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RESEARCH ARTICLE

THE BACTERIAL ACID-TOLERANCE MECHANISMS

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ABSTRACT

Most bacteria of medical interest grow and develop at pH values around neutrality (pH 7). However, many of these bacteria are also capable of maintaining themselves for long periods of time in acidic environments. The same goes for bacteria that thrive in the environment. Such is the case of some rhizobial bacteria associated with plants, where the acidic pH of the soil could represent a limiting aspect of their growth, however this is not the case because these bacteria can grow at low pH. These bacteria have different mechanisms to survive in acidic environments, which are presented in a general way in this work.

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INTRODUCTION

The bacteria are an example of organisms that are highly adaptable to different environments. Many of these bacteria are associated with humans and grow at pH values close to neutrality (Mir *et al*., 2017; Wang *et al*., 2020). However, there are bacteria as *Helicobacter pylori* that can grow in the acidic environments as gastric secretions (Benoit *et al*., 2013).

Another example is the acid-acetic bacteria *Acetobacer aceti* that develops in vinegar, or the lactic bacteria *Lactobacillus sp.* which is used in the production of fermented foods (Nakano and Ebisuya, 2016). Survival in acidic media depends on different mechanisms that have been studied in bacteria. In this work, some bacterial acid-tolerance mechanism are presented.

GENERAL MECHANISMS OF BACTERIAL ACID-TOLERANCE: Tolerance to acidic pH is an adaptation of cells that have been exposed to a gradual decrease in environmental pH and have managed to survive against lethal stress. It is known that bacterial tolerance to stress conditions varies depending on the environmental factors (Álvarez-Ordóñez *et al*., 2012). One of the most common mechanisms of resistance is the presence of a physical barrier in bacterial cells, which is represented by the cell wall and the cytoplasmic membrane. These bacterial elements are the first defense barrier against stressful environmental conditions, for example low or high temperatures, osmotic pressure, presence of oxidants and substances of microbial origin and the extreme pH values (Krulwich *et al*., 2011). Different mechanisms of bacterial acid-tolerance are currently known (Mubarak and Soraya, 2018). One of those mechanisms is changing the composition of the cytoplasmic membrane producing a decrease in membrane unsaturated fatty acids (Álvarez-

Ordóñez *et al*., 2012). The presence of membrane efflux pumps, which remove the protons outside the cell and maintaining an optimal cytoplasmic pH has been reported. This mechanism is key in the regulation of acid stress by excluding protons (Lei *et al*., 2011, Gaca and Lemon, 2019). Another mechanism that has been reported is the production of extracellular polymeric substances which protect to bacteria against extreme environments (Paitán *et al*., 2019). Next, some cases of the mechanisms that bacteria use to maintain themselves in environments where pH is acid are shown.

THE CASE OF *Rhizobium* sp. Some rhizobia are recognized to be acid pH-tolerant bacteria. Rhizobial strains are isolated from acid soils growing beans, cowpea beans and lotus (Shi *et al*., 2022; Soares *et al*., 2014). It has been reported that rhizobia in the soil have tolerance at pH ranges from 3.5 to 7.8 (Hernández-Forte *et al*., 2017). Smriti *et al*., (2014) reported that *Rhizobium* sp. had good growth at pH 6.0 and 7.0, while at pH 5 and 5.5 had regular growth and poor growth at pH 7.5 and 8.0. It has been reported that also in free-living conditions some species of rhizobia, such as *R. meliloti* and *R. tropici* can grow under high salinity conditions by accumulation of molecules and protons (Lei *et al*., 2011; Keneni *et al*., 2010). It has been observed that the acid-resistance mechanisms in rhizobia including proton pumping systems and regulators of two-component signal transduction systems, which alter the cell membrane producing lysyl-phosphatidylglycerol and alanyl-phosphatidylglycerol. These membrane lipids protect cells modifying the architecture, composition, stability, and activity of the cell (Lei *et al*., 2011). It has been observed that rhizobia have developed these mechanisms because they are very sensitive to acid environments. The acidity of soil limits the survival of rhizobia affecting the root infection and colonization, and the formation of nodules in the roots (Keneni *et al*., 2010). An isolate of *Rhizobium* sp. from *Abies procera* shown a powerful tolerance to acid; it survived at pH as low as 5.0 (Hernández-Forte *et al*., 2017; Smriti *et al*., 2014). There is evidence that *Rhizobium* strains block the entry of protons during acid shock by altering the structure of outer membrane and accumulating protons outside the cell (Ferreira *et al*., 2012; Lei *et al*., 2011). It has been reported also that *Rhizobium* strains in soils of the Amazon region showed better growth at pH 5.0 than at pH 6.0 or 6.9. In that case, the production of exopolysaccharides by rhizobia (as acid-resistance mechanism) was determinated. The production of exopolysaccharides is an adaptive response to stressful environmental conditions for microbial growth (Ferreira *et al*., 2012).

THE CASE OF *Enterococcus* sp. The enterococci group consist of ubiquitous microorganisms commonly found in dairy products and other foods (Morandi *et al*., 2005). *Enterococcus* species are acid-tolerant and acidify the pH of their environment as a result of their metabolism (Gaca and Lemon, 2019; Mubarak and Soraya, 2018). They can grow at pH values of pH 3 to 7. An example is the bacterium *Enterococcus faecium* which is used as a probiotic additive in food production (Castillo *et al.,* 2018). Mubarak and Soraya (2018) reported that the *Enterococcus faecalis* strains shown acid tolerance by contacting an extract of lime (*Citrus aurantiifolia*). The lime extract has a highly acidic pH range (1.7–3.1) and the acidity is generated by citric acid and amino acids; the essential oils contribute also to maintaining its acidic pH (Hugenholtz, 1993; Mubarak Z and Soraya, 2018; Wongkhantee *et al*., 2006). It has been reported that *E. faecalis* acid-tolerance is attributed to an increase in the production of lipoteichoic acids and biofilm formation (Fabretti *et al*., 2006; Mubarak and Soraya, 2018). The gene *EfCitH* of *E. faecalis* encodes a citrate transporter protein which is on the surface of the cell membrane and acts maintaining the balance between the citric acid generated by bacterium and the environment (Blancato *et al*., 2006; Mubarak and Soraya, 2018). It has been reported also that in acidic media, there is a decrease in the fluidity of the membrane and there is no increase in unsaturated or branched chain fatty acids (unlike at alkaline pH) (Kanno *et al*., 2015). Another mechanism of acid-tolerance has been described in *Enterococcus hirae*, which operates by proton extrusion carried out by F1Fo-ATPase, enzyme capable of hydrolyzing ATP and pumping protons outside the intracellular medium, overproducing protein under low pH conditions (Gaca and Lemon, 2019). The cytoplasmic buffer by NH_3 is another mechanism of acid tolerance that has been characterized in *E. faecalis* and *E. hirae*. At pH 3.5, in *E. faecalis* is has been reported that arginine and agmatine are metabolized by the arginine and agmatine deiminase and converted to NH_3 and other products as carbamoylputrescine. NH_3 produces NH_4^+ interacting with protons of acidified cytoplasm, raising the cytoplasmic pH (Gaca and Lemon, 2019; Ladero, 2010; Llacer *et al*., 2007; Suárez *et al*., 2013).

THE CASE OF *Salmonella* spp. The most common reservoir of *Salmonella* spp. is the intestinal tract of domestic and wild animals, and has been detected more frequently in fresh chicken, turkey, and pork meat (Chaudhuri *et al*., 2018). *Salmonella* spp. tolerates different stressful conditions, both in its natural niche, such as the gastrointestinal tract of hosts and in the environment. In this context, *Salmonella* spp. it is subjected to thermal and osmotic stress and extreme changes in pH (Ryu and Beuchat, 1998). *Salmonella typhimurium* is mainly associated in humans due to the consumption of contaminated pork, poultry, and bovine meat (Álvarez-Ordóñez *et al*., 2012). It has been reported that *S. typhimurium* produce alterations in the composition of membrane lipids to support an acid pH. It is characterized by a decrease in the ratio of membrane unsaturated to saturated fatty acids and in the relative concentration of octadecenoic acid (Álvarez-Ordóñez *et al*., 2012; Al Tayib and Al-Bashan, 2007). The changes in the fatty acid composition of membrane produce cells with decreased membrane fluidity, which generally showed a greater ability to survive under lethal acid exposures and heat treatments (Álvarez-Ordóñez *et al*., 2013; Brenneman *et al*. 2013).

The enteric bacteria such as *E. coli* and *Salmonella* spp. can colonize and cause disease in the human intestinal tract. They have to combat acidic environments during the process of invading the host surviving at pH values as low as 1.5-2.5 in the stomach (Foster, 2004; Xu *et al*., 2020). This also occurs in the passage of *E. coli* into the small intestine finding a less acidic environment (pH 4.0–6.0) due presence of organic acids produced by the normal intestinal flora (Lin *et al*., 1996). So, *E. coli* and *Salmonella* spp. have developed variable acidic stress response systems as the acid resistance (AR) systems that response to extreme acid stress and the acid tolerance response (ATR) system for mild and moderate acid stress (Brenneman *et al.,* 2013; Lund *et al*., 2014; Xu *et al*., 2020). The ATR system, though poorly understood, is induced by exposing bacterial cells to moderate acid stress (pH 4.5-5.8), and will protect cells from a subsequent challenge of extreme acid pH (pH 2.0-3.0) (Foster, 2001; Lin *et al*. 1995). ATR can be activated during adaptation at mild acidic pH by the regulators Fur and PhoPQ in exponential phase cells and by RpoS and OmpR in stationary phase cells, but the stationary phase cells are much more tolerant to acid than the log phase cells (Foster, 2001; Lund *et al*., 2014; Xu *et al*., 2020). Another important mechanism in *S. typhimurium* for pH maintenance is the presence of the inducible enzymes lysine decarboxylase and arginine decarboxylase system that maintain intracellular pH under acid conditions (Álvarez-Ordóñez *et al.*, 2012). This system is composed by a transcriptional regulator (CadC), an operon *cadBA*, a lysine decarboxylase enzyme (CadA) and a lysine-cadaverine antiporter (CadB) (Viala *et al.*, 2011). Under conditions of low external pH in the presence of lysine, CadC acts as a signal sensor and as a transcriptional regulator that activates the transcription of the cadBA operon. After induction, the enzyme CadA converts intracellular lysine to cadaverine with the consumption of a proton increasing the intracellular pH (Han *et al.*, 2018). CadC also controls the expression of 36 proteins during the presence of acid, demonstrating the importance of the lysine decarboxylase system (Lee and Kim, 2017). On the other hand, in *Salmonella* spp. there is an active arginine decarboxylase system, composed of an arginine decarboxylase (AdiA) which converts arginine to agmatine in the cytoplasm producing the consumption of a proton (Álvarez-Ordóñez *et al.*, 2012; Kieboom and Abee, 2006). It is known that there are other mechanisms of protection of *Salmonella* spp. in acidic environments, such is the case of induced acid shock proteins to prevent or repair the macromolecular damage caused by acid stress (Álvarez-Ordóñez *et al.,* 2013). Acid shock proteins are induced in different phases of bacterial growth and are expressed in stress of both organic and inorganic acids (Álvarez-Ordóñez *et al*., 2012; Hu *et al*., 2020). It has been shown that a moderate acidic pH promotes the transcription of several genes regulated by the system of two components PhoPQ involved in the logarithmic phase of ATR of *S. typhimurium*, conferring protection against inorganic acid stress by the induction of four acid shock proteins (Hu *et al.*, 2020, Álvarez-Ordóñez *et al.*, 2012). The alternative sigma factor RpoS helps also the survival of *Salmonella* spp. in stationary phase as a general response to stress and at acidic pH (Brenneman *et al*. 2013; Hu *et al*., 2020). This RpoS factor is involved in the acid-inducible log-phase ATR of *S. typhimurium*, controlling the expression of least 10 ASPs that protect the cell from acid stress and other stress conditions (Álvarez-Ordóñez *et al.*, 2012). Another tolerant mechanism is the Fur protein, which is induced at low pH, controlling a subset of ASPs that contribute to the log-phase ATR of *S. typhimurium*, providing protection against stress by organic acids (Brenneman *et al*. 2013).

CONCLUSION

Acid-tolerant bacteria can be found in different environments. Their presence in foods of some of them is very important because they are a source of contamination, and since they can develop at low pH values, they guarantee their permanence, representing a risk to human health (for example, in the case of *Salmonella* spp.). In other cases, tolerance to acidic pH gives soil bacteria such as rhizobia the possibility of maintaining themselves in stressful environments. Therefore, it is interesting to learn about the mechanisms that allow bacteria to maintain themselves in acidic environments.

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REFERENCES

Al Tayib Y.A.M and Al-Bashan M.M. 2007. Experimental studies on the potential for acid tolerance, growth and survival of *Salmonella enterica serovar typhimurium* and *E. coli* O157: H7 in orange juices. Adv. Biol. Res. 1:99-107.

Álvarez-Ordóñez A., Prieto M., Bernardo A. Hill C. and López M. 2012. The acid tolerance response of *Salmonella spp.*: an adaptive strategy to survive in stressful environments prevailing in foods and the host. Food Res. Internat. 45:482-492.

Álvarez-Ordóñez A., Valdés L., Bernardo A., Prieto M. and López M. 2013. Survival of acid adapted and non-acid adapted *Salmonella typhimurium* in pasteurized orange juice and yogurt under different storage temperatures. Food Sci. Technol. Internat. 19:407-414.

Benoit S.L., Miller E.F. and Maier R.J. 2013. *Helicobacter pylori* stores nickel to aid its host colonization. Infect. Immun. 81:580-584.

Blancato V.S., Magni C. and Lolkema J.S. 2006. Functional characterization and Me^{2+} ion specificity of a Ca^{2+}- citrate transporter from *Enterococcus faecalis*. The FEBS J. 273:5121-5130.

Brenneman K.E., Willingham C., Kong W., Curtiss R. and Roland K.L. 2013. Low-pH rescue of acid-sensitive *Salmonella enterica serovar typhi* strains by a rhamnose-regulated arginine decarboxylase system. J. Bacteriology. 195:3062-3072.

Castillo Arroyo P.L., Betancur Hurtado C.A and Pardo Pérez E. 2018. Caracterización de microorganismos con potencial probiótico aislados de estiércol de terneros Brahman en Sucre, Colombia. Rev. Investig. Vet. del Perú. 29:438-448.

Chaudhuri D., Roy Chowdhury A., Biswas B. and Chakravortty D. 2018. *Salmonella typhimurium* infection leads to colonization of the mouse brain and is not completely cured with antibiotics. Frontiers in Microbiol. 9:1632.

Fabretti F., Theilacker C., Baldassarri L, Kaczynski Z., Kropec A., Holst O. and Huebner J. 2006. Alanine esters of enterococcal lipoteichoic acid play a role in biofilm formation and resistance to antimicrobial peptides. Infect. and Immun. 74:4164-4171.

Ferreira P.A.A., Bomfeti C.A., Soares B.L and de Souza Moreira F.M. 2012. Efficient nitrogen-fixing *Rhizobium* strains isolated from amazonian soils are highly tolerant to acidity and aluminium. World J. Microbiol. Biotechnol. 28:1947-1959.

Foster J.W. 2001. Acid stress responses of *Salmonella* and *E. coli*: survival mechanisms, regulation, and implications for pathogenesis. J. Microbiol. 39:89-94.

Foster J.W. 2004. *Escherichia coli* acid resistance: tales of an amateur acidophile. Nat. Rev. Microbiol. 2:898-907.

Gaca A.O. and Lemos J.A. 2019. Adaptation to adversity: the intermingling of stress tolerance and pathogenesis in enterococci. Microbiol. Mol. Biol. Reviews. 83:e00008-19.

Han L., Yuan J., Ao X., Lin S., Han X. and Ye H. 2018. Biochemical characterization and phylogenetic analysis of the virulence factor lysine decarboxylase from *Vibrio vulnificus.* Frontiers Microbiol. 9:3082.

Hernández-Forte I., Nápoles-García M.C., Rosales-Genqui P.R., Ramírez-Pedroso J.F. and Ponte-Betancourt S. 2017. Tolerancia a la acidez de rizobios provenientes de nódulos de *Canavalia ensiformis*. Cultivos Tropicales. 38:55-57.

Hugenholtz J. 1993. Citrate metabolism in lactic acid bacteria. FEMS Microbiol. Rev. 12: 165-178.

Kanno M., Tamaki H., Mitani Y., Kimura N., Hanada S. and Kamagata Y. 2015. pH-induced change in cell susceptibility to butanol in a high butanol-tolerant bacterium, *Enterococcus faecalis* strain CM4A. Biotechnol. Biofuels. 8:1-6.

Keneni A., Assefa F. and Prabu P.C. 2010. Characterization of acid and salt tolerant rhizobial strains isolated from faba bean fields of Wollo, Northern Ethiopia. J. Agricult. Sci. Technol. 12:365-376.

Kieboom J. and Abee T. 2006. Arginine-dependent acid resistance in *Salmonella enterica serovar Typhimurium.* J. Bacteriol. 188:5650-5653.

Krulwich T., Sachs G. and Padan E. 2011. Molecular aspects of bacterial pH sensing and homeostasis. Nat. Rev. Microbiol. 9:330-343.

Ladero V. 2010 Toxicological effects of dietary biogenic amines. Curr. Nutr. Food Sci. 6:145-156.

Lee Y.H. and Kim J.H. 2017. Direct interaction between the transcription factors CadC and OmpR involved in the acid stress response of *Salmonella enterica*. J. Microbiol. 55:966-972.

Lei Z., Jian-Ping G., Shi-Qing W., Ze-Yang Z., Chao Z. and Yongxiong Y. 2011. Mechanism of acid tolerance in a *Rhizobium* strain isolated from Pueraria lobata Willd. Ohwi. Canadian J. Microbiol. 57: 514-524.

Lin J., Lee I.S., Frey J., Slonczewski J.L. and Foster J.W. 1995. Comparative analysis of extreme acid survival in *Salmonella typhimurium*, *Shigella flexneri*, and *Escherichia coli*. J. Bacteriol. 177:4097-4104.

Lin J., Smith M.P., Chapin K.C., Baik H.S., Bennett G.N. and Foster J.W. 1996. Mechanisms of acid resistance in enterohemorrhagic *Escherichia coli*. Appl. Environ. Microbiol. 62:3094-3100.

Llacer J.L., Polo L.M., Tavarez S., Alarcon B., Hilario R. and Rubio V. 2007. The gene cluster for agmatine catabolism of *Enterococcus faecalis*: study of recombinant putrescine transcarbamylase and agmatine deiminase and a snapshot of agmatine deiminase catalyzing its reaction. J. Bacteriol. 189:1254-1265.

Lund P., Tramonti A. and De Biase D. 2014. Coping with low pH: molecular strategies in neutralophilic bacteria. FEMS Microbiol. Rev. 38:1091-1125.

Mir B., Serrano I., Buitrago D., Orozco M., Escaja N. and González C. 2017. Prevalent sequences in the human genome can form mini i-motif structures at physiological pH. J. Amer. Chem. Society. 139:13985-13988.

Morandi S., Brasca M., Alfieri P., Lodi R. and Tamburini A. 2005. Influence of pH and temperature on the growth of *Enterococcus faecium* and *Enterococcus faecalis*. Le Lait. 85:181-192.

Mubarak Z. and Soraya C. 2018. The acid tolerance response and pH adaptation of *Enterococcus faecalis* in extract of lime *Citrus aurantiifolia* from Aceh Indonesia. F1000Research. 7:287-295.

Nakano S. and Ebisuya H. 2016. Physiology of *Acetobacter* and *Komagataeibacter spp.*: acetic acid resistance mechanism in acetic acid fermentation. In Acetic Acid Bacteria pp. 223-234. Springer, Tokyo.

Paitán E., Santos R., Sotelo A., Zúñiga D. and Vílchez C. 2019. Caracterización molecular de bacterias con potencial probiótico aisladas de heces de neonatos humanos. Rev. Peruana de Biología. 26:119-130.

Ryu J.H. and Beuchat L.R. 1998. Influence of acid tolerance responses on survival, growth, and thermal cross-protection of *Escherichia coli* O157: H7 in acidified media and fruit juices. Internat. J. Food Microbiol. 45:185-193.

Shi H., Sun G., Gou L., and Guo Z. 2022. Rhizobia–legume symbiosis increases aluminum resistance in alfalfa haifan. Plants. 11:1275-1284.

Smriti K., Rakesh P., Dash D. and Chowdhury T. 2014. Detection and characterization of *Albizia procera-Rhizobium* for stress tolerance. Internat. J. Plant Sci. Muzaffarnagar. 9:349-352.

Soares B.L., Ferreira P.A.A., Oliveira-Longatti S.M.D., Marra L.M., Rufini M., Andrade M.J.B.D. and Sousa Moreira F.M.D. 2014. Cowpea symbiotic efficiency, pH and aluminum tolerance in nitrogen-fixing bacteria. Sci. Agric. 71:171-180.

Suárez C., Espariz M., Blancato V.S. and Magni C. 2013. Expression of the agmatine deiminase pathway in *Enterococcus faecalis* is activated by the AguR regulator and repressed by CcpA and PTS Man systems. PLoS One. 8:e76170.

Viala J.P., Meresse S., Pocachard B., Guilhon A.A., Aussel L. and Barras F. 2011. Sensing and adaptation to low pH mediated by inducible amino acid decarboxylases in *Salmonella.* PLoS One. 6:e22397.

Xu Y., Zhao Z., Tong W., Ding Y., Liu B., Shi Y., Wang J., Sun S., Liu M., Wang Y., Qi Q., Xian M. and Zhao G. 2020. An acid-tolerance response system protecting exponentially growing *Escherichia coli*. Nat. Commun. 11:1496-1509.

Wang S.P., Rubio L.A., Duncan S.H., Donachie G.E., Holtrop G., Lo G. and Flint H.J. 2020. Pivotal roles for pH, lactate, and lactate-utilizing bacteria in the stability of a human colonic microbial ecosystem. Mol. Biol. Physiol. 5:e00645-20.

Wongkhantee S., Patanapiradej V., Maneenut C. and Tantbirojn D. 2006. Effect of acidic food and drinks on surface hardness of enamel, dentine, and tooth-coloured filling materials. J. Dent. 34:214-220.

Xu Y., Zhao Z., Tong W., Ding Y., Liu B., Shi Y. and Zhao G. 2020. An acid-tolerance response system protecting exponentially growing *Escherichia coli.* Nature Commun. 11:1-13.

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