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Analysis of whole cell protein profiles of Salmonella serovars isolated from chicken, turkey and sheep faeces by SDS-PAGE

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ABSTRACT: This study was carried out to determine the whole cell protein profiles of Salmonella serovars from chicken, turkey and sheep faeces by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). A total of 34 *Salmonella* strains were included in the study, 14 of them were isolated from chicken, 14 from turkey and six from sheep. SDS-PAGE was carried out using 12% (w/v) separating and 4% (w/v) stacking gels. The results showed more than 30 protein bands ranging in size from 97 kDa (kilodaltons) to below 14.4 kDa as determined by visual assessment of their approximate molecular masses. Protein bands of 78.1, 51.2, 41.5, 37.3, 35.1, 33.9, 30.7, 27.6, 25.4, and 24 kDa were detected in all Salmonella serovars. *Salmonella* strains used in this study were closely related and could not be differentiated depending on the whole cell protein profiles using SDS-PAGE.

Keywords: whole cell protein; *Salmonella*; chicken; turkey; sheep; SDS-PAGE

Salmonella species are members of the *Enterobacteriaceae* family and are classified into more than 2500 serovars using the Kauffmann-White schema. Individual serovars are determined on the basis of somatic (O), flagellar (H), and capsular (Vi) antigens present in the cell walls of *Salmonella* organisms (Rementeria et al., 2009). Although all Salmonellae are recognized as major zoonotic pathogens of considerable clinical and economic importance and cause important infections both in animals and humans, there exists a remarkable difference in the invasive capacites of different Salmonella serotypes in different hosts. Salmonella (S) Typhi is highly invasive in humans, S. Gallinarum and S. Pullorum in chicken, S. Dublin in cattle, S. Abortus-equi in horse, S. Abortus-ovis in sheep and S. Choleraesuis in swine (Carraminana et al., 1997). Contamination of poultry with salmonellae results from infected breeders, contaminated feed and environmental factors. Contaminated food of animal origin, especially poultry, remains the major source of these pathogens for humans. (D'Aoust, 1994; Solano et al., 1998). S. Enteritidis and S. Typhimurium are common pathogens in human food poisoning due to consumption of contaminated poultry meat (Carraminana et al., 1997; Solano et al., 1998).

Biochemical properties, serotyping and phage typing are routinely used in reference laboratories for the identification and characterization of *Salmonella* isolates, but their overall low discriminative power mean that these methods are of limited use as discriminative tools in epidemiological studies (Heir et al., 2002; Rementeria et al., 2009). A number of genotyping and genetic methods represent the major techniques for the characterization of bacteria from food and other biological substances. Electrophoretic separation of whole cell and outer membrane proteins, or lipopolysaccharides by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), pulsed field gel electrophoresis (PFGE), plasmid profiling, DNA amplification finger printing (DAF), random amplified polymorphic DNA analysis (RADP-PCR) and restriction fragment length polymorphism (RFLP) analysis are the molecular techniques used for the characterization of bacterial macromolecules (Mare et al., 2001; Heir et al., 2002; Ngwai et al., 2005; Durrani et al., 2008; Foley et al., 2009). In addition, molecular techniques could also be an important tool to reveal epidemic patterns, trace sources of infection and aid the development of reasonable intervention strategies to reduce the presence and spread of *Salmonella* infections in animals (Heir et al., 2002). Several studies have been carried out on Salmonella serovars using SDS-PAGE to evaluate the whole cell lysate (Nakamura et al., 2002; Acik et al., 2005; Ngwai et al., 2005; Begum et al., 2008; Hassanain, 2008).

The aim of this study was to determine the whole cell protein profiles of Salmonella serovars from chicken, turkey and sheep faeces by SDS-PAGE.

MATERIAL AND METHODS

Bacterial isolates

Thirty-four strains, biochemically and serologically identified as Salmonella serovars, were tested in this study. The strains were provided by the Bacterial Culture Collection of the Microbiology Department at the Yuzuncu Yil University, Van, Turkey. Their clinical sources are listed in Table 1. All *Salmonella* strains were isolated by classical bacteriological methods (Holt et al., 1994). These strains were serotyped in the Refik Saydam National Public Health Agency, Ankara, Central Veterinary Control and Research Institute-Etlik, Ankara, Turkey and Hamburg Hygiene Institute, Germany. All institutes are reference laboratories. Out of a total of 34 *Salmonella* strains analyzed in this study, 14 were isolated from chicken, 14 from turkey and six were from sheep. In addition, S. Enteritidis *538* strain obtained from the Refik Saydam National Public Health Agency was used as a positive control.

Samples preparation

Whole cell lysates of Salmonella serovars for SDS-PAGE analysis were prepared essentially as described by Nakamura et al. (2002). Briefly, one colony was picked from MacConkey agar (MCA, Merck), inoculated into 100 ml of Tryptic Soy Broth (TSB, Merck) and incubated overnight at 37°C. Subsequently, the broth culture was centrifuged (Sigma, 3-18K, Germany) at 15 000 rpm for 15 min at 4°C. The sediment was resuspended in 10 ml of phosphate buffer solution (PBS, pH 7.2). One ml of the suspension was transferred into 1.5 ml microcentrifuge tubes and centrifuged at 15 000 rpm for 15 min at 4°C. The sediment was suspended in 10 µl of 10% SDS (AppliCem) and an equal volume of loading buffer [0.125M Tris (hydroxymethyl) aminomethane (Tris, AppliCem), 4% SDS, 10%

Table 1. The Salmonella serovars used in this study

Strain No.	Salmonella serovars	Source of isolation	Serogroup
1	S. Enteritidis	chicken	D_1
2	S. Enteritidis	chicken	D_1
3	S. Enteritidis	chicken	D_1
4	S. Enteritidis	chicken	D_1
5	S. Enteritidis	chicken	D_1
6	S. Enteritidis	chicken	D_1
7	S. Enteritidis	chicken	D_1
8	S. Typhimurium	chicken	B
9	S. Agona	chicken	B
10	S. Corvallis	chicken	C_3
11	S. Corvallis	chicken	C_3
12	S. Corvallis	chicken	C_3
13	S. Corvallis	chicken	C_3
14	S. Enteritidis	chicken	D_1
15	S. Enteritidis	turkey	D_1
16	S. Enteritidis	turkey	D_1
17	S. Virchow	turkey	C_1
18	S. Augustenborg	turkey	C_1
19	S. Augustenborg	turkey	C_1
20	S. Augustenborg	turkey	C_1
21	S. Enteritidis	turkey	D_1
22	S. Enteritidis	turkey	D_1
23	S. Enteritidis	turkey	D_1
24	S. Enteritidis	turkey	D_1
25	S. Cambridge	turkey	E_2
26	S. Anatum var. O15	turkey	E_2
27	S. Anatum var. O15	turkey	E_2
28	S. Anatum var. O15	turkey	E_2
29	S. Saintpaul	sheep	B
30	S. Saintpaul	sheep	B
31	S. Saintpaul	sheep	B
32	S. Saintpaul	sheep	B
33	S. Saintpaul	sheep	B
34	S. Saintpaul	sheep	B
35	S. Enteritidis *538*	positive control	D_1

2-mercaptoethanol (Merck), 0.2% bromophenol blue (AppliCem); pH 6.8] was added. After vigorous shaking by vortex, the prepared samples were boiled for 10 min at 100°C, centrifuged for 1 min (15 000 rpm at 20°C) and the supernatants were stored at –20°C until use.

Electrophoresis

The SDS-PAGE was carried out using the method of Laemmli (1970) using 12% (w/v) separating and 4% (w/v) stacking gels. The protein concentrations of the whole cell lysates were measured according to the method of Lowry et al. (1951). Five µl of samples were electrophoresed on 12% acrylamide (Sigma) gel for 3 h at 30 mA using a small electrophoresis chamber (Thermo EC120 Mini Gel Vertical System, USA). In each gel a wide range molecular weight marker (Sigma) was included. The gels were stained in 0.25% Coomassie Brilliant Blue R250 (Sigma) in methanol:acetic acid:distilled water (5 : 1 : 5) for 90 min with gentle shaking. Then the gels were destained in methanol:acetic acid:distilled water (2 : 3 : 35) overnight and visualized with the Gl-5000 visualization system (Spectronics Co., USA).

RESULTS AND DISCUSSION

The whole cell protein profiles of the Salmonella serovars were determined using SDS-PAGE using 12% (w/v) separating and 4% (w/v) stacking gels. More than 30 protein bands could be resolved ranging in size from 97 kDa to below 14.4 kDa as determined by visual assessment of their approximate molecular masses. Seventy-eight point 1, 51.2, 41.5, 37.3, 35.1, 33.9, 30.7, 27.6, 25.4, and 24 kDa protein bands were detected in all Salmonella serovars. Protein bands of 78.1, 51.2, and 41.5 kDa appeared as major bands in all strains.

When the protein profiles of S. Enteritidis originating from chickens and turkeys were compared, no differences were found among the isolates within this serovar (Figure 1). Similarly, when the isolates of the serogroups B, C_1, C_3, and E_2 were compared, there were no differences among all the isolates examined (Figure 2).

Conventional identification and typing methods still have an important role in routine microbiological diagnosis (Durrani et al., 2008). The bacterial protein profiles are a reflection of the genome of the strain; therefore, determination of the whole protein content plays an important role in classification, identification, typing, and comparative

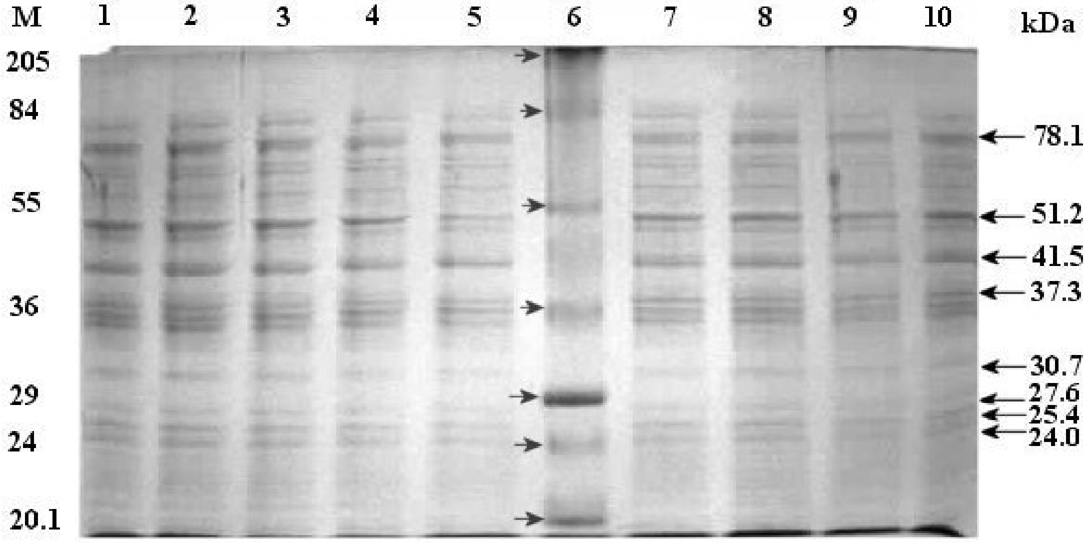

Figure 1. The WC proteins of S. enteritidis from chicken and turkeys. Lane 1 = S. Enteritidis (strain No. 3), Lane 2 = S. Enteritidis (strain No. 4), Lane 3 = S. Enteritidis (strain No. 7), Lane 4 = S. Enteritidis (strain No. 14), Lane 5 = S. Enteritidis *538* (strain No. 35), Lane 6 = M (molecular weight marker), Lane 7 = S. Enteritidis (strain No. 16), Lane 8 = S. Enteritidis (strain No. 21), Lane 9 = S. Enteritidis (strain No. 22), Lane 9 = S. Enteritidis (strain No. 23), ➔ marker, 🡐 strains

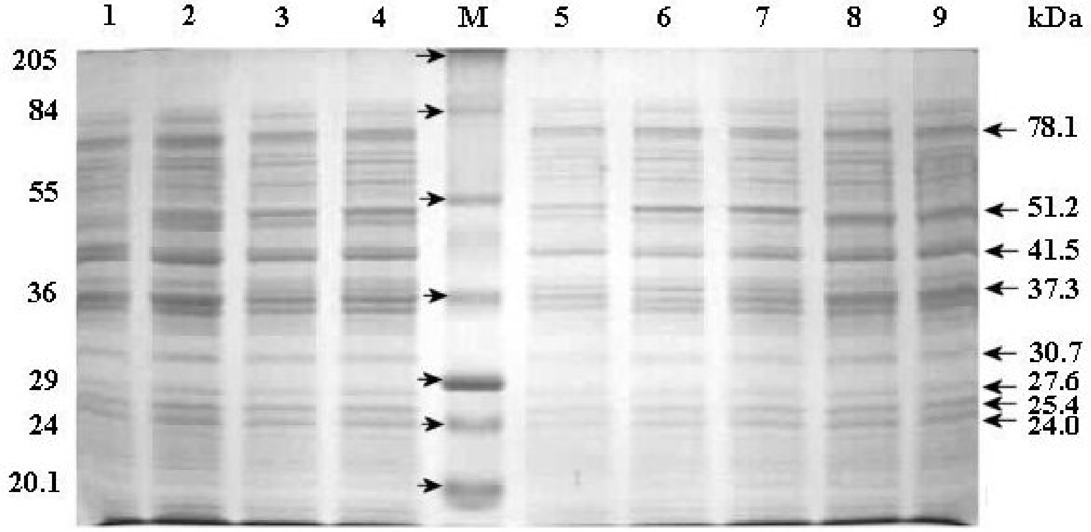

Figure 2. The WC proteins of Salmonella serovars according to serogroups. Lane 1 = S. Corvallis (strain No. 10), Lane 2 = S. Augustenborg (strain No. 19), Lane 3 = S. Typhimurium (strain No. 8), Lane 4 = S. Agona (strain No. 9), Lane 5 = M (molecular weight marker), Lane 6 = S. Enteritidis *538* (strain No. 35), Lane 7 = S. Enteritidis (strain No. 1), Lane 8 = S. Enteritidis (strain No. 21), Lane 9 = S. Cambridge (strain No. 25), Lane 10 = S. Anatum var. O15 (strain No. 26), ➔ marker, ← strains

studies of bacteria (Kustos et al., 1998). SDS-PAGE is also an important molecular technique used for identification at the species level (Durrani et al., 2008). In microbiological analysis associated with the epidemiological investigation of outbreaks, it is often necessary to obtain a more detailed identification and characterization of the organisms involved than can be provided by conventional methods such as plasmid analysis and whole cell protein analysis.

Several studies have been carried out on Salmonella serovars using SDS-PAGE to evaluate the whole cell lysate (Nakamura et al., 2002; Acik et al., 2005; Ngwai et al., 2005; Begum et al., 2008; Hassanain, 2008). Nakamura et al., (2002) reported that the whole cell proteins of S. Typhimurium and S. Enteritidis showed similarity in analysis by SDS-PAGE. Both strains yielded major bands at 71.4, 67.7, 44.0, and 30.3 kDa. In another study, Ngwai et al. (2005) reported analysis of the whole cell proteins of S. Typhimurium strains by SDS-PAGE and detected 36.5 and 65 kDa proteins while the protein profiles of all strains were similar. Hassanain (2008) noted that protein profiles of *Salmonella* strains show many bands between 77.5 and 11.4 kDa and protein bands of 77.5, 55.2, 33.1 and 16.2 kDa are common. The whole cell protein profiles of 54 Salmonella serovars, including S. Typhimurium, S. Enteritidis, S. Agona, S. Anatum, S. Virchow, and S. Corvallis, have also been compared using SDS-PAGE (Begum et al., 2008). A protein band of 37.8 kDa was detected in all serovars and protein profiles did not differ among the serovars. Also, Acik et al. (2005) have argued that electrophoretic banding patterns obtained using the SDS-PAGE method are insufficient for reliable differentiation of *Salmonella* species.

In this study, 78.1, 51.2, 41.5, 37.3, 35.1, 33.9, 30.7, 27.6, 25.4, and 24 kDa protein bands were detected in all Salmonella serovars and the 78.1, 51.2, and 41.5 kDa bands were observed to be major bands in all strains. Unfortunately, the *Salmonella* strains could not be differentiated according to whole cell protein profiles using SDS-PAGE, similar to previous reports (Nakamura et al., 2002; Acik et al., 2005; Ngwai et al., 2005; Begum et al., 2008).

Our results suggest that *Salmonella* strains serotyped in reference laboratories are closely related. However, for the definitive characterization and differentiation of Salmonella serovars, alternative molecular techniques such as plasmid analysis, PFGE or RFLP should be employed.

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REFERENCES

Acik L, Temiz A, Celebi A, Arslan S, Yilmaz R (2005): Protein patterns and plasmid profiles of the bacterial strains isolated from a poultry slaughterhouse in Ankara, Turkey. Food Technology and Biotechnology 43, 255–262.

Begum F, Adachi Y., Khan MSR (2008): Characterization of *Salmonella* serovars in comparison with some *Enterobacteria* by SDS-PAGE analysis. Bangladesh Journal of Veterinary Medicine 6, 169–174.

Carraminana JJ, Yanguela J, Blanco D, Rota C, Agustin AI, Herrera A (1997): Potential virulence determinants of Salmonella serovars from poultry and human sources in Spain. Veterinary Microbiology 54, 375–383.

D'Aoust JY (1994): *Salmonella* and the international food trade. International Journal of Food Microbiology 24, 11–31.

Durrani R, Abubakar M, Javed Arshed M, Saleha S, Ullah I, Ali Q (2008): Biological characterization and protein profiles of two model bacteria by SDS-PAGE and FT-IR. ARPN Journal of Agricultural and Biological Science 3, 6–16.

Foley SL, Lynne AM, Nayak R (2009): Molecular typing methodologies for microbial source tracking and epidemiological investigations of Gram-negative bacterial foodborne pathogens. Infection, Genetics and Evolution 9, 430–440.

Hassanain NA (2008): Detection of antibodies against zoonotic food borne pathogens in sera of food handlers. Global Veterinaria 2, 285–289.

Heir E, Lindstedt BA, Nygai I, Vardund T, Hasseltvedt V, Kappeerud G (2002): Molecular epidemiology of Salmonella Typhimurium isolates from human sporadic and outbreak cases. Epidemiology and Infection 128, 373–382.

Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST (1994): Facultatively anaerobic gram-negative rods. In: Bergey's Manual of Determinative Bacteriology. 9th ed. Williams and Wilkins, USA. 175–290.

Kustos I, Kocsis B, Kerepesi I, Kilar F (1998): Protein profile characterization of bacterial lysates by capillary electrophoresis. Electrophoresis 19, 2317–2323.

Laemmli UK (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685.

Lowry OH, Rosebrough J, Farr AL, Randall RJ (1951): Protein measurement with the folin phenol reagent. Journal of Biological Chemistry 193, 265–275.

Mare L, Dicks LMT, van der Walt ML (2001): Characterization of South African isolates of *Salmonella enteritidis* by phage typing, numerical analysis of RAPD-PCR banding patterns and plasmid profiles. International Journal of Food Microbiology 64, 237–245.

Nakamura A, Ota Y, Mizukami A, Ito T, Ngwai YB, Adachi Y (2002): Evaluation of aviguard, a commercial competitive exclusion product for efficacy and after effect on the antibody response of chickes of *Sallmonella*. Poultry Science 81, 1653–1660.

Ngwai YB, Ochi K, Ogawa Y, Adachi Y (2005): Analysis of the protein profiles of the antibioticresistant *Salmonella typhimurium* definitive phage type (dt) 104. African Journal of Biotechnology 4, 727–737.

Rementeria A, Vivanco AB, Ramirez A, Hernando FL, Bikandi J, Herrera-Leon S, Echeita A, Garaizar J (2009): Characterization of a monoclonal antibody directed against *Salmonella enterica* Serovar Typhimurium and Serovar [4,5,12:i:-]. Applied and Environmental Microbiology 75, 1345–1354.

Solano C, Sesma B, Alvarez M, Humphrey TJ, Thorns CJ, Gamazo C (1998): Discrimination of strains of *Salmonella enteritidis* with differing levels of virulence by an *in vitro* glass adherence test. Journal of Clinical Microbiology 36, 674–678.

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