

Metals and Metalloids by Actinobacteria

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Contributor: Alessandro Presentato, Elena Piacenza, Raymond Turner, Davide Zannoni, Martina Cappelletti

Actinobacteria include high GC bacteria that inhabit a wide range of terrestrial and aquatic ecological niches, where they play essential roles in recycling or transforming organic and inorganic substances. The metal(loid) tolerance and/or resistance of several members of this phylum rely on mechanisms such as biosorption and extracellular sequestration by siderophores and extracellular polymeric substances (EPS), bioaccumulation, biotransformation, and metal efflux processes, which overall contribute to maintaining metal homeostasis.

Keywords: metal resistance mechanisms, actinobacteria ; metal stress response ; metal-based nanostructures ; biogenic nanoscale materials

1. Introduction

Metals [e.g., silver (Ag), aluminum (Al), cadmium (Cd), cobalt (Co), chromium (Cr), cesium (Cs), copper (Cu), mercury (Hg), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), strontium (Sr), zinc (Zn), uranium (U)] and metalloids [i.e., arsenic (As), selenium (Se), tellurium (Te)], although naturally occurring throughout the Earth's crust, are not generally present in soluble forms, but suspended as colloids, or in association with organic matters, minerals, and rocks ^[1]. The release of metals and metalloids (henceforth indicated as metal(loid)s) into the environment derives from geochemical alterations, the type and age of the rock material, as well as natural events, such as volcanic eruptions, atmospheric deposition, leaching of the Earth, earthquakes, typhoons, and climate changes. Some metal(loid)s (e.g., Cu, Co, Fe, Mn, Se, Zn) are essential trace micronutrients needed to support several biological functions in all the domains of life, acting as cofactors featuring important structural and catalytic properties in enzymes and proteins, electron carriers, and regulators of the cellular osmotic pressure ^[2]. As opposed to this scenario, the presence of “*non-physiological*” levels of metal(loid)s into the environment has nowadays gained growing concern worldwide, and the reasons behind this phenomenon must be sought elsewhere. Anthropogenic activities (e.g., alloy production, use of pesticides, leather tanning, battery production, and electroplating industries) inexorably determined the buildup of wastes containing metal(loid)s during the 20th century. Moreover, metal(loid) anthropogenic source is more bioavailable and (bio)reactive as compared to those normally found in nature, being this aspect strictly correlated to the enhanced metal(loid) mobility and accumulation at different levels of the trophic chain ^[3]. Indeed, the reduction or destruction of these substances is a challenging task, making the excess of metal(loid)-containing compounds in the environment a persisting long-term problem ^[4]. In the case of metal(loid)s, they can cause severe environmental and human health issues even at low concentrations, exerting mutagenic and carcinogenic effects and impairing, among others, the central and peripheral nervous system or the circulatory one ^[5]. The toxic effects of metal(loid)s depend on the chemical element itself and the load at which it is encountered, as well as a variety of physical-chemical (e.g., temperature, pH, redox-potential, presence/absence of chelating compounds) and biological parameters (e.g., the metal(loid)-lipid solubility, physiological state of the organism, presence/absence of metal interacting proteins, organism adaptation phenomena), including also the way all these factors interplay with each other ^[6]. Furthermore, the overexposure to metal(loid)s can both compromise soil and water micro-flora fitness, severely worsening the chances of recovering environments contaminated by new xenobiotics ^{[7][8]}, and contribute to the emergence, maintenance, and transmission of antimicrobial-resistant pathogens ^{[9][10][11]}. Among possible solutions for metal(loid) removal or recovery, chemical precipitation, ultrafiltration, ion-exchange, reverse osmosis, and electro-dialysis are procedures that generate toxic wastes and are economically disadvantageous, mostly considering the large-scale application necessary to face the diffuse contamination of metal(loid)s ^{[12][13]}. Conversely, bioremediation strategies represent promising eco-friendly, economically feasible, efficient, and pollutant-selective approaches, involving the use of metal(loid) tolerant and/or resistant microorganisms ^[14], which have evolved specific metabolic processes and adaptation mechanism(s) associated with their survival under different stress conditions ^[15]. In particular, the survival and development of bacteria in extreme and harsh environments (e.g., in the presence of toxic metals) is allowed by the evolution of inducible mechanisms associated with specific genetic traits (i.e., referred to as resistance) and/or adaptation ones related to intrinsic biochemical features (i.e., referred to as

tolerance) [15]. These metabolic activities can be exploited to lower metal(loid) concentration to acceptable and safe levels in order to restore contaminated ecological niches and metal transformation for their recovery (e.g., metal-based nanostructure bioproduction) [16].

Among bacteria that are frequently recovered from toxic metal(loid) and, in turn, utilized for bioremediation strategies, members of Actinobacteria phylum have acquired extensive research attention. Actinobacteria are distributed among 221 phenotypically and morphologically different genera that are highly ubiquitous on Earth and inhabit diverse and extreme environments (e.g., water, soil, plant surface, arctic, and deserted areas) [17]. This bacterial group includes microorganisms exhibiting highly variable physiological and metabolic properties as a reflection of their wide genomic heterogeneity [17]. Among Actinobacteria, members of *Streptomyces* genus and the CMNR group (*Corynebacterium*, *Mycobacterium*, *Nocardia*, and *Rhodococcus*) possess large genomes and multiple linear and circular megaplasms, which contribute to their extraordinary metabolic capabilities and toxic metal resistance [18][19][20]. One interesting aspect of combining bioremediation with metal recovery strategies involves the utilization of microbes to produce nanostructures (NSs) containing metal(loid)s, which are typically more toxic in bulk. Metal(loid) nanomaterials (MeNMs) play a central role in nanotechnology, the field of science dealing with materials that possess at least one dimension in the nano range (10^{-9} m), where they hold physical-chemical characteristics (high surface-to-volume ratio, large surface energy, and spatial confinement) from which a plethora of intriguing properties—e.g., size-dependent, optical, catalytic, electronic, mechanical—arise as compared to the corresponding bulk materials. This aspect has enabled the extensive use of metal(loid) nanomaterials (MeNMs) at a multidisciplinary level in various application fields [21][22].

2. Metal(loid) Nanomaterial Biosynthesis by Actinobacteria

Although diverse biochemical strategies could be exploited by bacteria to regulate metal(loid) concentration in the environmental niches they live in, these microorganisms elicit metabolic traits aimed at detoxifying their habitat, generally involving the reduction of metal(loid) ions to less toxic and bioavailable (e.g., elemental) forms. This process results in a high intra- or extracellular localized concentration of metal(loid) atoms, which, to counteract their thermodynamic instability, tend to aggregate each other, eventually assembling in defined and uniform metal(loid) nanostructures (MeNSs; [Figure 1](#) and [Figure 2](#)) [23][24][25][26]. Thus, the proficiency of several bacterial genera, including Actinobacteria, to cope with metal(loid) ions makes them ideal candidates in the microbial nanotechnology field—i.e., the exploitation of microorganisms for the *green* and *eco-friendly* production of valuable Me nanomaterials (MeNMs). Indeed, a wide spectrum of Actinobacteria showed a strong attitude in biosynthesizing intra-, extracellular, or membrane-bound MeNMs with different sizes, morphologies, and properties. However, parameters and biochemical processes influencing the production of MeNSs and their assembly, in combination with a lack of their extensive physical-chemical characterization and potential applications, are still the black holes of this emerging field, underlining some critical concerns about the possibility of scaling-up the biogenic route behind NMs production stream. In this regard, key aspects to consider in designing innovative MeNM production methods are the choice of reducing agents, metal(loid) precursor-to-reducing agent ratio, temperature, pH, and reaction time. Considering the biogenic synthesis, controlling the reducing agent(s) is a very demanding task, especially when whole cells or EPS are used, as several and complex biomolecules could participate in these bioprocesses [22]. Moreover, whole-cell mediated synthesis also forces to keep constant the temperature and pH of the system, parameters that are optimized for the bacterial strain investigated. Particularly, a temperature between 30 and 37 °C and pH values close to neutrality are the optimum conditions to favour the growth of Actinobacteria [27]. Alternatively, when the metal(loid) reduction occurs through cell-free extracts or EPS, a higher flexibility for these two parameters can lead to an improved optimization of the NM production. For instance, a temperature of 70–100 °C mediated the fast reduction of Au^{3+} or Ag^+ generating MeNSs using cell-free extracts derived from *Nocardia farcinica* [28] and *Gordonia amarae* [29], as well as the EPS recovered from *Arthrobacter* sp. B4 [30].

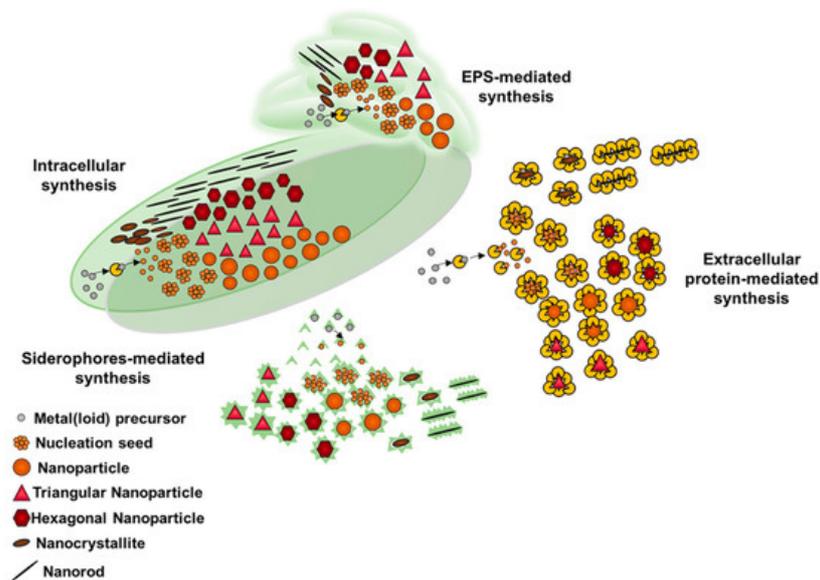


Figure 1. Schematic representation of the diverse biogenic nanomaterial morphologies obtained by actinobacterial biotransformation of metal salt precursors through intra- or extracellular (i.e., mediated by siderophores, extracellular proteins, and extracellular polymeric substances (EPS)) processes.

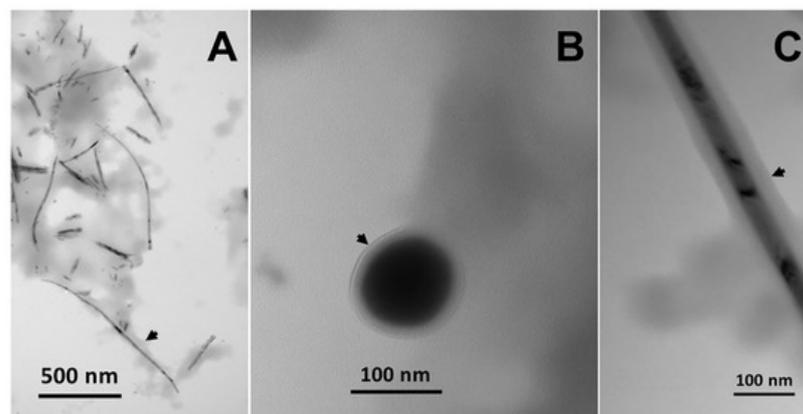


Figure 2. Biogenic nanomaterials in the form of tellurium nanorods (TeNRs) (A), selenium nanoparticles (SeNPs) (B), and selenium nanorods (SeNRs) (C) produced by the actinomycete *Rhodococcus etherivorans* BCP1 cells grown in liquid-rich medium amended with either potassium tellurite or sodium selenite as metal salt precursors. Arrowheads point toward the slight electron-dense organic coating featuring biogenically produced nanostructures.

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