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Green synthesis of gold and silver nanoparticles by an actinomycete *Gordonia amicalis* HS-11: Mechanistic aspects and biological application

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ABSTRACT

A bacterial isolate obtained from an oil-contaminated soil sample was identified as *Gordonia amicalis* on the basis of morphological features, biochemical traits and 16S rRNA gene sequence. The cell free supernatant (CFS) of this bacterium (cultivated on *n*-hexadecane) when incubated with 1 mM chloroauric acid ($HAuCl_4$) or silver nitrate ($AgNO_3$) at pH 9.0 in a boiling water bath mediated the synthesis of gold and silver nanoparticles, respectively. It was hypothesized that the CFS contained some thermostable biomolecule that mediated metal reduction reactions. Extraction of CFS with chloroform:methanol (2:1), subsequent column chromatography and thin layer chromatography led to the activity-guided purification of a glycolipid. The glycolipid was hydrolysed and the glycone (glucose) and aglycone components (three saturated fatty acids (myristic, palmitic and stearic) were identified by gas chromatography mass spectrometry. The reduction of glucose to gluconic acid under alkaline conditions provided the reducing equivalents needed for nanoparticle synthesis. The purified glycolipid and CFS mediated nanoparticles were characterized by using a UV–vis spectrophotometer, X-ray diffractometer and Transmission Electron Microscope. The nanoparticles displayed free radical scavenging activities. This paper thus highlights nanoparticle synthesis by a hitherto unreported actinomycete culture, identifies the biomolecule involved in the process and describes the associated antioxidant activity.

1. Introduction

Conventional chemical and physical methods used for the synthesis of metal nanoparticles are generally expensive. Moreover, wastes or by-products generated during these processes affect the environment adversely [1]. On account of the aforementioned issues, research in the field of green synthesis and the development of eco-friendly protocols has become relevant [2]. Although different types of biological forms are being used for this purpose, microbial systems due to their diversity are emerging as promising options [3]. Among microorganisms, a variety of bacterial cells have been exploited for developing nanobiosynthetic procedures [4]. In general, bacteria offer advantages with respect to the ease with which they can be handled and genetically manipulated [5]. Moreover, diverse types of nanostructures (cell-associated or extracellular) can be obtained by using bacterial forms. Their well-defined morphological features have been exploited for generating nanoparticles with specific shapes (metal microspheres and nanotubes) that can be used in drug delivery, catalysis, optoelectronics and biomedical imaging [6]. The utility of metal nanostructures derived from bacterial systems is thus well-documented.

Literature survey on this topic revealed that a variety of bacteria (*Pseudomonas aeruginosa, Morganella* sp., *Bacillus subtilis, Bacillus licheniformis, Rhodopseudomonas capsulate, Brevibacterium casei* and *Lactobacillus* sp.) mediate extracellular or intracellular synthesis of metal nanoparticles [2,7–12]. Moreover, this survey also highlighted the fact that there are very few reports describing the role of specific biomolecules mediating such reduction reactions. In most of the cases, the probable role of certain functional groups

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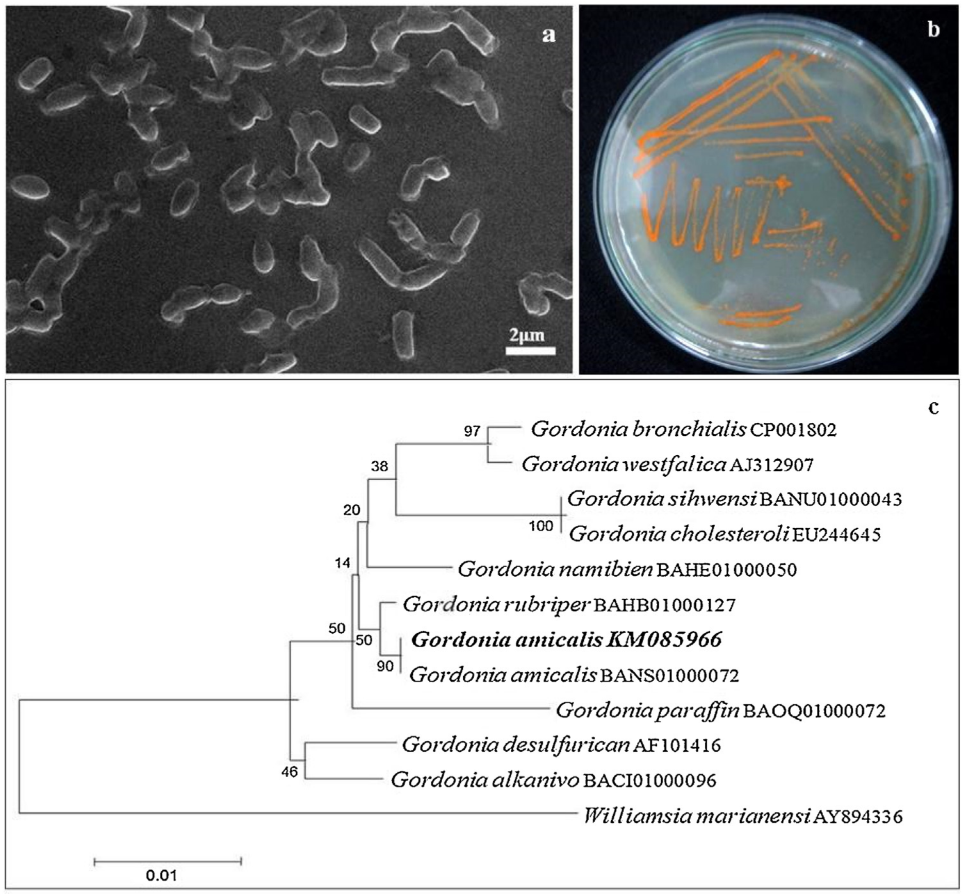

Fig. 1. Morphological characterization and taxonomic features of the tropical soil isolate identified as *Gordonia amicalis* (a) Scanning electron image showing the presence of rods (b) growth of the bacterium on LB agar showing orange colored pigment (c) Phylogenic tree of *G amicalis* HS-11 (GenBank accession no. KM085966). Phylogenetic analysis was carried out using the Blastn, multiple sequence analysis by using CLUSTAL X software (version 2.0.9), Unrooted phylogenetic tree by using MEGA 5 software. Bootstrap analysis was carried out to check the reliability of tree topology. The numbers at the nodes indicate the bootstrap supportive values for percent of 1000 replicates.

(based on FTIR data) in nanoparticle synthesis has been suggested [13,14]. Only on some occasions, certain enzymes such as sulphite reductases, nitrate reductases and lactate dehydrogenases are reported to mediate nanoparticle synthesis [15–17]. On the basis of this background, the current work on nanoparticle synthesis by a hitherto unreported actinomycete was undertaken. We describe here (i) the isolation of a tropical soil bacterium (ii) the identification of this bacterium as *Gordonia amicalis* (iii) the synthesis of extracellular gold and silver nanoparticles by this actinomycete (iv) the characterization of the glycolipid (v) propose the mechanism involved in the synthetic process (vi) and describe the antioxidant activity of these nanoparticles.

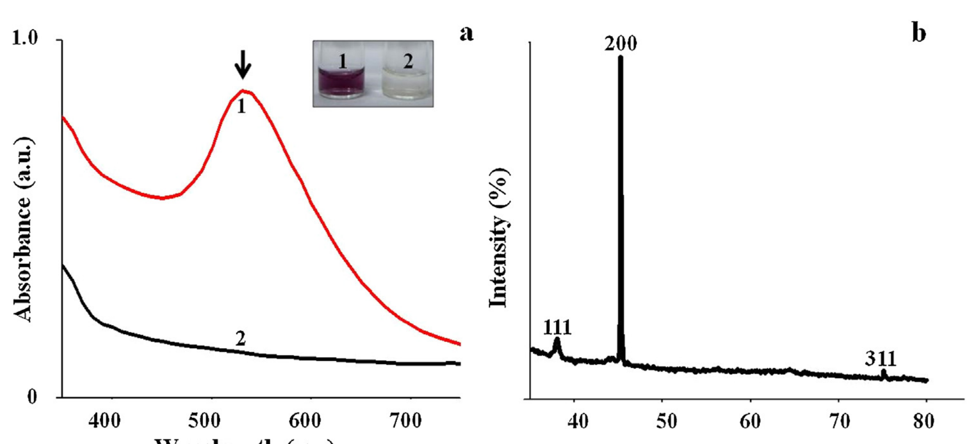

Fig. 2. Characterization of gold nanoparticles mediated by the CFS of *G. amicalis* (a) UV–vis spectra of reaction mixtures containing equal proportions of CFS and 1 mM $HAuCl_4$ incubated in a boiling water bath for 10 min at pH 9.0 (line 1); control without 1 mM $HAuCl_4$ (line 2). Inset: visual observations of a test reaction (tube 1) and control reaction (tube 2). (b) Representative XRD profile of AuNPs.