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MICROBIAL SYNTHESIS OF SILVER NANOPARTICLES BY USING FISH PATHOGEN *AEROMONAS SALMONICIDA*

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ABSTRACT

Microbial synthesis of silver nanoparticles using bacteria has received profound interest because of their potential to synthesize nanoparticles of various size, shape and morphology. In the present study, synthesis of silver nanoparticles was done by bacterial strain *Aeromonas salmonicida*. Nanoparticles are used immensely due to its small size, orientation, physical properties, which are reportedly shown to change the performance of any other material which is in contact with these tiny particles. These particles can be prepared easily by different chemical, physical, and biological approaches. But the biological approach is the most emerging approach of preparation, because, this method is easier than the other methods, ecofriendly and less time consuming. The synthesis of silver nanoparticles was successfully observed by the formation of colourless aqueous solution to reddish brown colour after the 48hrs of incubation with bacterial strain *Aeromonas salmonicida.* Synthesis of silver nanoparticles takes place due to excitation of Surface Plasmon Resonance (SPR) of silver ions. UV-VIS spectroscopy also recorded and the spectra showed a maximum absorption peak at a visible range of 300nm that confirms the formation of silver nanoparticles. Silver nanoparticles shows greatest antibacterial and antifungal activity and also useful in aquatic and biomedical applications.

KEYWORDS: Silver Nanoparticles, *Aeromonas salmonicida*, Microbial Synthesis, UV-Visible Spectrophotometer.

INTRODUCTION

Silver nanoparticles allow very intense antibacterial activity opposed to gram positive as well as gram negative bacteria inclusive of multi resistant strains, and also it was elevated in few studies (Esaki, 1999). Nanotechnology deals with materials in the size of 0.1 to 100 nm; however it is also inherent that these materials should display different properties such as electrical conductance chemical reactivity, magnetism, optical effects and physical strength, from bulk materials as a result of their small size. Nanotechnology will play a critical role in coming 50 years by protecting the environment and providing sufficient energy for a growing world. Nanotechnology can help in developing new ecofriendly and green technologies that can minimize undesirable pollution.

Among the various methods of nanoparticle synthesis, biological methods are much promising because of its effectiveness, flexibility and environment friendly approach. Considering the diverse biological applications of silver nanoparticles (AgNPs), it is necessary to explore various biological systems for silver nanoparticle production. Many microbes including bacteria, yeast and fungi have been found to be capable of synthesizing silver nanoparticles. Among these microbes, bacterial systems are excellent option because they are easy to handle and can be manipulated genetically without much difficulty (Vaidhyanathan et al., 2010).

Aquaculture continues to be the fastest growing animal food-producing sector and can help in maintaining the socioeconomic status. Aquaculture has the ability to contribute significantly to food and nutrition security in the society. It has been reported that about 20 percent per capita intake of animal protein. It is highly rich source of micronutrients, minerals, proteins and essential fatty acids (Mahajan, 2011). Nanotechnology has a wide usage potential in aquaculture and seafood industries (Handy et al., 2012; Bhupinder Singh Sekhon, 2014). Direct use of silver nanoparticles in water to treat a fungal disease has been found toxic to young trout whereas a water filter coated with silver nanoparticles can prevent the fungal infections in rainbow trout fish in the fish culture (Solomon et al., 2007).

Aquaculture industry has suffered tremendous losses due to disease caused by bacteria (Huang et al., 2015). Silver nanopaticles are employed for their antimicrobial and biocidal properties. Silver is well-known for its toxic properties to organisms. In the last decade, an increasing number of studies have focused on silver nanoparticle antibacterial activity (Nowack and Bucheli, 2007).

Nanoparticles Silver is generally used in the nitrate form to induce antimicrobial effect, but when silver nanoparticles are used, there is a huge increase in the surface area available for the microbe to be exposed to. Though silver nanoparticles find use in many antibacterial applications, the action of this metal on microbes is not fully known. It has been hypothesized that silver nanoparticles can cause cell lysis or inhibit cell transduction. There are various mechanisms involved in cell lysis and growth inhibition (Gong et al., 2007).

Nanotechnology has a wide usage potential in aquaculture and seafood industries (Handy et al., 2012; Bhupinder Singh Sekhon, 2014). Less knowledge on the effect of nanoparticles on aquatic organisms. It has been observed that young carp and sturgeon exhibits a faster development due to effect of iron nanoparticle. It has also been observed that the nano selenium-supplemented diet could improve the fish weight, relative gain rate, antioxidant status of the fish, and increases the glutathione peroxidase activities and muscle selenium concentrations of crucian carp (*Carassius auratus gibelio*) (Zhou et al., 2009).

The problem with most of the chemical and physical methods of nanosilver production is that they are extremely expensive and also involve the use of toxic, hazardous chemicals, which may pose potential environmental and biological risks. It is an unavoidable fact that the silver nanoparticles synthesized have to be handled by humans and must be available at cheaper rates for their effective utilization; thus, there is a need for an environmentally and economically feasible way to synthesize these nanoparticles (Prabhu and Poulose, 2012).

In natural environment also, microbes produce nanomaterials as part of their metabolism and hence, can be utilized for various applications discussed in this paper. The microbes reproduce fast; therefore this characteristic can be well exploited for their use in various aspects. Their use in various applications is well known to everyone in the field of biological sciences. Biotechnology has joined hands and has emerged as an initiative for the study of microbes, and its various characteristics in the form of "microbiology". Microorganisms are of size 10^{-6} nm, and they are referred to as nanofactories, meaning generators of nanoparticles. Since they are present in nature, they are also called as biofactories (Vibha Saklani et al., 2012). Nanoparticles are nowadays becoming very popular in various field of research, and are useful in combating various diseases in the form of their early and fast detection.

Microorganisms like Bacteria, Virus and Fungi are known to synthesize Silver nanoparticles. The produced nanoparticles have different size and shape. Silver nanoparticles are one of the most effective nanoparticles because they have a good antimicrobial efficacy against some bacteria, viruses and other eukaryotic microorganisms (Gong et al., 2007). Nanoparticles resulting from some microbial processes are composite materials and consist of inorganic component and special organic matrix (proteins, lipids, or polysaccharides) and they have unique chemical and physical properties different from the properties of conventionally produced nanoparticles and of other microorganisms even when they are incubated in the same medium under the same conditions (Nelly Ya et al., 2011).

Silver is known for its antibacterial properties and has been used for years in the medical field for antimicrobial applications and even has shown to prevent HIV binding to host cells (Elechiguerra et al., 2005). The Ag-NPs are also reported to be nontoxic to human and effective against bacteria, viruses, and other eukaryotic micro-organisms at very low concentration and without any side effects (Jeong et al., 2005).

Furunculosis was reported for the first time as early as in 1894 in Germany (Emmerich and Weibel, 1894). The name furunculosis was given because the diseased fish had furuncle-like swellings, which were ulcerative at a later stage of the disease. In the earlier literature the causative agent was referred to as Bacterium or *Bacillus salmonicida* (Mc Craw, 1952), but it was later named "*Aeromonas salmonicida*" by Griffin et al. (1953). Diseased and dead fish are the major contamination source of waterborne *A. salmonicida* infection (Enger et al., 1992). It has been shown that large numbers of the bacterium will be present in water during, and for a long time after, an epizootic (Mc Carthy, 1977a).

Aeromonas salmonicida comprises facultative anaerobic, Gram-negative, non-encapsulated, non-motile coccobacilli which produce catalase and oxidase, and grow optimally at 22 to 25°C (Popoff, 1984). The colony size of *Aeromonas salmonicida* is between 1-2nm. Four sub-species, salmonicida, achromogenes, masoucida, and smithia have been described. Among these, *A. salmonicida* is the most important fish pathogen. The bacterium readily ferments and oxidizes glucose, and is catalase- and cytochrome oxidase-positive.

In the present study Silver Nanoparticles are microbially synthesized by the fish pathogen *A.salmonicida*. To our knowledge extracellular synthesis of Ag particles by bacterial strain *A.salmonicida* has not been reported so far and it is the first time.

MATERIALS AND METHODS

Synthesis of silver nanoparticles using *Aeromonas salmonicida*

24 hours old *A.salmonicida*; Sterile Nutrient broth; Distilled water; 1 mM Silver nitrate.

Experimental strain

Aeromonas salmonicida, a Virulent Strain, was obtained from MTCC 14174, Chandigarh, India. From this parent culture, sub cultures of *Aeromonas salmonicida* were prepared and doses were made under aseptic conditions. Culture and doses of *Aeromonas salmonicida* was done following the method of Pelczar (1993). The bacterial suspension was prepared to $1x10^9$ Colony Forg Units as determined using a Neubauer haemocytometer.

Preparation and Synthesis of Silver nanoparticles

Nutrient broth medium was prepared and bacteria *A. salmonicida* was inoculated in to broth along with silver nitrate and kept on an orbital shaker for the proper mixing of compounds in the broth at 150rpm and kept at room temperature for 72 hours. If the solution turns in to reddish brown indicates the synthesis of silver nanoparticles takes place.

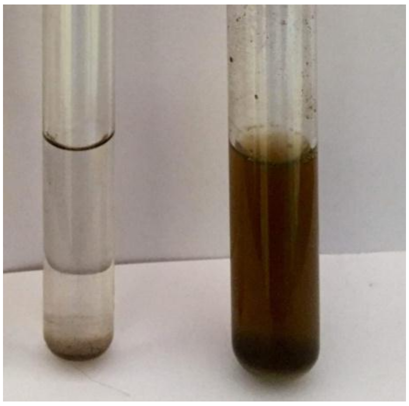
UV-Visible Spectrophotometry

The reduction of pure Ag^+ ions was monitored by measuring the UV-Visible spectrum of the reaction medium at 5 hr after diluting a small aliquot of the sample into distilled water. UV-Visible analysis was done by using UV-VIS spectrophotometer **(Nanotrope, Model No - 8000)**. UV-VIS spectroscopic analysis was done at DST-PURSE, Sri Venkateswara University, Tirupati.

RESULTS AND DISCUSSION

In the Present study the synthesis takes place due to the provocation of Surface Plasmon Resonance (SPR) vibrations of silver nanoparticles. *Aeromonas salmonicida* when placed in concentrated aqueous solution of silver nitrate played a important role in the reduction of Ag^+ ions and the formation of silver nanoparticles with in periplasmic space of the bacteria. Aqueous coloured silver nitrate solution along with *A. salmonicida* transformed in to reddish brown colour after 48 hrs of incubation period results in the formation of silver nanoparticles due to the trapping of metal ions on the surface or internally in microbe and the trapped metal ions are then reduced to nanoparticles in the existence of enzymes.

a. Control without silver nitrate. (No colour change was observed after 48hrs remains aqueous colour).



b. Reddish brown colour in testube was observed after 48hrs indicates formation of AgNPs.

Fig-1: Microbial synthesis by *A.salmonicida* showed synthesized silver nanoparticles after incubation period of 48hrs.

In our present study the fusion/synthesis of Nanoparticles in the medium changes from white colour to reddish brown colour is the primary confirmation. The formation of reddish brown will be shown by adding Ag^+ ions to the pellet and supernatant. In **Fig-1** the intensity of the colour increases with the incubation period due to reduction mechanism. The nanoparticles production directly proportional to intensity of colour. The control has not showed any colour formation when incubated for same period and condition without Ag^+ ions. After the incubation period the test tube with bacteria (*A. salmonicida*) with Ag^+ ions shows colour changes in supernatant but pellet takes long period to colour formation. So the silver nanoparticles synthesis depends on culture incubation period and the synthesis of silver nanoparticles takes place due to excitation of surface Plasmon vibrations of silver ions.

The first report of nanoparticles mechanism that is responsible for the formation of metallic nanoparticles and how it can vary in different bacteria (Johnston et al., 2013). He et al. (2007) group suggested an alternative method for gold nanoparticles synthesis by bacteria, the extracellular formation of gold nanoparticles of 10-20nm by the bacterium *Rhodopseudomonas capsulata* and suggested that these nanoparticles were synthesised via an NADH - Dependant Reductase. Nanoparticles were found to be formed on the cell envelope of the bacteria which makes them attractive as they are easily accessible. The colour of *Bacillus licheniformis* culture after the addition of silver ions turned a dark brown indicating the formation of silver nanoparticles (Kalimuthu et al., 2008).

Synthesis of Silver nanoparticles by microbes is due to their resistance mechanism, and the synthesized nanoparticles produced are useful to us. The defensive mechanism caused by the bacterial cell for silver ions are responsible for the synthesis of silver nanoparticles. Silver ions are acidic and highly toxic to bacterial cells whereas microbes are basic in nature, so their cellular machinery helps in the conversion of reactive silver ions into stable silver atoms.

From our results the formed silver nanoparticles showed transmission peak at 300nm was recorded **(Fig-2).** The formation of colour is allowed to calculate the wavelength to conform the formation of silver nanoparticles. The aggregation of the silver nanoparticles indicated by the plangency presence in the solution. The formation of silver nanoparticles in the reaction mixture and silver ions reduction is unambiguous but in the synthesis of silver nanoparticles proteins, molecules and enzymes including nitrate reductase enzyme acts as good regulating agent. Hence it is observed that the reduction of Ag^+ ions occurs extracellularly than the intracellular culture. Earlier reports suggested that silver nanoparticles are formed due to the transverse Plasmon vibrations after exposure to UV light (Shankar et al., 2003).

UV-Vis Spectroscopy observations shows the silver nanoparticles do not assemblage due to the balanced posture of absorbance peak (**Fig- 2**). The amount of silver nanoparticles increases indicates the increase of wavelength absorption. If the particles size increases, the absorption peak habitually relocates in the direction to red wavelengths. Silver nanoparticles hold negative charge due to the presence of citrate ions, offensive force created along silver particles and interrupted aggregation.

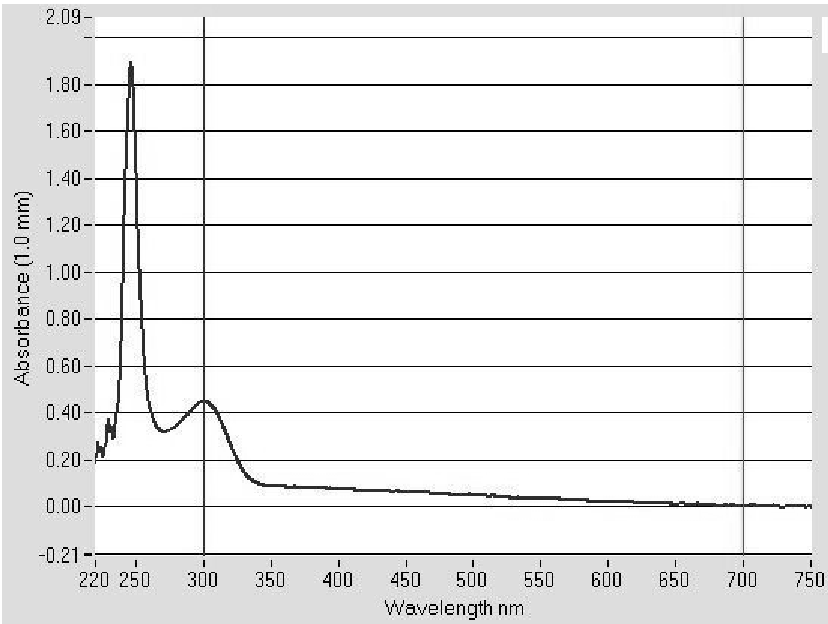


Fig-2: UV-VIS absorption spectrum showing transmission peak at 300nm are recorded and confirms the microbial synthesis of silver nanoparticles takes place.

Earlier reports showed that AgNPs may grow in a process involving rapid bio-reduction and that they strongly influence the Surface Plasmon Resonance in the water extract (Huang et al., 2007 and Jeong et al., 2005). Silver nanoparticles, because of their large specific surface area, are highly active and can play a crucial role in inhibiting bacterial growth in aqueous and solid media. The antimicrobial activity of colloidal silver is influenced by the size of the particles. Smaller the particle size more is its antimicrobial activity.

CONCLUSION

We understood that silver ions are very reactive, and are known to attach with the essential cell components, encouraging cell death. Synthesis of silver nanoparticles through chemical method is very lackluster, whereas, through microbes *Aeromonas salmonicida*, is rapid and an eco-friendly approach. Nanotechnology further be useful in various medical and industrial applications.

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