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ORIGINAL PAPER

Bacterial Biosynthesis of Gold Nanoparticles Using *Salmonella enterica* subsp. *enterica* serovar Typhi Isolated from Blood and Stool Specimens of Patients

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Abstract Salmonella is one of the most common causes of gastroenteritis. In this study, a PCR assay was developed for the rapid detection of microbial cultures of *Salmonella enterica* sub species of *enterica* serovar Typhi in samples of patients suspected typhoid. The assay was based on duplicate the STY4669-hypothetical protein gene. The gene primers were designed and blast. Then the bacteria strains were employed for the green synthesis of gold nanoparticles (AuNPs), and one of the strains represented ability to extracellular synthesis of gold nanoparticles. The nanoparticles were characterized using UV–visible spectrophotometer, X-ray powder diffraction, Transmission electron microscopy. The synthesized nanoparticles had a maximum absorption in UV–vis spectra at 556 nm, a crystalline structure, and an average size of 42 nm.

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Introduction

Diarrhea is a disease, which is a health/social dilemma in all parts of the world, especially in developing countries [1]. In these countries, diarrheal diseases causes nearly 5 million deaths, and Salmonella bacteria have an important contribution in this regard [2]. This disease is common among children and old people. Most species of Salmonella are potential pathogens for humans and many of animals [3, 4]. This species are placed in the gut of inflammatory diarrhea and food poisoning are considered [5]. The species are common in older children and adults. The bacteria have settled in the digestive system of vertebrates, including mammals, birds and fish and depending on the species, conditions and multiple factors of the host, can cause diseases with different symptoms and complications [3, 6–8]. Salmonella belongs to Entereobacteriaceae family and consists of 2200 species and more than 90% of species are human pathogens and other are animal pathogens, including typhi, typhimurium, enteritidis, Aynfantys species, etc. This is one of the most common bacteria transferred from animals to humans which has a variety of host species. It is also considered as one of the most important factors in diseases transmitted via food and one of the major public health problem around the world [3]. Therefore, early detection of diseases caused by these bacteria is extremely important [9, 10].

The field of nanobotechnology mainly encompasses with medicine, environment sciences and it develops novel therapeutic nanostructures for biomedical applications [11–18]. The biosynthesized nanomaterials such as copper, silver, gold, palladium, platinum, zinc oxide and magnetite nanoparticles have been effectively controlling the various pathogens [19–27]. In last decade, many studies have proved that the microbial extracts act as a potential precursor for the synthesis of nanomaterial in non-hazardous ways [28].

Due to the unique physicochemical properties of gold nanostructures such as optical, mechanical, and high surface to volume ratio, they have various applications in different fields including biosensors, microbiology, biotechnology, pharmacy, catalysis, etc. [29–37]. Biosynthesis of gold nanoparticles is important due to needing lower energy, simple equipments, and lower costs [38–46].

In present study, PCR was used for rapid detection of clinical samples of *Salmonella enterica* subsp. *enterica* serovar Typhi. Then, this species was employed as a biological source for the biosynthesis of gold nanoparticles which has never approached.

Materials and Methods

Chemicals and Biologicals

All chemicals were purchased from Merck (Germany) and used as received.

Blood samples and applied stools of patients with diarrhea were received from Baghiat-Allah hospital (Tehran, Iran). The samples were transferred to selenite F medium and incubated for 10 h at 37 °C. The samples were then transferred to the medium of Salmonella-Shigella agar (SS), Xylose-Lysine Deoxychola agar (XLD) and MacConky agar, and incubated for 24 h at 37 °C. Suspected Salmonella colonies were identified and isolated by standard biochemical tests, TSI citrate Lysine Iron Agar, Urea and Methyl Red/Voges Proskauer (MR-VP). After those tests, the serotype tests were performed with antiserum [47]. First, the blood samples were cultured for 24–48 h in a biphasic (solid and liquid) medium and then were transferred to the medium of Blood Agar, Salmonella-Shigella agar (SS), Xylose-Lysine Deoxychola agar (XLD), and MacConky agar. After 24 h, the suspected colonies were separated and then identified with the biochemical tests. To separate and confirm *S. enterica* serovar Typhi, the Serotyping test was conducted to determine the O and H antigens with specific antiserum of Staten Serum Institute (Denmark). After preparing the bacterial suspensions and adjacent to antiserum, in case of positive results, it was observed on the slide agglutination for 2 min [48–50].

Molecular Identification

The sequences of forward and reverse primers are designed for molecular identification of bacteria STY4669-hypothetical protein gene. The genome DNA of clinical strains that was identified by biochemical and serologic methods were extracted and PCR was performed. To investigate the designed primers, the genomic DNA of other gram-positive Enterobacteriaceae bacteria strains was used.

Culturing Bacteria for DNA Extraction

After isolating and identifying, the bacteria were grown in Luria broth (LB) medium and incubated for 24 h in a shaking incubator. The turbidity of the tubes containing a liquid culture of bacteria was measured using a spectrophotometer. To isolate the cell mass of bacteria, the medium containing bacterial mass was centrifuged at 9000 × *g* for 7 min. The supernatant phase containing medium was removed and deposited bacterial cell mass was collected. 3 mL proteinase K (20 mg/mL), 13 mL sodium dodecyl sulfate (SDS, 20%) and 620 mL tampons lysis were added to the cell mass of the bacteria deposited in microtubes, and incubated for 1 h at 60 °C in bain-marie medium. This solution was relatively clear, indicating lysis of bacterial cells. Then, 620 mL of phenol–chloroform–isoamylic alcohol were added to microtubes containing bacterial lysate solution from the previous step with ratios of 1:24:25. The microtubes were shaken gently to attain milky solutions. The solutions were then centrifuged at 10,000 × *g* for 10 min. At the end of this phase, three-phase was formed: bacterial genomes in upper phase, proteins in interphase, and phenol–chloroform–isoamylic alcohol at the lower region. The upper and clear liquid supernatant was separated and the residues of phenol was eliminated by adding 620 mL of chloroform and then centrifuged. The upper phase was transferred to microtubes, cold pure ethanol was added and gently inverted several times to appear DNA strands. The microtubes were centrifuged at 10,000 × *g* for

10 min at 4 °C. Finally, the upper phase was discarded and 100 mL TE solution was added to sediment and gently shacked [51, 52]. The purified DNA was stored at −20 °C. Concentration and purity of DNA samples were evaluated by UV absorption.

Primers Design and PCR Procedures

Firstly, the gene sequence of STY4669-hypothetical protein was extracted from NCBI database and the primers were designed by Primer3 software (Table 1). The accuracy and specificity of the designed primer sequences were checked in Blast database and eventually synthesized Blast.

PCR steps (Master cycler gradient) included as follows [53]:

- Denaturation at 94 °C for 5 min
- Repeating 30 temperature cycle to amplify DNA. Each cycle consists of:
  1. 95 °C for 1 min
  2. 59 °C for 1 min
  3. 72 °C for 1 min
- Storing at 72 °C for 10 min
- Storing at 4 °C for 2 h

5 mL of the PCR product was electrophoresed to verify the amplification reaction. After seeing the amplified fragment with a size of about 489, PCR product was sequenced by Bioneer (Korea).

Biosynthesis of Gold Nanoparticles

After isolation and identification of *S. enterica* subsp. *enterica* serovar Typhi, the cells were dissolved in 5 mL double distilled water. 10 mL of 1 mM $HAuCl_4$ solution was added to 1 mL bacteria lysed cells at 25 °C and was kept in dark. The color of the mixture gradually changed to reddish-purple.

Characterization of the Gold Nanoparticles

The optical properties of biosynthesized gold nanoparticles were studied by using UV–visible Spectrophotometer (Nanodrop, Analytik jena, Germany). The phase

Table 1 The properties of used pair primers

Size (bp)	T_M (°C)	Length (b)	Primers sequence	Gene type
489	62	21	F: 5′- TGTCCGCTGTCTGAAGTCATC-3′	STY4669 - hypothetical protein gene
	68/2	22	R: 5′-ATCTCAGGCAAACTCACAAGG G-3′	

formation of gold nanoparticles were examined by XRD technique using a X-ray diffractometer (Phillips, Holland) over a wide range of Bragg angles 20–80°, 2θ with CuKa radiation k = 1.5405 A°. The size, distribution and shape of synthesized gold nanoparticles were characterized using TEM (Carl ZIESS, Germany).

Results

The extracted DNA samples were confirmed to be pure by electrophoresis without RNA contamination (Supplementary material S1). In addition, the ratio of optical density measured at 260–280 nm was obtained as 1.73, further confirmed the purity of the extracted DNA samples.

First, the best PCR conditions were optimized and the best temperature (59°), and $MgCl_2$ concentration (5.0 mL) was determined (Fig. 1).

The amplified genes (Fig. 2) were sequenced. The obtained sequences from the NCBI BLAST database indicated 100% similarity in *S. enterica* subsp. *enterica* serovar Typhi. The effect of PCR reaction with the designed primers was studied on some of Enterobacteriaceae bacteria (*Escherichia coli*, Shigella, Proteus, Klebsiella) and gram-positive cocci of *Staphylococcus aureus*. The results are presented in Fig. 3 and indicated that the primer sets were unique and can be reproduced only in *S. enterica* subsp. *enterica* serovar Typhi.

During the biosynthesis of the gold nanoparticles, reddish-purple color was firstly observed (Fig. 4b). UV–vis spectrophotometry of the synthesized solution showed absorption peak with a maximum at 556 nm, as shown in Fig. 4.

X-ray diffraction pattern of the gold nanoparticles is presented in Fig. 5 containing strong peaks at 2θ values of 38, 44, 64 and 77°. These peaks are assigned to the crystal planes of (1 1 1), (2 0 0), (2 2 0), and (3 1 1), respectively.

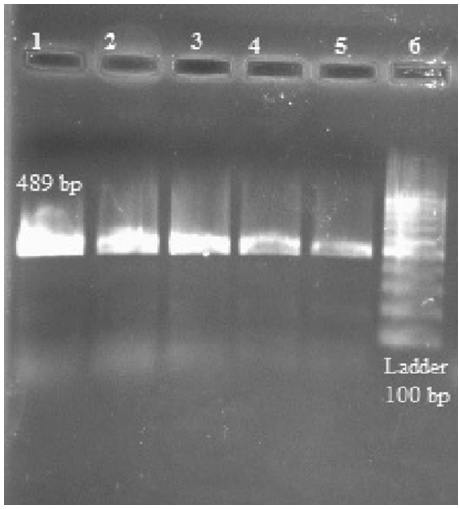

Fig. 1 *Salmonella enterica* subsp. *enterica* serovar Typhi band and species-specific PCR product bp 489 reproduced in different concentrations in order from left to right, 1, 9.0, 5/0, 4.0 and 3.0 mL $MgCl_2$. In addition, in the pit Ladder No. 6 bp100. After PCR reaction in optimum condition, the fragment containing the sequence STY4669 -hypothetical protein of *S. enterica* subsp. *enterica* serovar Typhi was designed from clinical strains

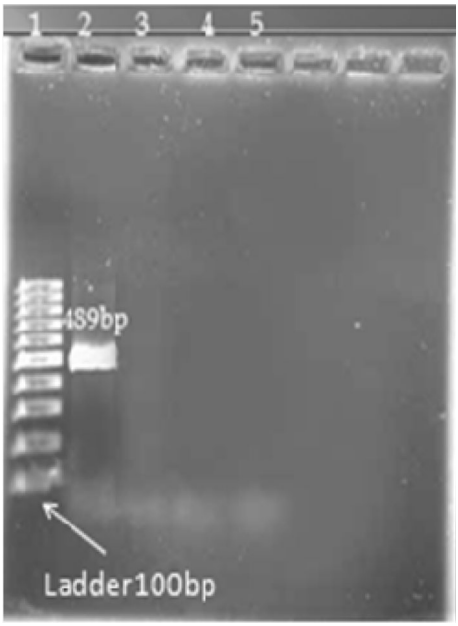

Fig. 2 The image of amplified *S. enterica* subsp. *enterica* serovar Typhi from 489 bp strain (Well 2), and 100 bp markers (Well No. 1)

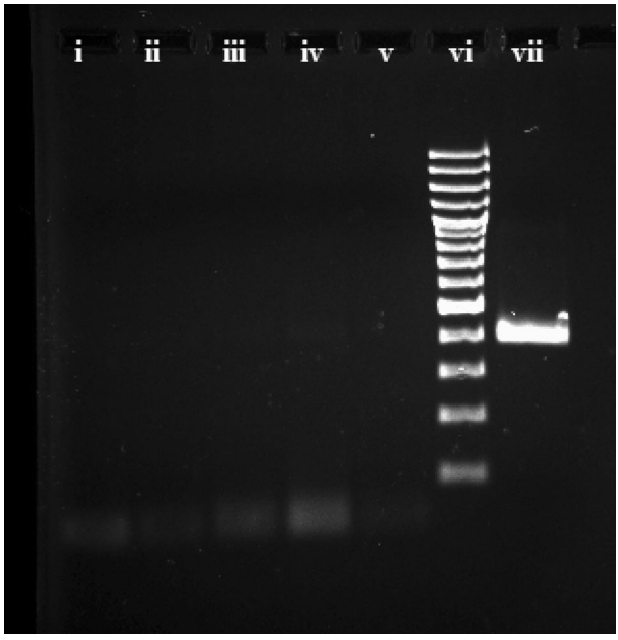

Fig. 3 PCR of designed primers on the genetic material of other bacterial species. (*i*) *Escherichia coli,* (*ii*) *Proteus bacteria,* (*iii*) *Shigella,* (*iv*) *Klebsiella* bacteria, (*v*) *Staphylococcus aureus* bacteria, gram-positive cocci, (*vi*) Ladder 100, (*vii*) and the positive control

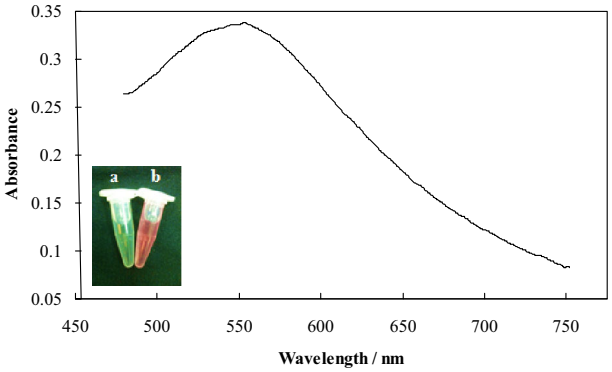

Fig. 4 UV-Vis spectrum of the gold nanoparticles (*b*) synthesized using extracellular content of *Salmonella typhimurium* (*a*)

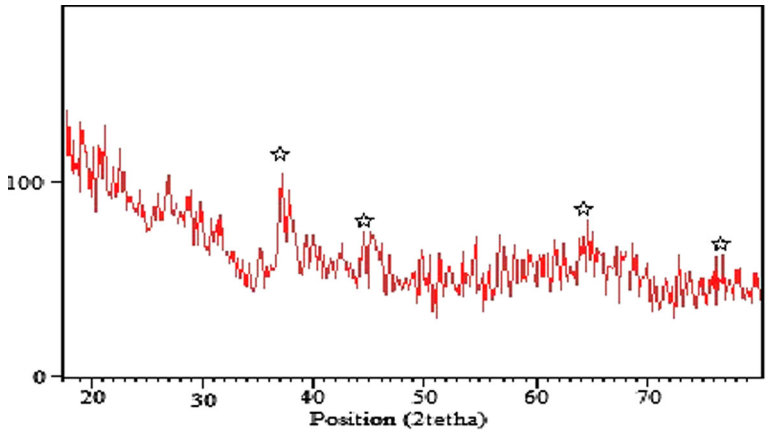

Fig. 5 X-ray diffraction pattern of the gold nanoparticles synthesized using *S. enterica* subsp. *enterica* serovar Typhi

A TEM image of the nanoparticles is shown in Fig. 6. The gold particles had an average size of 42 ± 2 nm.

Discussion

Salmonella is one of the most important causes of gastroenteritis in the world. Fast and accurate detection of these bacteria in food and patients can prevent the spread of these bacteria. Traditional methods of detection of the bacterium are often time consuming and always problematic for microbiologists, especially when quick results announcement is economically and medically crucial. The PCR method is one of the fast and secure methods of molecular identification to amplify and detect a separated genetic locus.

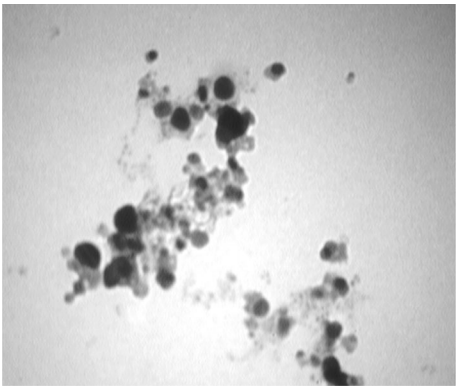

Fig. 6 A TEM image of gold nanoparticles synthesized using extracellular content of *S. enterica* subsp. *enterica* serovar Typhi

Trokv et al. [54] used srRNA 16 (16srRNA) and Pathmanathan et al. [55] used *hilA* gene to identify *Salmonella* without identification of species, while specific detection of bacteria in clinical samples is required. In recent years, Au nanoparticle were used widely in order to design biosensors for the detection of pathogens [56]. These results provided a situation for future studies and scientists can use Au nanoparticles and biosensors for the rapid detection of microbial strains like Chang et al. [57] who recognized *Staphylococcus aureus* in 1 and half hour since Au nanoparticles attached to the aptamer. Also Wu et al. [58] have designed and reported two rapid and sensitive detection methods for *S. enterica* subsp. *enterica* serovar Typhi by Au nanoparticles attached to antibodies.

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Compliance with Ethical Standards

Conflict of interest The authors confirm that this article content has no competing interests.

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