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Engineering membrane and cell-wall programs for tolerance to toxic chemicals: Beyond solo genes

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Metabolite toxicity in microbes, particularly at the membrane, remains a bottleneck in the production of fuels and chemicals. Under chemical stress, native adaptation mechanisms combat hyper-fluidization by modifying the phospholipids in the membrane. Recent work in fluxomics reveals the mechanism of how membrane damage negatively affects energy metabolism while lipidomic and transcriptomic analyses show that strains evolved to be tolerant maintain membrane fluidity under stress through a variety of mechanisms such as incorporation of cyclopropanated fatty acids, *trans*-unsaturated fatty acids, and upregulation of cell wall biosynthesis genes. Engineered strains with modifications made in the biosynthesis of fatty acids, peptidoglycan, and lipopolysaccharide have shown increased tolerance to exogenous stress as well as increased production of desired metabolites of industrial importance. We review recent advances in elucidation of mechanisms or toxicity and tolerance as well as efforts to engineer the bacterial membrane and cell wall.

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Introduction — microbial metabolite toxicity remains a limiting bottleneck in the production of fuels and chemicals

The repertoire of metabolites that can be produced as commodity or specialty chemicals and biofuels or biofuel precursors through microbial processes is ever expanding, and includes organic acids, alkanes, long-chain alcohols and aldehydes, and free fatty acids [1]. Most industrially attractive chemicals are toxic to microbes, an issue that limits the development of economically feasible bioprocesses [1,2]. Engineering or adapting organisms to tolerate high titers of toxic chemicals under realistic metabolite-production conditions aim to make them capable of producing toxic metabolites faster and at higher titers. Thus, understanding and developing microbial tolerance to a broad spectrum of chemicals is a problem of major industrial significance [1]. It is necessary that tolerant strains are capable of producing the desirable toxic metabolites at higher rates, and titers, and not merely tolerate their toxicity [3,4].

The focus of this review: bacterial membranes and cell wall as they relate to metabolite toxicity

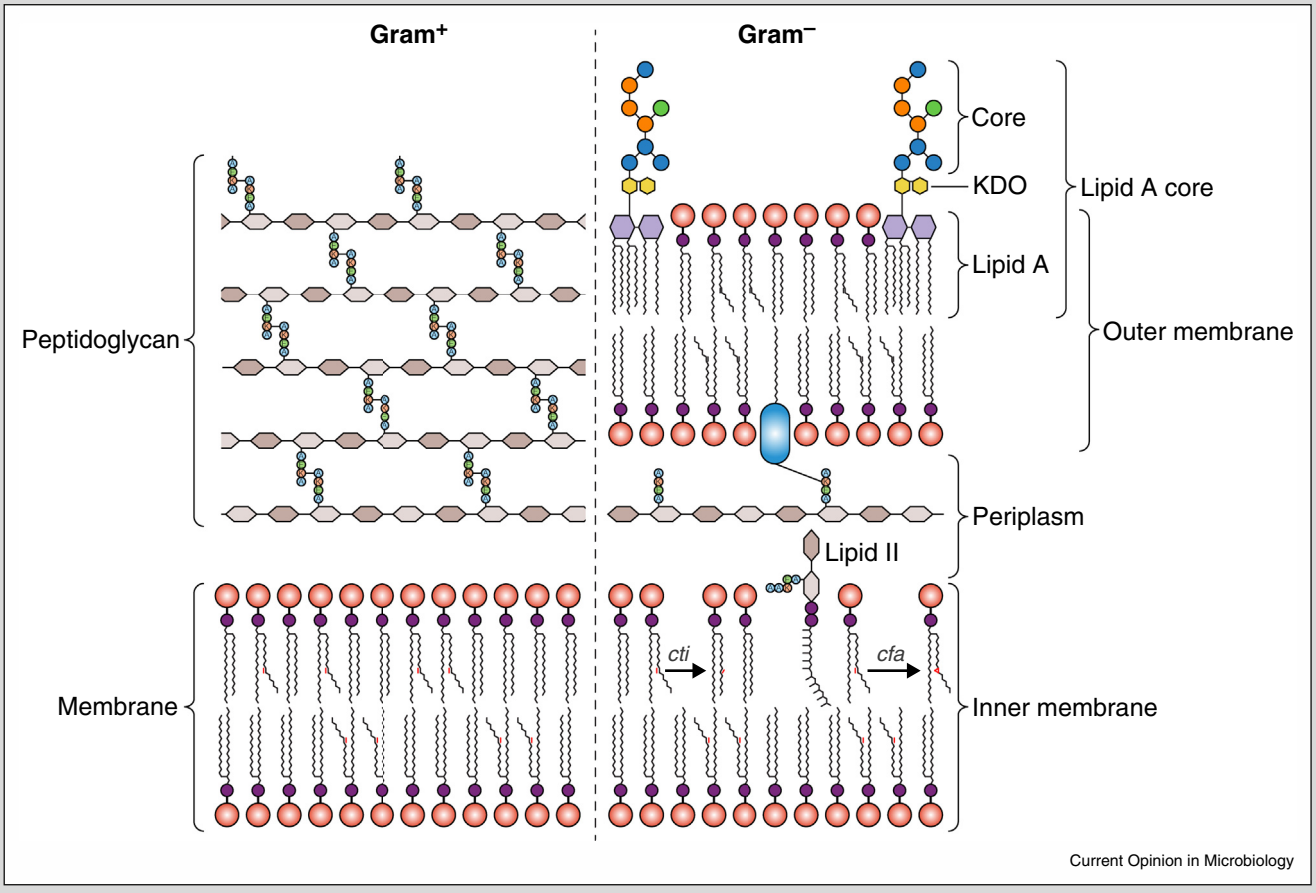
The last 10 years have witnessed a large activity in tolerance engineering, including the use of genetic and genomic screens and methods, and synthetic approaches, including focus on the role and impact of membrane transporters [2,4,5–11]. With few exceptions, the emphasis has been on deleting or overexpressing one gene/protein at a time, where the improvement in tolerance has been small to modest. Little effort has been placed on designing new or modified cellular programs or systems as a means to combat toxicity. Thus, this review focuses on precisely this possibility with emphasis on the understanding and engineering the bacterial membrane and cell wall at the systems level.

Insights and inspiration for engineering tolerance can come from a cell's natural response to toxicity, the differences between a tolerant mutant and the wild type, and even comparing the response mechanisms between strains and species which have varying degrees of naturally occurring tolerance. Beyond comparisons, use of heterologous sources of genetic material, from pure culture or metagenomic sources, can confer tolerance to a toxicity that the host strain may not be able to attain by manipulating only the endogenous genetic material. These are the guiding principles as we navigate through the literature to understand and engineer the tolerance phenotype.

Native membrane-adaptation mechanisms aim at constant membrane fluidity to combat the impact of toxic environments

Microbial membranes are permeable to small neutral molecules needed for growth (e.g. water, oxygen) as well as waste products for passive expulsion (e.g. carbon dioxide, hydrogen), but create a large free energy barrier to diffusion of large and highly polar/charged molecules (Box 1). In addition to these 'gatekeeper' functions, the ability of the membrane to maintain a chemical potential

Box 1 The bacterial membrane and cell wall of peptidoglycan and lipopolysaccharide (for Gram$^-$) provide a first line of defense against exogenous threats to the cell. The cytoplasmic membrane consists of phospholipids from the fatty acid biosynthesis pathway (Figure 1) with varying degrees of *cis*-unsaturation which can be modified to cyclopropanated fatty acids or *trans*-unsaturated fatty acids. Peptidoglycan, or murein, is a polymer network comprising amino acids and sugars formed in lipid II biosynthesis (Figure 2a) that is bound to the membrane by lipoprotein. The peptidoglycan layer is thick in Gram$^+$ bacteria (>20 nm) compared to Gram$^-$ bacteria (~7 nm), where it is situated in the periplasm. Lipopolysaccharide (LPS) is the outermost feature of Gram$^-$ bacteria and consists of the lipid A from the outer membrane and a polysaccharide chain which varies from strain to strain which forms a monolayer on the cell surface (Figure 2b). While these peptidoglycan and LPS related genes are often implicated in tolerance to various stressors, engineering these structures is an area ripe for exploration.



is crucial for energy generation and other cellular functions. Damage to the cell membrane has long been implicated in the toxicity of various chemicals, with the degree of toxicity correlating to the hydrophobicity [12,13]. Short alcohols and hydrophobic compounds, such as ethanol or butanol, can partition into lipid bilayer membranes [14,15]. These compounds displace water at the membrane/water interface which reduces surface tension. As concentrations and the alcohol carbon chain length rise, the membrane becomes overly fluid, which compromises its integrity [14]. Unsaturated fatty acids allow short alcohols to partition the membrane more than saturated fatty acids, which affects membrane phase behavior [14,16]. Exogenous free fatty acids, common in biodiesel-derived crude glycerol feedstocks, can partition into membranes and have a detrimental effect on growth and fermentation [16–18].

Under changing environmental conditions or stress, such as chemical stress from solvents, acids, and temperature changes, microbes engage several mechanisms for maintaining membrane integrity and fluidity; this is largely done by modifying the fatty acid profile of the phospholipid bilayer, termed the homeoviscous response. Modulating the fatty acid profile is not only important in maintaining membrane stability and integrity, but also for proper function of proteins and cofactors associated with the membrane. Generally, saturated fatty acids increase membrane stability and provide resistance to solvents and high temperatures by packing tightly together due to increasing van der Waals interactions between the acyl chains [19]. In contrast, by breaking up this tightly packed configuration, *cis*-unsaturated acyl chains increase membrane fluidity [19]. Regarding chain length, longer acyl chain lengths lead to an increase in the