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Mechanisms of acid tolerance in bacteria and prospects in biotechnology and bioremediation

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ABSTRACT

Acidogenic and aciduric bacteria have developed several survival systems in various acidic environments to prevent cell damage due to acid stress such as that on the human gastric surface and in the fermentation medium used for industrial production of acidic products. Common mechanisms for acid resistance in bacteria are proton pumping by F_1–F_0–ATPase, the glutamate decarboxylase system, formation of a protective cloud of ammonia, high cytoplasmic urease activity, repair or protection of macromolecules, and biofilm formation. The field of synthetic biology has rapidly advanced and generated an ever-increasing assortment of genetic devices and biological modules for applications in biofuel and novel biomaterial productions. Better understanding of aspects such as overproduction of general shock proteins, molecular mechanisms, and responses to cell density adopted by microorganisms for survival in low pH conditions will prove useful in synthetic biology for potential industrial and environmental applications.

Contents

1. Introduction

Bacteria play important roles in the industrial production of various products, in human health, and in environmental governance. In order to survive sudden, potentially lethal challenges, microorganisms must possess effective mechanisms against various environmental stresses such as antibiotics, acids, organic solvents, and heat (Winfield and Groisman, 2003). Among these, an acidic condition is the most common condition encountered by several bacteria; therefore, biologists have focused a lot of attention on acid resistance in microorganisms. Acid mine drainage causes serious environmental problems (Akcil and Koldas, 2006), strongly affecting routine human life. Several environmental pollutants such as mine drainage basins contaminated with polycyclic aromatic hydrocarbons (PAHs) are acidic in nature, owing to the utilization of HCl or H_2SO_4. Moreover, industrial production of products such as lactic acid (Michelson et al., 2006; Zhang et al., 2014) and amino

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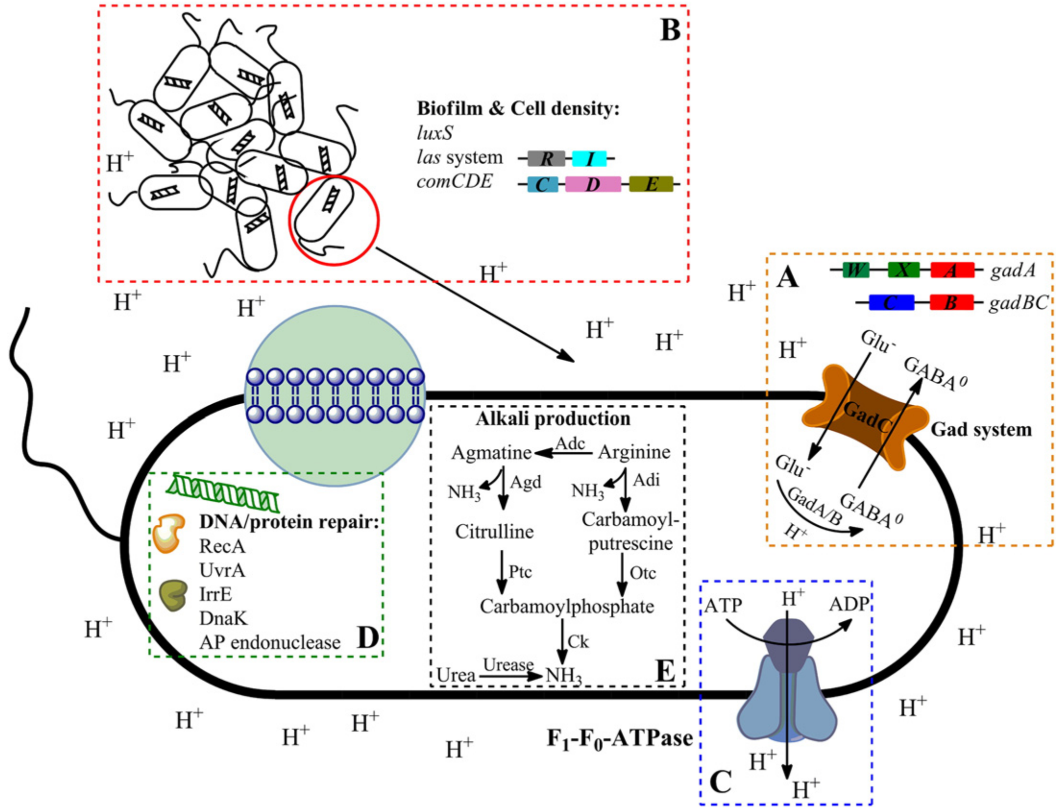

Fig. 1. Common acid-resistance mechanisms in microorganisms (partly adapted, with permission, from Matsui and Cvitkovitch, 2010). A: Gad system; *gadA*/*B* encode glutamate decarboxylase GadA/B, which convert Glu to GABA, and GadC (encoded by *gadC*), which acts as the Glu/GABA antiporter (Capitani et al., 2003). B: Biofilm and cell density; *comCDE* (Li et al., 2001) and *luxS* (Wen and Burne, 2004) are involved in a quorum sensing system that is essential for biofilm formation, and the *las* system is necessary for the exogenous *Pseudomonas* quinolone signaling molecule to stimulate biofilm formation (Williams and Camara, 2009). C: F_1–F_0–ATPase; as a proton pump, this complex pumps H^+ out to increase intracellular pH. D: Protection of macromolecules; RecA (Van der Veen and Abee, 2011) and UvrA (Croteau et al., 2008), as well as AP endonuclease (Hahn et al., 1999), are involved in DNA repair; DnaK (Tomoyasu et al., 1998) is a protein repair chaperone, and IrrE (Earl et al., 2002) is a global regulatory protein that can stimulate *recA* transcription. E: Alkali production; urea is transformed into NH_3 by urease to neutralize protons (Maroncle et al., 2006); The Adi and Agd systems can also produce NH_3 through several reactions (Griswold et al., 2004). Glu: glutamate; Adc: arginine decarboxylase; Agd: agmatine deiminase; Ptc: putative putrescine transcarbamylase; Adi: arginine deiminase; Otc: ornithine transcarbamylase; Ck: carbamate kinase.

acids (Shih and Van, 2001) have limiting acidic conditions such as a low pH environment, which negatively influence the growth of microorganisms. It is therefore mandatory to add chemicals such as calcium carbonate to neutralize the protons of acids to increase the pH in the medium. It is important to understand the acid-resistance mechanisms (ARMs; the survival systems used in acidic conditions) in microorganisms to determine their adaptive modifications. Strains such as *Escherichia coli*, *Salmonella enterica*, and *Shigella flexneri* are highly resistant to low pH and can survive in acidic conditions of the mammalian stomach as well, which can decrease rapidly to an approximate pH of 2.0 (Kanjee and Houry, 2013; Spector and Kenyon, 2012; Waterman and Small, 2003). These microorganisms can remain viable for several hours at a pH as low as 2.5 in a stationary phase, mainly owing to their ARMs (Waterman and Small, 2003). The two general ARMs adopted by microbes for maintaining their pH homeostasis during growth are as follows: i) use of H^+ antiport systems such as H^+-ATPase activity, acid end-product efflux, and decreased proton permeability to maintain a low intracellular concentration of protons; and ii) synthesis of alkali products to neutralize acid generated during extracellular metabolism. Several bacteria have evolved diverse resistance or tolerance mechanisms against the normally lethal pH values of ≤ 2.5, mainly through acid-tolerance responses (ATRs), which provide the ability to sense, respond and adapt to an acidified environment, and ARMs (Spector and Kenyon, 2012). In addition, *Pseudomonas aeruginosa* (Williams and Camara, 2009) and *Streptococcus mutans* (Li et al., 2001) form dense biofilms to protect cells against extracellular acid shock.

In this article, we review some model acid-resistant bacteria and their related ARMs (Fig. 1). Breakthroughs in genetic engineering and genomic research related to acid-resistance systems of microorganisms can supply genetic devices and biological modules for applications in industrial biotechnology and bioremediation. In addition, promising applications of ARMs in synthetic biology are discussed.

2. Common mechanisms of acid resistance

Microorganisms developed various effective mechanisms to survive the acidic environment, among which the most common mechanisms are the Gad system, biofilm formation and cell density, the F_1–F_0–ATPase proton pump, protection or repair of macromolecules and alkali production. We discuss these acid resistance mechanisms in detail below.

2.1. The glutaminase and Gad system

The Gad-dependent acid-resistance system is present in a variety of bacteria such as *E. coli* (Kanjee and Houry, 2013), *S. flexneriis* (Waterman and Small, 2003), *Listeria monocytogenes* (Cotter et al., 2005), and *Lactobacillus reuteri* (Su et al., 2011). The Gad system plays important roles in the ARMs of these strains (Capitani et al., 2003; Su et al., 2011; Teixeira et al., 2014). In addition, the acid resistance system in *E. coli* relies on L-glutamine (Gln), which is converted to L-glutamate (Glu) by the acid-activated glutaminase YbaS, with concomitant release of dissolved ammonia. YbaS and the amino acid antiporter GadC are sufficient for *E. coli* survival in extremely acidic environments (Lu et al.,