S1-4

Acid Tolerance Response of *Salmonella typhimurium*: Comparative Analysis in Aerobic and Anaerobic Environment

In Soo Lee
Han Nam University

Many bacteria including *Salmonella* experience life-threatening stresses in pathogenic and natural environments. In order to increase the chance of surviving in stress environments, *Salmonella* will sense a changing environment and undergo a molecular response by which specific stress-inducible proteins are synthesized. These stress-induced proteins presumably act to prevent or repair macromolecular damages. One of the most frequently encountered hostile conditions is an acid stress. *Salmonella* encounters low pH stress in the natural environment as well as during passage through the stomach to the intestine. Fecal material after defecation presents another problem for the enteric bacteria, as the material acidifies because of increased fermentation. The acid survival system has been referred as the acid tolerance response (ATR) in *Salmonella*.

The ATR of *Salmonella typhimurium* is a complex inducible phenomenon in which exposures to slight or moderate low pH will produce a stress response capable of protecting the organism against more severe acid challenges. There are two discernable pH-dependent ATR systems in this organism: a log phase ATR in which acid shock (shift to pH <4.5) adaptation triggers the synthesis of acid shock proteins (ASPs) and a stationary phase ATR. These ASP protein sets are essential for low pH inducible acid tolerance (1,3). Log phase also provides some protections against acid as well as against heat, salt and oxidative stresses. The phenomenon that acid shock induces cross-resistance to a variety of stresses suggests that cells undergoing acid shock in the stomach will be well prepared to endure the environmental stresses subsequently confronted in the intestine.

The alternative sigma factor σ^S, encoded by *rpoS*, regulates one aspect of acid tolerance. RpoS is known to be a putative RNA polymerase of stationary phase but its importance to log phase cells is being recognized (2). A connection to acid tolerance was discovered after noting that virulent strains of *Salmonella* (UK1) exhibited an acid tolerance superior to that of an avirulent laboratory strain of LT2. The cause was traced to a mutation in the *rpoS* allele of LT2 by showing that the acid tolerance phenotypes of virulent and avirulent stains, as well as virulence itself, could be exchanged simply by swapping *rpoS* alleles (4). Western blot and 2-dimensional PAGE analysis of ASPs revealed that RpoS is itself an ASP and that it controls the expression of eight other ASPs. *Salmonella* possesses also RpoS independent ATR systems, which known as low temperature (5) and anaerobic ATR system. The *rpoS* gene and its RpoS are critically important for aerobic ATR but are of no important for the anaerobic and low temperature ATR system which is totally RpoS independent.

References

1. Bearson, S., Bearson, B. and Foster J.W. (1997) Acid stress responses in enterobacteria. FEMS Microbiol. Letters. 147, 173-180
2. Hengge-Aronis, R. (1996) Back to log phase: Hengge-Aronis, R. (1996) Back to log phase: RpoS as a global regulator in the osmotic control of gene expression in *Escherichia coli*. Mol. Microbiol. 21, 887-893.
3. Lee, I.S., Slonczewski, J.L. and Foster, J.W. (1994) A low-pH inducible stationary phase acid tolerance response in *Salmonella typhimurium*. J. Bacteriol. 176, 1422-1426.
4. Lee, I.S., Lin, J., Hall, H.K., Bearson, B. and Foster, J.W. (1995) The stationary-phase sigma factor σ^S (RpoS) is required for a sustained acid tolerance response in virulent *Salmonella typhimurium*. Mol. Microbiol. 17, 155-167
5. Song, S.S., Lee, S., Lee, K.M., Lim, S.Y., Cho, M.H., Park, Y.K., Park, K.R., and Lee, I.S. (2001) Low temperature inducible acid tolerance response in virulent *Salmonella enterica* serovar Typhimurium. Kor. J. Microbiol. 37, 228-233

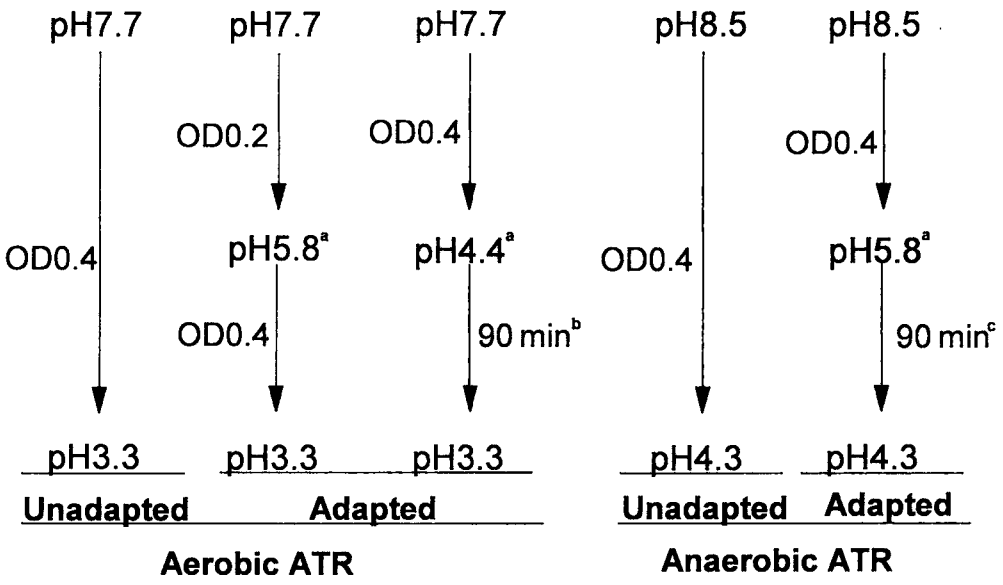

Figure 1. Schematic representation of acidification tolerance response. Bacterial cells were grown in SG minimal media. (a); The pHs of cultures were adjusted pH5.8 or 4.4 with inorganic acid. (b); For acid adaptation in pH4.4, cells were incubated in shaker for 90 min. (c); pH-adjusted cells were cultured in anaerobic chamber without shaking for 90 min.

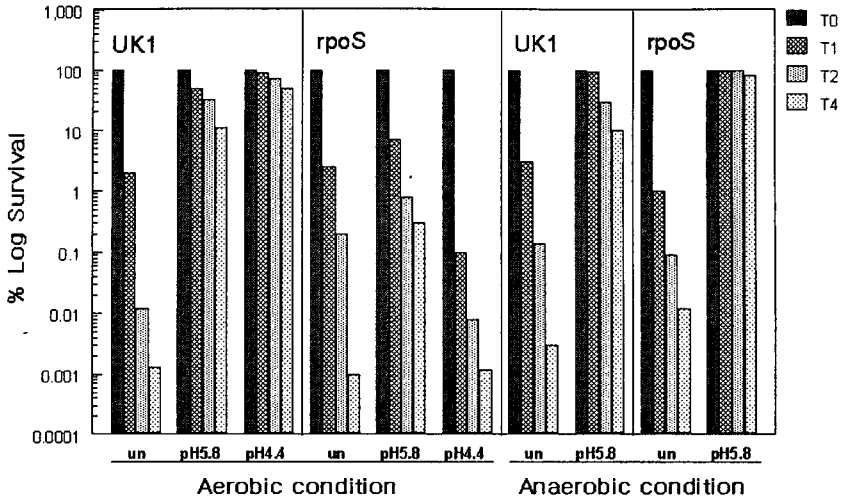

Figure 2. Log-phase acid tolerance responses of *rpoS* and wild type. Cells grown to mid log phase in minimal glucose medium were subjected to various adaptation protocols prior to acid challenge at pH3.3 for aerobic or pH4.3 for anaerobic. Unadapted cultures were adjusted directly to challenge pHs. Adaptation to pH5.8 in aerobic was allowed to occur for one cell doubling prior to acid challenge. Acid shock adaptations at pH4.4 for aerobic and pH5.8 for anaerobic were conducted for 90min before acid challenge, respectively. The bars represent average percent survival at pH3.3 condition after 0, 1, 2, and 4h. In the case of anaerobic ATR, percent survivals were calculated after 0, 2, 4, and 6h at pH4.3.