Polish Journal of Microbiology
2005, Vol. 54, No 2, 91–95

# Numerical Analysis of Electrophoretic Periplasmic Protein Patterns of *Aeromonas* sp. Strains

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Received 1 December 2004, received in revised form 1 March 2005, accepted 4 March 2005

A b s t r a c t

A total of 103 strains of *Aeromonas* spp. isolated from clinical and from environmental samples was compared by using SDS-PAGE of periplasmic proteins patterns. Strains isolated from Polish children suffering from gastroenteritis did not appear similar to strains isolated from human living in Hong-Kong. *Aeromonas* sp. strains did not show a tendency to cluster according to their origin. Our results have demonstrated no species-specific periplasmic protein profiles. A significant protein electrophoretic heterogeneity was observed within the species *A. hydrophila*, *A. bestiarum*, *A. salmonicida*, *A. caviae*, *A. media*, and *A. veronii* biotype sobria.

K e y w o r d s: *Aeromonas* spp., electrophoretic periplasmic protein patterns

## Introduction

Members of the genus *Aeromonas* occur widely in the aquatic environment including freshwater, estuaries and marine (Altwegg, 1999). *Aeromonas* spp. are also isolated from diseased cold- and warm-blooded animals and from humans (Altwegg, 1999). The most common infection in humans is gastroenteritis, with frequent isolation of *Aeromonas* spp. from diarrhoeal stool (Altwegg, 1999). On the basis of DNA-DNA hybridization, 18 hybridization groups (HG) of *Aeromonas* sp. have been identified (Kaznowski, 1998; Altwegg, 1999; Pidyar *et al.*, 2002). A variety of methods have been used to type *Aeromonas* sp. for epidemiological and ecological purposes (Altwegg, 1996). Genotyping methods such as RAPD, ERIC-PCR and PFGE of total chromosomal DNA after restriction with rare-cutting endonucleases have been used with success in differentiation of isolates (Talon *et al.*, 1996; Hänninen and Hirvelä-Koski, 1997; Davin-Regli, *et al.*, 1998; Szczuka and Kaznowski, 2004).

Gargallo-Viola and Lopez (1990) have proposed electrophoretic periplasmic protein patterns for epidemiological study. The objective of our investigation was to evaluate the potential of this method for identification and differentiation of strains within *Aeromonas* spp. isolated of various origins. We also wanted to compare clinical strains isolated in Europe and Asia. We wanted to found out if strains showed a tendency to cluster according to their origin.

## Experimental

### Materials and Methods

**Bacterial strains.** The study was performed on 103 *Aeromonas* spp. strains, including type and reference strains (Tables I and II).

**Preparation of periplasmic protein samples, electrophoresis and staining.** The methods used for cultivation of bacteria, preparation of periplasmic protein samples, gel preparation and staining have been described previously (Ames *et al.*, 1984; Costas, 1992). The gels were visualized on a UV light transilluminator and documented with V.99 Bio-Print system (Vilber Lourmat, France). Computer analyses was determined using GelCompar II version 3.0 software (Applied Maths, Belgium). Cluster analysis was performed using the unweighted pair group method with average linkages (UPGMA).

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Table I
Strains of *Aeromonas* sp. isolated from human

| Genospecies | HG | Strain no | Source of isolation |
|---|---|---|---|
| *A. hydrophila* | 1 | RK 70363, RK 226254, RK 217215<br>ATCC 49140 | human stool, Hong Kong<br>human |
| *A. caviae* | 4 | AK 375, AK 376, AK 377, AK 378, AK 379, AK 380, AK 383, AK 384, AK 385, AK 386, AK 388, AK 390, AK 393<br>RK 25447, RK 27611, RK 65541, RK 66942, RK 77620, RK 217455, RK 220132 | human stool, Poland<br>human stool, Hong Kong |
| *A. veronii* biotype sobria | 8/10 | AK 382, AK 387, AK 389, AK 391, AK 392<br>RK 43939, RK 66113, RK 77343 | human stool, Poland<br>human stool, Hong Kong |
| *A. veronii* biotype veronii | 10/8 | ATCC 35624$^T$ | sputum |
| *A. jandaei* | 9 | ATCC 49568$^T$ | human stool |
| *Aeromonas* sp. | 11 | ATCC 35941 | abscess |
| *A. schubertii* | 12 | ATCC 43700$^T$ | abscess |
| *A. trota* | 14 | ATCC 49657$^T$ | human stool |
| *Aeromonas* sp. | * | RK 61871 | human stool, Hong Kong |

AK, Culture Collection of Department of Microbiology A. Mickiewicz University, Poznań, Poland; RK, strains obtained from Dr R. Kong, Hong Kong University; ATCC, American Type Culture Collection, Manassas, Va.; USA; * isolate not included in any of *Aeromonas* sp. HG.

Table II
Strains of *Aeromonas* sp. isolated from environmental sources

| Genospecies | HG | Strain no | Source of isolation |
|---|---|---|---|
| *A. hydrophila* | 1 | AK 44<br>ATCC 7966$^T$ | lake water<br>canned milk |
| *A. bestiarum* | 2 | AK 1, AK 41<br>AK 115<br>ATCC 23213<br>ATCC 23211<br>ATCC 13444<br>ATCC 51108$^T$ | lake water<br>drinking water, Konin<br>river water<br>drinking water<br>surface water<br>diseased fish |
| *A. salmonicida* | 3 | AK 46, AK 50, AK 76, AK 130, AK 131<br>AK 106, AK 117, AK 125<br>AK 400, AK 401, AK 402<br>AK 409, AK 410<br>CDC 0434-84 | lake water<br>rolling-mill emulsion<br>sea water<br>drinking water, Poznań<br>fresh water |
| *A. caviae* | 4 | AK 48, AK 335, AK 338, AK 339<br>AK 276, AK 296<br>AK 104, AK 126<br>AK 220, AK 232<br>AK 404, AK 405<br>AK 406, AK 407, AK 408<br>ATCC 15468$^T$ | lake water<br>sewage<br>drinking water, Konin<br>rolling-mill emulsion<br>river water<br>sea water<br>guinea pig |
| *A. media* | 5A | AK 42<br>AK 403<br>CDC 0862-83 | lake water<br>sea water<br>fish |
| | 5B | ATCC 33907$^T$ | fresh water |
| *A. eucrenophila* | 6 | AK 65<br>ATCC 23309$^T$ | lake water<br>fresh water |
| *A. sobria* | 7 | CIP 7433$^T$ | fish |
| *A. veronii* biotype sobria | 8/10 | AK 12, AK 59<br>AK 100, AK 102, AK 120<br>AK 156, AK 160, AK 164, AK 180<br>AK 165, AK 167, AK 176<br>AK 411, AK 412, AK 413<br>CDC 0437-84 | lake water<br>drinking water, Konin<br>dead fish<br>healthy fish<br>drinking water, Poznań<br>fish |

Table II continued

| Genospecies | HG | Strain no | Source of isolation |
|---|---|---|---|
| *A. allosaccharophila* | 15 | CECT 4199$^T$ | diseased eel |
| *A. encheleia* | 16 | CECT 4342$^T$ | fish |
| *A. popoffii* | 17 | LMG 17541$^T$ | drinking water, Belgium |

AK, Culture Collection of Department of Microbiology A. Mickiewicz University, Poznań, Poland; ATCC, American Type Culture Collection, Manassas, Va., USA; LMG, Culture Collection, Laboratorium voor Microbiologie Universiteit Gent, Belgium; CECT, Coleccion Espanola de Cultivos Tipo, Universitad de Valencia, Spain; CIP, Collection bacterienne de l˙Institut Pasteur, Paris, France; CDC, Centers for Disease Control, Atlanta, Ga., USA

## Results and Discussion

One dimensional SDS-PAGE of periplasmic protein extracts of 103 cultures of *Aeromonas* sp. strains produced patterns containing 10–26 discrete bands corresponding to molecular size of 20 to 100 kDa (Fig. 1). The reproducibility limits of protein patterns from different gels were r = >0.92. We identified seven clusters at the 90% S level (Fig. 1). Two clusters (2 and 7) contained strains isolated from Polish children suffering from gastroenteritis. Our previous genetic study using RAPD and ERIC-PCR methods demonstrated that strains included in cluster 2 and 7 are genetically different, (Szczuka and Kaznowski, 2004) which is in disagreement with the results obtained by SDS-PAGE method. Protein patterns of the remaining 13 strains isolated from Polish children suffering from gastroenteritis were clearly different. Strains isolated from stool of people living in Hong-Kong also showed distinct patterns. It is interesting that none of clinical strains isolated in Poland showed high degree of similarity to clinical strains originated from Hong-Kong. This is in accordance with our previously genetic analysis (Szczuka and Kaznowski, 2004).

Our results demonstrated that the majority of protein patterns of strains isolated from Polish children suffering from gastroenteritis did not match with those of the strains isolated from water supply. The present study revealed the existence of strains of *A. veronii* biotype sobria AK 411, AK 412, and AK 413 with very similar protein patterns in drinking water collected from city distribution system in Poznań (cluster 3). Domination of these strains in the water distribution system could be a result of being a component of biological membranes (Gavriel *et al.*, 1998). Our results also suggested colonisation of local industrial water distribution system in Konin by widespread strains of *A. veronii* biotype sobria AK 102, AK 100, and AK 120 (cluster 4).

We found that seven strains of *A. veronii* biotype sobria isolated from healthy and dead fish *Rutilus* collected from the same lake did not form a separate group. Only two strains, AK 176 and AK 167 (cluster 5), had very similar protein patterns. However they are not clonally related (Szczuka and Kaznowski, 2004). Patterns of the rest of strains isolates from fish were strain-specific. We observed considerable heterogeneity in protein profiles among strains isolated from the lake. Seven strains originated from seawater generated highly distinct profiles. No specific profile was obtained for strains isolated from sewage. However, we identified two clusters, cluster 1 and cluster 6, containing strains isolated from rolling emulsion. Strains of *A. salmonicida* AK 106, AK 125 and AK 117 (cluster 1) are clonally related as previously determined by RAPD and ERIC-PCR methods (Szczuka and Kaznowski, 2004). Strains of *A. caviae* AK 232 and AK 220 belonging to cluster 6 are not clonally related. This indicated that genetically different strains produce very similar proteins when they are isolated from very specific sources. This study demonstrated also that protein patterns reflect genome information of the bacteria because strains belonging to different species isolated from rolling emulsion did not group together.

We observed considerable heterogeneity in periplasmic protein profiles of isolates within *A. hydrophila, A. bestiarum*, *A. salmonicida*, *A. caviae*, *A. media*, and *A. veronii* biotype sobria. Previous analysis also revealed genetic heterogeneity within these species (Szczuka and Kaznowski, 2004). We did not obtain protein bands specific for the species and therefore the analysis of SDS-PAGE of periplasmic proteins can not be used for distinguishing *Aeromonas* species.

By using SDS-PAGE of periplasmic protein patterns we were able to recognise related strains (cluster number 1, 3, 4). However, some genetically different strains isolated from the same source also showed very similar patterns. Ruimy *et al.* (1994) suggested that bacteria contain the products of many regulated

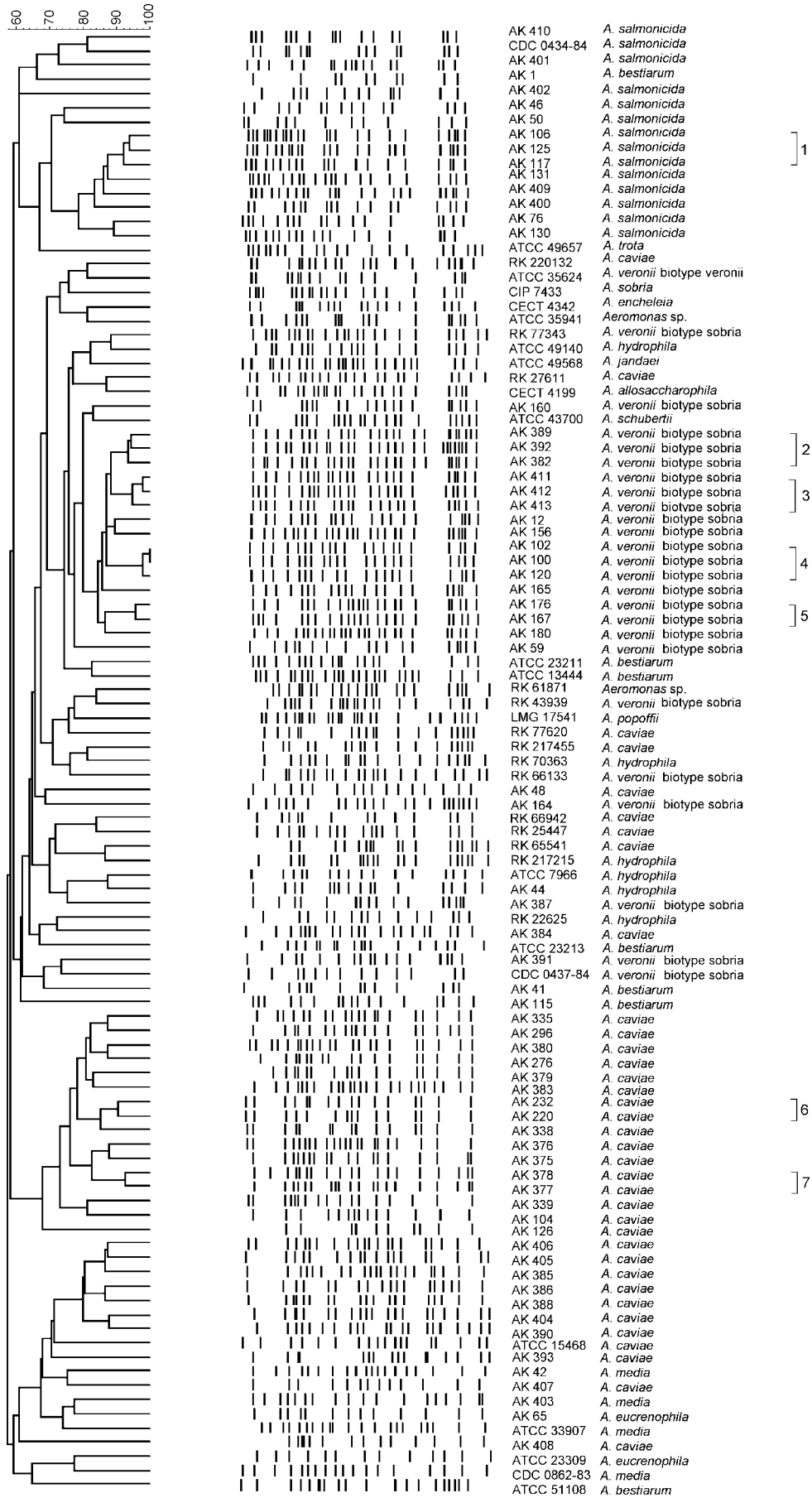


Fig. 1. Dendrogram showing periplasmic protein similarity of 103 strains of *Aeromonas* sp. determined by the SDS-PAGE protein pattern analysis using Dice similarity coefficient and UPGMA cluster method

genes that are expressed according to the environment in which they grew. Environmentally-linked phenotypes could be reflections of the ecosystems from which the bacteria were obtained and they could occur for more than several cell cycles.

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