was loaded in each lane of the gel. The gel was run at 80V for 12 hrs, then stained with coomassie brilliant blue R-250(sigma) staining solution for 8 hrs and finally, destained with destaining solution. Calculation of molecular weight(s) (MW) of the peptide(s) was done by extrapolation of relative mobility of the unknown samples against that of standard molecular weight markers. Details of reagents used for SDS-PAGE are given in table-2.

Results and Discussion

The separation of OMP was carried out by culturing *Aeromonas* strains in brain heart infusion broth at 37^0C with shaking, followed by ultrasonification and centrifugation. Inner and outer membranes were separated by incubation with sarkosyl. This separation was necessary because OMP profiles are reported to be influenced by temperature and air supply to bacterial cultures (Statner *et al.*, 1988). This method was able to recover the whole OMPs from *Aeromonas* strains (Kuijper *et al*., 1989). OMP of 16 strains were separated and protein concentration estimated is presented in table 2. In this study protein concentrations were in the range of 11.3 mg/ml to 16.2 mg/ml.

Table-2: SDS-PAGE reagents

Reagent used	Components of reagent	Amount of components
1. 1M Tris HCl (pH 8.8)	Tris	12.1g
	Distilled water	50ml
	Adjustment of pH 8.8 with concentrated HCl and 100 ml volume was made with distilled water.	
2. 1M Tris HCl (pH 6.8)	Tris	12.1g
	Distilled water	50ml
	Adjustment of pH 8.8 with concentrated HCl and 100 ml volume was made with distilled water.	
3. Electrophoresis buffer (pH 8.3)	Tris	3.025 g
	Glycine	14.413 g
	SDS	1.0 g
	Distilled water to make	1000 ml
4. Sample buffer (5X)	1 M Tris HCl (pH 6.8)	0.6 ml
	50% Glycerol	5 ml
	10% SDS (w/v)	2 ml
	2-Mercaptoethanol	0.5 ml
	1% Bromophenol blue	1 ml
	Distilled water	0.9 ml
5. Acrylamide solution	Acrylamide	29.2 g
	Bisacrylamide	0.8 g
	Distilled water to make	100 ml
6. Running Gel (12.5%)	Acrylamide solution	12.5 ml
	1M Tris HCl (pH 8.8)	11.2 ml
	Distilled water	6.2 ml
	10% SDS	0.3 ml
	TEMED	20 µl
	10% ammonium per sulphate (w/v)	100 µl
7. Staking Gel	Acrylamide solution	1.67 ml
	1M Tris HCl (pH 6.8)	1.25 ml
	Distilled water	7.03 ml
	10% SDS	0.4 ml
	TEMED	10 µl
	10% ammonium per sulphate (w/v)	50 µl
8. Staining solution	Coomassie brilliant blue	0.15%
	Methanol	45%
	Acetic acid	10%
	Distilled water	45%
9. Destaining solution	Methanol	45%
	Acetic acid	10%
	Distilled water	45%

SDS-PAGE analysis of OMPs: SDS-PAGE analysis of OMP of different *Aeromonas* strains revealed up to 4 major and 4 to 6 minor polypeptide bands (Fig. 1 & 2). The molecular weight (MW) of the major polypeptide bands, estimated by comparison with standard MW markers run parallel was in the range of 14 to 55kDa. Large smearing of the bands in the MW range of 40 to 45 kDa was observed. In addition a few minor bands in the lower molecular weight range (10-12 kDa) were also observed in certain strains.

Major polypeptides of 14 and 35 kDa and minor polypeptide 25 kDa were common in most of the *Aeromonas* spp. irrespective of serogroup or species. Heterogeneity in protein profile was observed among *Aeromonas* strains of different serogroups as well as of same serogroups.

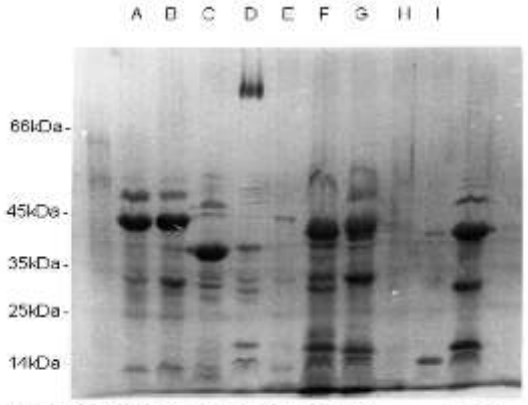

Fig 1 SDS-PAGE analysis of OMP of different *Aeromonas* strains
Lanes:A,VPI5;B,10;C,BuffD,C19;E,69;F,F2;G,P6;H,AC5;I,G3

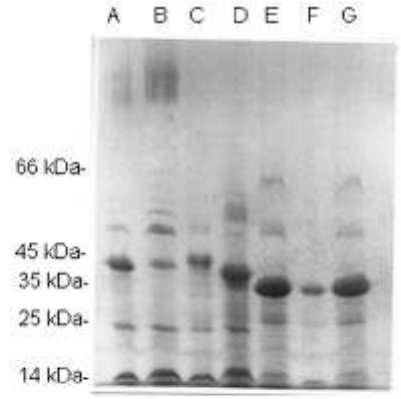

Fig 2. SDS-PAGE analysis of OMP of different *Aeromonas* strains
Lanes:A,F17;B,F7;C,F14;D,Floor2;E,C5;F,C11F2;G,C23

However, some similarity was observed for some species of *Aeromonas*. Protein profile was identical for 2 (F 7 and F 17) *A. caviae* strains and for 2 (VPH 5 and 10) *A. hydrophila* strains of rough serogroup.

The SDS–PAGE analysis revealed heterogeneity in protein banding pattern both within and among the species and serotypes, except for a few exceptions. Similar variability in OMP profiles has been described by Santos *et al*., (1996) who observed that *Aeromonas* strains belonging to different serogroups and as well as to same serogroup exhibited differences in their protein banding pattern. Aoki and Holland (1985) also observed wide variation in the profiles of *A. hydrophila* proteins and reported them to be due to serogroup differences, variation in habitat, host range and virulence. They also reported that OMP profile of *A. hydrophila* differed from *A. salmonicida*. In an interesting study Kuijper *et al*., (1989) analysed the OMP of 46 faecal *Aeromonas* strains from hybridization groups (HGs) 1 (*A. hydrophila;* n=10*)*,4 (*A. caviae*, n=16) and 8(*A. veronii;* n=20) and reported that every isolate of HG-1 and HG-8 had a unique OMP profile, in contrast to isolates of HG-4, which were separated into 5 different OMP types. Our study also revealed some homogeneity among *A .hydrophila* strains.

It may also be pointed out that 2 of the 3 rough strains tested in this study had identical OMP profile. Detailed studies on more number of strains are needed to confirm these findings. The study revealed 4 major and 4 to 6 minor protein bands within the MW range of 14 to 55 kDa. In addition some minor bands were also observed in the range of 10 to 12 kDa and 66 kDa with a few strains. This is in agreement to reports of earlier researchers who have noted major protein bands in the range of 30 and 45 kDa (Kuijper *et al*., 1989). Similarly Santos *et al*., (1996) observed major proteins to be in the range of 55 and 28 kDa. Dooley and Trust (1988) studied the OMP of highly virulence group of strains. They observed that major proteins of MW of 30 kDa and proteins in the molecular weight range of 45 to 55 kDa to be predominant. These variations are not unexpected due to the complex and diverse nature of *Aeromonas* spp.

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Conflict of interest

Authors declare that they have no conflict of interest.

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