Turkish Journal of Biology

Volume 27 | Number 1 Article 4

1-1-2003

# Acid Adaptation Protects Salmonella typhimurium From Environmental Stresses

HALİL TOSUN

ŞAHİKA AKTUĞ GÖNÜL

Turk J Biol
27 (2003) 31-36


# Acid Adaptation Protects *Salmonella typhimurium* From Environmental Stresses

Halil TOSUN, Şahika AKTUĞ GÖNÜL
Department of Food Engineering, Faculty of Engineering, Ege University, İzmir - TURKEY

Received: 02.05.2002

**Abstract:** *Salmonella typhimurium* can encounter a wide variety of environments during its life cycle. *S. typhimurium* can experience and survive dramatic acid stress. However, in vitro the organism is very sensitive to acid.

*S. typhimurium* was adapted to acid by exposure to HCl at pH 5.8 ± 0.1 for one growth cycle. Acid-adapted cells were much better protected from acid death than control cells.

The relationship between acid adaptation of *S. typhimurium* and tolerance to other environmental stresses was also examined. Acid-adapted cells were found to have increased tolerance to various stresses including heat, salt and organic acids. No cross-protection was noted for cold (3 ± 0.1 °C).

Decimal reduction times of acid-adapted and control cells at 50 °C ($D_{50}$) were also examined. The $D_{50}$ value of acid-adapted cells was considerably higher than that of the control cells.

These results show that acid adaptation alters cellular resistance to a variety of environmental stresses.

Key Words: *Salmonella typhimurium*, acid adaptation, environmental stresses.

## *Salmonella typhimurium*'da Aside Adaptasyonun Çevresel Streslere Karşı Koruma Sağlaması

**Özet:** *Salmonella typhimurium* yaşam sürecinde çok farklı ortamlarla karşı karşıya kalır. Konakçı ortamı dışında aside oldukça duyarlı olan *Salmonella typhimurium* buna rağmen etkili asit stresinde canlılığını sürdürebilir.

*S. typhimurium* pH 5.8 ± 0.1'e ayarlanmış HCl'ye bir generasyon süresi boyunca maruz bırakılarak aside adapte edildi. Aside adapte edilen hücreler, kontrol hücreleriyle karşılaştırıldığında, asitten kaynaklanan ölümden korundukları görüldü.

*S. typhimurium*'da aside adaptasyon ile diğer çevresel streslere karşı kazanılan tolerans arasındaki ilişkide incelendi. Aside adapte edilen hücreler sıcaklık, tuz, organik asitler gibi farklı streslere karşıda tolerans kazandılar. Soğuğa karşı ise çapraz koruma tespit edilemedi.

Aside adapte edilen hücrelerin ve kontrol hücrelerinin 50°'deki D değerleri ($D_{50}$) saptandı. Aside adapte edilen hücrelerin $D_{50}$ değeri kontrol hücrelerine göre oldukça yüksek bulundu.

Bu sonuçlar aside adaptasyonun hücrenin bir çok farklı strese karşı direncini artırdığını göstermiştir.

Anahtar Sözcükler: *Salmonella typhimurium*, aside adaptasyon, çevresel stresler.

## Introduction

The incidence of foodborne diseases is increasing yearly despite increasing information regarding foodborne pathogens. Additionally, in recent years many foodborne outbreaks involving acidic foods have been reported, and epidemiological studies have demonstrated that the causative agents were *Salmonella* species, which are considered neutrophiles (1,2).

*Salmonella* spp. are known to induce adaptive responses to various stresses including acids, salts and temperature, and these adaptive responses may enhance survival in deleterious environments (3).

Foster and Hall (4) demonstrated that *S. typhimurium* expresses an acid tolerance response (ATR) when exposed to mild pH.

Acid adaptation in *S. typhimurium* is described as a two-stage process that begins with an initial preshock exposure to a mild pH in the range of 5.0 to 6.0 followed by acid challenge exposure to a pH below 4.0 (acid shock). The ATR system was found to require protein synthesis and as such represents a newly described genetic response to environmental stress (5,6).

ATR also induced cross-protection against environmental stresses that may be encountered during food processing. Foster and Hall (4) have not noted cross-protection for hydrogen peroxide and heat shock, but Leyer and Johnson (7) have shown that acid adaptation in *S. typhimurium* induces cross-protection against stresses including heat, salt, an activated lactoperoxide system, the surface active agents, crystal violet and polymyxin B.

ATR is an important point which should be of concern to both food processors and clinicians, since pathogens may tolerate the acidic environments both in food processes and in the human body, and in organic acids frequently used to inhibit the growth and survival of pathogens. Foodborne pathogens may survive in various acidic foods as well as acid sprays or acid dips. Acid is also one of the barriers employed by the body to defend itself against microbial attack. Acquired acid resistance may also protect the pathogens during passage through the stomach or during transient residence in the macrophage phagosome (8,9).

The goal of this study was to determine the acid tolerance of *S. typhimurium* and cross-protection against some environmental stresses.

## Materials and Methods

**Bacterial strains and growth conditions:** *S. typhimurium* NRRL B 4420 was maintained on nutrient agar slants at 4 °C and revived by transferring a loop inoculum into E medium (Vogel Bonner Minimal Medium) (10) supplemented with 0.4% glucose (pH 7.0) and incubating at 37 °C. Three consecutive 24 h transfers were carried out before inducing acid adaptation.

The culture medium used for bacterial growth was plate count agar (PCA).

**Chemicals:** Chemicals were purchased from Sigma Chemical Co., St. Louis, MO. Complex media were purchased from Difco Laboratories, Detroit, MI.

**Measurement of Acid Tolerance Response:** The procedure of Foster and Hall (4) for acid tolerance response was used with minor modifications. A fresh, overnight culture of cells grown in minimal E glucose medium was used to inoculate medium E with 0.4% glucose and grown statically at 37 °C. Cells to be adapted to pH 5.8 ± 0.1 were grown until an optical density at 600 nm of 0.2 and at this point the medium was adjusted with 10 N HCl. The control cells remained at pH 7.0. After pH adjustment, both cultures were grown up to $2.10^8$ cells/ml and immediately adjusted to pH 3.45 ± 0.1 and further incubated at 37 °C for 60 min by dilution in E buffer (medium E without glucose) and plating on PCA at 37 °C.

**Determination of resistance to environmental stresses:** Acid-adapted and control cultures were grown up to $2.10^8$ cells/ml and the cells were harvested by centrifugation. The acid-adapted and control cell pellets were washed once in an equal volume of E buffer and resuspended in 2 ml of E buffer. These cells were used to determine resistance to environmental stresses.

**Survival of *S. typhimurium* towards heat:** Acid-adapted and control cells were added to E buffer. The tubes were placed in a water bath heated to 45 °C, 50 °C and 55 °C and cell viability was determined at various time intervals by diluting the cells and plating them on PCA.

**Survival of *S. typhimurium* towards salt:** Survival of acid-adapted and control *S. typhimurium* in the presence of 8% NaCl in E buffer was determined at 4 °C and 25 °C. Cell viability was determined at various time intervals by plating on PCA.

**Survival of *S. typhimurium* towards organic acids:** Acid-adapted and control cells were added to 10 ml of preacidified E buffer by using lactic and acetic acids. Final pH was 4.2 ± 0.1. The tubes were incubated at 37 °C. Viability was determined at various times by serial dilutions in E buffer and plating on PCA.

**Survival of *S. typhimurium* at 3 ± 0.1 °C:** Survival of acid-adapted and control cells in E buffer incubated at 3 ± 0.1 °C was determined. Viability was determined at various times by serial dilutions the cells in E buffer and plating them on PCA.

**Determination of Decimal Reduction Time at 50 °C:** 1 ml of acid-adapted and control cell suspensions were added to 99 ml of E buffer (pH: 7.0) and incubated at 50 °C in a water bath. Samples (1 ml) were withdrawn after heating for various time intervals. Serial dilutions in E buffer were prepared and appropriate dilutions were plated on PCA.

## Results

**Measurement of Acid Tolerance Response:** *S. typhimurium* was acid-adapted with HCl as described in Materials and Methods. The results (Figure 1) showed that the adapted cells were dramatically protected from acid death compared to control cells by several orders of magnitude.

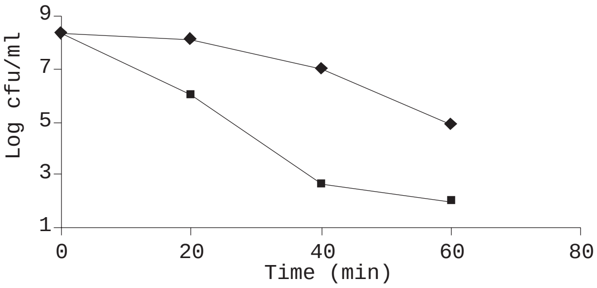



Figure 1. Survival of acid-adapted (◆) and control (■) *S. typhimurium* after exposure to HCl (pH 3.45 ± 0.1).

The acid-adapted cells declined in number about $10^3$-fold after 60 min, but there was a more rapid inactivation of the control cells. Cell death resulted in a 1000-fold difference in the number of survivors after 60 min. These results indicated that the acid-adapted cells were more acid tolerant than the control cells. This phenomenon is referred to as ATR.

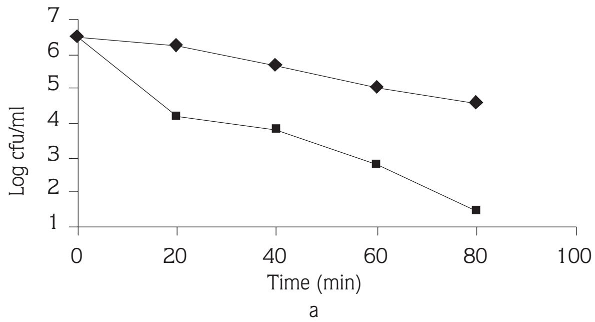
**Tolerance of acid-adapted *S. typhimurium* towards thermal stress:** Acid-adapted *S. typhimurium* was inoculated to E buffer (pH: 7.0) at 45 °C, 50 °C and 55 °C and viability was determined during 80 min exposure by plating the cells on PCA. Acid-adapted cells were more thermally tolerant than control cells at 50 °C (Figure 2a). At 45 °C during 80 min exposure few differences in survival were observed between the two cultures (Figure 2b).

At 50 °C control cells died off more rapidly than the acid-adapted cells. Cell death resulted in a 2 log decrease in the number of survivors after 20 min. This difference was about 1400-fold after 80 min of exposure. This trend was not observed at 55 °C.

**Tolerance of acid-adapted *S. typhimurium* towards osmotic stress:** *Salmonella* can experience osmotic stress in their natural environments or inside the host (11) and they can adapt high osmolarity (12). To investigate whether acid adaptation affected resistance to salt stress, survival of acid-adapted and control *S. typhimurium* in E buffer containing 8% NaCl was determined at 4 °C and 25 °C.

Acid-adapted cells displayed increased tolerance to salt compared to the control cells at both 4 °C and 25 °C. Control cells died off more rapidly compared to the acid-adapted cells at 25 °C of incubation. The number of viable acid-adapted cells declined $10^3$-fold in number after two days, indicating that they were about $10^4$ times more salt tolerant than the control cells. The acid-adapted cells still had significant numbers of survivors (< 100 cfu/ml) at the $18^{th}$ day of sampling (Figure 3a).

At 4 °C acid-adapted cells showed increased survival compared to the control cells during the incubation period. At the $18^{th}$ day of sampling the acid-adapted



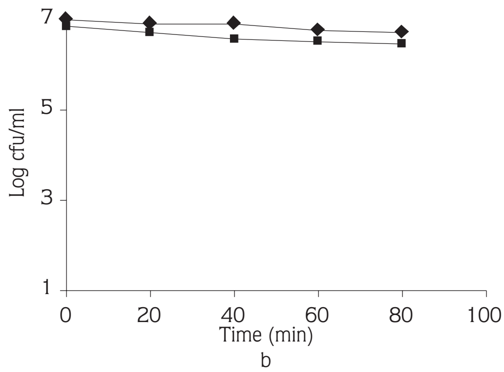




Figure 2. Survival of acid-adapted (◆) and control (■) *S. typhimurium* at 50 °C (a) and 45 °C (b).

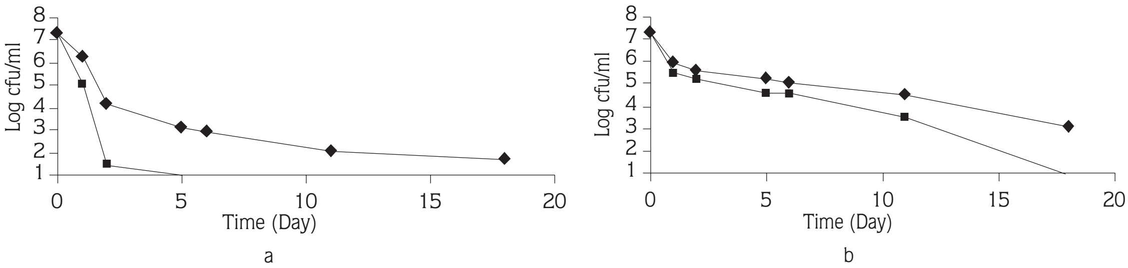



Figure 3. Survival of acid-adapted (◆) and control (■) *S. typhimurium* after exposure to 8% NaCl at 25 °C (a) and 4 °C (b).

population declined $10^4$-fold while control cells declined $10^7$-fold (Figure 3b).

**Adaptation of *S. typhimurium* to acids:** Acid-adapted and control cells were incubated in E buffer acidified with lactic and acetic acids at pH value of 4.2 ± 0.1. With each acid the adapted cells survived better than control cells. In the presence of lactic acid, acid-adapted cells declined in number about $10^2$-fold but control cells declined $10^6$-fold after 40 min (Figure 4a). Increased resistance of adapted cells was also found with acetic acid. The acid-adapted cells survived some 100-fold better than the control cells after 80 min (Figure 4b). These results show that acid adaptation to HCl also provides increased tolerance for lactic and acetic acids.

**Survival of *S. typhimurium* at 3 ± 0.1 °C:** Acid-adapted and control cells were incubated in E buffer at 3 ± 0.1 °C. There were no differences between the survival of acid-adapted and control cells during the incubation period (Figure 5).

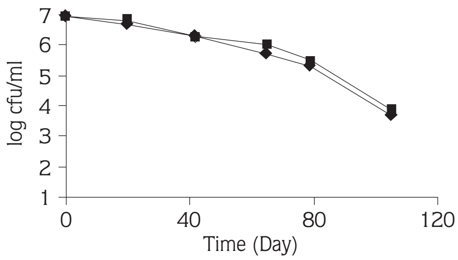



Figure 5. Survival of acid-adapted (◆) and control (■) *S. typhimurium* at 3 ± 0.1 °C.

**Determination of Decimal Reduction Time at 50 °C:** $D_{50}$ values of acid-adapted and control cells were 30.1 and 17.6 min respectively (Figure 6). These results also showed that acid adaptation affects the heat tolerance of *S. typhimurium*.

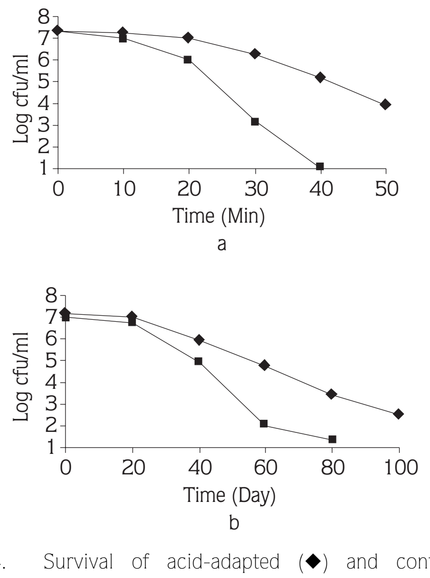



Figure 4. Survival of acid-adapted (◆) and control (■) *S. typhimurium* after exposure to lactic (a) and acetic acid (b). Cells were exposed to E buffer acidified with lactic and acetic acid (pH 4.2 ± 0.1).

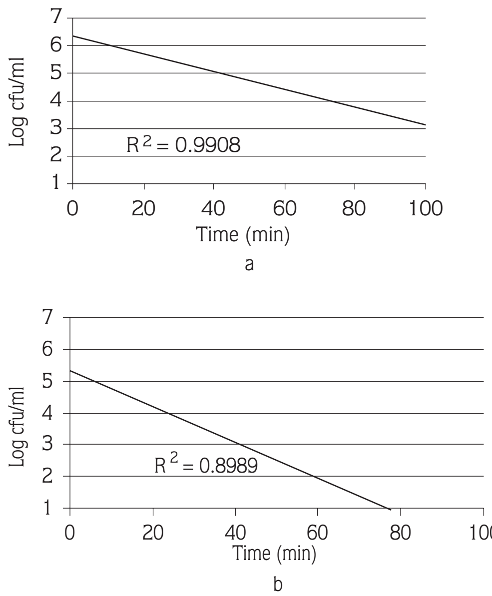



Figure 6. $D_{50}$ values of acid-adapted (a) and control cells (b).

## Discussion

Acidic pH is one of the most frequent stress conditions encountered by microbial systems and perpetuation of the species depends on the ability to survive during these encounters (13).

*S. typhimurium* has evolved several inducible systems to permit it to survive in acidic conditions as well as log phase and stationary phase acid tolerance responses (4). ATR is triggered in *Salmonella* species at pH values between 6.0 and 5.5, but protects cells against much stronger acid (pH 3.0 to 4.0), whereas nonadaptive pH homeostasis normally fails (14). The ATR system in *S. typhimurium* requires protein synthesis and as such represents a newly described genetic response to environmental stress (5).

In this study for acid adaptation we used the procedure of Foster and Hall (4) with minor modifications. We found that *S. typhimurium* has the ability to survive extreme low pH (pH 3.45 ± 0.1) if first adapted to mild pH (pH 5.8).

Acid tolerance is important to *Salmonella* species in surviving acid encounters both in the environment and in the infected host. The ability of foodborne pathogens including *S. typhimurium*, *E. coli O157: H7* and *L. monocytogenes* to adapt to acidic conditions is a concern of food safety. Leyer and Johnson (3) demonstrated that acid-adapted *S. typhimurium* survived better than control cells during milk fermentation and had increased resistance to inactivation by organic acids commonly present in cheese, including lactic, propionic and acetic acids.

In the present study we also examined whether acid adaptation cross-protected cells against environmental stresses commonly used in food preservation. We have shown that acid adaptation in *S. typhimurium* induced cross-protection against stresses including salt, heat and organic acids.

Thermotolerance has been demonstrated in *S. typhimurium* (15). Adaptation to a number of stresses or starvation increases thermal tolerance in *Salmonella* by a mechanism involving heat shock proteins (7). In our study acid-adapted *S. typhimurium* can enhance thermotolerance at 50 °C. Acid adaptation produces cross-protection to heat. Leyer and Johnson (7) also reported that acid adaptation induced cross-protection against heat. However, heat shock did not increase acid resistance, as shown by Foster and Hall (4).

*Salmonella* spp. are known to respond adaptively to conditions at high osmolarity (12). Glycine, betaine, proline, glutamate, and trehalose are the most efficient osmoprotectants in *S. typhimurium* (13). Leyer and Johnson (7) reported that the outer membrane proteins that respond to osmotic stress, OmpC and OmpF, were expressed in mildly acidic conditions. In this study we found that acid-adapted cells had increased tolerance towards osmotic stress.

In this study we also examined whether acid adaptation affected the sensitivity of *Salmonella* to lactic and acetic acid encountered in fermented food products. We found that acid-adapted cells had increased resistance to lactic and acetic acids.

Many foods depend on acidity for the elimination of *Salmonella* and some other sensitive pathogens. In mayonnaise, yoghurt and salad dressing lactic and acetic acids were reported to be the main inhibitory factor (16). Acid-adapted *S. typhimurium* may survive better than nonadapted cells in these types of food products.

In this study no cross-protection was noted for cold. Acid-adapted and control *S. typhimurium* were present throughout 105 days of incubation. Both acid-adapted and control cells were tolerant to cold (3 ± 0.1 °C).

We also examined $D_{50}$ values of acid-adapted and control cells. The $D_{50}$ value in acid-adapted *S. typhimurium* was considerably higher than the $D_{50}$ value in control cells. These results also show that heat tolerance can be substantially enhanced by acid adaptation.

In summary we have shown that acid adaptation in *S. typhimurium* induced cross-protection against stresses including salt, heat and organic acids. Acid adaptation is an important mechanism for the survival of cells in foods and in food processing environments. In conclusion, an understanding of the mechanism of adaptations to stress is important in order to design effective food safety systems and to prevent foodborne disease.

## References


1. Parish, M.E., Narciso, J.A., Friedrich L.M., Survival of Salmonella in orange juice. J. of Food Safety 17: 273-281, 1997.
2. Tsai, Y.W., Ingham, S.C., Survival of *Escherichia coli* O157: H7 and Salmonella spp. in acidic condiments. J. of Food Protection 60: 751-755, 1997

3. Leyer, G.J., Johnson, E.A., Acid adaptation promotes survival of Salmonella spp. in cheese. Appl. and Env. Micr. 2075-2080, 1992.

4. Foster, J.W., Hall, H.K., Adaptive acidification tolerance response of *Salmonella typhimurium*. J. Bacteriology 172: 771-778, 1990.

5. Foster, J.W., Salmonella acid shock proteins are required for the adaptive acid tolerance response. J. Bacteriology 173: 6896-6902, 1991.

6. O'Driscoll, B., Gahan, C.G.M., Hill C., Adaptive acid tolerance response in *Listeria monocytogenes*: Isolation of an acid tolerant mutant which demonstrates increased virulence. Appl. and Envir. Mic. 1693-1698, 1996.

7. Leyer, G.J., Johnson, E.A., Acid adaptation induces cross-protection against environmental stresses in *Salmonella typhimurium*. Appl. and Envr. Micr. 1842-1847, 1993.

8. Hill, C., O'Driscoll, B., Booth I., Acid adaptation and food poisoning microorganisms. Int. J. of Food Micr. 28: 245-254, 1995.

9. Riesenberg, M.R.W., Bearson, B., Foster, J.W., Curtis, R., Role of the acid tolerance response in virulence of *Salmonella typhimurium*. Infection and Immunity 1085-1092, 1996.

10. Vogel, H.J., Bonner, D.M., Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. 93: 273-284, 1956.

11. Archer, D.L., Preservation microbiology and safety: Evidence that stress enhances virulence and triggers adaptive mutations. Trends in Food Science and Technology. 91-95, 1996.

12. Csonka, L.N., Physiological and genetic responses of bacteria to osmotic stress. Microbiology Rev. 53: 121-147, 1989.

13. Foster, J.W., Spector, M., How Salmonella survive against the odds. Annual Rev. Microbiology 49: 145-174, 1995.

14. Foster, J.W., Hall, H.K., Inducible pH homeostasis and the acid tolerance response of *Salmonella typhimurium*. J. Bacteriology 173: 5129-5135, 1991.

15. Lindquist, S., The heat shock response. Annual Rev. Biochemistry 55: 1151-1191, 1986.

16. Rubin, H.E., Nerad, T., Vaughan, F., Lactate acid inhibition of *Salmonella typhimurium* in yoghurt. J. Dairy Sci. 65: 197-203, 1982.