

Original Research Article

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## Potential Application of *Bacillus* sp. SDNS Gold Nanoparticles

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### ABSTRACT

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Metallic nanoparticles have fascinated scientist for over a century and are now heavily utilized in biomedical sciences and engineering. They are a focus of interest because of their huge potential in nanotechnology. In this research, gold nanoparticles manufactured extracellularly by *Bacillus* sp. SDNS were evaluated for application as antibacterial agent and in glass staining. AuNPs(1-6 nm) showed antagonistic activity towards *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis* with highest activity against *Pseudomonas aeruginosa* recording MIC of 3.125 µg/ml. Glass pieces stained with AuNPs showed different colors hues.

### Introduction

Gold nanoparticles (AuNPs) gained attention due to their application in different fields of science (Ngo, *et al.*, 2012). The featured optical property of AuNPs makes them totally helpful in the biomedicine. The usage of metallic nanoparticles is a promised approach to disband the dilemma of antibiotic resistance (Hema *et al.*, 2012). Owing to their tiny size and high surface-to-volume ratio (S/V), nanoparticles have a large contact area with microorganisms and thus promote its antimicrobial efficiency. Metallic nanoparticles are also masterful in targeting various bacterial structures (Priyadarshini *et al.*, 2014).

AuNPs have been reported as non-toxic parallel to other metallic nanoparticles such as silver and platinum nanoparticles (Lee *et al.*, 2010; Asharani *et al.*, 2011).

Thirumurugan *et al.* (2012) produced AuNPs from *Bacillus subtilis* and were estimated for their antifungal and antibacterial efficacy. The potential activity of gold nanoparticles versus microbial pathogens depends fundamentally on the shape and size of the particles (Kagithoju *et al.*, 2012). It neatly connects to surface of the microorganisms causing apparent deterioration to the cells (Grace and Pandian, 2007).

AuNPs create holes in the cell wall, resulting in the seepage of cell contents leading to death, in another route it can bind to the bacterial DNA and inhibits its transcription (Rai *et al.*, 2010). Millenbaugh *et al.*, (2015) utilized gold nanospheres in targeting and killing of *Staphylococcus aureus*. Lima *et al.*, (2013) declared that AuNPs eliminate 90-95% of *Escherichia coli* and *Salmonella typhi* colonies. Prema and Thangapandiyam, (2013) stated that AuNPs could act as an efficient antibacterial agent and established as a substitution for the growth of novel antibacterial drugs to conflict resistance problem. The antibacterial activities of drugs-capped AuNPs against strains of Gram-negative and Gram-positive microorganisms were investigated (Sadowski and Maliszewska, 2011).

A well-known application of early nanotechnology is the ruby red color that was utilized for stained glass windows during the middle Ages (Horikoshi and Serpone, 2013). Au nanoparticles were successfully deposited on silica spheres by the electroless metal plating technique (Kobayashi *et al.*, 2005). Immobilizing the nanoparticles on supports such as powders and plates is a candidate to prevent the aggregation. An electroless metal plating technique can make metallic films plated on insulating support materials such as glass plates (Kobayashi and Ishii, 2013a,b).

It was aimed in this work to evaluate the potentiality of gold nanoparticles manufactured by a marine bacterium as an antibacterial agent and also its possible use in glass staining.

## **Materials and Methods**

### **Gold Nanoparticles (AuNPs)**

The gold nanoparticles used in this study

were biosynthesized extracellularly from the marine *Bacillus* sp. SDNS (Abouelkheir, 2015). Their size ranged between 1-6 nm.

### **Bacterial Indicator Strains**

The antibacterial activity of fabricated gold nanoparticles was examined against four indicator strains; the Gram negative *Pseudomonas aeruginosa*; ATCC ®: 15442, and *Escherichia coli*; 8739 and the Gram positive *Staphylococcus aureus*; 25923, and *Enterococcus faecalis*; 29212 obtained from the Public Health England (PHE) Culture Collections, Selectrol.

### **Preparation of AuNPs**

*Bacillus* sp. SDNS was cultured in nutrient broth in shaker incubator at pH 7 and 30 °C for 24–48 h and cells were separated by centrifugation at 5000 rpm for 20 min. Ten ml of bacterial supernatant were added to 20 mL of  $1 \times 10^{-3}$  M aqueous HAuCl<sub>4</sub> solution in a 250 ml Erlenmeyer flask. The mixture was left in a shaker incubator at 30°C for a further 24–72 h. The formed AuNPs were obtained by centrifugation, washed and suspended in distilled water. The gold nanoparticles were characterized and size was determined using FTIR, XRD, TEM and EDX (Abouelkheir, 2015).

### **Antibacterial Activity of Gold Nanoparticles (AuNPs)**

#### **Agar Diffusion Well–Method**

Bacterial indicator strains were cultured in nutrient broth till mid exponential phase. Inocula were uniformly spread on nutrient agar plates and wells (1cm diameter) were cut in the agar and filled with different concentrations (50, 100, 200 µL) of previously prepared AuNPs. Plates were incubated for 24 h at 37°C under aerobic conditions. Zone of inhibition was measured

in mm. Tests were performed in triplicate (Valgas *et al.*, 2007).

### **Minimum Inhibitory Concentration (MIC) Determination**

The microdilution method (Valgas *et al.*, 2007) was utilized. In each well, 990  $\mu$ l of nutrient broth were inoculated with 10  $\mu$ l of exponentially growing cells. From a stock solution (0.4 mg/ml), AuNPs were added to reach concentrations ranging from 0.2 to 0.39  $10^{-3}$  mg/ml, one well without inoculation was kept as control. Incubation was done for 24 h at 37 °C. MIC ( $\mu$ g/ml) was defined as the lowest concentration of AuNPs, which completely inhibited bacterial growth. All experiments were accomplished in triplicate.

### **Glass Staining with Gold Nanoparticles (AuNPs)**

#### **Synthesis of Gold Nanoparticle/ polyvinyl Alcohol (PVA) Solution**

PVA solid was slowly added to the warm gold nanoparticles solutions to make 4.5% (w/v) solution. The solutions were heated gently to dissolve the PVA and decanted into silicone bake molds (Duncan *et al.*, 2010).

#### **Preparation of the Nanostained Glass Pieces**

About 5 mL of AuNPs/PVA were poured into silicone bake molds, enough to cover the bottom of the mold.

Water was then evaporated by leaving to dry overnight or the solutions were heated in an oven at 200°C. Nanostained “glass” disks with different color hues (red to purple) were then removed from molds (Duncan *et al.*, 2010).

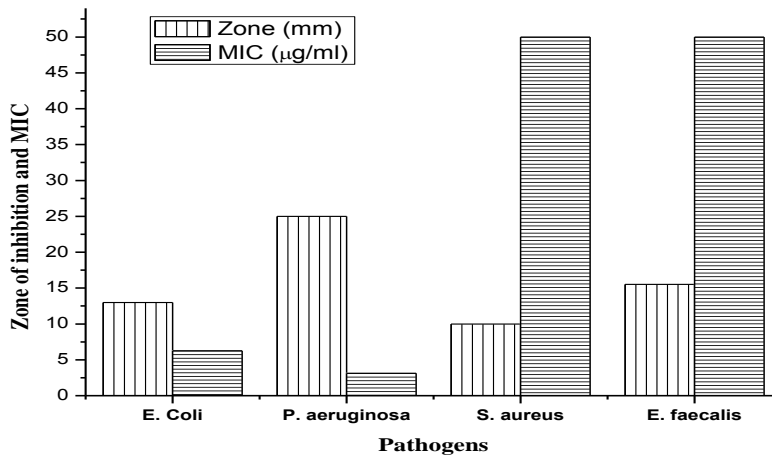
## **Results and Discussion**

Nanoparticles of gold and silver display a special optical feature as surface plasmon resonance (SPR) absorption, which mainly depends on particle shape, size and surface condition. Therefore, to take whole advantage of surface, a decrease in their particle size is important because total surface area of metal increases with the decrease in size (Kobayashi & Ishii, 2013a).

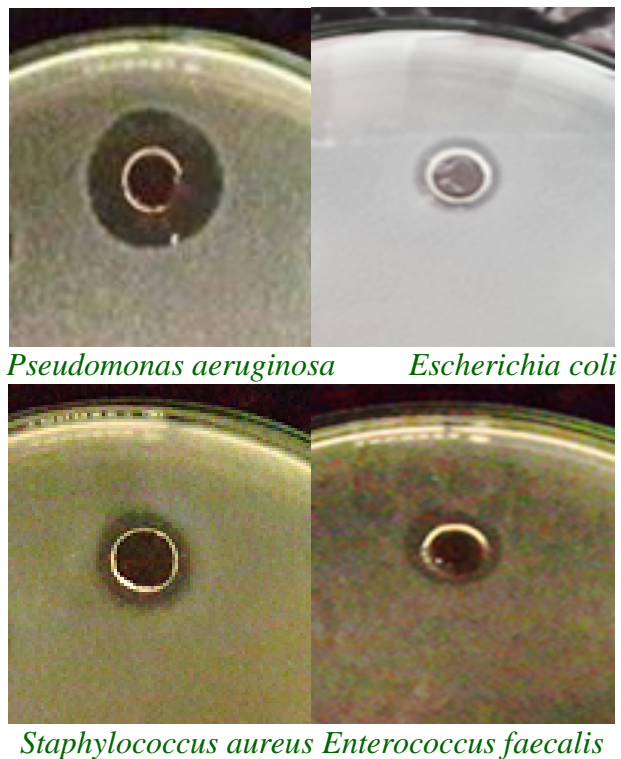
### **Antibacterial Activity**

The nanoscale allows the materials with the ability to permeate into different biological membranes, such as bacterial cell walls, elevating bactericidal effects (Nuñez-Anita *et al.*, 2014). Antimicrobials comprise not just antibiotics, but synthetically formed compounds (Patel *et al.*, 2012; Dixit *et al.*, 2015). AuNPs synthesized from *Bacillus* sp. SDNS exhibited antagonistic effect against all indicator strains tested with variable degrees depending on the bacterial species (Figs.1, 2). MICs of 3.125 and 6.25 were recorded for *Pseudomonas aeruginosa*, and *Escherichia coli*, respectively, whereas 50 $\mu$ g/ml was recorded for *Enterococcus faecalis* and *Staphylococcus aureus* (Fig.1). Grace & Pandian (2007) investigated the antibacterial efficiency of antibiotics-coated gold nanoparticles against diverse strains of Gram-positive and Gram-negative bacteria like *Staphylococcus aureus*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. However, in the present case the reason beyond the selective antibacterial activity might be imputed to the differences in the cell wall structure between gram positive and negative bacteria. Since Gram negative bacteria are less susceptible to antibiotics, it is thus possible that the gold nanoparticles synthesized by the marine bacteria may provide an effective means towards inhibiting the disease caused by the gram negative bacterial strains.

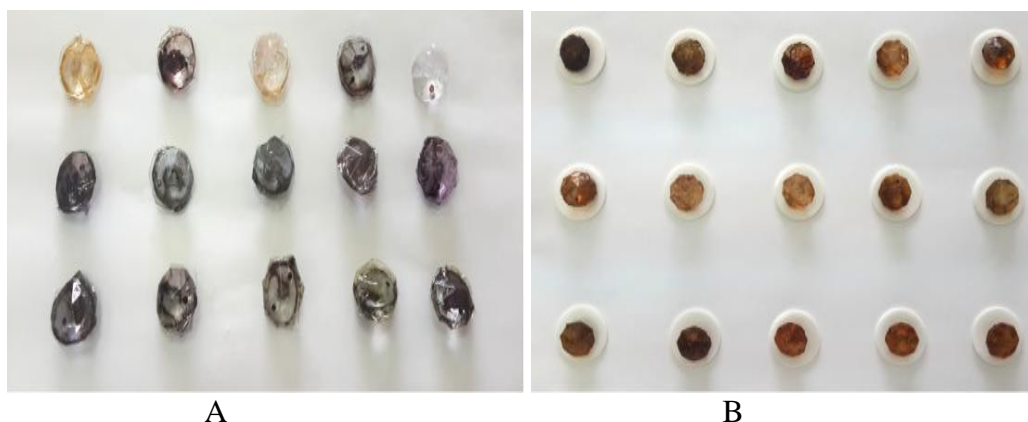
**Fig.1** Zone of Inhibition (mm) Produced by AuNPs Tested against Indicator Strains after 24 h Incubation at 37°C



**Fig.2** Effect of *Bacillus* sp. SDNS AuNPs on Bacterial Indicator Strains



**Fig.3** Au-glass Crystals, AuNPs were Deposited as Plastic Disks using Air (a), Au-glass Crystals using Oven-drying Process at 200°C for 2 h (b)



### Glass Staining

Glass is made from melted sand ( $\text{SiO}_2$ ) and need a high temperature, about  $1371.1^\circ\text{C}$  to melt so, artisans adding additional ingredients to help sand to melt at a much lower temperature. A mixture of sand, potash, soda ash, lime and lead oxide melt the sand to temperatures around  $815.6^\circ\text{C}$ . Once this mixture molten, coloring agents, or colorants, are added. Artisans observed that different compounds gave rise to different colors. For example, ruby glass was created by adding gold chloride, while green or brown glass was created by adding iron oxides (Zenner, 2008).

During the experimental work, a wide variation of colors was noticed for AuNPs colloidal solutions produced according to the different reaction conditions. Based on this observation different stained glass pieces were made using the colorant agent AuNPs. AuNPs/PVA metallic films plated on glass crystals were synthesized by air drying or heating in oven at about  $200^\circ\text{C}$  for 2 h (Fig.3). Similarly, Au nanoparticles were successfully deposited on silica spheres by the electroless metal plating technique (Kobayashi *et al.*, 2005) and on glass plates (Au-glass) (Kobayashi and Ishii, 2013b).

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