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Four psychrophilic bacteria from Antarctica extracellularly biosynthesize at low temperature highly stable silver nanoparticles with outstanding antimicrobial activity

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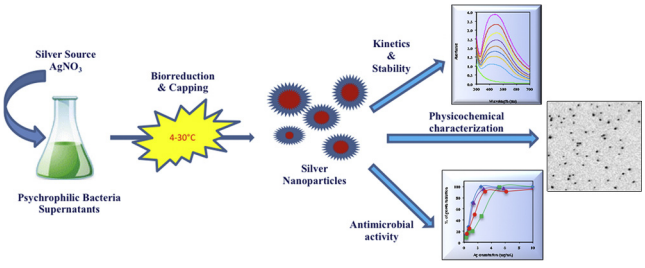
HIGHLIGHTS

- Four new psychrophilic Antarctic bacteria extracellularly biosynthesize nanosilver.
- Biosynthesis of AgNPs were successful at low (4 °C) and medium (30 °C) temperatures.
- AgNPs average sizes depend of the bacteria and temperature used and ranged 5–11 nm.
- AgNPs are excellent antimicrobials against Gram-negative and Gram-positive bacteria (very low IC50).
- AgNPs at 4 °C are stable and active antibacterials even after 10 months under light.

GRAPHICAL ABSTRACT



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ABSTRACT

Silver nanomaterials have been produced by chemical or biological methods requiring room to higher temperatures resulting in poor stable particles. Only a couple of reports use low temperature for their synthesis but, in these cases, nanoparticles aggregated in a short time. We hypothesized that psychrophilic bacteria could be found that produce stable nanoparticles at low temperature with antimicrobial activity that can be of use in biomedicine against pathogenic bacteria. Isolation of several bacteria from Antarctica was performed to obtain different psychrophilic bacteria, and their capability to extracellularly synthesize silver nanoparticles tested. Different conditions, including presence of sodium chloride and a low (4 °C) and a medium (30 °C) temperature, were used and the structure of the nanoparticles physicochemically characterized. The antimicrobial activity of freshly prepared and aged nanoparticles was determined against Gram-negative and Gram-positive bacteria. Nanoparticles were synthesized by all four psychrophilic bacteria at 4 °C and 30 °C. NaCl highly decreased the synthesis of nanosilver particles. Depending on the bacteria and temperature used, nanoparticles were spherical with sizes 5.0–11.1 nm, and stable after several months of incubation under light. The most stable nanoparticles were those kept at 4 °C and the highest detected activity was against Gram-positive bacteria.

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1. Introduction

Nano scale materials have unique size and shape-dependent properties such as surface plasmon resonance (SPR), enhanced Rayleigh scattering and surface enhanced Raman scattering (SERS), among others [1]. These amazing properties are generating a great number of applications in different fields such as cosmetics, electronics, catalysis, chemical and biological sensing, tissue and tumor imaging, environmental remediation, agriculture, medicine, etc. [2,3]. In special, silver nanoparticles (AgNPs)[2] have been shown to have properties useful in different applications and as antimicrobial agents. The synthesis of metal nanoparticles has been achieved mainly by non eco-friendly methods including expensive high-energy physical procedures and chemical methods involving the use of reducing agents and different stabilizing, and sometimes non-biodegradable, substances. A widening line of research in this field is the development of greener and cheaper methods for nanomaterials production [4]. Following this idea a number of methods had been reported in which nanomaterials have been obtained using different biocompatible organics [5]. However, most of the green methods propose the use of biological extracts or organisms, including plants, algae, fungi, bacteria and even viruses [5–8]. These methods include the intracellular or extracellular production of nanoparticles of different elements, mainly metals, oxides or sulphides and noble metal alloys by a variety of microorganisms [7] and plants extracts [6]. Also mixed methods using microorganisms and other added biocompatible materials have been reported [9]. An invertebrate's extract has also been used recently for silver and gold nanoparticles synthesis [10]. The conditions used for nanoparticles biosynthesis include the use of temperatures from 4 °C to room temperature and up to 200 °C, and in some cases the addition of accelerators such as NaOH, ammonia or triethanolamine. Several procedures used room temperature, but, as far as we know, low temperatures have only been used in a couple of reports using psychrophilic microorganisms [11,12]. In one of these [11] it was demonstrated that the phase of growth of the bacteria can affect to the size and shape, and consequently the biological activities, of the nanoparticles produced. In the other [12], nanoparticles were produced in a fast way, but they were not much stable, mainly when kept under light. The analysis of the published biosynthesis of silver nanomaterials indicates that their size range is wide, from 1 to 400 nm, with very few cases of monodisperse size [13]. The use of plant extracts has been considered as more suitable than that of microorganisms, mainly due to the higher costs of microorganism's culture. However, microorganisms grow usually fast and it is easy to obtain high amount of biomass or cell-free broths, which can be produced using different nutrients and temperature conditions. Most bacteria able to produce nanoparticles synthesizes them extracellularly, which facilitates recovery, others have the possibility of synthesizing them intracellularly, and a few can do it intra and extracellularly [14]. Moreover, there is a great experience in growing microorganisms for industrial uses. In addition, microorganisms can be easily genetically manipulated to optimize their use and they are phylogenetically and metabolically so diverse that the probability to find those producing nanoparticles and growing on cheap substrates and at non-costly, moderate or low, temperatures is high. However the organisms most reported as used for AgNPs production are plants and only a few synthesis using new species of bacteria have been reported lately. For these reasons more microorganisms from different environments should be tested for nanoparticles production, in particular those growing at low temperatures. Exploring the capabilities of these microorganisms from different origins would help to find the better candidates for AgNPs production by cheap and eco-friendly methods, with the possibility of making nanoparticles with different sizes and coating biomolecules that may lead to the discovery of nanomaterials with new properties and applications or higher efficiencies and less toxicity. In this article we explore the capabilities of strains of four bacterial species isolated from the ice-melting waters of Antarctica to produce AgNPs at low (4 °C) and medium (30 °C) temperatures, resulting in the production of AgNPs actives against Gram-positive and Gram-negative bacteria. This open up the possibility of synthesizing bioactive nanoparticles in a wide range of temperatures from low to moderate ones, with low energy costs. The synthesis of AgNPs with bacterial broths obtained at different temperatures could lead to the identification and use of different compounds acting as reductants and stabilizers since the metabolism of the bacteria has to adapt to the temperature probably producing different metabolites or at different concentrations. We describe here the synthesis, physicochemical characterization and antimicrobial properties of these nanoparticles, in particular of those synthesized at 4 °C, suggesting also ideas about their mechanism of action and the possible effect of the corona.

[2] AgNPs stands for silver nanoparticles.

2. Materials and methods

2.1. Bacterial isolates

Four bacteria were chosen from a collection of bacterial isolates obtained from ice-melting waters collected at the St George Island in Antarctica. The bacteria were isolated at 4 °C using nutritive medium (meat extract (Merck) 3 g/L, bacto-peptone (Difco) 10 g/L, NaCl (Merck) 5 g/L). Bacteria were identified based on their 16S rDNA sequences. For this, a colony of each isolate was suspended in lysis Buffer (SDS (Merck) 0.005%, Proteinase K (Roche) 400 ng/μL) and treated as previously described [15]. This DNA containing solution was used as the template for the amplification by the polymerase chain reaction (PCR) of most of the 16S rDNA sequence using the 27F and 1492R bacteria universal primers [16]. The amplicons were purified by the polyethylenglycol-NaCl method [17] and sequenced by Macrogen (http://www.macrogen.com).

Sequences obtained were edited using Finch TV (http://www.geospiza.com/Products/finchtv.shtml), and used for the phylogenetic adscription of the isolates by comparison with sequences in GenBank using the BLAST tool at the NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.2. Biosynthesis of AgNPs

Psychrophilic bacteria were grown in whole or NaCl-deprived nutritive medium at 4 °C or 30 °C until the cultures achieved stationary phase. For testing the capability of broths to synthesize AgNPs, cells were eliminated by centrifugation at 17.530 × *g* at 4 °C and supernatant filtered through 0.22 μm pore size filters (Millipore). Supernatants were kept at 4 °C until used in the next few days.

For AgNPs biosynthesis, equal volumes of supernatant and 2 mM $AgNO_3$ (Merck) solution were mixed and kept at the required temperature and irradiated under fluorescent household lamps or in the dark. For kinetics studies, aliquots were taken at different times and the UV–vis spectrum recorded. Controls of 1 mM $AgNO_3$, and medium supplemented with $AgNO_3$ were set and treated in the same light and temperature conditions.