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Toxic metal resistance in biofilms: diversity of microbial responses and their evolution

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Abstract

Since biofilms are an important issue in the fields of medicine and health, several recent microbiological studies have focused on their formation and their contribution to toxic compound resistance mechanisms. In this review, we describe how metals impact biofilm formation and resistance, and how biofilms can help cells resist toxic metals. First, the organic matrix acts as a barrier isolating the cells from many environmental stresses. Secondly, the metabolism of the cells changes, and a slowly-growing or non-growing sub-population of cells known as persisters emerges. Thirdly, in the case of multispecies biofilms, metabolic interactions are developed, allowing cells to be more persistent or to have greater capacity to survive than a single species biofilm. Finally, we discuss how the high density of the cells may promote horizontal gene transfer processes, resulting in the acquisition of new features. All these crucial mechanisms enable microorganisms to survive and colonize toxic environments, and probably accelerate ongoing evolutionary processes.

Keywords: Adaptation; Metal tolerance; Sequestration; Acid mine drainage; Persisters; Variants

1. Introduction

In many environments, populations of microorganisms concentrate at the solid/liquid or liquid/gas interfaces, resulting in formation of periphytons (biofilms forming on submerged solid surfaces), stromatolites (mushroom- and tower-shaped biofilms in quiescent waters) or aggregates of cells not attached to a solid surface (named flocs) or streamers (filamentous biofilms in flowing water), which have similar characteristics [1,2]. All these types of biofilms consist mainly of cells embedded in an organic matrix composed of hydrated biopolymers such as proteins (including enzymes), exopolysaccharides, glycolipids and extracellular DNA [3]. Considerable interest has focused during the last few years on the process of formation of surface-adherent communities and their dispersion, partly because of the advent of microscopic methods such as confocal microscopy and the development of new cell culture systems (which were recently reviewed in [1,3–6]). Briefly, planktonic bacteria first adhere to a biotic or abiotic surface; this first step often requires, but is not restricted to, the presence of flagella or pili (Fig. 1). Once they have become firmly attached, the cells migrate together using a process of twitching motility or recruit planktonic cells and grow to form microcolonies. Sessile bacteria excrete extracellular polymeric substances (EPS) forming a heterogeneous matrix interspersed with open water channels. The specific components of the matrix determine the architecture and its stability, and influence the functional efficiency of the biofilm, in particular, its adhesive properties, molecular exchange processes, matrix water content, charge and sorption properties [3]. The composition of the EPS depends on the microorganisms present and the environmental conditions, including the temperature and availability of nutrients. Cell lysis may be

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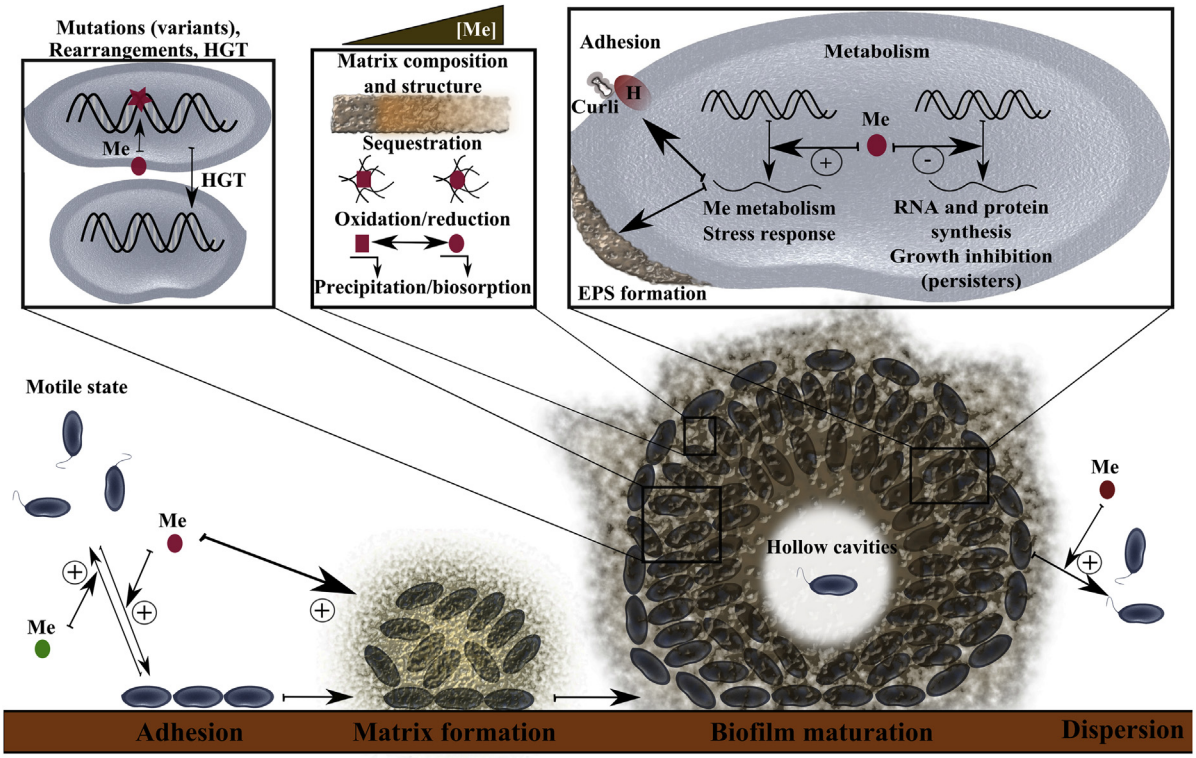

Fig. 1. Overview of biofilm formation and factors that contribute to toxic metal resistance or tolerance. The reduced sensitivity to toxic metals in biofilms is due to multiple factors, including changes in biofilm formation efficiency, matrix structure, metabolism and gene expression, as well as the onset of mutations. (Me: metal; H: hemagglutinin).

responsible for the formation of hollow cavities in a mature biofilm (Fig. 1). Other cells can be passively or actively (via a process of motility-driven dispersal) detached from the sessile community and disperse either in the fluid (via their swimming motility) or on the surface (by swarming on moist surfaces or via a process of gliding or twitching motility) and colonize other sites (Fig. 1). A link between the regulation of biofilm formation and swarming, swimming or twitching motility processes has been recently described and reviewed [1,7]. Biofilm formation is a coordinated developmental process based on cell-to-cell communications which involves several regulation processes such as quorum sensing (QS), two-component systems- or c-di-GMP-mediated regulation [2,7,8].

There exist several lines of evidence suggesting that biofilm formation protects cells from a wide range of environmental challenges [9–11]. In the field of microbial ecology, the role of biofilms in cell resistance to toxic compounds such as metal ions is of particular interest [2,10]. Several metal ions are toxic since they block many crucial cellular processes in all the domains of life [12,13]. In eukaryotic and prokaryotic cells, the toxicity of metals depends on whether or not they are essential metals, their speciation and their concentration [12,13]. Essential redox-active metals such as iron (Fe), copper (Cu), chromium (Cr) and cobalt (Co), which undergo cycling reactions involved in electron transfer processes, are toxic when they are present in excess, whereas non-essential metals such as arsenic (As), mercury (Hg), lead (Pb) and cadmium (Cd) are toxic even at very low concentrations. The mechanisms involved in the toxicity of metals have been recently reviewed [12,13]. Briefly, metals can directly or indirectly produce reactive radicals via Fenton and Haber-Weiss reactions, resulting in the formation of reactive oxygen and nitrogen species (ROS or RNS). This mechanism was observed in the case of Fe, As, Cr, tellurium (Te), Co, Hg, silver (Ag) and Cu. ROS, such as the hydroxyl radical ($^{\bullet}$OH) in particular, react with DNA, resulting in damaged bases or strand breaks, lipid peroxidation (leading indirectly to damaged bases) or protein modifications. In addition, metal toxicity may be linked to glutathione metabolism, which plays an important antioxidant role in cells and induces the chelation of several metals. The presence of excess As, Ag, Cd, Co, Cr or Te induces glutathione depletion, affecting the cellular redox balance. Metals interfere with protein activity by binding to thiols or other functional groups, catalyzing the oxidation of amino acid side chains, displacing essential metals ions in metalloproteins and interfering with folding processes and promoting protein aggregation [12–14]. Some metals can also inhibit cellular activity because they present a structural homology with enzyme substrates. This occurs, for example, with pentavalent arsenic (arsenate), which substitutes for phosphate used in many cellular processes including oxidative phosphorylation. Lastly, in microorganisms, some metals such as Ag and aluminium (Al) impair membrane function by interfering with nutrient uptake processes and disrupting the electron transport chain and/or the proton gradient force