Acidic pH

Enemy or ally for enteric bacteria?

Francisco Ramos-Morales
Departamento de Genética; Facultad de Biología; Universidad de Sevilla; Sevilla, Spain

Keywords: acidic pH, Salmonella, virulence, type III secretion, acid tolerance response, STM1485

Abbreviations: AR, acid resistance; ATR, acid tolerance response; ASP, acid shock protein; SCV, Salmonella-containing vacuole; T3SS, type III secretion system; SPI, Salmonella pathogenicity island

The first stress that foodborne pathogens find upon ingestion is the very acidic pH of the stomach of the host. In addition, intracellular pathogens like Salmonella are submitted to low pH inside the host cells. Two general acid survival systems are found in these organisms: acid resistance mechanisms and acid tolerance responses. These mechanisms involve the synthesis of a series of acid shock proteins. Only a subset of these proteins is directly involved in acid survival. This is related to the fact that low pH is not only a stress to cope with, but it is also an important signal that indicates to the bacterium that it is in a potential host environment and that triggers the induction of many virulence genes. Asr is an acid shock protein that supports growth of Escherichia coli at moderate acidity. In this issue of Virulence, Allam et al. investigate the role of STM1485, the homologous of asr in Salmonella enterica serovar Typhimurium, in acid survival and virulence. Although STM1485 is not required for acid survival of S. enterica, it is necessary for intracellular replication in human epithelial cells and murine macrophages, and to prevent the progression of the Salmonella-containing vacuole along the degradative pathway. In addition, Allam et al. are able to show that the defects of the STM1485 mutant at the cellular level correlate with reduced virulence in the mouse model.

All food-borne pathogens, including Escherichia coli O157:H7 and Salmonella enterica serovar Typhimurium, are exposed to multiple stresses to which they must respond to survive. These potentially harmful conditions include heat, low pH, osmotic shock, bile, shift to microaerobiosis or anaerobiosis, interaction with gut microflora, and cationic antimicrobial peptides. The ability to sense these conditions and respond by turning on a set of appropriate genes is an essential feature that enables enteric bacteria to survive in different environments.

The first stress that these bacteria find upon ingestion is the very acidic pH of the stomach of the host. The pH value of this section of the gastrointestinal tract is in fact variable. A pH as low as 1.3 was observed in healthy humans in the fasted state while the pH increased to 4.9 after meal ingestion due to the buffering effects of food. Measurements of gastric pH in rats and mice gave values of 3.9 and 4.0, respectively, in the fasted state. Surprisingly, pH in the fed state was lower (3.2 and 3.0, respectively), although this result was attributed to differences in the meal types. In addition, significant individual variability has been observed in humans and in rodents. This low gastric pH can, in principle, quickly kill enteric bacteria. Different bacterial species have developed mechanisms that are more or less efficient to promote survival during passage through the stomach. Two interesting set of data underscore the importance of acid survival in pathogenesis, the first related to pathogen variability and the second to host variability: (1) the disparate oral infective doses of related pathogens like Vibrio cholerae (10⁴–10⁵), non-typhoid S. enterica (10³) and Shigella flexneri (10²) correlate with their different levels of acid resistance; (2) hypochlorohydric people are more susceptible to diseases like cholera due to reduced protection afforded by gastric acid.

The essentiality of resisting acidity is emphasized by the variety of systems to cope with this form of environmental stress that are present in enteric bacteria like E. coli and S. enterica, considered neutralophilic. The distinction between acid survival mechanisms is confusing because the laboratories working on them use different media, exponential or stationary-phase cells, and different pH or temperatures. An initial attempt to carry out a direct comparison classified acid survival systems in these organisms in two general categories: (1) acid resistance (AR) mechanisms, which require some form of supplementation for either induction or function; (2) acid tolerance responses (ATR), that can be induced and function in unsupplemented minimal medium.

Pathogenic and non-pathogenic strains of E. coli are remarkably resistant to extreme acid stress and can survive at pH 2 for hours. Three acid-resistant systems, AR1, AR2 and AR3, have been characterized in
E. coli, whose activity depends on the media used for growth and for acid challenge.\(^9\) AR1 is active when bacteria are grown to stationary phase in LB medium, without glucose, buffered to pH 5.5, and requires the stationary phase sigma factor RpoS and the global regulatory protein Crp. The other two systems depend on the presence of specific amino acids in the media, glutamate for AR2 and arginine for AR3, and have similar simple mechanisms of action. In the AR2 system, the glutamate decarboxylase isozymes GadA and GadB use a cytoplasmatic proton to replace the \(\gamma\)-carboxyl group of glutamate and produce CO\(_2\) and \(\gamma\)-amino butyric acid (GABA). Then, the antiporter GadC, expels GABA and imports glutamate. The AR3 system uses the arginine decarboxylase AdiA to decarboxylate arginine and produce agmatine, which is then exported by AdiC in exchange of arginine. In addition, a fourth, less efficient system induced by lysine has also been described.\(^7\) Components of this system are the decarboxylase CadA and the lysine/cadaverine antiporter CadB. These AR systems contribute to pH homeostasis so that, when the external pH is 2.5, the internal pH is maintained at 4.5. The strategy of resistance includes also a change in the electrical potential, from negative to positive, and a decrease in metabolic activity.\(^8\)

Direct comparisons under identical conditions indicate that S. enterica is much less acid resistant than E. coli.\(^1\)\(^1\)\(^0\) However, three ATR mechanisms have been demonstrated in S. enterica serovar Typhimurium.\(^4\) In fact, ATR, although also exist in E. coli,\(^1\)\(^1\)\(^1\) has been best characterized in S. enterica. The exponential phase ATR is induced when bacteria are grown in minimal medium at a moderately acid pH (4.5–5.8). These bacteria are then able to survive a subsequent pH 3 challenge. In addition, S. enterica serovar Typhimurium possesses two independently regulated, stationary phase ATR. The first is pH independent and RpoS dependent and is part of a general stress response that is induced when cells enter stationary phase. The second is RpoS independent, is induced by exposition to pH 4.5 in minimal medium, and provide tolerance to longer exposures to pH 3 than the RpoS dependent system.

Salmonella ATR mechanisms involve the synthesis of a series of acid shock proteins (ASP). At least 60 ASPs are synthesized during acid shock of exponentially growing bacteria. Different sets of these ASPs are regulated by RpoS,\(^1\)\(^2\)\(^-\)\(^4\) the major iron regulatory protein Fur,\(^1\)\(^5\)\(^-\)\(^6\) or the two-component system PhoP-PhoQ.\(^1\)\(^7\)\(^-\)\(^8\) More than 40 ASPs are associated to the stationary phase ATR and the master regulator of this system is OmpR, the response regulator of the two-component system involved in response to osmolarity.

Although ATR in Salmonella occurs in the absence of amino acids, an increased acid resistance is observed when adapted bacteria are challenged in minimal medium pH 2.5 in the presence of arginine, lysine or ornithine. In fact, S. enterica also possesses amino acid decarboxylases that contribute to survival at low pH in the presence of their cognate amino acid.\(^1\)\(^9\)\(^-\)\(^2\)\(^0\) AdiA, CadA and SpeF decarboxylate arginine, lysine and ornithine to produce agmatine, cadaverine and putrescine, respectively, that are expelled by specific antiporters. The three systems contribute to survival at pH 2.3 with the following order of efficiency: AdiA > CadA > SpeF.\(^2\)\(^0\)

Only a subset (not completely identified) of the above-mentioned ASPs is directly involved in acid survival. This is related to the fact that low pH is not only a stress to cope with; it is also an important signal that indicates to the bacterium that it is in a potential host environment and that triggers the induction of many virulence genes. S. enterica, as a facultative intracellular pathogen that can cause self-limited gastroenteritis or systemic diseases, has the ability to enter non-phagocytic cells, like intestinal epithelial cells, using a type III secretion system (T3SS1) encoded by genes in Salmonella pathogenicity island 1 (SPI1). A second system, called T3SS2, encoded in SPI2, is necessary for intracellular survival and proliferation. Inside the host cells, Salmonella resides in a membrane-bound compartment known as Salmonella-containing vacuole (SCV). The pH of the SCV in macrophages decreases to \(<\) pH 5 within 1 h\(^2\)\(^1\) and the rate of acidification in these cells does not seem to be significantly delayed by Salmonella. Although, in epithelial HeLa cells the acidification of the SCV is delayed by the presence of live wild-type Salmonella (in contrast with E. coli or dead Salmonella), by 2 h post-infection the median pH of the SCV is 4.9.\(^2\)\(^2\) T3SS2-related genes and other virulence genes are induced in the acidified SCV.\(^2\)\(^3\) In fact, an acidic environment is necessary for survival and replication of the bacteria within the macrophage,\(^2\)\(^1\) and disruption of genes involved in regulation of the acid response, fur, phoP or rpoS, also causes virulence attenuation.\(^2\)\(^4\)

The function of acid-induced proteins has been dissected only in a limited number of cases. In this issue of Virulence, Allam et al.\(^2\)\(^5\) investigate the role of a S. enterica serovar Typhimurium ASP, STM1485, in acid survival and virulence. Transcriptional analysis revealed that STM1485 is the most highly upregulated gene in S. enterica serovar Typhimurium 4 h and 12 h after infection of macrophages.\(^2\)\(^6\) The orthologous gene in S. enterica serovar Typhi, STY1582, is also highly expressed inside macrophages.\(^2\)\(^7\) Comparisons between E. coli and Salmonella genomes using a 70% DNA sequence similarity cut-off\(^2\)\(^8\) failed to find an ortholog in E. coli. Interestingly, however, the most similar E. coli gene is asr (for acid shock RNA). Acid shift of the growth media from pH 7 to \(<\) 5 strongly increases the amount of the RNA corresponding to this gene.\(^2\)\(^9\) In fact, this is the most highly induced gene in E. coli under low pH conditions.\(^3\)\(^0\) The Asr protein is an ASP that supports growth of E. coli at moderate acidity (pH 4.5) and is required for the exponential phase ATR.\(^3\)\(^1\) This protein must be processed to an 8 kDa derivative to become functional. STM1485 codes for a protein of 82 amino acids, whereas Asr has 102 amino acids. Comparison between both proteins reveals 58% of amino acid identity with conservation of the cleavage sites used for processing.\(^3\)\(^1\) Sequence alignment of the promoter regions of asr, STM1485 and homologous genes in Yersinia pestis, S. flexneri and Enterobacter cloacae identified a highly conserved region that explain a similar pH-dependent induction.\(^3\)\(^2\)

With all these data in mind, Allam et al. study now the role of STM1485 in the survival of Salmonella during acid stress,
inside the host cells and in the mouse model. They reach two interesting conclusions: the STM1485 mutant shows a defect in the correct assembly of T3SS2. Some contradiction exists, however, in the literature about the role of T3SS2 in the SCV maturation.33,34 In addition, Allam et al. are able to show that the defects of the STM1485 mutant at the cellular level correlate with reduced virulence in the mouse model.

The comparison between the homologous acid-inducible genes asr and STM1485 is a perfect example of the two sides that acidic pH has for enteric bacteria: asr is a component of the exponential phase ATR that allows the growth of E. coli under potentially harmful acidic conditions; STM1485 is not involved in growth at low pH but in the detection of acidity as a signal that helps Salmonella in triggering expression of virulence genes appropriate to the intracellular environment.

Acknowledgments

The work in the laboratory of the author is supported by grant SAF2010–15015 from the Spanish Ministry of Science and Innovation and the European Regional Development Fund, and grant P08-CVI-03487 from the Consejería de Economía, Innovación y Ciencia, Junta de Andalucía, Spain.

References

1. Rychlik I, Barrow PA. Salmonella stress management and its relevance to behaviour during intestinal colonisation and infection. FEMS Microbiol Rev 2005; 29:1021-40; PMID:16023758; http://dx.doi.org/10.1016/j.femsre.2005.03.005
2. Russell TL, Berardi RR, Barnett JL, Dementzoglou LC, Jarvenpaa KM, Schimatz SP, et al. Upper gastrointestinal pH in seventy-nine healthy, elderly, North American men and women. Pharm Res 1993; 10:187-96; PMID:8456064; http://dx.doi.org/10.1023/A:1018970323716
3. McConnell EL, Basit AW, Mordan S. Measurements of rat and mouse gastrointestinal pH, fluid and lymphoid tissue, and implications for in vivo experiments. J Pharm Pharmacol 2008; 60:63-70; PMID:18088506; http://dx.doi.org/10.1211/jpp.60.1.0008
4. Audia JP, Webb CC, Foster JW. Breaking through the acid barrier: an orchestrated response to proton stress by enteric bacteria. Int J Med Microbiol 2001; 291:97-106; PMID:11437344; http://dx.doi.org/10.1016/S1438-4221(00)00106-2
5. Kohary MH, Babu US. Infective dose of foodborne pathogens in volunteers: a review. J Food Saf 2001; 21:49-68; http://dx.doi.org/10.1080/02701260.2001.10785163
6. Levine MM, Black R, Clemens ML. Pathogenesis of enteric infections caused by Vibrio. In: Colwell RR, ed. Vibrios in the environment. New York: John Wiley & Sons, Inc, 1984:109-22
7. Lin J, Lee IS, Frey J, Slonczewski JL, Foster JW. Comparative analysis of extracellular acid survival in Salmonella typhimurium, Shigella flexneri, and Escherichia coli. J Bacteriol 1995; 177:4097-104; PMID:7608084
8. Foster JW. Escherichia coli acid resistance: tales of an amateur acidophile. Nat Rev Microbiol 2004; 2:898-907; PMID:15494746; http://dx.doi.org/10.1038/nrmicro1021
9. Iyer R, Williams C, Miller C. Arginine-aminoguanine antiproton in extreme acid resistance in Escherichia coli. J Bacteriol 2003; 185:6556-61; PMID:14594828; http://dx.doi.org/10.1128/JB.185.22.6556-6561.2003
10. Kousounis RM, Sofos JN. Comparative acid stress response of Listeria monocytogenes, Escherichia coli O157:H7 and Salmonella Typhimurium after habitation at different pH conditions. Lett Appl Microbiol 2004; 38:321-6; PMID:15214733; http://dx.doi.org/10.1111/j.1472-765X.2004.00491.x
30. Tucker DL, Tucker N, Conway T. Gene expression profiling of the pH response in *Escherichia coli*. J Bacteriol 2002; 184:6551-8; PMID:12426343; http://dx.doi.org/10.1128/JB.184.23.6551-6558.2002

31. Seputiene V, Motiejunas D, Suziedelis K, Tomenius H, Normark S, Melefors O, et al. Molecular characterization of the acid-inducible *asr* gene of *Escherichia coli* and its role in acid stress response. J Bacteriol 2003; 185:2475-84; PMID:12670971; http://dx.doi.org/10.1128/JB.185.8.2475-2484.2003

32. Seputiene V, Suziedelis K, Normark S, Melefors O, Suziedeliene E. Transcriptional analysis of the acid-inducible *asr* gene in enterobacteria. Res Microbiol 2004; 155:535-42; PMID:15313253; http://dx.doi.org/10.1016/j.resmic.2004.03.010

33. Gallois A, Klein JR, Allen LA, Jones BD, Nauseef WM. *Salmonella* pathogenicity island 2-encoded type III secretion system mediates exclusion of NADPH oxidase assembly from the phagosomal membrane. J Immunol 2001; 166:5741-8; PMID:11313417

34. Uchiya K, Barbieri MA, Funaro K, Shah AH, Stahl PD, Grossman EA. A *Salmonella* virulence protein that inhibits cellular trafficking. EMBO J 1999; 18:3924-33; PMID:10406797; http://dx.doi.org/10.1093/emboj/18.14.3924