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Microbial Selenium Nanoparticles (SeNPs) and Their Application as a Sensitive Hydrogen Peroxide Biosensor

Kumar Suranjit Prasad[1] · **Jayraj V. Vaghasiya**[2] · **Saurabh S. Soni**[2] · **Jitesh Patel**[3] · **Rinkesh Patel**[3] · **Madhu Kumari**[4] · **Falguni Jasmani**[5] · **Kaliaperumal Selvaraj**[6]

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Abstract The cell-free extract, a crude enzyme (cytosolic and membrane fraction) obtained from an environmental isolate, *Bacillus pumilus* sp. BAB-3706 worked as excellent in reducing as well as stabilizing agent and facilitated the formation of stable colloidal selenium nanoparticles (SeNPs). Resulting nanoparticles were characterized using UV-vis spectrophotometer, TEM, EDAX, FT-IR and XRD, respectively. A working electrode was modified by coating the surface of indium tin oxide (ITO) with colloidal SeNPs. Successive additions of H_2O_2 (100 to 600 μM) in conventional three electrodes system, cyclic voltammeter with potential scan rate 25.0 mV/s, in 0.1 M phosphate buffer solution (PBS) yielded increase in current. A perpetual amperometric response at fixed potential (−1.0 V) and at selected time interval of 100 s showed different magnitude of current at every addition of H_2O_2. The linear range of detection of H_2O_2 was from 5 to 600 mM (R^2=0.9965), while the calculated limit of detection was found to be 3.00 μM. The current study suggested that microbial SeNPs can be used for fabrication of low cost, sensitive H_2O_2 biosensor.

Keywords Selenium nanoparticles · Hydrogen peroxide · Biosensor · TEM · EDAX · UV-vis spectroscopy

✉ Kumar Suranjit Prasad
suranjit@gmail.com

[1] Department of Environmental Studies, Faculty of Science, The M. S. University of Baroda, Vadodara 390002 Gujarat, India

[2] Department of Chemistry, Sardar Patel University, Anand 388120 Gujarat, India

[3] ARIBAS, New Vallabh Vidyanagar, Anand 388120 Gujarat, India

[4] Department of Botany, B. R. A. Bihar University, Muzaffarpur 842002 Bihar, India

[5] Gujarat Biodiversity Gene Bank, Udyogbhavan, Gandhinagar, Gujarat 382010, India

[6] National Chemical Laboratory, Pune 411008, India

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Introduction

Recently, hydrogen peroxide (H_2O_2) received much attention as an analyte since it has wide application in various fields such as food, industry, environment and medicine. In biological system, H_2O_2 plays vital role in the human metabolism and any imbalance in that can produce damage in genomic DNA, lysosomal membrane and induction of apoptosis [1–3]. Hence, reliable and quick monitoring of H_2O_2 in biological fluid, food and waste water is highly desirable [4]. Therefore, an accurate highly sensitive, quick, reliable and cost-effective sensors for determination of H_2O_2 has practical importance. Among all the sensors developed so far, electrochemical sensors for detection of H_2O_2 have been widely used due to their high accuracy and sensitivity [5]. While other sensors exhibit their own drawbacks that includes time consumption, susceptibility to interferences, electrodes modified with biological catalyst such as enzyme, a non-enzymatic naoparticles based sensors for H_2O_2 can be considered as a better alternative than priviously described sensors [6]. Nanomaterials play an important role in the development of biosensor, as they have conspicuous physicochemical properties which remarkably differ from bulk materials. In major cases, sensors respond through interaction of the surface parameters such as surface area with analyte. Hence, sensitivity is governed by the surface area of sensors which interact with analyte. Various nanomaterials have been extensively used in the development of electrochemical sensor, such as porous cuprous oxide nanotubes [7], composite of C@SiO_2 with gold nanoparticales (NPs) [8], ruthenium oxide-gold NPs [9] and catalase modified electrode with MgO NPs [10], etc., and detections of H_2O_2 that have been fabricated. Selenium (Se) is well known for its photo electric and semiconductor properties; hence, it finds use in solar cell, rectifiers, photographic exposure meters, xerography and sensing device [11]. In addition, Se is one of the important elements for exerting anti-oxidative and pro-oxidative effects [12, 13] in mammalian elements. In the past few years, many methods for synthesis of SeNPs, such as Se nanowire of diameter 65 nm using ascorbic acid and β cyclodextrin [14] amorphous (70 nm) and crystalline selenium 8 nm by γ-irradiation at room temperature [15] Se NPs (50–100 nm) using acrylonitrile in presence of PVA [16] Se rods with diameter 400 nm and length 1 μm by reduction of selenious acid by hydrazine [17] NPs of size 30–150 nm by using cell-free extract (cytosolic and membrane fraction) of bacteria *Microbacterium* sp. ARB05 [18] NPs of size 150–200 nm produced in growth media of *Bacillus cereus* [19] NPs of size 60–80 nm by leaf extract of lemon plant [20] and NPs of size 910–80 nm by *Terminalia arjuna* [21] have been attempted. In the majority of cases, SeNPs synthesis has been achieved by either physical or chemical methods, which suffer from drawback like high pressure, low yields and longer growth times which cannot be regarded as an eco-friendly process [22]. Hence, development of eco-friendly and simple method for stable SeNP synthesis with good biological activity is still a challenge.

Biogenic synthesis of various nanomaterials is a clean, nontoxic and environmentally friendly method which gives higher productivity with low cost [23]. Attempts have been made to synthesize metal NPs from microorganisms like bacteria [24], fungi [25] and yeast [26]. Previously, SeNPs prepared using chitosan polymer with horseradish peroxidase [11] and *Bacillus subtilis* [27] have been used for construction of H_2O_2 sensor. The SeNPs were synthesized using later method that produced nanoparticles having the size in the range of 50–400 nm, and their sensitivity towards H_2O_2 detection is in the order of 8×10^{-6} M. Hence, for better catalytic activity, smaller size SeNPs produced using biological process can be considered as a new approach for construction of H_2O_2 biosensor. In the present paper, a green and economical biological method for SeNPs synthesis is attempted with the help of crude enzyme obtained from environmental isolates *Bacillus pumilus* sp. BAB-3706. These SeNPs of size 10–80 nm, with high surface area/volume ratio, are used to fabricate the biosensor to detect the H_2O_2. The proposed sensor exhibited good performance and low detection limit.

Materials and Method

Isolation, Identification, Cell-Free Extract Preparation and SeNP Synthesis

The process of isolation of microbe, identification of *B. pumilus* sp. BAB-3706 and SeNPs synthesis has been adopted from the method described elsewhere [18]. Briefly, one pure colony was selected, and its 16S ribosomal RNA (rRNA) gene was PCR amplified for molecular identification. Cell-free extract obtained by lysozyme-treated cell pellet, followed by sonication, was added in to the 20 ml solution of Na_2SeO_3 (Sigma-Aldrich) under magnetic stirring followed by incubation of the solution in the dark and at shaking (110 rpm) condition. The reduction of selenite ions was monitored by measuring optical density from wavelength 350 to 700 nm using UV-vis spectrophotometer (OPTIZEN 3220, Mecasys, Korea). A 0.1 M phosphate buffer solution (PBS; pH 7.4) consisting of NaH_2PO_4 and Na_2HPO_4 (Sigma-Aldrich, USA) was employed as supporting electrolyte. Water used in the experiment was double-distilled water. All reagents were used without further purification.

Characterization of SeNP

The size and morphology of the synthesized SeNPs were analysed by using TEM measurements with Techni20 (Philips, Netherland). The powder of SeNPs was subjected to elemental analysis using SEM (Philips, Netherland) equipped with energy dispersive accelerated x-ray (EDAX) XL-30 system operated at an accelerating voltage at 15–25 kV. X-ray diffraction (XRD) analysis of the samples was carried out on a Phillips PW 1710 diffractometer with Co-Kα radiation operating at 40 kV, 35 mA with step scan mode between 5 and 75° and with a counting time 8 s per step. The optical characteristics of synthesized SeNPs were measured using UV-vis spectrophotometer.

The Preparation of Biosensor and Characterization

Cleaned indium tin oxide (ITO) glass electrode using solution of detergent and distilled water was further cleaned with ethanol and air dried. The modified electrode was prepared using dispersion of SeNPs (100 mg) in 10 ml of organic polar solvent (Ethanol: DMSO, 1:02) and dried under vacuum at 60 °C for 20 min. The active area of electrode was about 2 cm^2. Electrochemical experiments (cyclic voltammetry and H_2O_2 sensing) were performed under the N_2 condition. Cyclic voltammetry (CV) were performed in 1 ml of 0.1 M PBS (phosphate buffer saline, pH 7.4), that acted as supporting electrolyte and operated from 0 to −2 V (vs SCE) with a scan rate of 25 mV/s. Current responses were obtained at a fixed potential by adding different concentration of H_2O_2 (0.02, 0.1, 0.2, 0.5, 1, 2, 2.5 and 3 mM).

Results and Discussion

Identification of Selenium-Resistant Bacteria and UV-vis Spectroscopy

The characteristic red colour showing confluent growth on selenite supplemented growth media suggested that selenite was reduced to elemental selenium [18] (Fig. 1a). A partial nucleotide sequence of 16S rRNA (submitted to gene bank with accession no. KJ 464963) was analysed by constructing a phylogenetic tree. The sequence analysis of the isolate showed

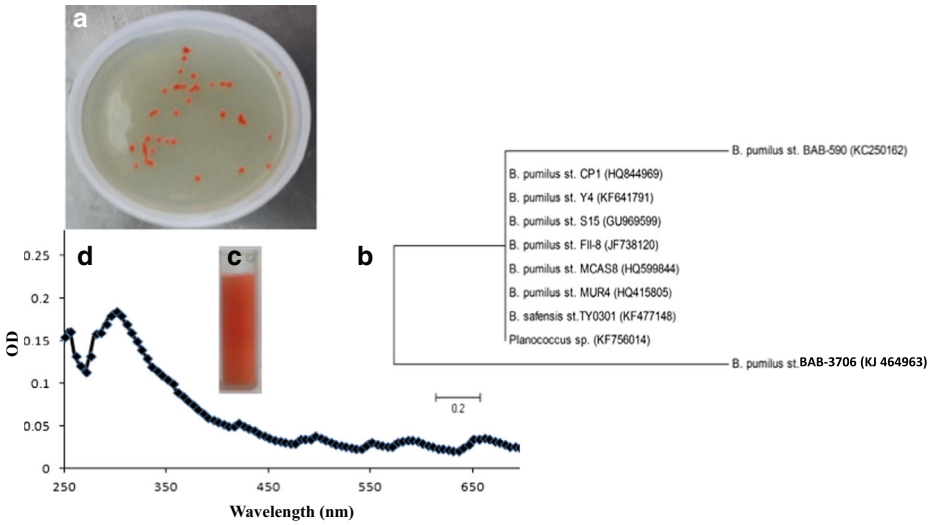

Fig. 1 *Bacillus pumilus* sp. BAB-3706 showing reduction of selenite to elemental selenium (**a**), an unrooted phylogenetic tree (**b**) and change in colour during selenium nanoparticle formation (**c**). UV-vis spectra of SeNPs synthesized using cell-free extract at 48 h (**d**)

close homology with *B. pumilus* strains (Fig. 1b). Aqueous selenite ions were reduced to SeNPs after addition of the strain's cell-free extract (cytosolic and membrane fraction). Initially, the mixture remained whitish yellow which ultimately turned in to dark red colour after 48 h (Fig. 1c). The absorption spectrum pattern recorded after 48 h of incubation showed a significant increase in the absorbance at 300 nm (Fig. 1d). In a study, glucose-stabilized Se nanosphere yielded an absorption maximum at similar wavelength (295 nm) [17].

TEM, SEM, XRD and EDAX Analysis of SeNPs

The dimension and morphology of SeNPs obtained from biological synthesis were examined by SEM and TEM. Figure 2a, c showed the image obtained from TEM and SEM, respectively. A perusal of figures shows that the synthesized SeNPs were spherical in shape and size ranges from 10 to 80 nM, which is much smaller than the SeNPs synthesized using *B. subtilis* [27]. Further, EDAX were recorded to investigate the purity and compositions of the SeNPs. The analysis revealed the presence of Se (63 %) in synthesized NPs. The composition and phase of the resultant samples were examined by SAED and XRD. The pattern in Fig. 2b of synthesized SeNPs indicates that particles were crystalline in nature. Figure 2d showed the XRD pattern of the sample. The XRD diffractogram contained eight prominent peaks with 2θ values of 23.69, 29.73, 41.32, 43.57, 45.76, 51.81, 56.51 and 61.53 corresponded to the crystal planes of 100, 101, 110, 102, 111, 201, 112 and 103 crystalline Se which were in good agreement with JCPDS (File No. -06-0362) for SeNPs. The peaks are strong but not sharp, which shows that particles are smaller in size.

Electrochemical Properties and Amperometric Response to H_2O_2

The electrocatalytical behaviour of the SeNP-coated ITO electrode towards the electrochemical reduction of H_2O_2 was studied using cyclic voltammetry. Figure 3a shows the CVs of SeNP-based working electrode in the absence (inset) and presence of H_2O_2 with different concentrations with

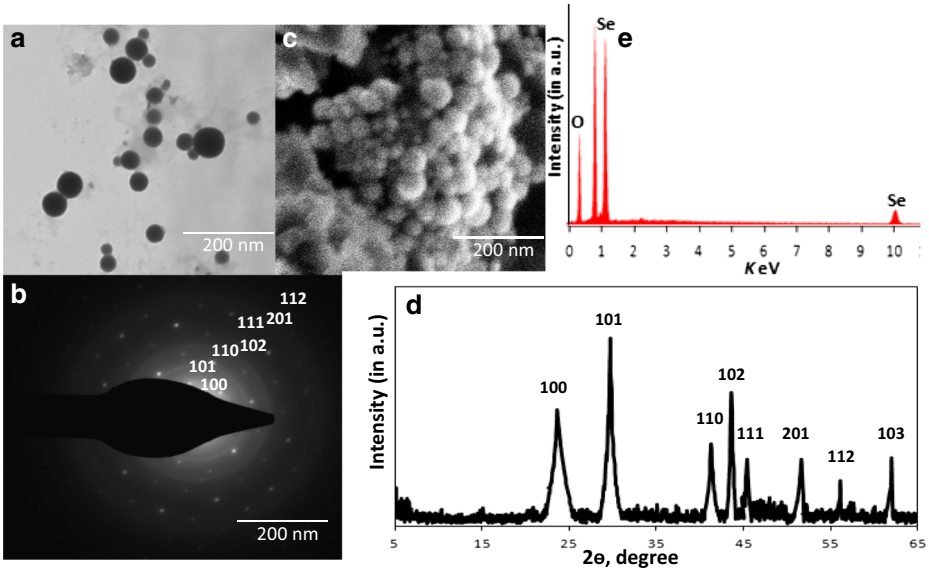

Fig. 2 TEM (**a**), SAED pattern (**b**), SEM micrograph of freeze dried SeNPs (**c**), XRD diffractogram (**d**) and EDAX spectrum (**e**)

increasing concentration of H_2O_2 (from 100 to 600 μM). The H_2O_2 reduction current increased gradually, suggesting the excellent catalytic activity of SeNPs for H_2O_2 reduction. The presence of SeNPs on electrode surface facilitates the electron transfer. The mechanism of the electrochemical reduction of H_2O_2 on SeNPs electrode surface can be summarized as follows [28]:

$$H_2O_2 + e^- \rightarrow OH_{ad} + OH^-$$
$$OH_{ad} + e^- \rightarrow OH^-$$
$$2OH^- + 2H^+ \rightarrow 2H_2O$$

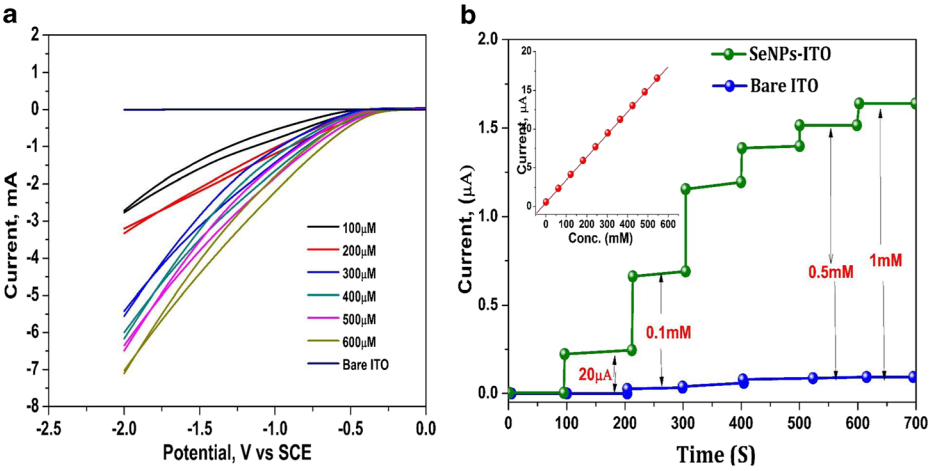

Fig. 3 CVs of SeNP electrode in presence of variation concentration of H_2O_2 in aqueous solution at 25 mV/s and *inset*: CV for selenium NP electrode in absence of H_2O_2 (**a**) and amperometric response of SeNPs electrode upon addition of H_2O_2 at −0.1 V (**b**). *Inset*: the corresponding calibration curve between the current response and concentration of H_2O_2

Table 1 Comparison of electrochemical sensing H_2O_2 on various electrodes

Sr. no.	Electrodes	Detection limit	Sensitivity	Applied potential (V)	Ref.
1.	Ag nanorods	1.0 mM	0.77 μA mM^{-1}	−0.5	[29]
2.	Ag nanoparticles	1.0 mM	0.23 mA mM^{-1}	−0.4	[30]
3.	$Ni(OH)_2$ nanoplates	1.0 mM	–	−0.7	[31]
4.	Electrochemically roughened silver	0.05 mM	–	−0.3	[32]
5.	Carbon nanotubes flower-like gold	0.02 mM	0.8 μA mM^{-1}	+0.95	[33]
6.	CuO nanoflowers	0.05 mM	88.4 μA mM^{-1}	−0.2	[34]
7.	Gold nanowire assembling sphere	0.1 mM	–	−0.2	[35]
8.	Glassy carbon electrode modified with HRP and bacterial SeNPs	8 μM	–	−0.6	[27]
9.	ITO electrode modifies with *B. pumilus* SeNPs	3 μM	16.54 μA mM^{-1}	−1	Present study

The detection of H_2O_2 using chronoamperometry on the basis of direct electrochemical reduction for H_2O_2 on SeNPs was performed. Figure 3b shows the typical amperometric response of the SeNPs upon successive addition of H_2O_2 into 0.01 M buffer solution. Selenium electrode responded (under operating potential −1.0 V) quickly to change of H_2O_2 concentration and reaches steady state signal within 2 s at higher (3.5 mM) concentration. The corresponding calibration curve for the H_2O_2 sensor is shown in the inset of Fig. 3b. The sensor displayed a linear range of responses from 5 to 600 μM having sensitivity of 16.54 μA mM^{-1} cm^{-2} (R^2=0.9965) with detection limit of 3 μM. A comparative account on nanoparticles based H_2O_2 electrochemical sensor has been presented in Table 1 that includes operating conditions, sensitivity and the detection limit. The perusal of data showed that the method used in our experiment (based on SeNPs) were simple, low cost, low detection limit and environmental benign. The result of the experiment revealed the excellent performance of SeNPs as a promising electrochemical sensor in H_2O_2 detection. The stability and reproducibility of the developed SeNP-based sensor was also investigated.

Conclusions

A selenium-resistant bacterium identified as *B. pumilus* sp. BAB-3706 cell-free extract has been implicated in the synthesis of selenium nanoparticles. The colloidal selenium solution showed an absorption maximum at 300 nm. TEM and SEM analysis indicated the presence of polydispersed nanosphere in the range of 10–80 nm, while SAED and XRD studies revealed a crystalline selenium nanoparticle formation. An ITO glass, modified by coating with colloidal SeNPs, was examined for amperometric response at fixed potential and at selected time interval of 100 s. Signals of differential current at every addition of H_2O_2 indicated a transformation of elemental selenium into selenite and selenate ions. Hence, microbial SeNPs can be a promising source for the development of H_2O_2 biosensor.

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Conflict of Interest The authors declare that they have no conflict of interests.

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