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Research Paper

Biosynthesis of gold and selenium nanoparticles by purified protein from *Acinetobacter* sp. SW 30

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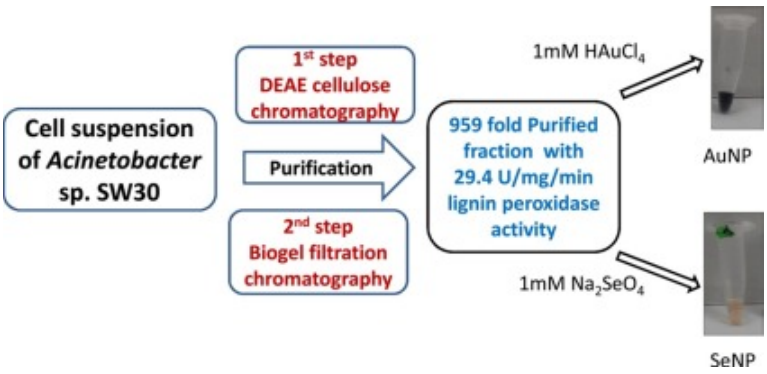
Abstract

Synthesis of nanoparticles is an enzymatic reduction process in microorganisms. In the present study, a protein, lignin peroxidase has been purified by DEAE-Cellulose anion exchange chromatography and Biogel P-150 gel filtration chromatography from the cell suspension of *Acinetobacter* sp. SW30 responsible for the synthesis of gold nanoparticles (AuNP) and selenium nanoparticles (SeNP). The purified fraction has a specific activity of 29.4U/mg/min with 959 fold purification. Native and SDS PAGE confirmed that purified lignin peroxidase is monomeric enzyme with 97.4KDa molecular weight. The enzyme synthesized spherical crystalline AuNP (10±2nm) and amorphous SeNP (100±10nm). It has maximum activity at pH 2 and temperature 40°C, with 1.0mMKm value, when *n*-propanol was used as a substrate. Activity was completely inhibited by sodium thiosulphate and zinc sulphate. This is the first report on association of lignin peroxidase in the synthesis of AuNP and SeNP from *Acinetobacter* sp. SW30.

Graphical abstract



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Introduction

Microorganisms, such as bacteria, fungi and algae, and their products play an important role in biosynthesis of nanoparticles. Enzymes, extracellular polysaccharides, DNA, rhamnolipids etc. are involved in biosynthesis of nanoparticles, [1], [2], [3], [4], which lead to the formation of polydispersed nanoparticles owing to the presence of multiple organic components in cell suspension or cell free extract. Proteins may cover the surface of such bio-nanoparticles in its native form and may confer stability [2], [5].

Microoragnisms produce metal nanoparticles either as the by-product of respiration process or as a survival mechanism against metal toxicity. It is a reduction process followed by capping with the proteins. It has been observed that nanoparticles synthesized by purified protein are monodispersed and smaller in size. Hence, purification and identification of proteins involved in the synthesis of metal nanoparticles are essential to predict the possible mechanisms of synthesis. Moreover, additional procedures for extraction of nanoparticles from microbial cells are not required which reduces recovery and purification cost [2].

Enzymes such as laccase, peroxidases, proteases, reductases and fibrinolytic enzyme (URAK) are known for the reduction of salts into insoluble elemental particles in microorganisms [2], [3], [6], [7]. ABC transporter from thermophilic bacterium *Thermus scotoductus* SA-01 is found to reduce Au (III) through an electron shuttle mechanism involving a cysteine disulphide bridge [8]. In viable cells, Au reduction is a three-electron two-step reduction process from Au^{+3} to Au^{+} followed by its reduction to Au^{00}. The emergence of methylated Au^{+3} supported defence mechanism against metal toxicity [9], [10]. Similarly, selenate reduction is also a two-step reduction process: (i) selenate (SeO_4^{-2}) to selenite (SeO_3^{-2}) catalyzed by selenate reductases and (ii) selenite to insoluble elemental selenium (Se^0) catalyzed by nonspecific selenite reductases, which predominantly include nitrite and sulphite reductases [11].

Acinetobacter is a diverse group of organisms present ubiquitously in nature [12], [13], [14], [15]. They are commonly found in water, soil, foods and skin of humans and animals [16], [17], [18], [19]. They have an excellent ability to form biofilm [19], [20]. *Acinetobacter* can survive in extreme environmental conditions [21]. Bioremediation potential of *Acinetobacter* is well studied with respect to textile dyes, where an active involvement of lignin peroxidase was reported [25].

Acinetobacter sp. can synthesize various metal nanoparticles such as gold, silver, platinum and selenium [22], [23], [24]. Proteins responsible for PtNP production from *Acinetobacter calcoaceticus* has been purified [24]. In view of this background, the present work is focused on the purification and biochemical characterization of one of the proteins responsible for the synthesis of gold and selenium nanoparticles from *Acinetobacter* sp. SW 30. Further purified protein has been used for the synthesis of gold and selenium nanoparticles and the characterization of gold and selenium nanoparticles is done by various analytical techniques such as TEM, EDX and SAED.

Section snippets

Microorganism and culture conditions

The *Acinetobacter* sp. SW30 was isolated from activated sewage sludge and can synthesize gold nanoparticles (AuNP) and selenium nanoparticles (SeNP) [22], [26]. The culture was routinely sub-cultured and maintained on Luria Bertani (LB) (HiMedia, India) agar at 4°C and in glycerol stocks stored at −80°C.…

Preparation of crude enzyme

A loopful of culture of *Acinetobacter* sp. SW30 was inoculated into the 10ml LB broth medium and incubated at 30°C overnight to obtain the inoculum. The 250ml LB broth was inoculated with 1%…

Preparation of crude enzyme

The cell suspension incubated for 120h did not show laccase, tyrosinase and nitrate reductase activity checked after every 24h. However, it showed maximum lignin peroxidase activity incubated for 24h (Fig. 1). Hence, 24h incubated cell suspension was used for the preparation of crude enzyme. *Acinetobacter calcoaceticus* NCIM 2890 is reported to contain lignin peroxidase enzyme [25].…

Purification of protein

To study the role of lignin peroxidase in the synthesis of nanoparticles, it was purified by DEAE-

Cellulose anion…

Conclusion

This is the first report on the involvement of lignin peroxidase in the synthesis of AuNP and SeNP from *Acinetobacter* sp. SW30. Native and SDS PAGE confirmed that purified lignin peroxidase is 97.4 KDa, a monomeric enzyme. Lignin peroxidase was able to synthesize spherical crystalline AuNP and amorphous SeNP. Apart from this enzyme has maximum activity at pH 2 and temperature 40 °C, with 1.0 mM Km value, when *n*-propanol was used as a substrate.…

Conflict of interest

The authors declare that they have no conflict of interest.…

Acknowledgement

SW and RS acknowledge University Grants Commission (UGC), New Delhi, Indiafor awarding research fellowship. US is thankful to UGC for awarding UGC-DS Kothari post doctoral fellowship.…

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>
> …Biosynthesized gold and selenium nanoparticles could be purified from Acinetobacter sp. SW 30, indicating the ability of utilizing of Se in this specie (Wadhwani SA et al., 2018). Previous comparative studies of Se utilization have reported several horizontal gene transfer (HGT) events for both Sec and SeU traits, such as HGTs of the entire Sec utilization pathway observed between Treponema denticola (Spirochaetes) and Photobacterium profundum (Gammaproteobacteria), as well as between Pseudomonadale species (Gammaproteo bacteria) and Betaproteo bacteria (Romero et al., 2005; Zhang et al., 2006).…

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> …The lignin peroxidase purified from Acinetobacter sp. SW30 was demonstrated to be responsible for the synthesis of gold nanoparticles (AuNPs) and SeNPs in vitro (Wadhwani et al., 2018). Recently, Se(IV)/Se(VI) were shown to be reduced to Se(0) nanoparticles and Se(-II) organoselenium by two Ascomycete fungal strains, Paraconiothyrium sporulosum and Stagonospora sp. under aerobic conditions, by simultaneously coupling the opposite redox bioreaction of mycogenic Mn(II) oxidation to Mn oxides (Rosenfeld et al., 2020).…

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