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Research Article

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Biosynthesis of Silver Nanoparticles By *Lactobacillus Sps* & Its Activity Against *Pseudomonas Auerogenosa.*

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Abstract: Tomorrow technology is going to depend on nanostructured metals. The impact of this technology will be felt greatly at the interphase of chemistry and biology. The desire to synthesize nanoparticles, using efficient and green chemistry approaches has led to the use of microorganisms. Among microbes prokaryotes have received most attention in the area of silver nanoparticles production. Here we report the ecofriendly production of silver nanoparticles assisted by *Lactobacillus* strain found in curd when exposed to appropriate ions. *Lactobacillus sps* isolated from curd was inoculated to Sterilized milk and latter (after 24hr) the whey was collected by coarse filtration (Whatman no.40). Silver nitrate (1 mg) was added to 5 ml of pale yellow filtrate then incubated at 37°C. UV-Vis measurements were done with Spectrophotometer (Thermo Spectronic Genesys 10 UV) for the samples drawn at every 12 hours. The silver nanoparticles of size ranging from 2 to 20nm were synthesized by *Lactobacillus sp* VRS2 which was confirmed by TEM. The biosynthesized silver nanoparticles (AgNPs) showed excellent antibacterial activity against *Pseudomonas auerogenosa* (MTCC strain no: 424).

Key words: Silver nanoparticles, Nosocomial pathogen, TEM, *Lactobacillus sps*, Antibacterial activity

INTRODUCTION:

Nanotechnology is dynamically emerging fields among modern sciences with potential applications in electronic and medicine [1]. Nanobiotechnology represents an economic alternative for chemical and physical methods of nanoparticles formation [2]. There is a growing interest in nanoparticles as they provide superior material properties with functional versatility. Silver nanoparticles (Ag-nps) are among the most commercialized inorganic nanoparticles due to their antimicrobial potential. The biomedical applications of silver nanoparticles can be effective by the use of biologically synthesized nanoparticles which minimize the factors such as toxicity and cost and are found to be exceptionally stable. Biological methods of nanoparticles synthesis using microorganism [3-5] enzyme [6] and plant or plant extract [7] have been suggested as possible ecofriendly alternatives to chemical and physical methods. It can also be suitably scaled up for large-scale synthesis of nanoparticles. The Gram-positive Lactobacilli are commensal inhabitants of the gastrointestinal (GI)

tract that also play important roles in the production and preservation of food. Based on their health-promoting effects, these bacteria are commonly marketed as probiotics [8].

Antimicrobial agents based on ionic silver (e.g., silver nitrate) have one major drawback: they are easily inactivated by complexation and precipitation and thus have a limited usefulness [9]. Zerovalent silver nanoparticles are considered as a valuable alternative for ionic silver. Due to their large specific surface-to-volume ratio, nanoparticles have different properties than bulk material [10]. It has been shown that silver nanoparticles are antimicrobial towards a broad spectrum of Gram-negative and Gram-positive bacteria [11, 12]. Furthermore, silver nanoparticles show antifungal [13] and antiviral activity [14]. In the present study, the *Lactobacillus sps* VRS-2 was subjected for silver nanoparticle production in whey. Further the synthesized silver nanoparticles were subjected to check its antibacterial activity against *Pseudomonas auerоginosa*.

MATERIALS AND METHODS

Pathogenic bacteria and reagents: *Pseudomonas auerоginosa* (MTCC-424) was procured from Microbial Type Culture Collection, Chandigarh, India. All reagents used during the present study were of analytical grade and were obtained from Himedia, Mumbai, India Ltd., unless specified otherwise.

Synthesis of silver nanoparticles: *Lactobacillus* strains used in the study was isolated from curd sample collected from the local vendor. After serial dilution the samples were plated over Modified Lactobacillus Agar plates (Hi-media, India). The bacterium was identified based on cultural and biochemical characteristics. The isolates were maintained in slants of tomato juice agar.

The isolated *Lactobacillus sps* designated as strain VRS2 was inoculated into sterilizes 250 ml of home delivered milk in 500 mL Erlenmeyer flask and incubated for curdling at 37°C for 24 hours. The whey was collected by coarse filtration (Whatman 40). The filtrate was pale yellow in appearance, and the pH was typically 4.4. To 5 mL of each sample solution taken in a test tube, 1 mg of $AgNo_3$ was added and kept in the laboratory under ambient conditions [5]. The solution became brown in about 12 h. A brown mass gets deposited at the bottom of the test tube after 24 h. Control was run along with experimental flask. The reaction mixture was analyzed periodically using UV-Vis spectrophotometer (Thermo Spectronic Genesys 10 UV Spectrophotometer). The absorbance was measured in the range 300-600 nm, which includes the Plasmon absorbance peak of the silver nanoparticles centered at 420 nm. Further the nanoparticles from the potent isolate were characterized by TEM analysis.

Transmission electron microscopic analysis: A drop of sample was placed on a piece of parafilm, carbon coated copper grid was placed and allowed for 5-10 minutes, and drained the excess with help of filter paper. Further again the preparation was washed with distilled water and stained with 2% uranyl acetate. The dried preparation was observed under transmission electron microscope at various magnifications (Model: Hitachi, H-7500) as per the standard protocol [15].

Analysis of the Antibacterial Activity of Silver Nanoparticles: Agar diffusion method was performed to check the antibacterial activity of silver nanoparticles [16]. The bacteria was seeded in agar plate by pour plate technique, cavities were made using a cork borer (5 mm diameter) at an equal distance and were filled with the silver nanoparticle solution (20 mL) produced by *Lactobacillus sps* VRS2 and then incubated at 37°C. The formation of a clear inhibitory zone around the cavity is an indication of antibacterial activity.

RESULTS AND DISCUSSIONS:

Production of Nanoparticles: Biological synthesis of silver nanoparticles by *Lactobacillus sp* VRS2 is primarily confirmed by change of the reaction mixture from pale yellow to brown color (Fig 1) indicating the production of silver nanoparticles (Ag^+ to Ag^0). It is reported that reduction of Ag^+ to Ag^0 occurs through nitrate reductase enzyme [17]. These enzymes released in the solution can reduce the silver nitrate to silver nanoparticles through capping agents such as proteins. The reaction mixture was analyzed using UV-Vis spectrophotometer. The absorbance was measured in the range 300-650nm, which includes the Plasmon absorbance peak of the silver nano particles centered at 430 nm (Fig-2). The silver nanoparticles formed were highly stable even after few weeks after the reaction [18]. Formation of colloidal silver particles can be easily followed by changes of UV-Vis absorption [19] optical absorption spectroscopy has proved to be a very useful technique for the analysis of nanoparticles. The characteristic brown color due to the excitation of Plasmon vibrations in the nanoparticles provides a convenient signature of their formation [20]. The surface Plasmon band remains in the range of 420-440 nm throughout the reaction period suggesting that the particles are dispersed in the aqueous solution with no evidence for aggregation after the complete of reaction**.**

Transmission Electron Microscopic Analysis: TEM picture of the silver nanoparticles formed by *Lactobacillus sp* VR-2 after 48 h is shown in Fig 3. The TEM Technique used to visualize size and shapes of biosynthesized silver nanoparticles have predominantly shown spherical shape structures

with size ranging between 2nm - 20 nm. Reduction followed by bioadsorption of silver nanoparticles is evident after the analysis of TEM micrograph [21].

The Antibacterial Activity of Silver Nanoparticles: The biosynthesized silver nanoparticles (AgNPs) showed antibacterial activity against *Pseudomonas aueroginosa* (Fig-4). The antibacterial activity of silver nanoparticles (AgNPs) is well known from ancient time. The major mechanism through which silver nanoparticles manifested antibacterial efficacy is by anchoring to and penetrating the bacterial cell wall and cause further damage by possibly interacting with sulphur and phosphorus containing compounds such as DNA [22]. We attribute this enhanced antibacterial effect of the nanoparticles to their stability in the medium as a colloid, which modulates the phosphotyrosine profile of the bacterial proteins and arrests bacterial growth.

CONCLUSION:

In the present study we have reported a simple biological extracellular, easy, low cost, non toxic economical and ecofriendly approach for synthesizing silver nanoparticles by using *Lactobacillus sps.* The characterizations of silver nanoparticles formed were characterized by UV-vis spectra and TEM studies. The size of the particles was of 2- 20 nm. The possibility of protein as a stabilizing material in silver nanoparticles is revealed by TEM studies. Study was carried out on *P. aeruginosa* and the effect of the nanoparticles was found to be significantly more pronounced.

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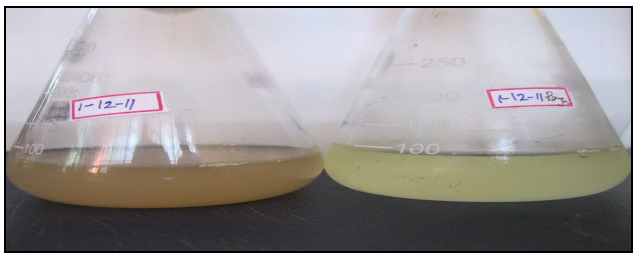

Test **Control**

Figure 1: (a) *Lactobacillus sps* in whey with (1 mg/5 ml) $AgNO_3$ (b*) Lactobacillus sps* VRS2 in whey without AgNO3 is taken as control

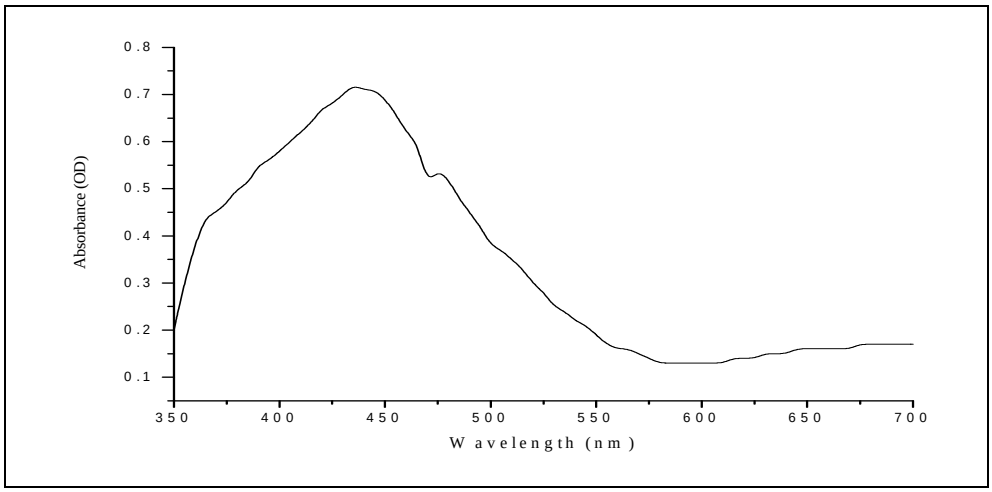

Figure 2: Absorbance spectrum of silver nanoparticles synthesized by *Lactobacillus sps* VRS2

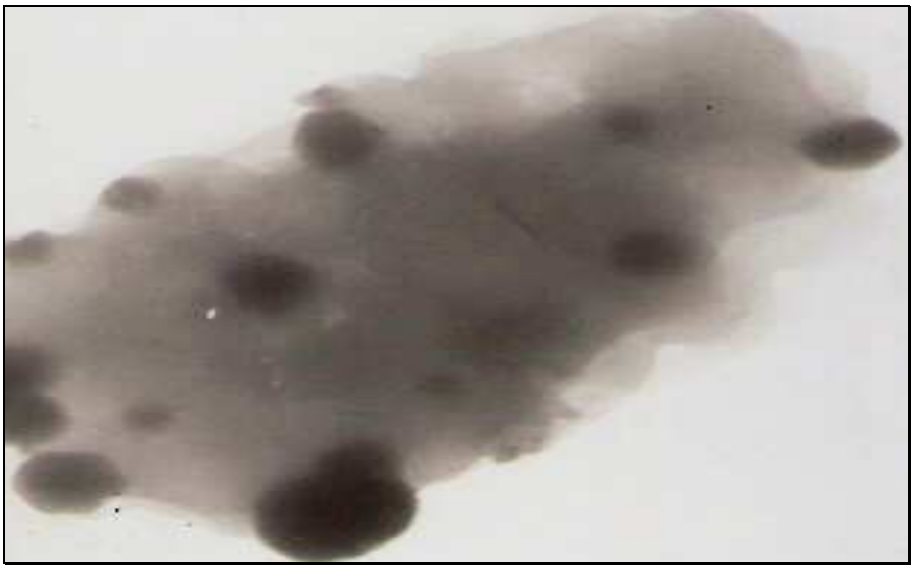

Figure 3: Transmission electron micrograph of silver nanoparticles embedded on *Lactobacillus sps* VRS2

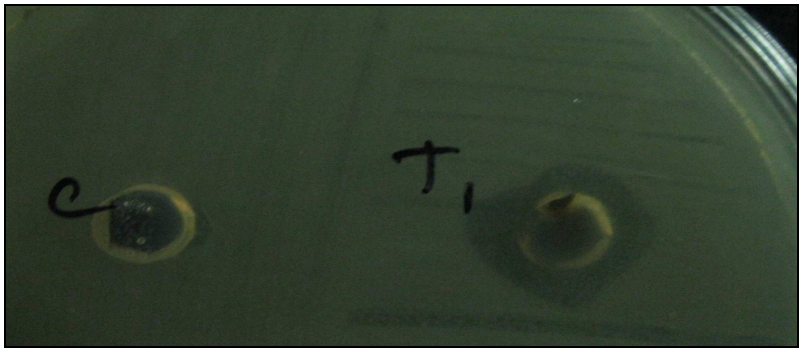

Figure 4: Nanosilver shows the zone of inhibition against *P. aeruginosa*

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