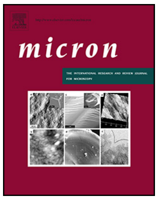
Micron 54–55 (2013) 1–27

Contents lists available at ScienceDirect

Micron

journal homepage: www.elsevier.com/locate/micron



Review

Biosynthesis and microscopic study of metallic nanoparticles

Katrin Quester[a], M. Avalos-Borja[b,1], E. Castro-Longoria[a,*]

[a] *Departamento de Microbiología, Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE), Ensenada, Mexico*
[b] *Centro de Nanociencias y Nanotecnología, Universidad Nacional Autónoma de México (UNAM), Ensenada, B.C., Mexico*

ARTICLE INFO

Article history:
Received 29 May 2013
Received in revised form 5 July 2013
Accepted 5 July 2013

Keywords:
Nanobiotechnology
Metallic nanoparticles
Biosynthesis
Bioreduction

ABSTRACT

Nanobiotechnology, bionanotechnology, and nanobiology are terms that have emerged in reference to the combination of nanotechnology and biology. Through the convergence of these disciplines, the production of metallic nanoparticles (NPs) using biological material as reducing agents is rapidly progressing. In the near future, the application of clean, non-toxic, and eco-friendly nanostructured material will be possible in industry and/or biomedicine. Currently, there is a wide range of organisms that have been reported to be useful in producing NPs. However, the development of finer protocols and the applicability of biosynthesized nanostructures are presently under study. Silver and gold are among the most studied metals due to their potential use in medical treatment. In fact, silver NPs have been evaluated as antimicrobial agents, having been successfully used against several types of fungi and bacteria. However, the use of such material in our daily life must be carefully evaluated. This article summarizes some of the most significant results using organisms to produce metallic NPs as well as the microscopic analyses used to characterize the nanostructured material obtained, providing a valuable database for future research.

Contents

1. Introduction

The use of biological material as reducing agents for the production of metallic NPs is currently the subject of various investigations. An increasing number of studies have demonstrated the utility of organisms and biomolecules to produce nanostructured material. However, for a biological process to successfully compete with chemical and physical nanostructure synthesis, strict control over average particle size in a specific size range and uniform particle morphology is required. The ongoing research is looking for more refined protocols to resolve the polydispersity and gain control over the size and shape of nanostructures; some studies have obtained very good results using biomolecules from algal solutions (Xie et al., 2007a,b).

While the control of nanostructure size and shape using biological material is being achieved, an important area of research in nanobiotechnology is to evaluate the potential applicability of

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* Corresponding author. Tel.: +52 646 175 05 00x27060; fax: +52 646 175 05 95x27052.
E-mail address: ecastro@cicese.mx (E. Castro-Longoria).

[1] On leave at IPICyT, División de Materiales Avanzados, San Luis Potosí, S.L.P., Mexico.

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http://dx.doi.org/10.1016/j.micron.2013.07.003

the eco-friendly NPs in industry and/or biomedicine. One of the first studies that proved the utility of biosynthesized NPs was carried out using *Schizosaccharomyces pombe* to synthesize cadmium sulfide NPs and using them to fabricate a diode, which operated at low voltage and had high forward current value (Kowshik et al., 2002). Important applications in fuel cell technologies can also be developed, as demonstrated in a study in which biosynthesized mesoporous zirconium phosphate using yeast as biotemplate was evaluated for the fabrication of air electrodes. The air electrode manufactured using the biosynthesized material exhibited significant electrocatalytic activity for oxygen reduction reactions, compared to that of the electrolytic manganese dioxide air electrode employed commercially (Tian et al., 2010).

The evaluation of the use of NPs in biomedical sciences has become indispensable as they might play an important role in drug delivery, diagnostics, imaging, and sensing among others. Therefore, in this field, the use of eco-friendly produced NPs could be of valuable importance. To our knowledge, the possible application of eco-friendly silver NPs as antimicrobial agents has been the most widely explored. Antimicrobial activity of silver NPs produced using supernatants of *Staphylococcus aureus* was tested against pathogenic organisms such as methicillin-resistant *S. aureus* (MRSA), methicillin-resistant *Staphylococcus epidermidis* (MRSE), *Streptococcus pyogens*, *Salmonella typhi*, *Klebsiella pneumonia*, and *Vibrio cholera*. The antibiotic activity of the bionanoparticles for MRSE was found to be maximal, followed by MRSA and *S. pyogenes* (Nanda and Saravanna, 2009). Furthermore, toxicity studies using silver NPs synthesized by cell-free filtrate of the bacteria *Escherichia coli* and the fungus *Aspergillus niger* against the gram-negative bacteria *E. coli* and *Shewanella oneidensis* as well as the gram-positive bacterium *Bacillus subtilis* showed that the biosynthesized silver NPs were found to be of higher toxicity compared to chemically synthesized silver NPs (Suresh et al., 2010).

The use of silver NPs in biomedicine is promising; however, this should be carefully evaluated. For instance, *Brevibacterium casei* was used for the synthesis of Au and Ag NPs and they showed anti-coagulative activity that was confirmed by the inhibition of blood clot formation in the test tubes containing blood along with silver and gold NPs (Kalishwaralal et al., 2010a). Besides, the possible use of eco-friendly NPs in food industry was assessed for silver NPs. Silver NPs synthesized using the fungus *Trichoderma viride* were incorporated into sodium alginate films for vegetable and fruit preservation. This film showed good antibacterial activity against test strains *E. coli* ATCC 8739 and *S. aureus* ATCC 6538 (Fayaz et al., 2009a). Recently, nano-TiO_2 was synthesized employing *B. subtilis* and was proposed as an eco-friendly method to control aquatic biofilm communities due to its photocatalytic activity (Dhandapani et al., 2012).

In addition, biosynthesized gold NPs have been tested for antimicrobial properties. Toxicity assessments of gold NPs synthesized using *S. oneidensis* were performed against the gram-negative bacteria *E. coli* and *S. oneidensis* and the gram-positive bacterium *B. subtilis*; however, they showed that the particles were neither toxic nor inhibitory to any of those microorganisms (Suresh et al., 2011). On the other hand, gold NPs synthesized by the plant *Terminalia chebula*, did show antibacterial activity towards *S. aureus* (Kumar et al., 2012a).

The control of the size and shape of biosynthesized NPs and their application are still two major challenges in bionanotechnology. This review provides a summary of existing works exploring the use of organisms for the synthesis of metallic NPs and presents some of the most significant results that can be used for further studies.

2. Production of metallic nanoparticles using biological agents

2.1. Production of nanoparticles using bacteria

Bacteria are the most abundant microorganisms on Earth; they are prokaryotic cells greatly diversified in size, shape, and means of gaining energy and live in all kinds of habitats, including extreme environments that exhibit, for example, extremely high or low temperatures, acidity, alkalinity, and salt or sulfur concentrations. The organisms that inhabit these extreme habitats, referred to as extremophiles, are so well adapted that they readily grow and multiply. Some species of bacteria have developed the ability to resort to specific defense mechanisms to quell stresses like toxicity of foreign metal ions or metals; even at high metal ion concentrations some of these organisms can survive and grow. Species like *Pseudomonas stutzeri* live in soil rich in silver (Haefeli et al., 1984) and others like *Pseudomonas aeruginosa* have developed resistance to that metal (Bridges et al., 1979). Moreover, there are well-known examples of bacteria that are able to synthesize inorganic materials like the magnetotactic bacteria, which synthesize intracellular magnetite NPs (Lovley et al., 1987; Spring and Schleifer, 1995). Consequently, a wide number of bacterial species have been used in nanobiotechnology to research alternative methods for the production of metallic NPs (Table 1). To explore bacteria's ability to reduce metal ions, most of the studies have used intact cells; however, the disadvantage of using this procedure is the need to get rid of excess biomass. Recent studies have started to use cell extracts instead, which are a suitable alternative since biosynthesis of metallic nanostructures using this method has proven to be successful as well.

Studies have shown that, while some bacteria are able to reduce metal ions and deposit them as NPs inside the cell, others can synthesize them both intra- and extracellularly. Yet few studies have determined if NPs are associated at a specific site or cell organelle; such information could be useful for optimizing biological methods. *P. strutzeri* was one of the first bacteria explored for the production of metallic NPs (Klaus et al., 1999). Micrographs of *P. strutzeri* sections revealed that silver-based crystalline NPs were located intracellularly at the cell poles. However, silver was also accumulated at other locations inside the cells; Ag-containing crystals were embedded in the organic matrix of the bacteria in the periplasmic space. No size range was reported, but the authors point out that NPs were up to 200 nm in size; nevertheless, from the published micrographs, a wide range of particle sizes can be observed. Other studies have reported that metallic NPs can be synthesized at the bacterial cell wall (De Windt et al., 2005; Lengke et al., 2006a; Zhang et al., 2007; Chen et al., 2009; Sinha and Khare, 2011) and on the cell surface (Lengke et al., 2006a; De Windt et al., 2005, 2006; Zhang et al., 2007; Lengke et al., 2007a,b; Deplanche and Macaskie, 2008; Lee et al., 2007; Law et al., 2008; Marshall et al., 2006; Gauthier et al., 2010; Bunge et al., 2010).

Independently of the bacterial species utilized as a reducing agent, the majority of the resulting synthesized NPs present polydispersity. Nevertheless, some studies report promising results with narrower particle size ranges; for example, the photosynthetic bacterium *Rhodopseudomonas palustris* successfully produced cadmium sulfide (CdS) NPs of 6–11 nm (Bai et al., 2009).

Attempting to optimize or control size and shape of synthesized NPs, studies have reported incubation assays using different ambient conditions varying temperature and pH, changing incubation time, and/or metal precursor concentration. Some studies have shown good results using protocols that work better at certain temperatures (Zhang et al., 2007; Lee et al., 2007; Lengke et al., 2007b; Ramanathan et al., 2011); others have obtained more efficiency at specific pH values showing that variation in pH results in dramatic changes in size and number of produced particles (Deplanche and