EFFECT OF pH ON GROWTH AND WHOLE CELL PROTEIN PROFILES OF *SALMONELLA* OF POULTRY ORIGIN

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ABSTRACT

A *Salmonella* isolate of poultry origin was grown at different adjusted pH LB-Broth and growth was monitored spectrophotometerically up to 96 hours. Maximum growth was observed in the medium at pH 7.4, while the normally used LB-Broth having the pH 6.7 showed diminished growth. No growth was observed at pH 9.2. LB-Broth at different pH values were prepared and after inoculation and incubation at 37°C for 24 hours, each of the sample of different pH was analyzed for the whole cell protein profiles using SDS-PAGE. Comparisons of whole cell protein profiles of different pH samples collected at definite intervals of time were analyzed. The results showed that after 4.5 hours a 24 kd band was absent at pH 5.0. Similarly a 43 kd band was absent at pH 6.7, 5.0 and 8.6. After 6 hours, a 73 kd band was present at pH 8.6 while absent in other pH values. At the same time a 54 kd band was absent at pH 5.5 and 7.4.

INTRODUCTION

Salmonella infections occur in many kinds of birds and mammals (Garg and Sharma, 1979). Both domestic and wild poultry are vulnerable to *Salmonella* infections (Javed *et al.*,1990; Hofstad *et al.*, 1990). More than 2300 known *Salmonella* serotypes have so far been reported, which suggest a ubiquitous nature of Salmonellae (Edwards and Ewing, 1989). In Pakistan, incidence of avian salmonellosis has increased considerably during the last decade. Nafees (1984) recorded overall incidence of 5.64 per cent in and around Lahore. In 1985, 6.81 per cent incidence was recorded in Faisalabad (Sajid *et al.*, 1986) whereas overall 4.63 per cent incidence of salmonellosis in breeder flocks has been recorded in the areas surrounding Rawalpindi-Islamabad, where 90 per cent of the total breeder population is kept (Tariq, 1992).

Acid adaptation increases the resistance of *Salmonella* species to various organic acids and also greatly increase their survival in certain foods (Leyer and Johnson, 1992).The relationship of acid adaptation to tolerance of other environmental stresses was examined in *S. typhimurium*, which was adapted to acid by exposing the cells to mildly acidic conditions (pH 5.8) for one or two cell doublings. Acid adapted cells were found to have increased tolerance towards various stresses including heat, salt, an activated lactoperoxidase systems and the surface active agents, crystal violet and polymyxin B. Acid adaptation increases cell surface hydrophobicity. Specific outer membrane proteins were induced by acid adaptation, but the lipopolysaccharide component appeared to be unaltered (Leyer and Johnson, 1993).

The study was conducted to analyze the most suitable pH for the growth of *Salmonella* in LB-Broth on shaking at 37°C. The second part of the experiment dealt with the analysis of acid shock proteins to see how *Salmonella* resist extreme pH conditions.

MATERIALS AND METHODS

A *Salmonella* isolate of poultry origin was obtained from the Molecular Biology Laboratory Quaid-i-Azam University, Islamabad. For the study of effect of pH on growth, 100 ml of LB-Broth, in different 250 ml conical flasks, at pH 5.0, 5.5, 6.7, 7.4, 8.6 and 9.2 were inoculated by 1 ml of *Salmonella* isolate, grown overnight at 37°C, and put on shaker at 100 rpm at 37°C. Five ml of sample from each flask was collected after 30 minutes of intervals, pH was recorded by pH meter and growth was monitored spectrophotometerically at 600 nm. Later on, whole cell protein profile by SDS-PAGE was done for each fraction collected.

SDS-PAGE was carried out by the method of Laemmli (1970), to analyze the bacterial proteins. Electrophoresis was performed in vertical slab gel apparatus with 0.75 mm thick spacer, using 12% resolving gel (30% acrylamide mixture, 1.5 M Tris (pH 8.8), 10% SDS, 10% ammonium persulphate, TEMED) and 5% stacking gel.

Whole Cell Protein Sample Preparation

One ml from overnight grown cultures at 37°C were harvested by spinning at 8000 rpm for 3 min.

The pellet after washing with 500 µl of stacking gel buffer (0.5 M Tris-HCl pH 6.8) was re-suspended in 230 µl of stacking gel buffer, 10 µl of ß-mercaptoethanol and 80 µl of sample buffer [50 mM Tris-HCl pH 7.0, 2%SDS (w/v), 0.7M ß-mercaptoethanol, 0.9% glycerol (v/v), 0.1% Bromophenol blue]. The samples were boiled in a water bath for 5 min., followed by immediate cooling on ice for 5 min. and centrifuged at 8000 rpm for 2 min., to remove the debris. The supernatants were separated and were prepared for loading on gel.

RESULTS

Effect of pH on Growth

The growth, at all given values of pH, which increased, with the passage of time, was observed up to 6 hrs. Normally used LB-Broth had pH 6.7. The maximum growth rate was observed for the media at pH 7.4, as is evident by its O.D. (Fig. 1). No growth was observed at pH 9.2.

Effect of pH on Whole Cell Protein Profiles

Samples from each broth, adjusted at different pH values, showed an increase of newly synthesized polypeptides with time. The comparison of the samples at pH values, collected after definite intervals of time are shown in Figs. 2-4.

After 3 hrs (Fig. 2), the comparison shows the major difference of the presence of an approximately 14.3 kd protein band at pH 5.0 (lane 3), which was strongly stained compared to others. Similarly, another band of 42.5 kd was strongly stained in culture at pH 6.7 (lane 2) and pH 7.4 (lane 6) after 3 hrs and weakly stained in the other lanes at other pH values. Fig. 3, after 4.5 hrs, show the absence of a 24 kd protein band at pH 5.0 and the presence of a 43 kd protein band at pH 5.5 and pH 7.4, which is absent at pH 6.7, 5.0 and 8.6. After 6 hrs (Fig. 4), a 73 kd weakly stained band was seen at pH 8.6 which is absent in others. A protein band of 54 kd was found absent at pH 5.5 and 7.4. A band of 36.5 kd was fairly stained at all pH values but very weakly stained at pH 5.5. Two closely associated bands of approximately 66 kd and 64 kd were absent at pH 5.5.

DISCUSSION

The *Salmonella* has an ability to survive at extreme low pH (5.5 to 6.0). This phenomenon has been referred to as acidification tolerance response (ATR) proteins. Neutrophilic organisms, such as *Salmonella typhimurium* can grow over a wide range from pH 5 to 9 because of physiologically triggered pH homeostasis mechanisms that maintain a relatively constant intracellular pH (pHi) over the broad range of growth pH values (pHo) (Foster, 1991).

In our experiments, the LB-Broth media at pH 7.4 was found to be the most effective for growth of *Salmonella* isolates. Normally used LB-Broth has a pH of 6.7. In the experiment (Fig.1), after 3.5 hrs, both the media had almost the same growth rate but afterwards the growth in media at pH 7.4 continued to increase up till 6 hrs, but the growth in media at pH 6.7 slowed down and became rather steady up to 6 hrs. So it is suggested to use the LB-Broth at pH 7.4 for efficient *Salmonella* growth within a limited time of 6 hrs of shaking at 37°C. The media at more acidic pH (5.0 and 5.5) and the more basic media (pH 8.6) showed very little growth. Highly basic media (pH 9.2) showed no sign of growth.

Foster (1992) compared protein synthesized in preshock-adapted versus unadapted cells which reveals a change in the levels of 18 polypeptides upon shifting to pH 5.8. Twelve proteins increased while six decreased. Many of these proteins appear predominantly in membrane fraction. Acid tolerance is more complex than initially perceived, with induction of acid tolerance being a two-stage process. The first stage (pre-acid shock), triggered at pHo below 6.0, induce synthesis of the ATR-specific pH homeostasis mechanism that augments pHi when external pH falls below 4. The second stage (post-acid shock) triggered below pHo 4.5, induces a different set of proteins that by themselves will not afford protection against severe acid (pH 3.3). However the acid shock proteins are important for survival when coupled with the inducible homeostasis system. The post-acid shock proteins may minimize DNA damage and internal protein denaturation, both of which occur when internal pH falls below 5.5. Several of the chaperonin class of stress proteins are induced during acid shock (Foster, 1992).

Synthesis of new polypeptides with the passage of time was common in all the pH media used. The comparisons of the whole cell protein profiles of *Salmonella* at different pH media show the synthesis of specific proteins at given pH values and in the same way, disappearance of specific bands in some cases. After 4.5 hrs of growth, a 24 kd protein band in the media at pH 5.0 disappeared and the appearance of 43 kd protein band in pH 5.5 and 7.4 media which were absent at pH 6.7, 5.0 and 8.6. Different pH media induced the synthesis of some additional polypeptides and also the consumption of some proteins. The appearance of new protein bands may have been due

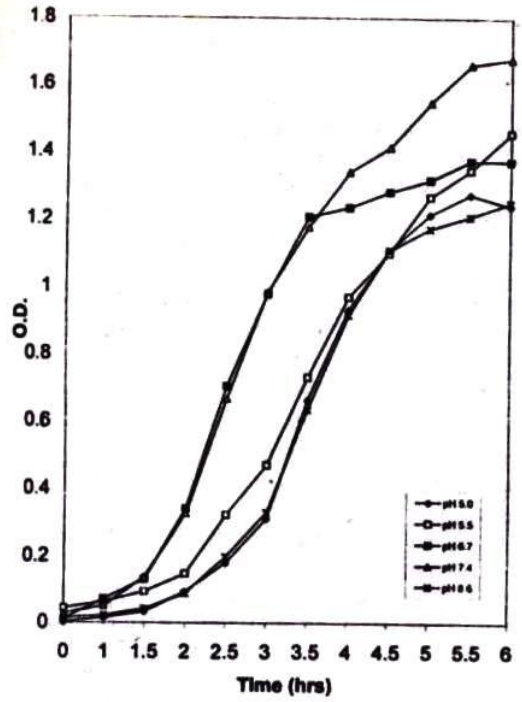

Fig. 1: Effect of pH on Salmonella growth

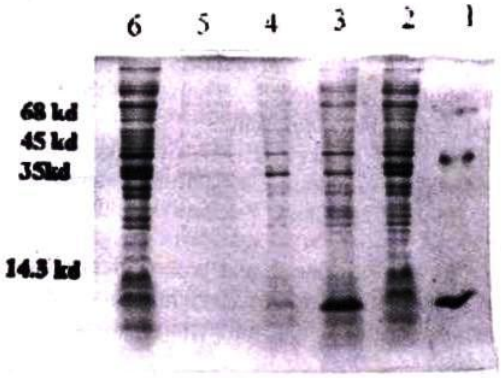

Fig.2: SDS-PAGE (12%) Coomassie blue stained gel showing the whole cell protein profiles of *Salmonella* exposed to different pH adjusted medium after 3 hours. Samples in each lane are as follows: --
Lane 1 Standard molecular weight markers,
Lane 2 Sample at pH 6.7
Lane 3 Sample at pH 5.0,
Lane 4 Sample at pH 8.6
Lane 5, Sample at pH 5.5,
Lane 6 Sample at pH 7.4

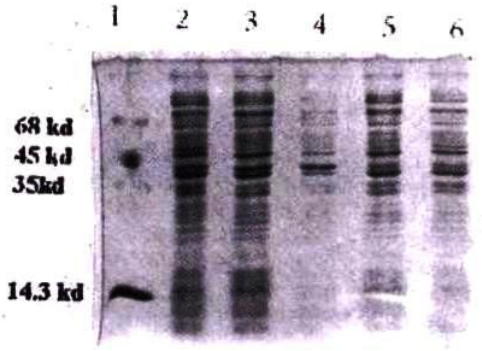

Fig.3: SDS-PAGE (12%) Coomassie blue stained gel showing the whole cell protein profiles of *Salmonella* exposed to different pH adjusted media after 4.5 hours. Samples in each lane are as follows:
Lane 1 Standard molecular weight markers,
Lane 2 Sample at pH 6.7,
Lane 3 Sample at pH 5.0 ,
Lane 4 Sample at pH 8.6
Lane 5 Sample at pH 5.5,
Lane 6 Sample at pH 7.4,

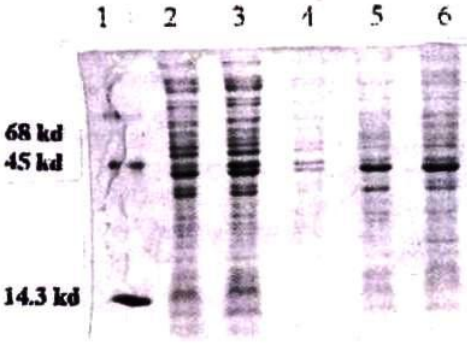

Fig.4: SDS-PAGE (12%) Coomassie blue stained gel showing the whole cell protein profiles of *Salmonella* exposed to different pH adjusted media after 6 hours. Samples in each lane are as follows: --
Lane 1 Standard molecular weight markers,
Lane 2 Sample at pH 6.7,
Lane 3 Sample at pH 5.0,
Lane 4 Sample at pH 8.6
Lane 5 Sample at pH 5.5,
Lane 6 Sample at pH 7.4,

the synthesis of new OMPs under the influence of acid adaptation as has also been reported by Leyer and Johnson (1993). Ikram (1993) subjected *Salmonella typhi* to pH of 7.5 to 5.5, 6.5, 8.5 and 9.5, and gel analysis indicated not only the appearance of more number of polypeptides found in elevated amounts, but also a relative increase in amounts of polypeptides as indicated by higher Coomassie blue staining.

The appearance of a comparatively strongly stained band of 14.3 kd at pH 5.0 (lane 3) after 3 hrs, might be an acid shocked protein, synthesized in large amount in response to acid adaptation. A 73 kd band at pH 8.6, after 6 hrs, which was absent in others, might be the one synthesized in response to the highly basic medium.

Most suitable pH of LB-Broth for the rapid growth of salmonellae was found to be 7.4 while routinely used LB-Broth has pH 6.7.In the extream acidic or basic conditions salmonellae synthesize some pH shock proteins e.g. at pH 8.6 after 6 hours of growth (Fig. 4) a 73 kd protein band is seen (lane 4) which is absent in others and in some cases some protein bands disappear e.g. at pH 5.5, 66 kd and 64 kd protein bands were absent (Fig. 4, lane 5). The newly synthesized proteins bands at definite pH values may be the acid shock protein or/and OMPs and the bands which disappear may be certain enzymes which are degraded at that pH value.

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