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Inhibition of microorganisms involved in deterioration of an archaeological site by silver nanoparticles produced by a green synthesis method

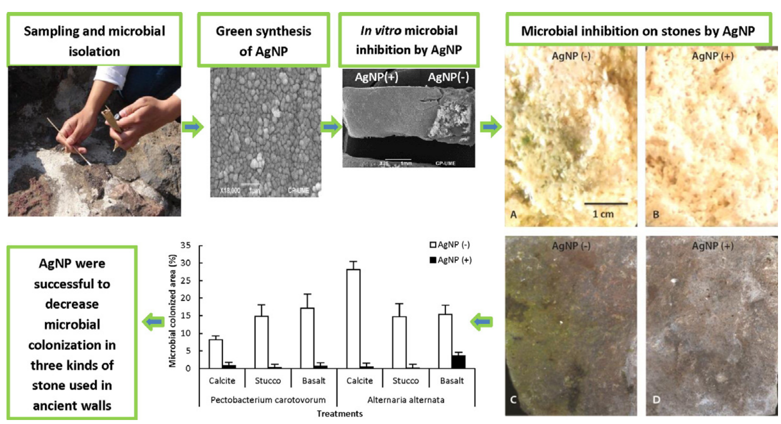
Rogelio Carrillo-González [a], Miriam Araceli Martínez-Gómez [a], Ma. del Carmen A. González-Chávez [a,*], José Carlos Mendoza Hernández [b]

[a] Colegio de Postgraduados, Campus Montecillo, Texcoco, México 56230, Mexico
[b] Benemérita Universidad Autónoma de Puebla, 4 sur 104, 72000 Puebla, Mexico

HIGHLIGHTS

- AgNP inhibited *in vitro* different microorganisms involved in stone deterioration.
- AgNP properties and microbe sensitivity influenced NP-biocide activity.
- AgNP synthesized from *F. vulgare* were more effective to control biodeterioration.
- AgNP was effective in decreasing microbial colonization on three kinds of stone.

GRAPHICAL ABSTRACT



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ABSTRACT

The Citadel, part of the pre-Hispanic city of Teotihuacan and listed as a World Heritage Site, harbors irreplaceable archaeological walls and murals. This city was abandoned by the 7th century and its potential deterioration represents a noteworthy loss of the world's cultural heritage. This research consisted of isolation and identification of bacteria and fungi contributing to this deterioration from walls of a pre-Hispanic city. In addition, silver nanoparticles (AgNP) produced, using a green synthesis method, were tested as potential inhibitors of microbes. AgNP of different sizes and concentrations were tested using *in situ* assays. Leaf aqueous extracts from two plants species (*Foeniculum vulgare* and *Tecoma stans*) and two extraction procedures were used in the NP synthesis. The potential of AgNP as preventive/corrective treatments to protect stucco materials from biodeterioration, as well as the microbial inhibition on three stone materials (stucco, basalt and calcite) was analyzed. Twenty-three bacterial species belonging to eight genera and fourteen fungal species belonging to seven genera were isolated from colored stains, patinas and biofilms produced on the surfaces of archaeological walls from the pre-Hispanic city, Teotihuacan. AgNP from *F. vulgare* were more effective for *in vitro* microbial growth inhibition than those from *T. stans*. Bacteria were less sensitive to AgNP than fungi; however, sensitivity mainly depended on the microbial strain and the plant extract used to prepare AgNP. The use of AgNP as a preventive or corrective treatment to

* Corresponding author.
E-mail address: carmeng@colpos.mx (M.C.A. González-Chávez).

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decrease microbial colonization in three kinds of stone used in historical walls was successful. Calcite was more colonized by *Alternaria alternata*, but less by *Pectobacterium carotovorum*. This is the first study at different scales (*in vitro* and tests on different stone types) of inhibition of biodeterioration-causing microorganisms isolated from an archaeological site by green synthesized AgNP.

1. Introduction

Biodeterioration, a worldwide problem caused by diverse microorganisms, is commonly observed in many areas designated as world cultural heritage sites. Deterioration of cultural heritage built with stone materials, such as exterior archaeological monuments, is a complex process caused by physical, chemical and biological agents. Particularly, microbial growth on stones of these monuments causes diverse damage (aesthetic, physical and chemical changes), which can become irreversible and result in destruction and permanent loss of stone monuments (Farooq et al., 2015).

Several alternatives, preventive and remedial measures, have been tested to control microorganisms causing biodeterioration of monuments with high archaeological or cultural heritage value (Ditaranto et al., 2011). For example, synthetic chemicals, waterproofing and damp-proofing agents, aqueous leaf extracts from plants with antimicrobial activity, inhibitors of microbial activity, and potent ionic antimicrobial agents based on silver, copper, zinc, etc. (Ascaso et al., 2002; Gomez-Villaba et al., 2011; Gupta et al., 2014). However, the use of synthetic chemicals on the monuments as biocides does not represent a viable alternative because it is not always successful and may cause unintended side-effects. Failed action has been reported to be due to the ability of microbial biofilms to prevent chemical penetration, which results in reduced effectiveness (Peraza-Zurita, 2004). These chemicals can also produce physical alterations or esthetical damage (color, structure, permeability) or chemical reactions (for example, solubilization of minerals, pH change) to the historical materials (Peraza-Zurita, 2004; Fonseca et al., 2010, Gomez-Villaba et al., 2011). In addition, these compounds are toxic and hazardous to the environment and to public health (Gupta et al., 2014). In general, most of the synthetic chemicals are products used in agriculture, these are toxic substances to control plagues and at the same time also affect unintended organisms, such as animals or human beings. Moreover, some of these are reported as carcinogenic, teratogenic or inducers of chronical health problems when accumulation occurs in several organisms (EPA, 2013). Some microbial species get resistance to these chemicals and remain growing on the walls.

Silver is widely recognized as a strong antimicrobial since ancient eras. Several properties such as thermal and chemical stability, health and environmental safety, and low toxicity to human cells are the basis for its preferential use (Bellissima et al., 2014). Silver has been successfully tested for biomedical applications, water and air purification, food production, cosmetics formulations, clothing, and bactericidal coatings on surfaces of diverse materials (Marambio-Jones and Hoek, 2010).

A greater effort has been undertaken to develop safer, environmental friendly, less costly and highly effective management methods that pose less risk to these monuments and, consequently, to humans (usually visitors) and the environment. Emerging alternatives, such as nanotechnology, more dedicated to overcoming deficiencies observed in conventional management, are being suggested. It is now recognized that nanoparticles (NP) with dimensions less than 100 nm possess distinctive physico-chemical properties, which may be useful in solving several world issues related to medicine, agriculture and environment.

Silver nanoparticles (AgNP) have different physicochemical characteristics than the bulk matter (Trans et al., 2013), which make them more reactive and more highly effective against a broad spectrum of microorganisms (Choi et al., 2008). The most relevant feature is that AgNP may prevent microbial resistance and it has been demonstrated AgNP are an effective biocide against drug-resistant strains. In addition, green synthesis methods are an environmental friendly chemistry approach that associates nanotechnology with plant biotechnology. Production of nanomaterials based on biological or natural components (green synthesis) is more acceptable as it offers diverse benefits over chemical methods (Narayanan and Sakthivel, 2010) and has low environmental impact.

Plant extracts can be used for an eco-friendly synthesis of NP, since these extracts contain proteins and other chemicals which act as reducing and capping agents forming stable AgNP (Hebbalalu et al., 2013). In addition, to reduce the side effects of the toxic chemicals remaining after silver reduction and compounds formed during reaction on the target organism, it is wished to have the lowest amount of chemicals used for synthesis of NP. These can be designed to decay to innocuous products using green synthesis.

AgNP, also termed as a new-generation antimicrobials (Feng et al., 2000), are used in consumer products such as cosmetics, textiles, dietary supplements and food packaging. They are used in medicine against multidrug susceptible bacterial strains and for surgical coatings and medical implants (Thiel et al., 2007; Lok et al., 2006; Gunawan et al., 2011, Choi et al., 2008). Other uses are related to water disinfection applications (Chandrakanth et al., 2014), control of agricultural fungal pathogens (Lamsal et al., 2011; Kim et al., 2012), seed borne pathogens (Kaur et al., 2012), among some possible uses (Trans et al., 2013).

Several studies on biodeterioration control have been conducted to analyze the inhibitory activity of AgNP on specific microorganisms, which may or may not be involved in the biodeterioration process. Tested microorganisms also come from clinically isolated multidrug resistant bacteria (Chandrakanth et al., 2014), or they were selected as model microorganisms, such as *Escherichia coli* (Maiti et al., 2014), *Staphylococcus aureus* (Agnihotri et al., 2014), and *Bacillus subtilis* (Bellissima et al., 2014). Mohammadi and Maghboli-Balasjin (2014) and Fonseca et al. (2010) argued that recognizing and characterizing biodeteriorants is necessary before any restoration and conservation treatments can be established. In consequence, little information available on the role of AgNP in microbial inhibition takes into account natural microorganisms that cause or participate directly in deterioration of historical monuments, archaeological sites or rock materials.

This research analyzed the use of green synthesized AgNP as an alternative for control of microorganisms that are thought to cause biodeterioration. The present study highlights the influence of these AgNP, synthesized from *Tecoma stans* L. and *Foeniculum vulgare* Mill extracts, on the *in vitro* inhibition of bacterial and fungal growth isolated from biofilms produced on stone walls of the archaeological complex, the Citadel. This research also tested the microbial inhibition on common rock materials from historic buildings (stucco, basaltic and calcite stones) by AgNP.

The objectives of this research were: 1) To isolate and identify microorganisms that colonized the stone surface of an archaeological monument at Teotihuacán, Mexico; 2) To evaluate the antimicrobial activity of silver nanoparticles (AgNP) synthesized from *F. vulgare* and *T. stans* leaf aqueous extracts; 3) To analyze the effect of sizes and doses of AgNP on *in vitro* biocontrol of microorganisms isolated from biofilm formation on stones of walls of a historic monument; 4) To test the preventive/corrective application of AgNP to control microbial establishment; and 5) To study microbial inhibition in three types of stones from historic monuments by green-synthesized AgNP.

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