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CHARACTERIZATION OF *SALMONELLA* SEROVARS IN COMPARISON WITH SOME ENTEROBACTERIA BY SDS-PAGE ANALYSIS

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ABSTRACT

Salmonella bacteria causes a wide variety of disease and disease syndrome in different animals, birds including human beings and remains as a serious problem with public health significance throughout the world. A suitable vaccine or suitable immunogen detection system is not yet still available. However, it is interesting to characterize of a common immunodominant surface protein from a wide variety of *Salmonella* serovars to get the protective measures of Salmonellosis. *Salmonella* surface protein characterization could be useful for development of protective measures against Salmonellosis and for analysis of the protein profile relationship among the *Salmonella* serovars. A common and immunodominant surface protein of *Salmonella* serovars was critically important. SDS-PAGE analysis during the period of January 2004 to December 2004 showed a target surface protein of 37.81 kDa among the 54 *Salmonella* serovars in comparison to some enterobactors. The protein profiles in SDS-PAGE of *Salmonella* serovars were not different among all *Salmonella* serovars examined in this study. In contrast to the protein band of 37.81 kDa in all serovars of Salmonella were compared and recorded with those of *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, and *Enterobacter cloacae* and were detected as 36.5 kDa. SDS-PAGE analysis showed different size of protein of Salmonella serovars and other tested enterobacters. However, it needs further investigation including Western blotting and 2-D PAGE analysis of the specific band of 37.81 kDa and 36.5 kDa protein.

Key words: *Salmonella* serovars, characterization, SDS-PAGE

INTRODUCTION

Salmonella enterica (*S. enterica*) serovar *S.* typhi cause systemic infection and typhoid fever, whereas other serovars such as *S*. typhimurium cause gastroenteritis (McClelland *et al*., 2001). *Salmonella* infection in calves remains to be a major problem. Substantial economic losses were through mortality and poor growth of infected animals as well as the hazard of transmitting food poisoning to humans. Many outbreaks of *Salmonella* infection have been reported world wide. The most frequently isolated serovars are *S. typhimurium*, *S. enteritidis*, *S. dublin*, *S. anatum*, *S. newport*, *S. cerro*, *S. montevideo* and *S. agona*. These are considered the major host-adapted *Salmonella* for cattle (Mitz *et al*., 1981, Konrad *et al*., 1994, Ritchie *et al*., 2001 and Veling *et al*., 2002). SDS-PAGE of only *S. typhimurium*, *S. enteritidis* and *S. dublin* was conducted by Nakajima, (1999) in order to observe the suitability of the technique. But comparative analysis of protein profiles was not performed among the members of Enterobacteriaceae which possess ECA (Erbel *et al*., 2003, Mier and Mayer 1985). SDS-PAGE with porin proteins or OmpF, OmpC, and OmpD of *S. typhimurium* was reported by Sing *et al*. (1995, 1992). SDS-PAGE of *S. typhimurium* (Udhayakumar and Muthukkaruppan, 1987), *S. typhi* (Tabaraie *et al*., 1994), *S. typhimurium* LT2 (Matsue and Arai, 1989), and *S. typhimurium* SH5014 (Kuusi *et al*., 1979) were also reported. However, these studies with the limited number of serovars did not give a conclusive idea regarding a common vaccine candidate against infections of a wide variety of *Salmonella* serovars.

Therefore, in the present study, SDS-PAGE of a wide variety of 54 *Salmonella* serovars was performed and compared with that of several Enterobacteria to find out a suitable common immunodominant surface protein regarding the control of Salmonellosis.

MATERIALS AND METHODS

The whole research work was performed in the Animal Health Laboratory, School of Agriculture, Ibaraki University, Ibaraki, Japan during the period of January 2004 to December 2004. To perform this study, a total of fifty four *Salmonella* serovars (A 1 Agona, A 2 Albany, A 3 Amage, A 4 Anatum, A 5 Bardo, A 6 Bareily, A 7

Blegdam, A 8 Blockley, A 9 Braenderup, A10 Brandenburg, A11 Bredeny, A12 Cerro, A13 Choleraesuis, A14 Colorado, A15 Corvalis, A16 Derby, A17 Dublin, A18 Duesseldorf, A19 Enteritidis, A20 Gaminara, A21 Give, A22 Grumpensis, A23 Hadar, A24 Havana, A25 Heidelburg, A26 Infantis, A27 Istanbul, A28 Johannesburg, A29 Kentucky, A30 Krefeld, A31 Lexington, A32 Liverpool, A33 Livingstone, A34 London, A35 Mbandaka, A36 Meleagridis, A37 Montevideo, A38 Muenchen, A39 Newport, A40 Ohio, A41 Oranienburg, A42 Orion, A43 Ouakam, A44 Panama, A45 Potsdam, A46 Rissen, A47 Sandiego, A48 Senftenberg, A49 Taksony, A50 Tennesse, A51 Thompson, L1338 Typhimurium, A53 Virchow and A54 Worthington) were obtained from the National Institute of Animal Health, Kannondai, Tsukuba, Japan and other Enterobacteria, namely *Escherichia* (*E.*) *coli*, *Enterobacter* (*Ent.*) *aerogenes*, *Klebsiella* (*K.*) *pneumonia*, and *Ent. cloacae*, were obtained from the repository of the Animal Health Laboratory, School of Agriculture, Ibaraki University, Japan.

The Culture of *Salmonella* serovars and Enterobacteria were prepared according to the procedure described by Hegazy and Adachi, 2000. Whole cell lysate of *Salmonella* serovars and Enterobacteria for SDS-PAGE analysiswas prepared from all serovars of *Salmonella* and Enterobacteria as described previously (Nakamura *et al*., 2002). The whole cell lysate protein concentration was measured according to the method of Lowry *et al*. (1951). Protein concentration was measured by a spectrophotometer at the wave length of 750 nm. Optimization of protein concentration for SDS-PAGE was done through a series of experimental SDS-PAGE. Then, SDS-PAGE was carried out as previously described by Laemmli, 1970 with some modification using all fifty four Salmonella serovars and some Enterobacteria. Briefly, the sedimented cells were suspended in 100 µl of physiological saline and mixed with an equal volume of dye buffer containing 0.125M Tris hydroxymethylamino methane, 4% SDS, 10% 2-mercaptoethanol, 20% glycerol, and 0.2% bromophenol blue. After shaking vigorously by vortex, the sample was boiled for 5 min and then centrifuged at 15,000rpm for 5 min at 16ºC. The supernatant was taken for SDS-PAGE using a rapid analytical slab electrophoretic experiment (Atto, Japan). Condition of a separation gel for SDS-PAGE was decided by using 10% and 12% separation gels. The samples were loaded with molecular size markers (Bio-Rad, U.S.A.) and run for 3 h at 10 mA. The gels after running were stained with 0.25% Coomassie Brilliant Blue R-250 (CBB, Sigma, USA) in methanol: acetic acid: distilled water (5 : 1: 5) with gentle shaking for 20 min and then destained with the solution containing 5% methanol and 7.5% acetic acid. All chemical reagents were purchased from Wako chemical Co. Ltd. Tokyo, Japan.

RESULTS AND DISCUSSION

The protein profiles of 54 *Salmonella* serovars were compared within the serovars and also with the some Enterobacteria (Fig.1, 2, 3, 4, 5 and 6). The suitability of the gel percentage was studied and it was shown that the over all resolution of the protein profiles including a 37.81 kDa protein in all *Salmonella* serovars using the 10% separation gel became most clear and detectable (Fig. 1, 2, 3, 4, and 5) as compared with 7% (data not shown) and 12% separation gels (Fig. 6). The purpose of this study was to identify protein profiles of a wide variety of *Salmonella* serovars by a suitable, reliable and common technique. The technique was SDS-PAGE which is widely used for the detection of protein. Fifty four serovars of *Salmonella* were subjected to well defined SDS-PAGE (Fig. 1, 2, 3, 4, and 5). The results demonstrated the presence of the common heavy protein band of 37.81 kDa among all 54 serovars. Although SDS-PAGE profile analyses of porin protein or OmpF, OmpC, and OmpD of *Salmonella typhimurium* (Sing *et al*., 1995 and 1992, Udhayakumar and Muthukkaruppan 1987, Tabaraie *et al*., 1994, Matsue and Arai, 1989, and Kuusi *et al*., 1979) was also reported. Comparative SDS-PAGE of a wide variety of *Salmonella* serovars with Enterobacteria is not still available. The present findings showed that there was the common heavy protein band of 37.81 kDa among all fifty four *Salmonella* serovars.

The present investigation describes the characterization of the immunodominant surface protein from a wide variety of *Salmonella* serovars and the study of protective potential of that particular protein in order to control Salmonellosis. The protective potential of *Salmonella* using an outer surface protein was studied by Tabaraei *et al*. (1994) Muthukkumar *et a*l. (1993) Udhaykumar and Muthukkaruppan (1987) Matsui *et al*. (1989) and Kuusi *et al*.(1981, 1979) in mice. They used only a limited number of *Salmonella* (*S. typhi* and *S. typhimurium*) strains. Moreover, the previous investigators focused on the outer membrane proteins of *S. typhimurium*, *S. enteritidis* and *S. dublin* which induced the strong humoral antibodies to the *Salmonellae*. However, Nakajima (1999) used only 3 serovars of *Salmonella*.

The selected Enterobacteria were included due to the presence of ECA among the members of the family Enterobacteriacae (Erbal *et al*., 2003, Meier *and* Mayer, 1985, Ramos *et al*., 2003,). The common protein of 37.81 kDa was found in all *Salmonella* serovars, while the 36.5 kDa of Enterobacteria was found through SDS-PAGE studies. Fifty four *Salmonella* serovars proved that the 37.81 kDa protein of *Salmonella* serovars was clearly different from the 36.5 kDa protein of Enterobacteria (Fig. 6) although there is a report of ECA present among the members of Enterobacteriacae (Erbal *et al*., 2003, Meier *and* Mayer, 1985, Ramos *et al*., 2003).

This is a first report for accurate comparasion of 54 *Salmonella* serovars with several Enterobacteria by using the well-defined SDS-PAGE.

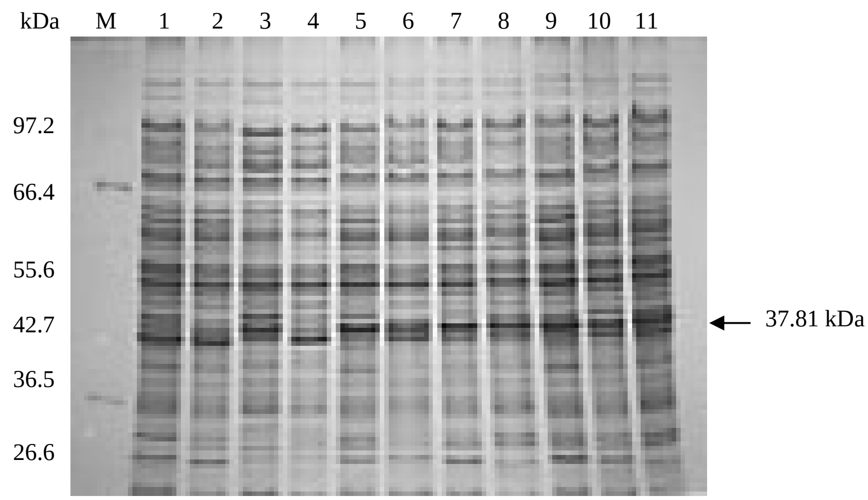

Fig. 1. Comparison of the protein profiles among *Salmonella* serovars. Lane 1, *S. agona* A1; Lane 2, *S. albany* A2; Lane 3, *S. amager* A3; Lane 4, *S. anatum* A4; Lane 5, *S. bardo* A5; Lane 6, *S. bareily* A6; Lane 7, *S. blegdam* A7; Lane 8, *S. blockley* A8; Lane 9, *S. braenderup* A9; Lane 10, *S. brandenburg*; Lane 11, *S. bredeny* A11. Arrow indicates 37.81 kDa. M, molecular size markers.

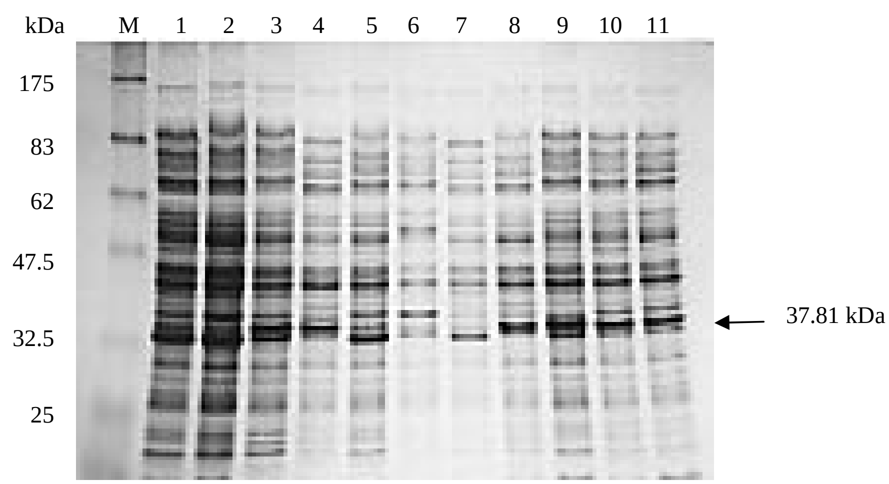

Fig. 2. Comparison of the protein profiles among *Salmonella* serovars. Lane 1, *S. cerro* A12; Lane 2, *S. choleraesuis* A13; Lane 3, *S. colorado* A14; Lane 4, *S. corvalis* A15; Lane 5, *S. derby* A16; Lane 6, *S. dublin* A17; Lane 7, *S. duesseldorf* A18; Lane 8, *S. enteritidis* A19; Lane 9, *S. gaminara* A20; Lane 10, *S. give* A21; Lane 11, *S. grumpensis* A22. Arrow indicates 37.81 kDa. M, molecular size markers.

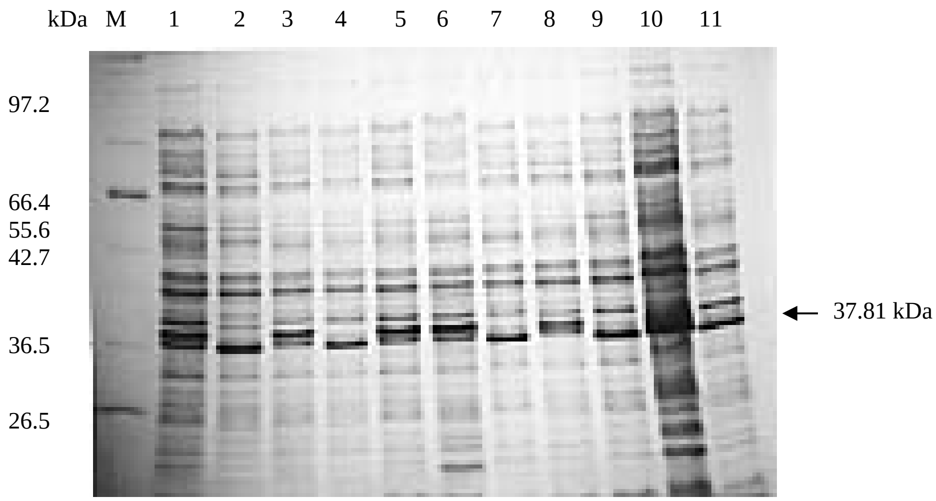

Fig. 3. Comparison of the protein profiles among *Salmonella* serovars. Lane 1, *S. hadar* A23; Lane 2, *S. havana* A24; Lane 3, *S. heidelburg* A25; Lane 4, *S. infantis* A26; Lane 5, *S. istanbul* A27; Lane 6, *S. johannesburg* A28; Lane 7, *S. kentucky* A29; Lane 8, *S. krefeld* A30; Lane 9, *S. lexington* A31; Lane 10, *S. liverpool* A32; Lane 11, *S. livingstone* A33. Arrow indicates 37.81 kDa. M, molecular size markers.

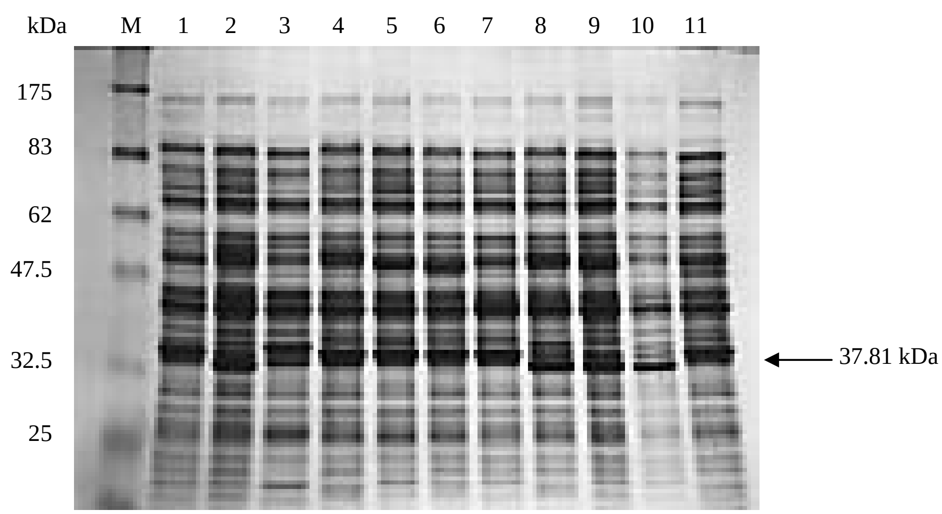

Fig. 4. Comparison of the protein profiles among *Salmonella* serovars. Lane 1, *S. london* A34; Lane 2, *S. mbandaka* A35; Lane 3, *S. meleagridis* A36; Lane 4, *S. montevideo* A37; Lane 5, *S. muenchen* A38; Lane 6, *S. newport* A39; Lane 7, *S . ohio* A40; Lane 8, *S . oranienburg* A41; Lane 9, *S. orion* A42; Lane 10, *S. ouakam* A43; Lane 11, *S. panama* A44. Arrow indicates 37.81 kDa. M, molecular size markers.

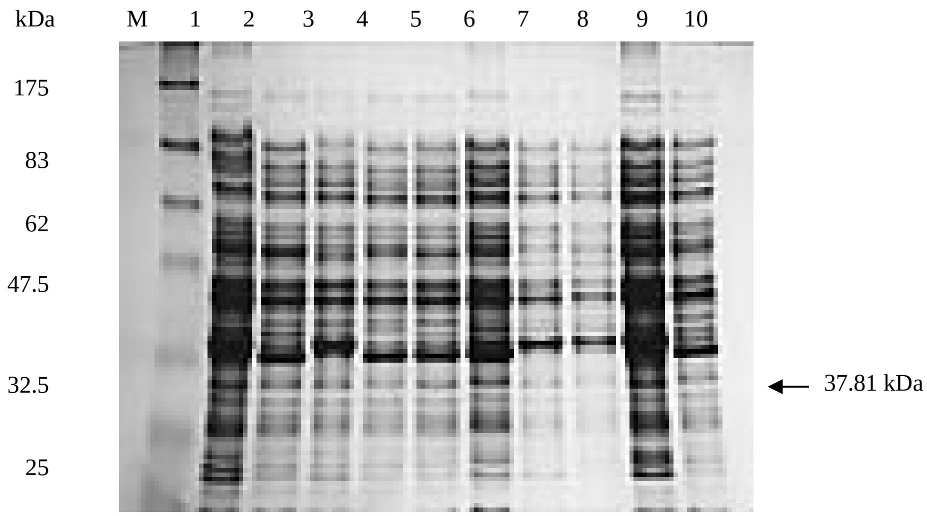

Fig. 5. Comparison of the protein profiles among *Salmonella* serovars. Lane 1, *S. potsdam* A45; Lane 2, *S. risen* A46; Lane 3, *S. sandiego* A47; Lane 4, *S. senftenberg* A48; Lane 5, *S. taksony* A49; Lane 6, *S. tennesse* A50; Lane 7, *S. thompson* A51; Lane 8, *S. typhimurium* L1338; Lane 9, *S. virchow* A53; Lane 10, *S. worthington* A54. Arrow indicates 37.81 kDa. M, molecular size markers.

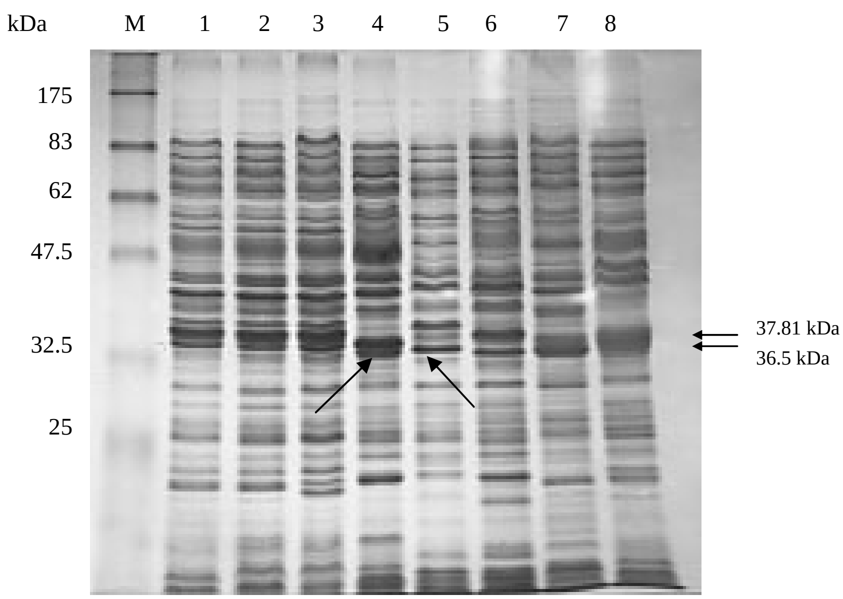

Fig. 6. Comparison of the protein profiles of three *Salmonella* Serovas and Enterobactria in SDS-PAGE. Lane 1, *S. typhimurium* L1338; Lane 2, *S. cerro* A12; Lane 3, *S. johannesburg* A28; Lane 4, *E. coli* v517; Lane 5, *Ent. aerogenes* ACLD0301; Lane 6, *Klebsiella pneumoniae* ACLT0201; Lane 7, *Ent. cloacae* ACLHa0901; Lane 8, *E. coli* ACLD2201. Arrows indicate 37.81 kDa and 36.5 kDa. M, molecular size markers.

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