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IMPACT OF METAL NANOPARTICLES ON THE PLANT GROWTH PROMOTING RHIZOBACTERIA

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Rhizosphere is a site with complex interactions between the root and associated microorganisms and high microbial diversity. Plant growth-promoting rhizobacteria (PGPR) are free-living, root colonizing, soil-borne bacteria exert phytostimulatory actions, when applied to seeds or crops by a combination of physiological attributes. Soil microorganisms play a very important role in maintaining soil health, ecosystem functions and crop productivity. So far, only a few eco-toxicological studies of nanotechnological products have been published. The present report is a review of scientific results on the potential negative or positive impact of engineered metal nanoparticles on the rhizobacteria. Here, we evaluated the effects of metal nanoparticles like as fullerens, gold, silver, aluminium and others on the PGPR. Altogether, the study suggests that metal nanoparticle could significantly produce ecotoxicity and killing of phytostimulatory soil bacteria. Thus, the engineered nanoparticle (ENPs) should be further tested as a possible ecofriendly agent.

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1. Introduction

The soil microbial world is the largest unexplored reservoir of microorganism on the Earth. In recent decades, it is an important frontier in biology under intensive investigations, since they perform numerous functions for the biosphere that include nutrient cycling and plant growth promotion. Soil being the ubiquitous habitat for almost all microorganisms, where microbes interact with their biotic components like rhizosphere and among themselves. Moreover, microbial population in rhizospheric soil is physiologically more active as compared to non-rhizospheric soil, since plant roots influence soil borne microbial communities via several mechanisms, including excretion of organic compounds, competition for nutrients, and providing a solid surface for attachment. Studies suggested population densities of bacteria in the rhizosphere depends on the physicochemical composition of the rhizospheric soil, changes in soil pH, water potential, partial pressure of oxygen and physical and chemical characteristics of plant exudation [1-2]. Rhizospheric microorganisms are considered as labile source of nutrients and play a critical role in organic matter synthesis and degradation. Furthermore, rhizobacteria exert beneficial effect on plant growth are called as plant growth-promoting rhizobacteria (PGPR) [3]. PGPR are free-living, soil-borne bacteria, isolated from the rhizosphere, when applied to seeds or crops, enhance the growth of the plant [3]. The PGPR are known to participate in many important ecosystem processes, such as the biological control of plant pathogens, nutrient cycling, and/or seedling growth [1-3]. PGPR help plant growth by a combination of physiological attributes such as asymbiotic N_2 fixation [4] phytohormones production namely indole-3-acetic acid (IAA), cytokinin, gibberellins [5] solubilizing insoluble mineral phosphate [6] and siderophore production [7]. Soil is the environmental matrix that is richest in natural nanoparticles, both as primary

particles and agglomerates/aggregates. This is due to constant physical/chemical weathering and re-arrangement of its geogenic constituents coupled with a high biological activity that transform both dead organic matter and minerals. Nano- and micron-scale particles, together with humic substances, give soils (and sediments) a high porosity and extremely high specific surface areas (tens to hundreds of square meters per gram).

2. Nanoparticles

Nanotechnology is a new, fast-developing industry, posing substantial impacts on economy, society and environment that likely will produce a huge number of new materials during the coming decades. Nanotechnology is estimated to far exceed the impact of the industrial revolution and is projected to become a $1 trillion market by 2015 and employ about 2 million workers [8] and Currently, more than 475 nanotechnology products, including tennis rackets, pants, and precision instruments, are available in the U.S. market. Thus, it generates both positive and negative responses from governments, scientists and social media throughout the world [9]. Particles in such a size (<100 nm) fall in the transitional zone between individual atoms or molecules and the corresponding bulk material, which can modify the physicochemical properties of the material (e.g., performing exceptional feats of conductivity, reactivity, and optical sensitivity). Therefore, such materials can generate adverse biological effects in living cells [10]. The term “nano(eco-)toxicology” has been developed as a separate scientific discipline with the purpose of generating data and knowledge about nanomaterials effects on humans and the environment [11]. Introduction of nanoparticles into the environment might have significant impacts as they may be extremely resistant to degradation and have the potential to accumulate in bodies of water or in soil. However, nanoparticles can act on living cells at the nano level resulting in biologically desirable effects. Recently, nanomaterials such as nanotubes, nanowires, fullerene derivatives and quantum dots have received enormous attention in the creation of new types of analytical tools for biotechnology and the life sciences [12]. The research field of PGPR and their interactions with plants is highly promising for possible applications to contribute to eco-friendly sustainable agriculture and environmental biotechnology. Furthermore, nanoparticles are introduced into the soil as a result of a number of human activities, including deliberate releases via soil and water remediation technologies, potential agricultural uses (e.g. fertilizers) and unintentional releases via air, water and sewage sludge applied to the land. However, ecotoxicological properties and the risks of these nanoparticles have not yet been fully characterized. Many nanoparticles have already been reported to have anti-microbial properties and thus directly affect microorganisms. Microbial toxicity has been reported for titanium dioxide and fullerene nanoparticles [13]. However, how nanomaterials affect living organisms remains unknown, though reactive oxygen species generation and oxidative stress are proposed to explain the toxicity of inhaled nanoparticles [14]. However, there are still a large number of pending problems related to the basic mechanisms of the underlying biological and chemical processes that occur both in the rhizosphere soil and in vivo (in plants and PGPR), which require systematic investigations at the molecular level using modern instrumental techniques. Only a very limited number of ecotoxicological studies have been performed on the effects of nanoparticles on environmentally relevant species. So far, scientific evidence show that some nanoparticles have toxic effects under laboratory conditions, but practically nothing is known about their mobility and uptake in organisms under environmental conditions. There is thus an urgent need for research on interactions between nanoparticles and environmental matrices (water, sediments and soils) and ecotoxicity studies that take into account the anticipated modifying effect of such matrices on uptake in organisms and toxicity.

(i). Fullerenes

C60 fullerens is a hydrophobic, carbon nanomaterial capable to adsorb various organic and inorganic compounds like vitamins, amino acids and minerals present in the soil [15-16]. Inhibitory effect of fullerens on the bacterial growth under pure culture conditions has been well

documented [17-18]. The effects of C60 aggregates on two common soil bacteria *Escherichia coli* (gram negative) and *Bacillus subtilis* (gram positive) was investigated by Fortner et al. (2005) on rich and minimal media, respectively, under aerobic and anaerobic conditions [18]. At concentrations above 0.4 mg/L growth was completely inhibited in both cultures exposed with and without oxygen and light. No inhibition was observed on rich media in concentration up to 2.5 mg/L, which could be due to that C60 precipitates or gets coated by proteins in the media. Furthermore, Lyon et al.(2006) explored the influence of four different preparation methods of C60 (stirred C60, THFC60, toluene-C60, and PVP-C60) on plant growth promoting (PGP) *B. subtilis* and found that all four suspensions exhibited relatively strong antibacterial activity ranging from 0.09 ± 0.01 mg/L- 0.7 ± 0.3 mg/L [19]. Tong et al. (2007) recently reported that introduction of fullerene nanoparticles in the soil had no influence on the soil bacterial diversity [20]. Nyberg et al. (2008) reported that C60 fullerene nanoparticles have no impact on anaerobic microbial communities [21]. Fullerenes have been found to inhibit the growth of commonly occurring soil and water bacteria [18, 22]. The inhibition of bacterial population might be due to antioxidant behavior of fullerens, which generate reactive oxygen species causing disruption of membrane lipids and DNA [23-24]. Alternatively, fullerenes indirectly limit the bacterial growth by adsorbing essential growth components like vitamins, trace metals, or mineral nutrients present in the soil. Which may ultimately leads to hazardous environmental effect of nanoparticles.

(ii). Gold nanoparticles

Gold nanoparticles (GNPs) have been considered for several potential biological applications going from drug delivery and imaging to therapy [25]. The chemical inertness and resistance to surface oxidation make gold an important material for use in nano-scale technologies and devices [26]. Only few studies deal with impact of GNPs on microbial cells. Moreover, nanocrystals of gold and their alloys have been synthesized within the cells of Lactic acid bacteria [27]. In other studies, bacteria, actinomycetes, archaea, and fungi have been shown to precipitate Au(I/III) complexes under a wide range of experimental conditions (28-33). For example, PGP *Bacillus subtilis* and *Pseudomonas aeruginosa* precipitate gold colloids intracellularly and extracellularly from $AuCl_4^-$ solutions [30-31]. Lengke and Southam [33] have shown active intracellular precipitation of gold particles from $Au(S_2O_3)_2^{3-}$ by *Acidothiobacillus thiooxidans*; they observed irregular, rounded gold particles and octahedral gold crystals that formed several months after bacterial growth had stopped. In field studies, structures resembling gold encrusted microfossils observed on numerous gold grains from the Americas and Australia have suggested that microbial processes contribute to the formation of secondary gold grains [34-35]. Furthermore, experimental evidences suggest gold toxicity on *R. metallidurans* has led to death of more than 90% of viable cells in an $AuCl_4^-$ (50mM) added medium, after 4 hours of inoculation and then increased to after 72 hours. Indicating that *R. metallidurans* harbors a resistance to $AuCl_4^-$ toxicity and is able to adapt to high gold concentrations. Initial gold precipitation by *R. metallidurans* was rapid and, after 8 hours of incubation, about 3mM gold had been precipitated. Subsequently, the precipitation of gold was slower, and at the end of the experiment, after 72 hours, 5.5mM gold had been precipitated. In another experiments, lysed, metabolically inactive *R. metallidurans* cells accumulated less than 50 wt % of gold relative to biologically active cells, indicating that *R. metallidurans* may be able to actively reduce $AuCl_4^-$ and accumulate metallic gold [36]. Moreover, Williams et al.,(2006) did not note significant decrease in bacterial growth of *E. coli* exposed to up to 1.1 10^{-4} g mL^{-1} PEG-coated GNPs, whereas, Huang and Zharov (2006) teams combined GNPs and near infra-red (NIR) light or laser pulses to potentially use GNPs for antimicrobial activities [37-38]. On the other hand Simon-Deckers et al (2008) has generated GNPs (30-40 nm diameters) which are not toxic to bacterial cells, and which did not accumulate in bacteria. These nanoparticles are ecotoxically safe, and will not be mobilized by bacteria, i.e. transferred in the ecosystems, leading to their dissemination [39].

(iii). Silver nanopartilces

Silver nanoparticles (SNPs); have been shown to have powerful bactericidal properties even in far lower concentration. *In situ* studies have demonstrated that silver, even in larger particle form, inhibits microbial growth below concentrations of other heavy metals. Toxicity of nanosilver has been reported in heterotrophic (ammonifying/nitrogen fixing/PGPR) and chemolithotrophic, soil formation bacteria [40]. However, the actual mechanism by which SNPs inhibit bacterial growth is still not unclear. Moreover, Soni and Bondi (2004) reported that SNPs damaged and pitted the cell wall of *E. coli* and accumulated in the cell wall, leading to increased cell permeability and ultimately cell death [41]. On the other hand reports suggest bactericidal effect of nanosilver by destroying the enzymes that transport the cell nutrient and weakening the cell membrane or cell wall, leading to increased cell permeability and cell death.[42]. However, other researchers believe nanosilver destroys the ability of the bacterial DNA to replicate. Size of nanosilver range from 1-50nm, at this size, the particles' surface area is large comparative to its volume, which enables its increased reactivity and toxicity against bacteria and various microbes. In addition, nanosilver of 1-10nm range exclusively attaching to the HIV-I virus and inhibiting it from binding to hosts cells [43]. The potential for nanosilver to adversely affect beneficial bacteria in the environment, especially in soil and water, is of particular concern. In recent years concerns have been mounting that SNPs pose an unacceptable toxicity risk to human health and the environment. Conversely, there is also a risk that use of SNPs will lead to the development of antibiotic resistance among harmful bacteria. As a powerful bactericide, SNPs threaten bacteria-dependent processes that underpin ecosystem function. Beneficial bacteria are of vital importance to soil, plant and animal health. Moreover, reports suggest silver mine- inhabiting PGP *Pseudomonas* sp. reduces silver ions to form SNPs [44].

(iv). Aluminum nanoparticle

Aluminum cation (Al_3^+) is very unfriendly to agriculture as it injures plant root cells and thus interferes with root growth and nutrient uptake in crops [45]. There are mainly two types of nanosized aluminium particles, with aluminum oxide, or carboxylate ligand coating, Alex and L-Alex, respectively has been used frequently to reveal the impact on environmental and soil microorganisms [43]. Phytotoxicity of ENPs has been demonstrated for Al as inhibition of seed germination and root growth. Effects of aluminum, alumina, nanoparticles on seed germination and root growth of six higher plant species (radish, rape, ryegrass, lettuce, corn, and cucumber) were investigated. Inhibition on root growth varied greatly among nanoparticles and plants [8]. However, No data are available on the ecotoxicity effect of Al nanoparticles on PGP bacteria.

(v). Other nanoparticles

Recently, Copper oxide nanoparticles (80 to 160 nm) were tested for antibacterial activity against PGP *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella paratyphi* and *Shigella* strains [46]. Moreover one of interesting finding suggests gram negative bacteria *serratia* mediate synthesis of copper/copper oxide nanoparticle composite. However, the process results in the killing of bacterial cells [47]. Iron and Copper based nanoparticles could be presumed to react with peroxides present in the environment generating free radicals. These radicals are known to be highly toxic to microorganisms [48]. On the other hand nanoparticles of zinc oxide (ZnO) and magnesium oxide have been shown to be effective in killing microorganisms, and are used as preservatives in food [48]. Moreover, ZnO nanoparticles are being used in personal care products, coating and paints, on account of their UV absorption and transparency to visible light. Thus, their potential harm to human health has attracted attention. Acute toxicity of ZnO nanoparticles has been observed on *E. coli* [49]. The ecotoxicity of TiO2 (APS 330 nm), SiO2 (APS 205 nm), and ZnO (APS 480 nm) ENPs to *B. subtilis* and *E.coli* bacteria in water suspensions containing citrate and low PO4 concentrations was investigated by Adams et al. (2006) [50].

3. Conclusions

However, the mechanisms underlying the nanoecotoxicity potential of ENPs are still not clear enough. Nanotechnology applications in food and agriculture are in its nascent stage. Moreover, some guidance is needed as to which precautionary measures are warranted in order to encourage the development of "green nanotechnologies" and other future innovative technologies, while at the same time minimizing the potential for adverse effects on human health and/or the environment. Thus there is urgent need for a systematic evaluation of the potential adverse effect of nanotechnology. It is therefore recommended that the ecotoxicological effect of nanomaterial be clarified before their application.

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