

Biosynthesis of Gold Nanoparticles by Human Microbiota from Healthy Skins

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Abstract

High moisture content of human allows prevalence of microbial flora as commensals. The present study aimed at isolation, purification, identification of human micro flora found in antecubital fosse in Indian adult individuals and their ability to produce metal nanoparticles. Swabs were collected from 80 healthy individuals consisting of 50% of male. Preliminary morphological identification of nine isolates (HACF 1, 4 -9, 11) was performed for the colonies growing on specific isolation medium. Isolates were identified by API 32 GN system and 16S r RNA gene sequencing. *Providencia* spp. HACF 11 exhibited the ability to produce cell bound gold nanoparticles by producing a 550nm in UV-Visible spectroscopy. It was further confirmed by TEM and SEM analysis. Temperature was found to exhibit profound effect on synthesis with increasing monodispersity producing spherical nanoparticles. With increase in temperature, the time required for synthesis of gold nanoparticles reduced to 24 h.

Keywords: Human microflora; Isolation; API 32GN system; 16S rRNA gene identification; UV-Vis spectroscopy; TEM; SEM; Gold nanoparticles

Introduction

The skin is the human body's largest organ, colonized by a diverse milieu of microorganisms, most of which are harmless or even beneficial to their host [1-12]. Metagenomic analysis has revealed that *Staphylococcus* and *Corynebacterium* spp. are the most abundant organisms colonizing moist areas [13,14]. Consistent with culture data suggesting that these organisms prefer areas of high humidity. These moist sites include the umbilicus (navel), the axillary vault, the inguinal crease (side of the groin), the gluteal crease (topmost part of the fold between the buttocks), the sole of the foot, the popliteal fossa (behind the knee) and the antecubital fossa (inner elbow) [12]. Study aimed at isolation, purification and identification microbiota from antecubital fossa and its ability to synthesize metal nanoparticles. There is an interest in the synthesis of nanomaterials because they exhibit unique physical, chemical, optical, electrical, magnetic, mechanical, thermal, dielectric, and biological properties, different from those of bulk materials, due to their distinct size- and shape-dependent characteristics [15] also the microbiota isolated is non-pathogenic and no detailed reports, yet been available on biosynthesis of gold nanoparticles from such microbiome were found. We also investigated the effect of reaction parameters on the rate of synthesis of AuNPs.

Materials and Methods

Subjects

Subjects in this study were students, faculty and employees of the University of Pune

Culture sites

Cultures were harvested from human antecubital fossa.

Microbiological method

A wet cotton swabs was used to collect bacteria from circular area of antecubital fossae [4] in sterile conditions. Samples were collected in summer and rainy season. Serial dilutions were made and culture

on various cultured medias like NA, LA, C.L.E.D, AMM etc. When it was found that the quantities were not significantly different only nine isolates were secured.

Characterization and identification of isolates

Isolates were characterized for size, shape, margin, opacity, elevation, consistency, Gram nature, catalase, oxidase, capsule staining, lactose fermentation.

Isolates were identified by API 32 GN system and 16S r RNA gene sequencing and are as under *Staphylococcus saprophyticus*, *Micrococcus luteus*, *Staphylococcus hominis*, *Staphylococcus gallinaium*, *Providencia* sp..

Growth media and inoculation

Isolates were maintained on LB agar slants (Himedia Mumbai India) agar plate/slant at 4°C and as glycerol stocks at -80°C. For inoculum preparation, a loopful of the culture was inoculated into 100 mL sterile Luria Bertani broth and incubated at 30°C/200 rpm for 24 hours to 7. A single colony of each of the respective isolates was inoculated in each of the respective flasks. The conical flasks were then kept on shaking incubator for 24 hrs at 37°C.

Screening of the isolates for nanoparticles synthesis of nanoparticles synthesis

All the identified isolates were screened for nanoparticles synthesis

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of different metals like gold, silver, platinum, palladium and copper respectively. Only the isolate HACF-11 i.e *Providencia sp* showed ability to produce AuNPs. So, futher experiment was carried out with isolate HACF-11 only (Table 1).

Synthesis of AuNPs and ultraviolet-visible (UV-Vis) spectra analysis

For screening, the Isolates (optical density [OD]₆₀₀ ≈ 1 × 10¹⁰ CFU/mL) were suspended into sterile Milli-Q® water (pH 7) after repeated rinses to remove the traces of media and incubated at 30°C/200 rpm for 72 hours. Synthesis of AuNPs was visually observed for a color change over a period of 72 hours. Reduction of Au⁺ ions was monitored by recording the UV-Vis spectrum between 300 and 800 nm at regular intervals up to 168 hours on SpectraMax M5 Multi-mode Microplate Reader (Molecular Devices LLC, Sunnyvale, CA, USA). The strain giving maximum absorbance was selected. All the isolates were screened for nanoparticles synthesis for different metals and those which were positive for nanoparticles synthesis.

Characterisation of AuNPs

After 72 hours of synthesis, the sample of AuNPs was centrifuged at 14,000 rpm for 30 minutes at room temperature. Repeated rinses were performed to remove impurities. The pellet of AgNPs was suspended in 1 mL sterile Milli-Q water. Samples for transmission electron microscopy (TEM) and high-resolution TEM (HR-TEM) were prepared by drop-coating the AgNPs solution on a carbon-coated copper grid and drying under infrared radiation. The analyses were performed on FEI Tecnai G² 12 BioTwin (FEI Company, Eindhoven, the Netherlands) TEM. A drop of AuNP sample was taken and dried on a glass slide for analysis under a scanning electron microscope (SEM) (JSM-6360; JEOL, Tokyo, Japan) at 20 kV accelerating voltage.

Effect of Reaction parameters on the morphology of AuNPs

To obtain the optimized reaction parameters giving maximum

synthesis of AuNPs, firstly, AuNO₃ ranging from 0.1 to 5 mM (final concentration) was added to the cell suspension and incubated at temperature from 20°C-50°C for 72 hrs. . Synthesis failed to be initiated at lower temperatures like 20°C while a moderate rate of reaction was observed at 30°C and 40°C. The rate of reaction was found to be maximum at 50°C which supported the fact that higher temperature plays a key role in enhancing the reaction rate. UV-Vis spectra and TEM analyses were carried out to study morphology with varying salt concentrations and reaction temperatures.

Result and Discussion

Synthesis and characterisation of AuNPs

A Study on the biosynthesis of AuNPs by 8 strains isolated from human skin microbiota was carried out. All of the strains showed positive results for the Synthesis of AuNPs. But *Providencia sp* was selected for further studies because it gave synthesis in minimum time. Visual observation of cells incubated with HAuCl₄.H₂O showed a color change from colorless to purple to dark violet, clearly indicating the formation of AuNPs (Figure 1). In the UV-Vis spectrum, a single, strong, and broad peak was observed at 500 nm, confirming the synthesis of AuNPs using isolates).

As temperature increases the rate of synthesis of nanoparticles also get increased levels that at higher temperature the synthesis is rapid. Hence temperature was optimized to 50°C (Figures 2 and 3).

Conclusion

This is the first report on biosynthesis of AuNPs by using *Providencia sp*, an human microbiome isolate from anticubital fosse, which was optimized for reaction parameters. Futher studies on the applications of these gold nanoparticles is been carried.

Acknowledgment

Metals	HACF-1	HACF-2	HACF-3	HACF-4	HACF-6	HACF-7	HACF-8	HACF-9	HACF-11
Au	-	-	-	-	-	-	-	-	+
Ag	-	-	-	-	-	-	-	-	-
Pt	-	-	-	-	-	-	-	-	-
Pd	-	-	-	-	-	-	-	-	--
Cu	-	-	-	-	-	-	-	-	-

[-] = negative ii = [+] positive

Table 1: Screening of the isolates for nanoparticles synthesis of nanoparticles synthesis.

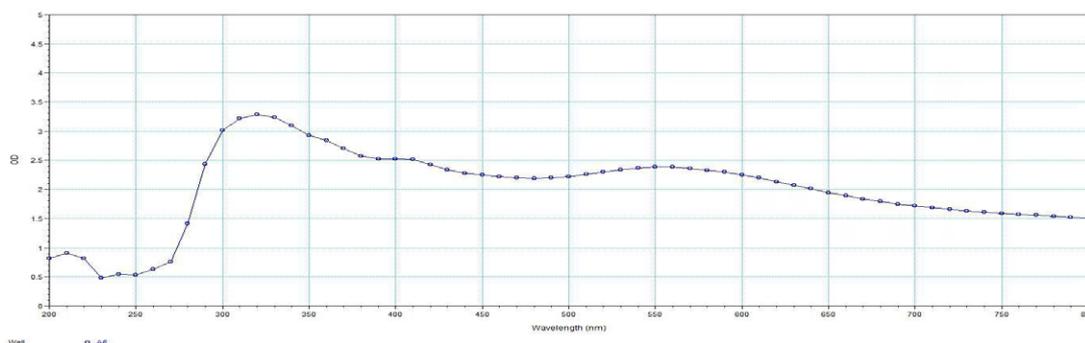


Figure 1: Synthesis of AuNPs using isolates.



Figure 2: Color change at various reaction temperatures.

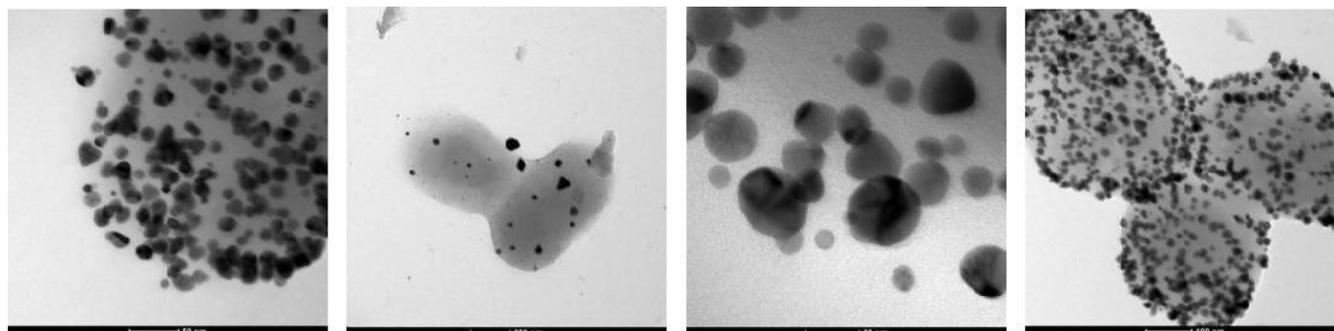


Figure 3: Morphology of AuNPs can be seen at 50°C in TEM analysis.

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