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Organic solvent adaptation of Gram positive bacteria: Applications and biotechnological potentials

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ABSTRACT

Organic-solvent-tolerant bacteria are considered extremophiles with different tolerance levels that change among species and strains, but also depend on the inherent toxicity of the solvent. Extensive studies to understand the mechanisms of organic solvent tolerance have been done in Gram-negative bacteria. On the contrary, the information on the solvent tolerance mechanisms in Gram-positive bacteria remains scarce. Possible shared mechanisms among Gram-(−) and Gram-(+) microorganisms include: energy-dependent active efflux pumps that export toxic organic solvents to the external medium; *cis*-to-*trans* isomerization of unsaturated membrane fatty acids and modifications in the membrane phospholipid headgroups; formation of vesicles loaded with toxic compounds; and changes in the biosynthesis rate of phospholipids to accelerate repair processes. However, additional physiological responses of Gram-(+) bacteria to organic solvents seem to be specific. The aim of the present work is to review the state of the art of responsible mechanisms for organic solvent tolerance in Gram-positive bacteria, and their industrial and environmental biotechnology potential.

Contents

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1. Introduction

Organic solvents can be extremely toxic to all life forms because they are able to bind to the cell membrane affecting its integrity. Disruption of membrane functions implies loss of the permeability barrier and the energy transducer; concomitantly leading to cellular metabolism damages, growth inhibition, and, finally cell death (Sardessai and Bhosle, 2002a; Heipieper et al., 2007). Despite this, for almost two decades, organic solvent-tolerant bacteria capable of thriving in the presence of these toxic compounds have been reported (Inoue and Horikoshi, 1989; Zahir et al., 2006). The first report of an organic-solvent-tolerant bacterium was described in 1989 on a *Pseudomonas putida* IH-2000 able to grow in the presence of very toxic toluene (Inoue and Horikoshi, 1989). Since that time, solvent-tolerant bacteria are being explored for their potential in industrial and environmental biotechnology (Sardessai and Bhosle, 2004). Their enzymes are expected to be stable and active in the presence of toxic solvents, representing one of the most promising tools for biocatalysis in non-aqueous systems (Castro et al., 1992; Ogino and Ishikawa, 2001; Fang et al., 2006; Takeda et al., 2006; Gupta and Khare, 2009).

Most of the studies on solvent-tolerant microorganisms were focused on Gram-(−) bacteria, which display a cascade of adaptive mechanisms used to acclimatize in the presence of toxic organic solvents. Two major mechanisms have been extensively described particularly in *Pseudomonas* sp. and *E. coli* species as typical models. The first one involves alterations of the cellular membrane composition in order to decrease solvent permeability (Pinkart et al., 1996; Aono and Kobayashi, 1997; Ramos et al., 1997; Tsubata et al., 1997; Heipieper et al., 2003). The second type reduces the accumulation of organic solvents in the inner membrane by transporting solvent molecules out of the lipid bilayer (Isken and de Bont, 1996). Likewise, solvent utilization at high rates or solvent biotransformation to a less toxic product was observed in some tolerant bacteria (Vangnai et al., 2002). In addition, modifications in the overall morphology of cells were reported in Gram-(−) microorganisms in response to organic solvents and other stressful environments (Shi and Xia, 2003; Neumann et al., 2005). However, limited studies have been done in order to understand the effects of organic solvents in Gram-(+) bacteria. Although microorganisms belonging to *Bacillus*, *Rhodococcus*, *Staphylococcus* and *Arthrobacter* species tolerant to very toxic organic solvents have been reported (Abe et al., 1995; Moriya et al., 1995; Baigorí et al., 1996; Kato et al., 1996; Paje et al., 1997; Torres and Castro, 2003; Na et al., 2005; Nielsen et al., 2005; Zahir et al., 2006).

In order to test solvent toxicity on cells and cellular components, a solvent hydrophobicity (log P) parameter was established. The log P is defined as the logarithm of the solvent partitioning coefficient between octan-1-ol and water (Laane et al., 1987). It is generally accepted that solvents with log P values below 5 are considered extremely toxic because of their high degree of partitioning into the aqueous layer surrounding the cells, and from there into the lipid membrane bilayer (Inoue and Horikoshi, 1991). Toxicity of organic solvents appears to be, at first instance, elated to their ability to dissolve into biological membranes, causing an increase of the cell membrane fluidity compromising the physiological functions of critical cell components (Sikkema et al., 1995; de Bont, 1998).

Additionally, solvent toxicity is directly related to the accumulation of solvent molecules inside the cell membrane. Each organism has its own intrinsic solvent tolerance level, which is genetically determined and environmentally influenced. Therefore, organic solvent tolerance is believed to be a strain-specific property (Kobayashi et al., 1998; Huertas and Duque, 1998).

The aim of the present work is to review the state of the art of the responsible mechanisms for organic solvent tolerance of Gram-(+) bacteria, and their industrial and environmental biotechnology potential.

2. Mechanisms of organic solvent tolerance in Gram-(+) bacteria

Unlike Gram-(−) bacteria, in which the mechanisms of tolerance to organic solvents have been extensively studied and reviewed, very little information regarding what makes Gram-(+) bacteria tolerant to toxic solvents is available. Due to the differences between the cell envelopes of Gram-(+) and Gram-(−) bacteria, one would expect

Table 1
Mechanisms of organic solvent-tolerance proposed in Gram-positive bacteria.

OS-tolerance mechanism	Microorganism (OS)	References
General stress response		
Sigma β genes: multidrug efflux proteins (proposed)	*B. subtilis* (ethanol)	Petersohn et al., 1999
Hsp33 stress protein	*B. psychrosaccharolyticus* (2-propanol)	Kang et al., 2007
Deactivation of organic solvents		
Biodegradation	*Bacillus* sp., *Rhodococcus* sp. (benzene, toluene, xylene)	Paje et al., 1997; Wang et al., 2008
	B. pallidus ST3 (2-propanol)	Bustard et al., 2002
Esterefication	*B. licheniformis* S-86 (3-methylbutan-1-ol)	Torres et al., 2009a
Changes in cell morphology		
Decrease in cell surface-to-volume ratio (filamentous growth)	*B. licheniformis* S-86 (3-methylbutan-1-ol)	Torres et al., 2009a
Unusual extracellular capsule	*Staphylococcus* sp. ZZ1 (toluene)	Zahir et al., 2006
Phenotypic adaptation: change in colonies' color	*R. erythropolis* (wáter-inmiscible solvents)	de Carvalho et al., 2004
Cell surface modifications		
Decreased cell surface hydrophobicity	*B. licheniformis* S-86 (3-methylbutan-1-ol)	Torres et al., 2009a
Increased cell surface hydrophobicity	*Mycobacterium frederiksbergense* (anthracene)	Wick et al. 2002
Cell membrane adaptations		
Increased membrane fluidity (changes in fatty acid)	*Staphylococcus haemolyticus* (toluene); *Rhodococcus erythropolis* DCL14 (short-chain alcohols)	Nielsen et al., 2005; Pepi et al., 2008
Increased membrane fluidity (changes in fatty acid)	*Bacillus* sp. ORAs2 (toluene); *Rhodococcus erythropolis* DCL14 (alkanes and long-chain alcohols)	de Carvalho et al. 2005; Pepi et al., 2008
Changes in membrane proteins	*Clostridium thermocellum* 27405 (ethanol)	Williams, et al., 2007
Solvent excretion		
Energy-dependent toluene efflux pump	*B. cereus* R1 (toluene)	Matsumoto et al., 2002

OS, organic solvent.