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Research Article

# The Characterization of Protein Profiles of the *Aeromonas hydrophila* and *A. caviae* Strains Isolated from Gull and Rainbow Trout Feces by SDS-PAGE

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**Abstract:** Ten motile Aeromonads, including one *Aeromonas hydrophila* ATCC 7512, were used. Of the strains, five were isolated from gulls (one *A. hydrophila* strain and four *A. caviae* strains) and four from rainbow trout (three *A. hydrophila* strains and one *A. caviae* strain). Whole cell protein profiles of these strains were analyzed by SDS-PAGE. Forty-five pairs were formed in comparing the protein profiles of each strain. Of the pairs, 17 (37.8%) were different, 18 (40%) were unclear and 10 (22.2%) were identical. When the strains belonging to the same species were compared within themselves, four *A. caviae* strains were found to be identical, while all four *A. hydrophila* strains were different or of uncertain relationship to the group. One *A. hydrophila* strain isolated from gulls was found to be identical to four *A. caviae* strains again isolated from gulls.

In this study, *A. hydrophila* and *A. caviae* strains were isolated from gull feces and their protein profiles were shown by SDS-PAGE for the first time in Turkey. These findings are expected to contribute to future studies in this field.

Key Words: *Aeromonas hydrophila, Aeromonas caviae*, SDS-PAGE, gull, rainbow trout, feces

## Martı ve Alabalık Dışkılarından İzole edilen *Aeromonas hydrophila* ve *Aeromonas caviae* Suşlarının Protein Profillerinin SDS-PAGE Yöntemi ile Karakterizasyonu

**Özet:** Bu çalışmada biri *Aeromonas hydrophila* ATCC 7512 olmak üzere toplam 10 hareketli aeromonas suşu kullanıldı. Bu suşların beşi martılardan (bir *A. hydrophila* ve dört *A. caviae* suşu), dördü alabalıklardan (üç *A. hydrophila* suşu ve bir *A. caviae* suşu) izole edildi. Bu suşlara ait tüm hücre protein profilleri SDS-PAGE yöntemi ile analiz edildi. Her bir suşa ait protein profillerinin karşılaştırılması sonucu 45 çift oluşturuldu. Bu çiftlerin 17'si (% 37,8) farklı, 18'i (% 40) şüpheli ilişkili, 10'u (% 22,2) identik bulundu. Aynı türe ait suşlar kendi içerisinde karşılaştırıldığında dört *A. hydrophila* suşunun tamamı farklı veya şüpheli ilişkili bulunurken, beş *A. caviae* suşunun biri dışında dördü identik bulundu. Martılardan izole edilen bir *A. hydrophila* suşu, yine martılardan izole edilen dört *A. caviae* suşu ile identik bulundu.

Ülkemizde, *A. hydrophila* ve *A. caviae* suşlarının martı dışkılarından izolasyonunun ve SDS-PAGE yöntemiyle protein profillerinin ilk defa ortaya konulduğu bu araştırma, konuyla ilgili bundan sonra yapılacak çalışmalara katkıda bulunacaktır.

Anahtar Sözcükler: *Aeromonas hydrophila*, *Aeromonas caviae*, SDS-PAGE, martı, gökkuşağı alabalığı, dışkı

## Introduction

Aeromonas spp. are members of the Vibrionaceae (1). Although they are common in fresh surface water, their presence has been shown in the feces of several animals and humans (2-4). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of whole-cell proteins has proved to be useful for typing several bacterial species, including Aeromonads (5-7). A more sensible approach might be to identify isolates as *Aeromonas hydrophila*, *A. sobria* or *A. caviae*, and to use an electrophoretic fingerprinting technique to further characterize them (8). MacInnes et al. (9) carried out the first DNA hybridization experiments with Aeromonas, and concluded that the genus consisted of two main evolutionary lines: a diverse group of motile Aeromonads and genetically more homogeneous non-motile Aeromonads. Fanning et al. (10) confirmed that all strains of Aeromonas were more closely related to each other than to species in other families, and found that motile Aeromonas species could be divided into at least 10 different DNA hybridization groups.

In this study, we aimed to show the whole cell proteins, using SDS-PAGE, of *A. caviae* and *A. hydrophila* strains isolated from gulls and rainbow trout, and to

determine the relationship of the protein profiles of each strain.

## Materials and Methods

### Bacterial strains

A total of nine strains, five *A. caviae* and four *A. hydrophila*, isolated from the feces of gulls and the intestinal contents of rainbow trout were identified by classical methods (8).

### SDS-PAGE method

*A. hydrophila* and *A. caviae* strains were cultured on sheep-blood agar and incubated overnight at 37 ºC in air, and then washed in phosphate buffered saline (PBS) (pH 7.2). The cells were sedimented at 3000 g for 15 min, resuspended in 15% glycerol, 1% sodium dodecyl sulfate (SDS), and 0.1 M Tris/HCl pH 6.8, and denatured by treatment at 100 ºC for 20 min. Nonsolubilized material was removed by centrifugation at 3000 g for 15 min and the resulting supernatant was diluted 1:1 with 20% glycerol, 10% 2-mercaptoethanol, 4% SDS, and 0.125 M Tris/HCl, pH 6.8 (11). The protein concentration of the supernatant was adjusted to 0.75 µg/µl with the same buffer (12). After incubation for a further 2 min at 100 ºC, the samples were stored at –20 ºCfor electrophoresis. Then 20 µl of the sample was loaded on to the gel. SDS-PAGE was carried out at constant current (300 V). The resolving gel contained 12% acrylamide/bis acrylamide in a ratio of 29:1 with a stacking gel of 4.75% with respect to total acrylamide (11). Other running conditions and buffers were used as previously described (13). After electrophoresis, the gel was dyed with Coomassie Blue (0.025% Coomassie Blue R-250, 40% methanol, 7% acetic acid) for 3 h. The gel was kept in Destaining Solution I (50% methanol, 10% acetic acid) for 1 h and then transferred to Destaining Solution II (7% acetic acid, 5% methanol) (14).

### Statistical analysis

The gel was analyzed in a light box. By considering each band formed per strain in the gel, the presence and absence of each band in the other strain was determined (coded as 1 and 0, respectively). Thus all strains were compared one by one, and their similarity coefficient was calculated as

$$SM(x,y) = \frac{a+d}{a+b+c+d} \qquad (15).$$

Similarity coefficients were ranked as identical (71%), different (51%), and uncertain (51-71%) (16). Strains were then subjected to cluster analysis by unweighted pair-group method using arithmetic averages (UPGMA) linkage. The dendogram was carried out with MINITAB.

## Results

As shown in Figure 1, protein bands between 14 and 40 were recognized in the gel analysis. A total of 45 pairs were formed in the comparison of each strain. Seventeen (37.8%) of them were different, 18 (40%) were uncertain and 10 (22.2%) were identical. When *A. hydrophila* and *A. cavia* strains of the same species were compared within themselves, four *A. hydrophila* strains were found to be totally different or to have an uncertain relationship to the group. Of the *A. caviae* strains, four isolated from the gulls were identical, and one isolated from rainbow trout was found to be either different from or of uncertain relation to four identical strains isolated

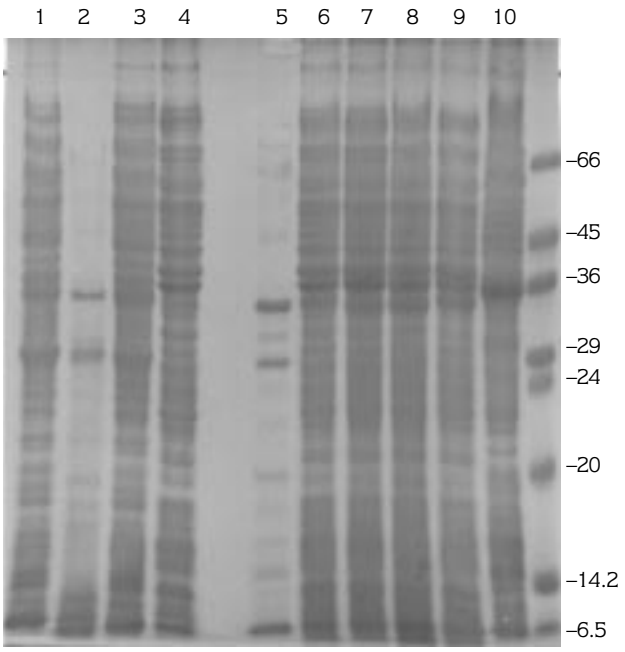


Figure 1. Protein profiles obtained from whole-cell extracts of *A. hydrophila* and *A. caviae* separated by SDS-PAGE.
Tracks 1,2,3 *A. hydrophila* rainbow trout isolates, 4 *A. hydrophila* Gull isolates, 5. *A. caviae*. Rainbow trout isolates 6,7,8,9 *A. caviae* Gull isolates, 10 *A. hydrophila* ATCC 7512.
* mol. wt. standards of 66, 45, 36, 29, 24, 20, 14.2, 6.5.

from gulls. In addition, one *A. hydrophila* strain isolated from gulls was found to be identical to four *A. caviae* strains isolated again from gulls. *A. hydrophila* ATCC 7512 standard strain was found to be either different from or of uncertain relationship to all strains (Table).

As shown in the dendogram, *A. caviae* strains isolated from gulls formed a group by showing over 80% similiarity, while the other strains formed single strain groups (Figure 2).

## Discussion

The utility of protein gel electrophoresis in microbial characterization has been established for 20 years. A second level of information for a microorganism is given by the cellular proteins, and different types of electrophoresis are used to explore the relationship at this level (17). Several studies have been carried out on Aeromonads by SDS-PAGE, and whole-cell protein profiles have been found useful for epidemiological

Table. Similarity coefficient (%) according to track no.

| Strain No. | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 49 | 70 | 58 | 45 | 62 | 60 | 60 | 60 | 55 |
| 2 | | 53 | 42 | 70 | 42 | 43 | 43 | 42 | 28 |
| 3 | | | 51 | 49 | 43 | 45 | 45 | 43 | 55 |
| 4 | | | | 45 | 77 | 75 | 75 | 77 | 47 |
| 5 | | | | | 53 | 55 | 55 | 53 | 42 |
| 6 | | | | | | 98 | 98 | 100 | 62 |
| 7 | | | | | | | 100 | 98 | 64 |
| 8 | | | | | | | | 98 | 64 |
| 9 | | | | | | | | | 62 |

Track 1, 2, 3 *A. hydrophila* rainbow trout isolates, 4 *A. hydrophila* gull isolates, 5 *A. caviae* rainbow trout isolates, 6, 7, 8, 9 *A. caviae* gull isolates, 10 *A. hydrophila* ATCC 7512.

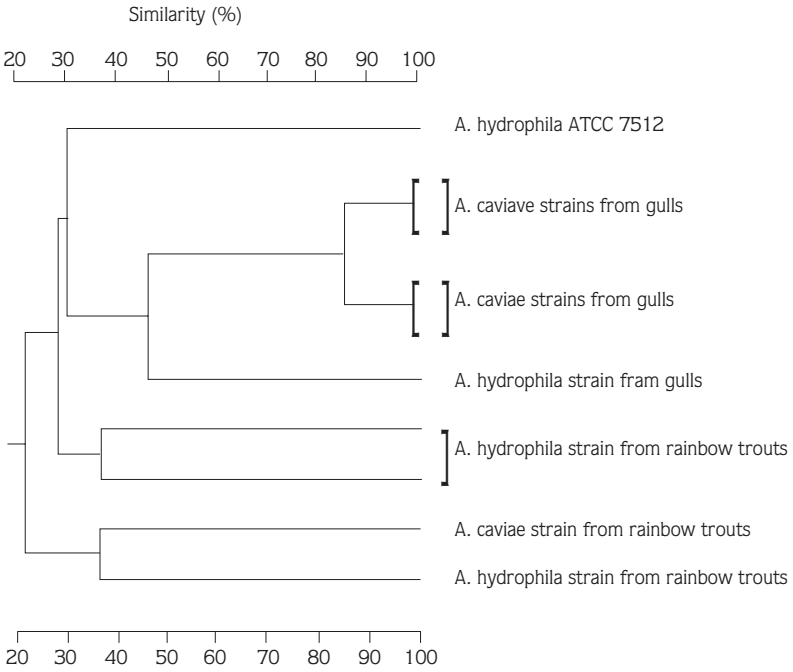


Figure 2. Dendogram based on unweighted pair group method with arithmetic averages algorithm (UPGMA) of the protein patterns of whole-cell of *A. hydrophila* and *A. caviae* strains from animal feces.

studies (6,18,19). A total of 91 isolates of Aeromonads, 51 from water and the rest from environmental specimens (39 from human feces and one from a perineal swab) were studied by Radio-PAGE. All isolates were typable and each yielded a labeled-protein of 40-60 visible bands. There was no clear pattern for *A. sobria*, but the similarity coefficients of 21% of pairs were <75% and for 75% of pairs were <80%. Water isolates of *A. hydrophila* and *A. caviae* did not appear to be similar to the isolates from human feces (6). By silver staining of 35 isolates of Aeromonad spp. previously typed by auto-radiography, the recognition of 30-50 protein bands was made possible. Similarity coefficients were calculated for 173 pairs of tracks for each method of protein staining. The majority (84.4%) of pairs were given the same classification (different, indistinguishable or of uncertain relationship) by both methods. Researchers also analyzed species according to the clusters of isolates identified within each species (13 *A. hydrophila*, 18 *A. caviae*, two *A. sobria*, and two *Aeromonas* spp.), The strains of *A. hydrophila* were divided into two groups of two and six isolates and five single strains by autoradiograph. Among 18 isolates of *A. caviae*, five single isolates, three clusters of two, four and five indistinguishable isolates, respectively, and a further two isolates of uncertain relationship to the largest cluster were inedtified. Two isolates of *A. sobria* and two not identifiable as any species were not related to any other isolate (16). In the examination of 60 isolates of Aeromonas spp. from the water, food and people in the London area, all the isolates yielded a protein fingerprint with 30-40 bands (19). Esteban et al. (18) compared whole-cell protein profiles of *A. hydrophila* strains isolated from wound and feces samples by SDS-PAGE with whole-cell protein profiles of outbreak strains. *A. hydrophila* strains isolated from clone biopsies and endoscopic lavage had similar protein profiles and these protein profiles were different from those obtained from strains of *A. hydrophila.*

*A. caviae* strains, particularly those isolated from gulls, were determined to belong to the same species based on the phenotypic diagnosis and SDS-PAGE of whole-cell protein.

Although the other test organisms (*A. hydrophila* strains and *A. caviae* strain from rainbow trout), particularly *A. hydrophila* strains, were phenotypically diagnosed to be the same species, it was interesting to see that all of them formed single-member groups. Likewise, four *A. caviae* strains isolated from gulls belonged to a single group while one *A. caviae* strain isolated from rainbow trout formed a single-member group.

Popoff et al. (20) were the first to fully show the complex nature of the *A. hydrophila* group and to clearly define three of its species. Unfortunately, at least four of the groups defined by DNA hybridization could not be separated by simple phenotypic tests, and all three of the named species were heteregeneous because they contained one or more additional DNA hybridization.

In addition, one *A. hydrophila* strain isolated from gulls was identical to four *A. caviae* strains isolated again from gulls, and although this strain was identified as *A. hydrophila* by phenotypic methods it may belong to the same DNA hybridization group as the four *A. caviae* strains.

The study reinforced the theory that since Aeromonads establish a highly heterogeneous group, isolates of very different phenotypes could have similar or identical protein fingerprints, whereas some of those of similar phenotypes had different fingerprints (16,19). The discriminatory level of protein electrophoresis in terms of taxonomy depends mainly on the type of protein extracted and on the electrophoretic system used.

In this study, four *A. caviae* strains isolated from gulls were found to be identical (similarity coefficients: 98-100%). We concluded that these strains might have been contaminated from the same source, and that SDS-PAGE could be a useful method for the characterization of *A. hydrophila* and *A. caviae*, and for the epidemiological assessment of findings.

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