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SCREENING OF DIFFERENT GROWTH MEDIA FOR EXTRACELLULAR SYNTHESIS OF SILVER NANOPARTICLES USING *ASPERGILLUS NIGER*

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ABSTRACT

In the present study screening of different growth media such as Czapex Dox, Yeast-Malt Extract and Potato Dextrose Broth were employed for the growth of *Aspergillus niger* capable of silver nanoparticles synthesis. Screening was done on the basis of color change and UV visible spectrometry and the results obtained suggested that Czapex Dox broth was the most suitable growth medium since it fulfills all the nutritional requirements of the fungi required for its growth and silver nanoparticle synthesis as depicted by the UV spectra with maximum absorption value of 1.22 at 430nm.

Key words: Czapex Dox, PD Broth, *Aspergillus niger*, Silver nanoparticles, UV Spectra

INTRODUCTION

Nanoparticles are nano-sized objects whose size is measured in nanometers (nm) (Ahmed *et al*., 2008) ranging from 0.1-100nm exhibiting distinct morphological properties compared to the bulk form of the same material. Numerous examples of biosynthetic methods for the synthesis of nanoparticles are available in the literature. These biosynthetic methods can be categorized as intracellular and extracellular depending on where these nanostructures are produced either within or outside the microbial cells respectively (Mourato *et al*., 2011). Biological organisms like plants, algae, fungi and bacteria make use of different mechanistic approaches for the biosynthesis of different metallic nanoparticles such as by means of bio-reduction using different reducing or stabilizing agents that contributes towards the synthesis and stability of nanoparticles. For example Cadmium nanoparticles have been synthesized by *E. coli* (Sweeney *et al*., 2004), *Bacillus cereus* (Babu and Gunasekran, 2009), *B. thuringiensis* (Jain *et al*., 2010) and *Corynebacterium* strain SH09 (Zhang *et al*., 2005) have been reported to produce silver nanoparticles (AgNPs). Not only bacteria but plants can also be used to produce nanoparticles of different types. Song and Kim (2008) reported the synthesis of silver nanoparticles using plant leaf extracts. Shankar *et al.* (2003) reported the production of spherical gold nanoparticles using Geranium and during his study revealed that the fungal polypeptides and the enzymes present in the plant extract were the reducing agents responsible for metal ion reduction. On the other hand Seshadri *et al* (2011) reported the biosynthesis of lead sulfide nanoparticles by *Rhodosporidium diobovatum*, which is lead resistant marine yeast. *Saccharomyces cerevisiae* and *Cryptococcus humicola* have been reported recently by Vainshtein *et al*. (2014) to be involved in the synthesis of magneto-sensitive nanoparticles, while *Penicillium* species are well known producers of gold nanoparticles (Zhang *et al*., 2005). Qian *et al*. (2013) reported the biosynthesis of silver nanoparticles using *Epicoccum nigrum*, which is an endophytic fungus and their activity against certain pathogenic fungi. These nanoparticles are known to show antifungal activity against various human pathogenic fungi (Gajbhiye *et al*., 2009; Rai *et al*., 2009)

Physiochemical conditions in which the biological organisms are cultured greatly influence the rate of biological synthesis of nanoparticles. Therefore, in order to produce large scale nanoparticles of definite size, shape and composition it is crucial to optimize the external environment to control the growth rate of the nano crystals by controlling the reaction parameters. Among other parameters, choice of media used for microorganism growth also influence the rate of nanoparticle synthesis. Therefore, in the current study screening of different growth media for silver nanoparticles synthesis using *Aspergillus niger* (Mubarak *et al*., 2012) was investigated.

MATERIALS AND METHODS

Following three different types of broth media were prepared for the production of silver nanoparticle synthesis using *Aspergillus niger*:

Czapex Dox (CD) Broth was prepared by dissolving glucose (10g), yeast extract (1g), ferrous sulphate (0.01g), zinc sulphate (0.01g), calcium chloride(0.5g), magnesium sulphate (0.5g), potassium dihydrogen phosphate(1g) and sodium nitrate (2g) in 1000ml of distilled water.

1. Yeast Extract-Malt Extract (YM) Broth was prepared by dissolving glucose (10g), malt extract (3g), peptone (5g), yeast extract (3g) in 1000mL of distilled water.
2. For Potato Dextrose (PD) Broth, extract from 200gm potato was prepared by boiling and mixed with 20gm sucrose. The final volume was adjusted to 1000mL with distilled water.

Hundred (100) mL of each broth media were dispensed in 250mL flask in triplicates and autoclaved for 15min at 121°C at 15psi (pounds/square inches). Using sterilized needle, culture of *Aspergillus niger* was inoculated into each of these three culture media and flasks were incubated on a rotatory shaker at 130rpm at room temperature (25±1ºC). The mycelia of *Aspergillus niger* were harvested after 4-5 days of incubation from each of the three types of broth media by filtration using Whatman's filter paper No. 42 followed by centrifugation at 13000 rpm for 15 min to obtain cell free fungal filtrate.

Silver nanoparticle synthesis

To synthesize silver nanoparticles (AgNPs) 10mL of culture filtrate incubated with 90mL of $AgNO_3$ (1mM) in dark at 25ºC in an incubator for 48h. Samples were analyzed for color change by visual observation to indicate AgNPs synthesis.

UV-visible spectroscopy was carried out for each sample using UV-Visible Spectrophotometer (JENWAY 6305). The spectrum analysis was carried out between 400nm to 500nm. Double distilled water was used as a blank for all measurements.

RESULTS AND DISCUSSION

Visual change in the color of each medium incubated with culture filtrate and silver nitrate solution was observed after 48h of incubation. In case of CD filtrate the color changes from pale yellow to light brown however appearance of dark brown color and grayish black color was observed for YM and PD filtrate respectively indicating the biogenesis of silver nanoparticles. Since silver nanoparticle synthesis was observed in all of the three types of fungal broth media inoculated with *Aspergillus niger* and incubated with silver nitrate solution. Thus it can be concluded from the obtained results that all these three types of growth mediums provide the essential macro and micronutrients required for the fungal mycelial growth. But by virtue of the fungal mycelia growth rate, sufficient mycelia biomass was seen in Czapex dox broth medium while moderate concentration of biomass was seen in yeast malt extract broth and lowest concentration of biomass was obtained in potato dextrose broth. The possible reason for this may be the difference in the composition of each broth medium as CD broth contains essential carbon and nitrogen source along with other vital macro and micronutrients such as magnesium, sodium, calcium, potassium, iron and zinc which are crucial for fungal growth. In contrast YM and PD broth growth medium lack these nutrients.

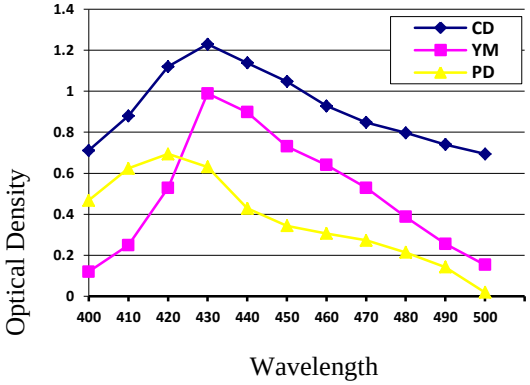

Fig. 1. UV -Visible Spectrum of nano-particle using three different fungal broth media.

UV visible spectrum obtained clearly depicted that maximum absorption value of 1.22 was obtained at 430nm for CD medium while absorption value of 0.98 was obtained at 430nm for YM medium. As it is well understood that greater absorption value (O.D) corresponds to greater concentration of colloidal silver nanoparticles because of

increased rate of bio reduction of silver metal ions by enzyme nitrate reductase present in the fungal filtrate. Therefore increased rate of silver nanoparticles synthesis is achieved using CD medium. On the other hand at 430nm absorption value of 0.63 was obtained for PD medium indicating lower concentration of silver nanoparticles i.e. slow reaction kinetics however optimum synthesis occurred at 420nm (Fig. 1)**.**

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