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RESEARCH ARTICLE

Protein Profiles in Different Strains of *Aeromonas hydrophila* Isolated from Retail Foods [1]

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Summary

In this study, whole cell protein profiles of 20 local strains of *Aeromonas hydrophila* isolated from different foods in Turkey and one reference strain (ATCC 7966) were analyzed by SDS-PAGE (sodium dodecylsulfate polyacrylamide gel electrophoresis). It was observed that there was variability among the strains. Molecular weight of proteins were found to be between 21-116 kDa. The protein polypeptide bands from 37.8 to 101.4 kDa were common in both local strains and reference strain of *A. hydrophila*. The results of this study indicated that there is a genetic similarity between strains of *A. hydrophila* and reference strain (ATCC 7966). These protein patterns are likely to be beneficial to differentiate between the strains in epidemiological studies.

Keywords: *Aeromonas hydrophila, SDS-PAGE, Whole cell protein, Food*

Perakende Gıdalardan İzole Edilen Farklı *Aeromonas hydrophila* Suşlarının Protein Profilleri

Özet

Bu çalışmada Türkiye'de çeşitli gıdalardan izole edilen lokal 20 *Aeromonas hydrophila* ve bir referans suşun (ATCC 7966) toplam hücre proteinleri SDS-PAGE (sodyum dodesilsülfat poliakrilamid jel elektroforezis) ile analiz edildi. Suşlar içinde değişkenlik gözlendi. Proteinlerin moleküler ağırlıkları 21-116 kDa arasında olduğu bulundu. *A. hydrophila*'nın hem referans hem de lokal suşlarında 37.8 den 101.4 kDa kadar protein polipeptid bantlarının yaygın olduğu görüldü. Bu çalışma *A. hydrophila* ve referans suş (ATCC 7966) arasında genetik bir benzerlik olduğunu göstermektedir. Bu protein patternleri epidemiyolojik çalışmalarda suş ayırımını kolaylaştırmada yararlı olabilir.

Anahtar sözcükler: *Aeromonas hydrophila, SDS-PAGE, Tüm hücre proteini, Gıda*

INTRODUCTION

Nowadays, rapid globalization of food production and trade has increased the potential likelihood of food contamination. The foodborne disease with a microbial origin constitutes a worldwide problem that still causes some worries. *Aeromonas* species have been recognized as pathogens which can cause a number of serious extra intestinal infections including bacteraemia, meningitis, pulmonary and wound infections [1,2]. The role of these bacteria in foodborne incidences is not firmly established, but *Aeromonas* spp. have the potential to emerge as significant food-borne pathogens. These microorganisms are found primarily in aquatic environments, they can be isolated from a variety of foods, including milk, milk products, meat, poultry, seafood, raw bovine, suine and lamb meat [3].

The need of a system for the identification and classification of *Aeromonas* isolates is justified by their ecological and clinical importance [4]. When a common

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organism is recovered from clinical or environmental sources, various traditional and molecular typing methods have been used to study the epidemiological links between cultures. Today, molecular typing methods are necessary for proving the similarity between the isolates [5].

Different methods as DNA-DNA hybridization [6], SDS-PAGE analysis of cell proteins [7], RAPD patterns [8], PCR [3], pulsed-field gel electrophoresis [9], 16S rDNA-RFLP analysis [10] and Plasmid DNA [11] have been used to type isolates. However, these methods are not generally accepted as standard systems for the evaluation of *Aeromonas* isolates, as a standard method should be simple, rapid, inexpensive, reliable, and applicable in any kind of routine laboratory [4].

SDS-PAGE of protein patterns has been widely used for typing strains within particular bacterial species [12]. *A. caviae* and *A. veronii* recovered from clinical and environmental samples were characterized by SDS-PAGE of whole cell proteins and all strains were typable and showed unique banding patterns [13]. SDS-PAGE remains a powerful method for structural studies and for typing and classification of microorganisms [14].

The aim of this study was to investigate the whole-cell proteins of *A. hydrophila* strains isolated from retail foods by SDS-PAGE and to determine the relationship among the protein profiles of *A. hydrophila* strains.

MATERIAL and METHODS

Fifty samples of raw calf meat, 50 samples of chicken carcasses and 80 minced meat samples were collected from randomly selected local retail shops and supermarkets in Kırşehir province (Turkey). Foods were purchased in regular consumer packages and immediately transferred to the laboratory for analysis. Approximately 20 g of meat was aseptically added to 180 mL of alkaline peptone water (APW) containing 30 µg/mL of ampicillin (A-9393 Sigma Chemical Co., St. Louis, Mo.) (APW, pH: 8.4) in a sterile stomacher plastic bag and homogenized for 2 min in Colworth Stomacher 400. After all strains were incubated at 28°C for 24 h, a loopful of enrichment broth was streaked on the glutamate starch phenol red agar (GSP agar, Merck, Darmstadt, Germany). Identification of the genus *Aeromonas* was done using standard tests including motility, Gram staining (-), cytochrome oxidase (+), d-glucose fermentation (37°C/24 h) (+), catalase (+) and sensitivity to O/129 (−) (Sigma St. Louis, MO, USA) after 24 h at 30°C. The species were identified by specific assays, such as esculin hydrolysis, l-arabinose utilization, gas production from glucose, indole, H_2S formation from cysteine and the capacity to grow under oxidative and fermentative conditions [15]. To control identification, the following ATCC (American Type Culture Collection, Rockville Maryland, USA) strains were used: *A. hydrophila* ATCC 7966.

SDS-PAGE method: Electrophoresis was performed with a discontinuous buffer system in a Consort N.V. Parklanaan 36B-2300, Turnhout, U.K. For SDS-PAGE analysis, *A. hydrophila* strains were cultured on Nutrient Broth and incubated overnight at 30°C for 24 h, and then washed in phosphate buffered salina (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°C for 20 min. Nonsolubilized material was diluted 1:1 with 20% glycerol, 10% 2-mercaptoethanol, 4% SDS, and 0.125 M Tris /HCl, pH 6.8 [16]. Sodium-dodecyl sulphate polyacrilamide-gel electrophoresis (SDS-PAGE) was performed according to [17,18], with a stacking gel containing 4.5% acrilamide and a resolving gel containing 12% acrilamide. Then 10 ml of the samples was loaded on to the gel. SDS-PAGE was performed at constant voltage of 80 V for stacking gels and 150V for resolving gel. We used SDS-PAGE protein Marker 6.5-200 kDa, Liquid Mix (SERVA-Germany), including myosin(200 kDa), ß-galactosidase (116.0 kDa), bovine serum albumin (67.0 kDa), ovalbumin (45.0 kDa), carboanhydrase(29.0 kDa) and trypsin (21.0 kDa). The bands were further characterized by designating them as Dark (D) and Light (L) and their staining intensities were recorded by + sign, one + indicating one unit.

Coding of data and computer analysis: Test results were classified as 1 for positive and 0 for negative. Statistical analyses were done using the NTSYS (Numerical Taxonomy and Multivariance Analysis System) program. Similarity between fingerprints was calculated with the Jaccard's Similarity Coefficient. Cluster analysis was performed using UPGMA (Unweighted Pair Group Method with Arithmetic Mean Analysis) with average linkages.

RESULTS

As seen from *Table 1*, 20 *A. hydrophila* strains were isolated from different sources (raw chicken (5/50), raw calf meat (2/50) and minced meat (13/80).

Table 1. *The strains of A. hydrophila used in the study*
Tablo 1. *Çalışmada kullanılan A. hydrophila suşları*

Strains	Sources of Isolation
Ah1, Ah3, Ah4, Ah5, Ah7, Ah9, Ah10	Minced meat
Ah12, Ah14, Ah15, Ah16, A18, Ah19	Minced meat
Ah2, Ah6, Ah11, Ah13, Ah8	Raw Chicken
Ah17, Ah20	Raw calf meat
ATCC 7966[T]	

[T] ATCC American Type Culture Collection, Rockville (USA)

As shown in *Table 2*, 20 strains of *A. hydrophila* (Ah1-Ah20) and the reference strain (ATCC 7966) yielded 7-14 polypeptide bands stained with Coomassie brilliant blue (R-250) on 12% SDS-PAGE electrophoresis The clear distinct bands as 88.1, 53.8 and 41.5 kDa in Ah1; 88.1, 59.1 and 53.8 kDa in Ah2; 96.8 and 76.5 kDa in Ah3; 51.3 and 41.5 kDa in Ah4; 74.7 and 57.7 kDa in Ah5; 74.7, 57.7 and 52.5 kDa in Ah6; 122.4, 101.4 and 57.7 kDa in Ah7; 101.4,

Table 2. *The molecular weight of the various polypeptide bands of A. hydrophila strains (Ah1-Ah20) and reference strain (Ah21)*
Tablo 2. *A. hydrophila suşlarının (Ah1-Ah20) ve referans suşun (Ah21) çeşitli polipeptid bantlarının moleküler ağırlığı*

Strains		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Ah1	MW in kDa X	134.5 L	125.3 L	101.4 L +	88.1 D	76.5 L+	59.1 L	53.8 D+	41.5 D+	38.7 L+	32.0 L	27.8 L			
Ah2	MW in kDa X	128.3 L	108.8 L	101.4 L	88.1 D+	76.5 L	59.1 D+	53.8 D+	45.6 L	41.5 L+	35.2 L+	31.3 L	27.2 L		
Ah3	MW in kDa X	141.0 L	128.3 L	101.4 L	96.8 D	76.5 D	59.1 L+	51.3 L+	43.5 L	40.5 L	33.6 L	29.8 L	25.9 L	18.2 L	
Ah4	MW in kDa X	141.0 L	122.4 L	101.4 L	99.1 L+	76.5 L	59.1 L+	51.3 D	41.5 D+	32.0 L+	27.8 L+	23.0 L	19.1 L	18.8 L	
Ah5	MW in kDa X	144.3 L	122.4 L	101.4 L	99.1 L	88.1 L	74.7 D	57.7 D							
Ah6	MW in kDa X	134.5 L	122.4 L	101.4 L+	88.1 L+	80.2 L+	74.7 D+	57.7 D+	52.5 D+	38.7 L+					
Ah7	MW in kDa X	134.5 L	122.4 D+	101.4 D+	80.2 L+	57.7 D+	74.7 L+	57.7 L+	23.0 L+	21.5 L+	18.6 L+	14.7 L+	10.6 L+		
Ah8	MW in kDa X	141.0 L+	128.3 L	101.4 D+	88.1 D+	74.7 L+	74.7 D+	57.7 L	52.5 L	38.7 D+	37.8 L+				
Ah9	MW in kDa X	141.0 L	128.3 L+	108.8 L+	90.2 L+	76.5 D+	74.7 L	57.7 D+	52.5 D	39.6 L	37.8 L	35.2 L			
Ah10	MW in kDa X	141.0 L	128.3 L	108.8 L+	99.1 L	76.5 D	74.7 D	57.7 L+	51.3 L	45.6 D+	37.8 L+	35.2 D+			
Ah11	MW in kDa X	141.0 L	128.3 L	108.8 L	99.1 L	78.3 L	73.0 L	57.7 D	53.8 L	51.3 D	46.7 D	37.8 L			
Ah12	MW in kDa X	141.0 L	128.3 L	108.8 L	103.8 D+	88.1 L	78.3 D+	57.7 L	50.1 L	44.5 D+					
Ah13	MW in kDa X	134.5 L	128.3 L	108.8 L	103.8 L	88.1 L	78.3 D+	57.7 D+	50.1 L						
Ah14	MW in kDa X	141.0 L	128.3 L+	108.8 L+	90.2 L+	88.1 L	84.0 L	57.7 D+	50.1 D						
Ah15	MW in kDa X	141.0 L	101.4 L	108.8 L	103.8 L+	88.1 D+	76.5 L+	57.7 D+	50.1 L+						
Ah16	MW in kDa X	134.5 L	128.3 L	108.8 L	103.8 L	88.1 L+	80.2 L	73.0 D	50.1 D						
Ah17	MW in kDa X	134.5 L	101.4 L	108.8 L+	103.8 L+	88.1 L+	84.0 D+	57.7 L	50.1 D+	44.5 D+	37.8 L	35.2 L+	23.0 L+	18.2 L	13.7 L
Ah18	MW in kDa X	141.0 L	128.3 L +	108.8 D+	103.8 L +	88.1 D+	76.5 D+	57.7 L +	50.1 L +	44.5 L +					
Ah19	MW in kDa X	141.0 L+	128.3 D+	108.8 L +	101.4 D+	88.1 D+	80.2 D+	73.0 L +	50.1 L +	44.5 L +					
Ah20	MW in kDa X	141.0 L	128.3 L+	108.8 L+	101.4 L+	88.1 L +	84.0 L +	57.7 D+	50.1 D+	44.5 D+					
Ah21	RS MW in kDa X	128.3 L	101.4 L	78.3 D+	50.1 L	43.5 L	37.8 L+	32.0 D+	21.0 L	14.7 L	13.4 L+	11.9 D+	10.8 D+		

MW, Molecular Weight; X, Characteristics of bands; Ah, Aeromonas hydrophila; D, Dark; L, Light; RS, Reference Strain (ATCC 7966: Ah21)

88.1,74.7 and 38.7 kDa in Ah8; 76.5, 57.7 and 52.5 kDa in Ah9; 76.5, 74.7, 45.6 and 35.2 kDa in Ah10; 57.7, 51.3 and 46.7 kDa in Ah11; 103.8,78.3 and 44.5 kDa in Ah12; 78.3 and 57.7 kDa in Ah13; 57.7 and 50.1 kDa in Ah14; 88.1 and 57.7 kDa in Ah15; 73.0 and 50.1 kDa in Ah16; 84.0,50.1 and 44.5 kDa in Ah17; 108.8, 88.1 and 76.5 kDa in Ah18; 128.3, 88.1 and 80.2 kDa in Ah19 and 57.7, 50.1 and 44.5 kDa in Ah20, which were unique and different from each other are tabulated *(Table 2)*. Representative photographs of each strain of *A. hydrophila* showing, gel electrophoretic band profiles are also shown in *Figure 1*.

A. hydrophila reference strain (ATCC 7966) yielded 12 clear and distinct polypeptide bands of molecular weight ranged from 10.8 to 128.3 to kDa *(Figure 2)*. Proteins of molecular mass 14.7, 37.8, 43.5, 50.1 78.3, 101.4 and 128.3 kDa were common in both the reference strain and in the local strains of *A. hydrophila.* Proteins of molecular weight of 141.0, 128.3 and 108.8 kDa were common in Ah3, Ah10, Ah12, Ah14 and Ah19 and this proved that strains are isolated from a common species minced meat.

When the Jaccard's similarity coefficiencies of 20

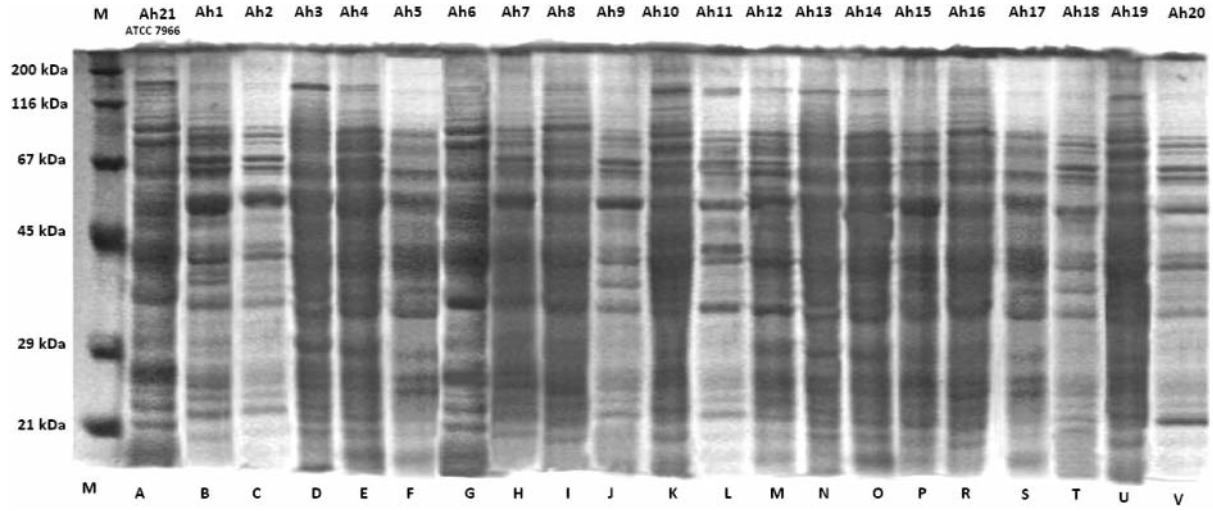

Fig 1. SDS-PAGE of whole-cell proteins of *A. hydrophila.*

Lane M: Molecular weight standard in kDa, ***Lane A:*** *A. hydrophila* ATCC 7966, (reference starin: Ah21), ***Lanes B-V:*** *A. hydrophila* from different food isolates, (Ah1-Ah20)

Şekil 1. *A. hydrophila*'nın tüm hücre proteinlerinin SDS-PAGE'si

Hat M: Moleküler ağırlık standardı kDa, ***Hat A:*** *A. hydrophila* ATCC 7966, (referans suş: Ah21), ***Hat B-V:*** Farklı gıdalardan *A. hydrophila* izolatları (Ah1-Ah20)

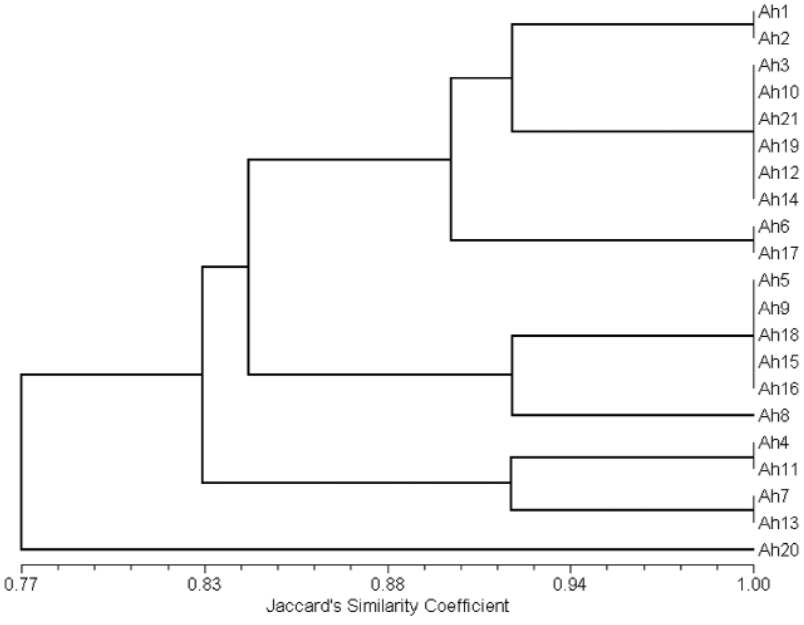

Fig 2. Dendrogram based on UPGMA cluster method of the phenetic similarity between twenty *A. hydrophila* strains

Şekil 2. Yirmi *A. hydrophila* suşu arasındaki UPGMA kümeleme yöntemiyle oluşan fenetik benzerliğin dendogramı

A. hydrophila strains evaluated, three basic clusters were seen to have formed in themselves. As seen in *Figure 2*, Ah1, Ah2, Ah3, Ah10, Ah19, Ah12, Ah14, Ah16 and Ah17 strains together with the reference strain (Ah21) took place in Cluster 1, Ah5, Ah8, Ah9, Ah18, Ah15, Ah16 strains in Cluster 2 and Ah4, Ah7, Ah11, Ah13 strains in Cluster 3. The most outstanding point in the dendogram is that Ah20 strain has been out of the three clusters.

DISCUSSION

Changes in protein expression have been described for a number of different food pathogens, including *A. hydrophila* and *A. caviae* [16,19]. Significant numbers of studies were carried out on *Aeromonas* by SDS-PAGE, and whole cell protein profiles have been found useful for epidemiological studies [12,13,20,21]. In the study conducted by Esteban [21], it was reported that *A. hydrophila* strains isolated from clone biopsies and endoscopic lavages had similar protein profiles, and these protein profiles were from different wound and feces samples obtained from strains of *A. hydrophila*.

Our observations show that there are minor variations in respect of the band numbers, intensity of bands and other properties of staining and electrophoretic mobilities in the whole cell proteins of the different strains of *A. hyrophila*. Das et al.[12] concluded that 19.5 and 86.2 kDa bands appear to be common to all species of *A. hydrophila* during SDS-PAGE of SDS extracted proteins of *A. hydrophila*. We decide here that there are 7 to 14 polypeptide bands in 20 strains of *A. hydrophila*. Out of them 101.4, 76.5 and 59.1 kDa in Ah1, Ah2, Ah3 and Ah4, 101.4, 74.7 and 57.7 kDa in Ah5, Ah6, Ah7 and Ah8, 141.0, 128.3, 108.8 and 57.7 kDa in Ah9, Ah10, Ah11 and Ah12, 108.8, 88.1, 57.7 and 50.1 kDa in Ah13, Ah14, Ah15 and Ah16, 108.8, 88.1, 50.1 and 44.5 kDa in Ah17, Ah18, Ah19 and Ah20 are common. This finding is consistent with the observations by Das et al.[12]. These results are similar to those of stated reports [12,19,21].

We conclude that there is a similarity among all strains of *A. hydrophila* 77%. The average similarity among the strains Ah3, Ah10, Ah12, Ah14 and Ah19 was 100% maximum and between Ah1 and Ah2 was 91%. Ah21 (reference strain ATCC 7966) showed 100% similarity with the former ones.

In a study, the major protein band was detected to have an approximate molecular weight of 24.0 kDa in 13 *A. hydrophila* isolates from swabs, fetuses and drinking water. In general, the protein patterns among all strains were quite similar [22]. In our study, the major protein band was detected to have an approximate molecular weight of 116.0 kDa in 20 *A. hydrophila* isolates. In general, the protein patterns among all strains were quite similar. Molecular masses of 116.0 kDa from 20 strains and below 21 kDa from in all strains were detected from food.

In conclusion, the SDS-PAGE of whole-cell proteins revealed that the majority of *A. hydrophila* strains were similar their source might have been the minced meat. These strains might have been contaminated from the same source. On the other hand, SDS-PAGE could be a useful method for the characterization of *A. hydrophila* strains, and for the epidemiological studies.

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