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COMPARISON OF PROTEIN PATTERNS AMONG SOME *SALMONELLA* SEROVARS AND *E. COLI* BY TWO-DIMENSIONAL POLYACRYLAMIDE GEL ELECTROPHORESIS

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ABSTRACT

The study was conducted to compare the protein patterns among some *Salmonella* serovars and *E. coli* using Two Dimensional Polyacrylamide Gel Electophoresis. The Two Dimensional Polyacrylamide Gel Electophoresis showed a 37.81 kDa well separated protein spots with all Salmonella serovars at the same time with *E. coli* a 36.5 kDa protein. However, these protein spots of Two Dimensional Polyacrylamide Gel Electophoresis were further tested with Immunoblotting analysis with specific antiserum against *Salmonella typhimurium* infected chicks. All selected *Salmonella* serovars successfully identified a common 37.81 kDa protein whereas *E. coli* spots identified as 36.5 kDa protein instead of 37.81 kDa. As a further monitoring of these proteins as to check the homogeneity and heterogeneity for N-terminal amino acid sequencing, the specific protein bands from all *Salmonella* serovars and *E. coli* were excised, purified and subjected to sequence analysis. The amino acid sequence alignment showed the 37.81 kDa proteins of some *Salmonella* serovars were identical or homologous among the *Salmonella* serovars. The N-terminal amino acid alignments of the 37.81 kDa proteins were determined as alanine-glutamine-valine-isoleucine-asparagine-threonine-asparagine. On the other hand, the N-terminal amino acid alignment of the 36.5 kDa protein of *E. cloi* ACLD2201 was found to be heterologous as alanine-proline-lysine-aspartic acid-asparargine-threonine-tryptophan. The findings of this study can be concluded that the 37.81 kDa protein of some *Salmonella* serovars and 36.5 kDa protein of *E. coli* were completely different though there is some identity of these organisms due to the presence of Enterobacterial common antigen.

Key words: Salmonella, 2D-PAGE, amino acid sequence

INTRODUCTION

The Gram-negative *Salmonella* has flagella, pili, fimbriae, envelope, capsule, outer membrane protein and porin proteins including other microstructures in their outer surface. Whole cell lysates of fifty four *Salmonella* serovars were analysed by SDS-PAGE and IB analysis to observe the protein profiles (Begum, 2005). However, SDS-PAGE and IB analysis studies were not enough to find out the distribution pattern of a specific protein band of a particular bacterium. Ochiai *et al*., 2000 used Two Dimensional Polyacrylamide Gel Electophoresis (2D-PAGE) for the separation of 22-kDa protein reacting with the sera in piglets experimentally infected with *Brachyspira hyodysenteriae*. Hence, 2D-PAGE was used to solve this type of problem. A total of eight *Salmonella* serovars were used for 2D-PAGE study to characterize a selective immunodominant common protein detected in SDS-PAGE and IB of 54 *Salmonella* serovars used by Begum 2005. The presence of the common protein in all *Salmonella* serovars was further proved by 2D-PAGE, its IB and sequencing of the common protein band of 37.81 kDa after excising and purifying from 37.81 kDa protein band as well as a whole cell lysate. There were few reports on the protein analysis of *Salmonella* serovars by 2D-PAGE of (Qi *et al*., 1996, Nakajima, 1999). In the present study several other *Salmonella* serovars and some Enterobacteria were used in order to get more precise information about a specific protein by 2D-PAGE and its IB.

MATERIALS AND METHODS

Salmonella serovars *S. typhimurium* L1338, *S. cerro* A12, *S. johannesburg* A28, *S. virchow* A53, *S. liverpool* A32, *S. meleagridis* A36, *S. newport* A39, *S. worthington* A54 and *E. coli* ACLD2201 were used for 2D-PAGE. These organisms were cultured according to the procedure described by Begum (2005). The work was performed during the period of July 2003 to June 2004 in Animal Health Laboratory, School of Agriculture, Ibaraki University, Chuou, Ami, Ibaraki 300-0393, Japan.

Out of eight *Salmonella* serovars, four *Salmonella* serovars namely *S. typhimurium* L1338 , *S. cerro* A12, *S. johannesburg* A28, and *S. virchow* A53 were further tested for 37.81 kDa protein bands after excising from the SDS-PAGE gel for further purification and characterization according to the procedure described by Miyazaki *et al*., (1994) with slight modification. The target proteins of 4 serovars of *Salmonella* were separated by SDS-PAGE with a 10% (w/v) gel. The gel containing the 37.81 kDa band was excised and extracted from gel fragments into a running buffer (0.1% SDS, 10mM Tris-HCL) electrically. For protein precipitation, double volumes of cold (-20ºC) acetone were added to the running buffer in which the 37.81 kDa protein was dissolved. The mixture was placed overnight at -20ºC and then centrifuged at 15,000 rpm for 15 min at 4ºC. The supernatant was removed. After air-drying the precipitated protein pellet, the precipitated protein was dissolved in the dye buffer and subjected to SDS-PAGE and IB for the confirmation of the target protein band. The target protein precipitated and confirmed and was used for 2D-PAGE. The 2-D PAGE gel was also subjected to IB.

2D-PAGE was carried out as described previously (Begum, 2005, Jiang *et al*., 2004, Toda, 1997, and O' Farrell, 1995). IB was carried out as described previously (Towbin *et al*., 1979).

The 37.81 kDa protein of *S. typhimurium* L1338, *S. cerro* A12, *S. johannesburg* A28, *S. virchow* A53, *S. liverpool* A32, *S. meleagridis* A36, *S. newport* A39, and *S. worthington* A54 and the 36.5 kDa protein of *E. coli*ACLD2201 were analyzed for N-terminal sequencing. N-terminal amino acid sequencing was carried out according to the method of Matsudaira (1987) with some modifications. The proteins were applied on 2D-PAGE as descrived previously (Jiang *et al* 2004, Toda, 1997, and O' Farrell, 1995). The gel was blotted to a PVDF membrane. The PVDF membrane was stained with 0.2% (wt/v) CBB for 5 min and quickly, destained with 60% (v/v) methanol for 2 min. After drying, the spot detected by the IB was cut out and sequenced by an amino acid sequencer (Model 491, ABI, U.S.A.).

RESULTS AND DISCUSSION

Comparison of the protein spot patterns among *S. typhimurium* L1338, *S. cerro* A12, *S. johannesburg* A28, *S. virchow* A53, *S. liverpool* A32, *S. meleagridis* A36, *S. newport* A39, *S. worthington* A54 and *E.coli* ACLD2201 was performed by 2D-PAGE using a 10% separation gel. Protein spot patterns of some *Salmonella* serovars and an Enterobacterium were analyzed for the selection of the protein of 37.81 kDa of *Salmonella* (Fig. 1, 2, 3, 4, 5, 6, 7 and 8) and the 36.5 kDa protein of Enterobacteria (Fig. 9).

By using convalescent chick sera, the 37.81 kDa protein spots of whole cell lysates of *S. typhimurium* L1338, *S. cerro* A12, *S. johannesburg* A28 , *S. virchow* A53, *S. liverpool* A32, S. *meleagridis* A36, *S. newport* A39, and *S. worthington* A54 more strongly reacted than other protein spots by IB (Fig. 10, 11, 12, 13, 14, 15, 16 and 17). In Enterobacteria, the 36.5 kDa protein spot also reacted with the convalescent chick sera (Fig. 18).

Alignments of N-terminal amino acid sequences of the 37.81 kDa proteins from *S. typhimurium* L1338, *S. cerro* A12, *S. johannesburg* A28, *S. virchow* A53, *S. liverpool* A32, *S. meleagridis* A36, *S. newport* A39, and *S. worthington* A54 were identical. The N-terminal amino acid sequence consisted of alanine-glutamine-valine-isoleucine-asparargine-threonine-asparagine (Table 1). However, N-terminal amino acid alignments of the 36.5 kDa protein of *E. coli* ACLD2201 was different from those of the *Salmonella* serovars and consisted of alanine-proline-lysine-aspartic acid-asparargine-threonine-tryptophan (Table 1). The amino acid sequence of 37.81 kDa protein has never been reported.

The present study was performed with eight *Salmonella* serovars in comparison to Enterobacteria in order to get more precise information about the 2D-PAGE patterns. The protein spot patterns of *S. typhimurium* L1338, *S. cerro* A12, *S. johannesburg* A28, *S. virchow* A53, *S. liverpool* A32, *S. meleagridis* A36, *S. newport* A39, *S. worthington* A54, and *E. coli* ACLD2201 were investigated by 2D-PAGE. The clear-cut protein spot patterns were confirmed and more distinctly detectable with the 10% separation gels (Fig. 1, 2, 3, 4, 5, 6, 7, 8 and 9) than with the 12% separation gel (data not shown). There was the distinction between these *Salmonella* serovars and *E. coli.* The 37.81 kDa protein spots (Fig. 1, 2, 3, 4, 5, 6, 7 and 8) of *Salmonella* serovars were compared with the 36.5 kDa (Fig. 9) protein spot of *E. coli*. Qi *et al*. (1996) studied the protein spot pattern biochemically using 2D-PAGE only with *S. typhimurium* SL1344. Nakajima (1999) also studied the protein spot patterns of *S. typhimurium, S. enteritidis*, and *S. dublin* using 2D-PAGE. The present results were consistent with their reports.

Table 1. N-terminal amino acid sequence alignments of the immunodominant surface proteins from some *Salmonella* serovars and *E. coli*

Salmonella serovars	Molecular size of protein	N-terminal amino acid sequence alignment
S.typhimurium L1338	37.81 kDa	Ala – Gln – Val – Ile – Asn – Thr – Asn
S. cerro A12	37.81 kDa	Ala – Gln – Val – Ile – Asn – Thr – Asn
S. johanesburg A28	37.81 kDa	Ala – Gln – Val – Ile – Asn – Thr – Asn
S. virchow A53	37.81 kDa	Ala – Gln – Val – Ile – Asn – Thr – Asn
S. liverpool A32	37.81 kDa	Ala – Gln – Val – Ile – Asn – Thr – Asn
S. meleagridis A36	37.81 kDa	Ala – Gln – Val – Ile – Asn – Thr – Asn
S. newport A39	37.81 kDa	Ala – Gln – Val – Ile – Asn – Thr – Asn
S. worthington A54	37.81 kDa	Ala – Gln – Val – Ile – Asn – Thr – Asn
E. coli ACLD2201	36.5 kDa	Ala – Pro – Lys – Asp – Asn – Thr– Trp

Ala, alanine; Gln, glutamine; Val, valine; Ile, isoleucine; Asn, asparagine; Thr, threonine; Pro, proline; Lys, lysine; Asp, aspartic acid; Trp, tryptophan.

Precipitation with TCA and then acetone resulted in the efficient sample concentration and desalting and much more clear of the 2D-PAGE protein spot patterns. The present experiments, although data not shown, reavealed that TCA was more powerful for 2D-PAGE sample preparation, which was supported by Jiang *et al*. (2004). The results of N-terminal amino acid sequencing were found to be identical after using 2D-PAGE of both whole cell lysates and the pure 37.81 kDa proteins of some *Salmonella* serovars (Table 1). The results were reproducible. Therefore, only the whole cell lysate was used for 2D-PAGE followed by IB. The results of IB and the amino acid sequencing in each step were found to be identical (Table 1). A further experiment was performed to analyze a difference between the 36.5 kDa protein of some Enterobacteria and the 37.81 kDa protein of *Salmonella* serovars. The sera of chicks experimentally infected with *S. typhimurium* specifically detected the 37.81 kDa spot in *Salmonella* serovars and the 36.5 kDa spot in Enterobacteria with IB after 2D-PAGE. However, the N-terminal amino acid sequence aligment of the 36.5 kDa protein of *E. coli* ACLD2201 was different from those of the 37.81 kDa proteins of *Salmonella* serovars. The protein spot patterns of *S. typhimurium* L1338, *S. cerro* A12, *S. johannesburg* A28 , *S. virchow* A53, *S. liverpool* A32, *S. meleagridis* A36, *S. newport* A39, *S. worthington* A54, and *E. coli* ACLD2201 were investigated by 2D- PAGE and / IB. The results showed that there was the distinction between *Salmonella* serovars and *E. coli* and that the N-terminal amino acid sequence of the 37.81 kDa protein of some *Salmonella* serovars was clearly distinct from that of the 36.5 kDa protein of *E. coli* ACLD2201.

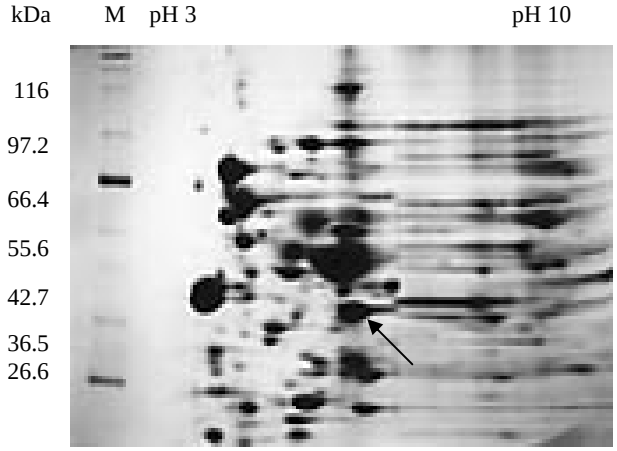

Fig. 1. 2D-PAGE of the whole cell lysate of *S. typhimurium* L1338 using a 10% separation gel. Arrow indicates a special protein spot of 37.81 kDa. M, molecular size markers. The gel diagram has the basic end to the right and the acidic end to the left.

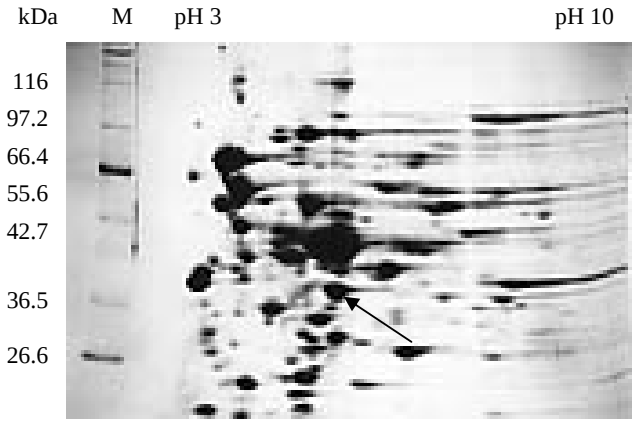

Fig. 2. 2D-PAGE of the whole cell lysate of *S. cerro* A12 using a 10% separation gel. Arrow indicates a special protein spot of 37.81 kDa. M, molecular size markers. The gel diagram has the basic end to the right and the acidic end to the left.

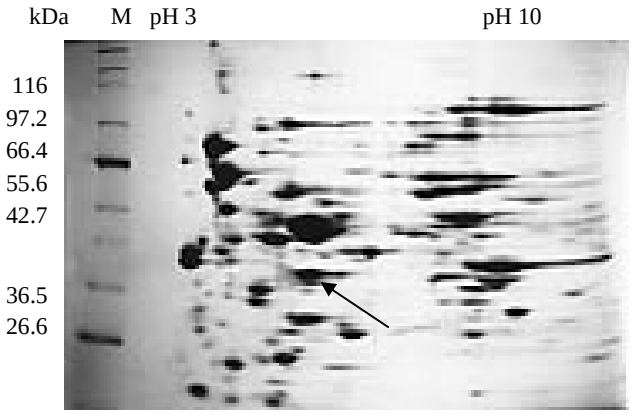

Fig. 3. 2D-PAGE of the whole cell lysate of *S. johanesburg* A28 using a 10% separation gel. Arrow indicates a special protein spot of 37.81 kDa. M, molecular size markers. The gel diagram has the basic end to the right and the acidic end to the left.

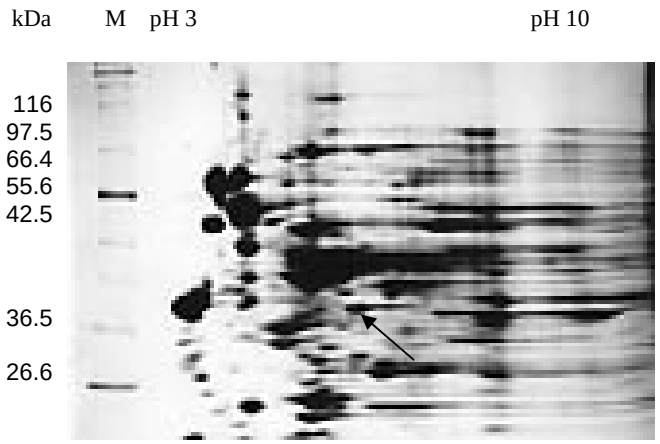

Fig. 4. 2D-PAGE of the whole cell lysate of *S. virchow* A53 using a 10% separation gel. Arrow indicates a special protein spot of 37.81 kDa. M, molecular size markers. The gel diagram has the basic end to the right and the acidic end to the left.

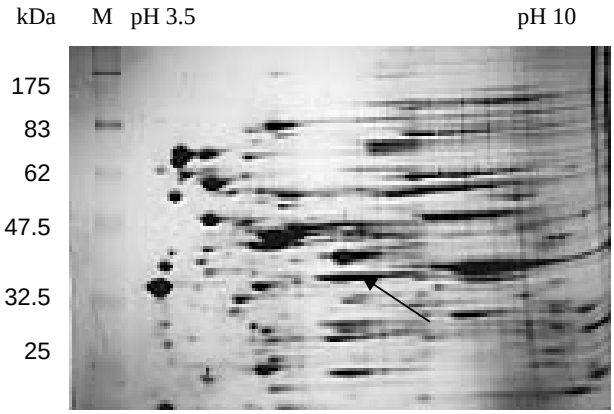

Fig. 5. 2D-PAGE of the whole cell lysate of *S*. Liverpool A32 using a 10% separation gel. Arrow indicates a special protein spot of 37.81 kDa. M, molecular size markers. The gel diagram has the basic end to the right and the acidic end to the left.

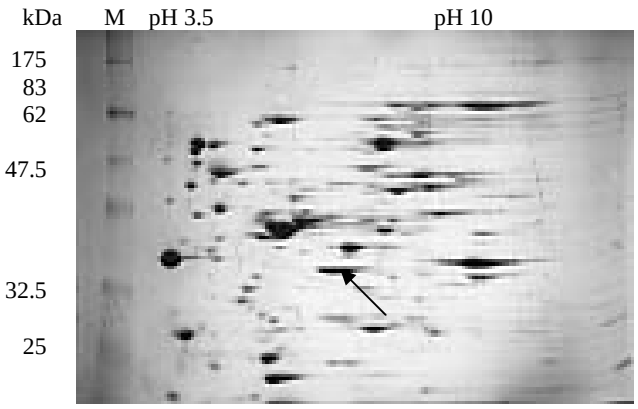

Fig. 6. 2D-PAGE of the whole cell lysate of *S. meleagridis* A36 using a 10% separation gel. Arrow indicates a special protein spot of 37.81 kDa. M, molecular size markers. The gel diagram has the basic end to the right and the acidic end to the left.

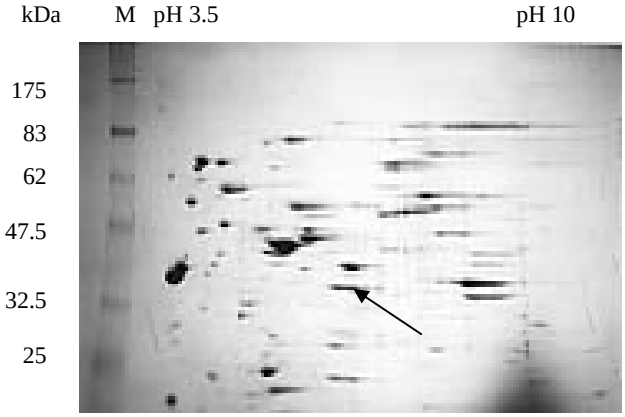

Fig. 7. 2D-PAGE of the whole cell lysate of *S. newport* A39 using a 10% seperation gel. Arrow indicates a special protein spot of 37.81 kDa. M, molecular size markers. The gel diagram has the basic end to the right and the acidic end to the left.

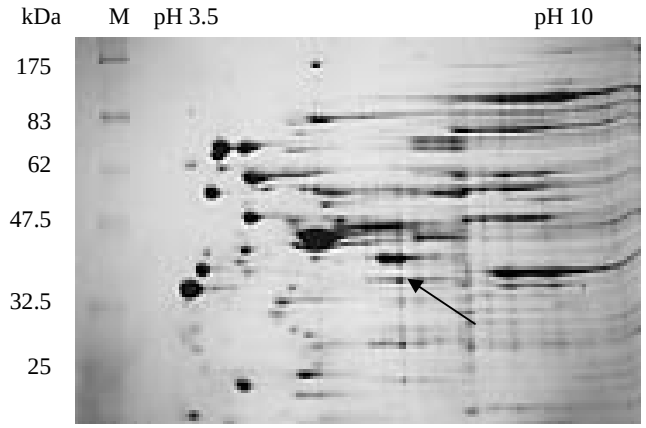

Fig. 8. 2D-PAGE of the whole cell lysate of *S. worthington* A54 using a 10% separation gel. Arrow indicates a special protein spot of 37.81 kDa. M, molecular size markers. The gel diagram has the basic end to the right and the acidic end to the left.

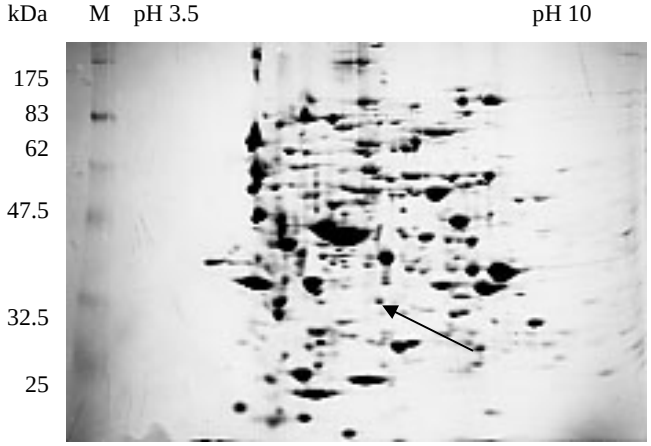

Fig. 9. 2D-PAGE of the whole cell lysate of *E. coli* ACLD2201 using a 10% separation gel. Arrow indicates a special protein spot of 37.81 kDa. M, molecular size markers. The gel diagram has the basic end to the right and the acidic end to the left.

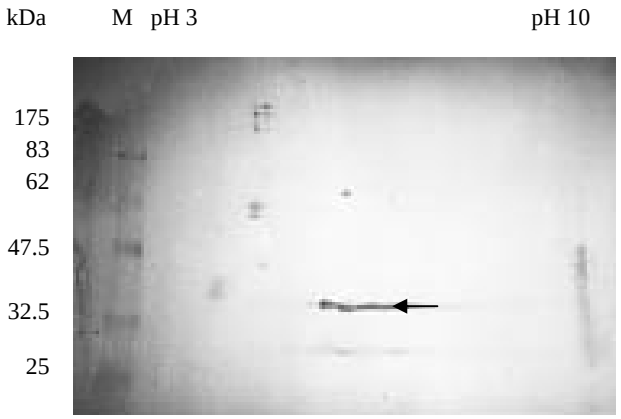

Fig. 10. IB profiles of 2D-PAGE of *S. typhimurium* L1338 with the sera of chicks infected with *S. typhimurium* using a 10% separation gel. Arrow indicates a special protein spot of 37.81 kDa. M, molecular size markers. The gel diagram has the basic end to the right and the acidic end to the left.

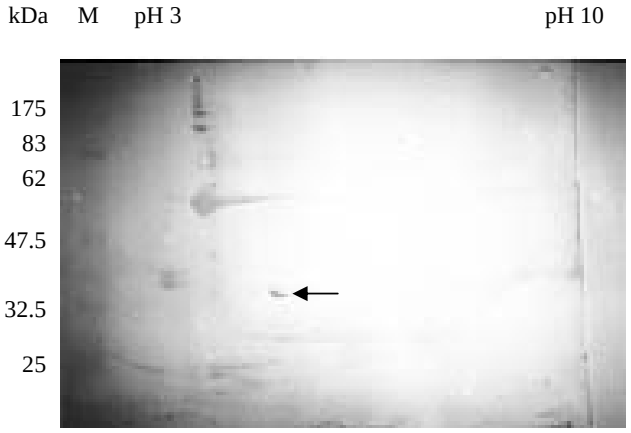

Fig. 11. IB profiles of 2D-PAGE of *S. cerro* A12 with the sera of chicks infected with *S. typhimurium* using a 10% separation gel. Arrow indicates a special protein spot of 37.81 kDa. M, molecular size markers. The gel diagram has the basic end to the right and the acidic end to the left.

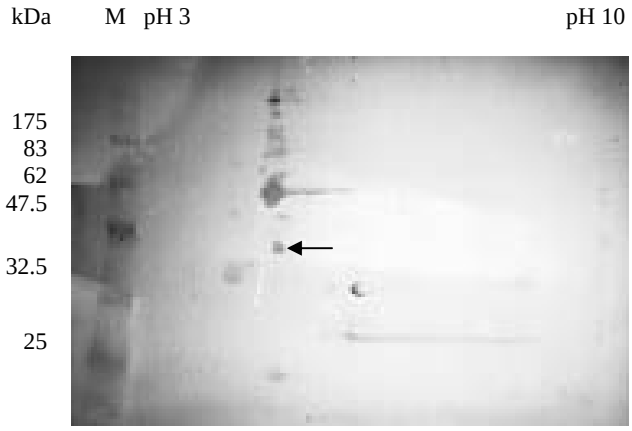

Fig. 12. IB profiles of 2D-PAGE of *S. johanesburg* A28 with the sera of chicks infected with *S. typhimurium* using a 10% separation gel. Arrow indicates a special protein spot of 37.81 kDa. M, molecular size markers. The gel diagram has the basic end to the right and the acidic end to the left.

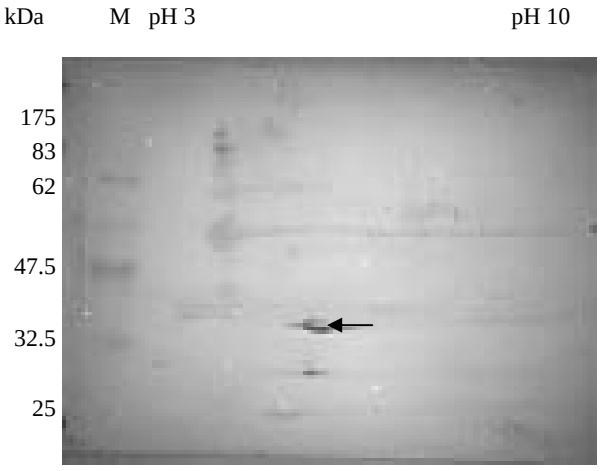

Fig. 13. IB profiles of 2D-PAGE of *S. virchow* A53 with the sera of chicks infected with *S. typhimurium* using a 10% separation gel. Arrow indicates a special protein spot of 37.81 kDa. M, molecular size markers. The gel diagram has the basic end to the right and the acidic end to the left.

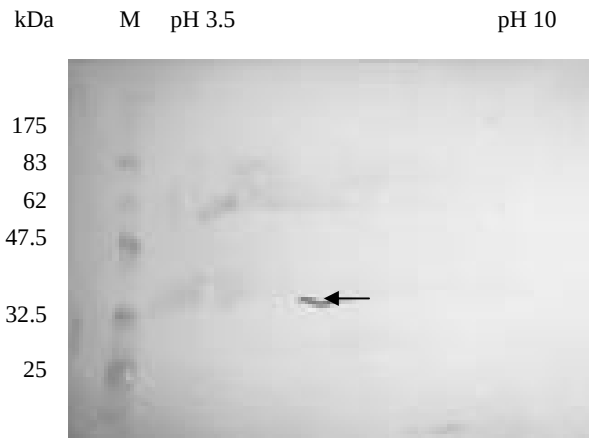

Fig. 14. IB profiles of 2D-PAGE of of *S. liverpool* A32 with the sera of chicks infected with *S. typhimurium* using a 10% separation gel. Arrow indicates a special protein spot of 37.81 kDa. M, molecular size markers. The gel diagram has the basic end to the right and the acidic end to the left.

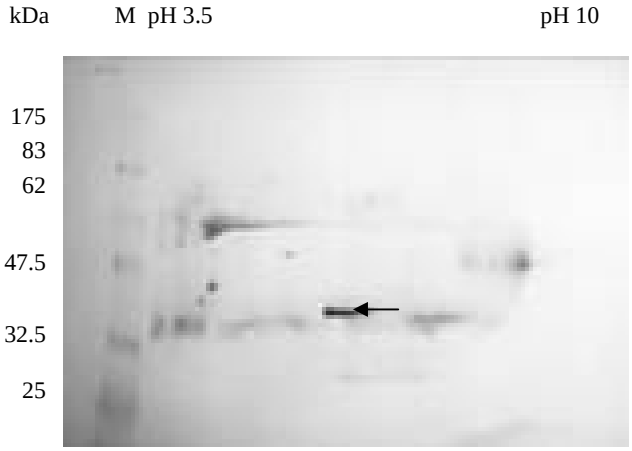

Fig. 15. IB profiles of 2D-PAGE of of *S. meleagridis* A36 with the sera of chicks infected with *S. typhimurium* using a 10% separation gel. Arrow indicates a special protein spot of 37.81 kDa. M, molecular size markers. The gel diagram has the basic end to the right and the acidic end to the left.

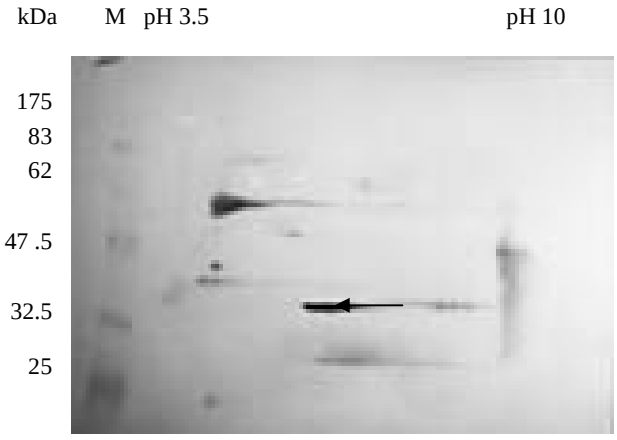

Fig. 16. IB profiles of 2D-PAGE of *S. newport* A39 with the sera of chicks infected with *S. typhimurium* using a 10% separation gel. Arrow indicates a special protein spot of 37.81 kDa. M, molecular size markers. The gel diagram has the basic end to the right and the acidic end to the left.

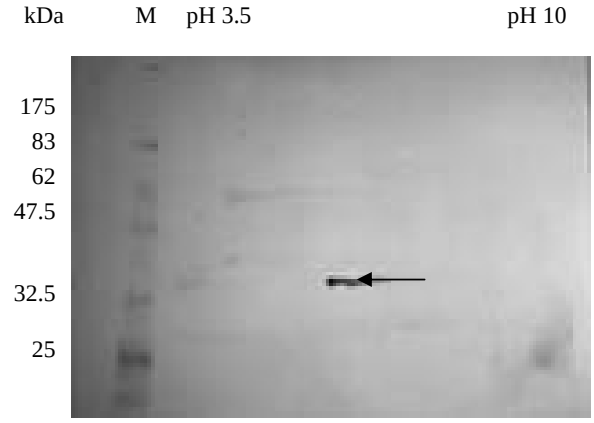

Fig. 17. IB profiles of 2D-PAGE of *S.* Worthington A54 with the sera of chicks infected with *S. typhimurium* using a 10% separation gel. Arrow indicates a special protein spot of 37.81 kDa. M, molecular size markers. The gel diagram has the basic end to the right and the acidic end to the left.

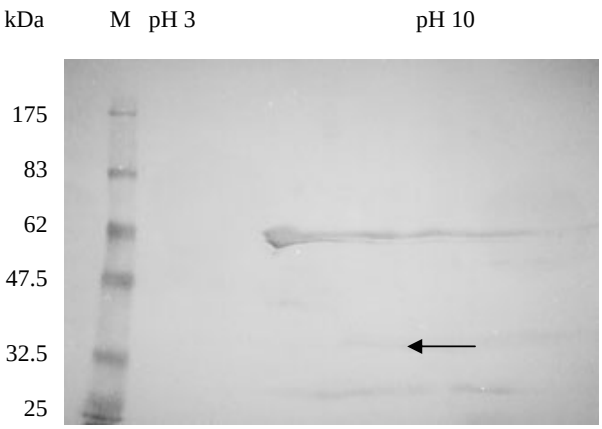

Fig. 18. IB profiles of 2D-PAGE of ***E. coli*** ACLD2201 with the sera of chicks infected with *S. typhimurium* using a 10% separation gel. Arrow indicates a special protein spot of 37.81 kDa. M, molecular size markers. The gel diagram has the basic end to the right and the acidic end to the left.

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