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MICROORGANISMS AS NANOFACTORIES: ADVANCES IN SYNTHESIS OF NANOPARTICLES BY BACTERIA

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ABSTRACT: Bio-nanotechnology explores a variety of promising approaches in the area of material sciences on a molecular level and is of leading interest in the world of science. It deals with the fusion of biology and nanotechnology based on the principles and chemical pathways of living organisms, and refers to the functional applications of biomolecules in nanotechnology. A vast array of biological resources available in nature including plants and plant products, algae, fungi, yeast, bacteria, viruses could all be employed for synthesis of nanoparticles. Bio-based methods are more useful for the production of highly stable, well characterized and safer nanoparticles than the chemical methods, which are usually not environmental friendly, less stable and not easy to scale up. Microorganisms produce nanomaterials as part of their metabolism and hence, can be utilized for various applications discussed in this review. 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. Bacteria, due to high growth rate, are useful tools in the production of NPs and their application in nanobiotechnology is expanding. However, generation of nanoparticles through chemical method is very tedious; whereas, through microbes it is a fast and an eco-friendly approach. These can further be useful in various industrial, as well as many biomedical applications. The large-scale synthesis of NPs using bacteria is interesting because it does not need any hazardous, toxic, and expensive chemical materials for synthesis and stabilization processes. It seems that by optimizing their action conditions and selecting the best bacteria, these natural nanofactories can be used in the synthesis of stable NPs with well-defined sizes, morphologies, and compositions.

Key words: - Nanoparticles, Microorganisms, Ag-Au Complexes, Nanobiotechnology.

INTRODUCTION:

Bio-nanotechnology is the key functional technology of the 21st century. It is a fusion of biology and nanotechnology based on the principles and chemical pathways of living organisms and refers to the functional applications of biomolecules in nanotechnology (BSilver chi D, 2013). This method of synthesis can be divided into intracellular and extracellular (Ahmad A, et al 2005). A vast array of biological resources are available in nature including plants and plant products, algae, fungi, yeast, bacteria and viruses could all be employed for synthesis of NPs (Thakkar 2010) (Figure 1). Nanotechnology has become the new arena for future technology

which is mainly dependent on nonmetals and semiconductors (Prakash et al, 2011). NPs are produced by chemical and biological methods. Chemical synthesis represents a key approach for the production of materials, which generally involves a number of steps taking place in liquid or gas phase (Yu CH et al 2011). In the chemical method, a nucleus is needed so that the other NPs bind to it. The formation of atoms can be accomplished by using chemical reaction(s) under controlled but mild reaction conditions.

In order to overcome the limitations posed by these conventional methods, there has been a growing demand to develop eco-friendly and rapid synthesis of nanomaterials with the desired size and shape. Consequently, researchers have developed biogenic principles to synthesize nanomaterials by using biological resources such as plants and microorganisms or their products (Schröfel et al. 2011, Prasad et al. 2018). Microbial synthesis of metal nanoparticles can take place either intracellularly or extracellularly (Jain et al, 2011). Intracellular synthesis of nanoparticles requires additional steps such as ultrasound treatment or reactions with suitable detergents to release the synthesized nanoparticles (Kalimuthu et al. 2008). At the same time extracellular biosynthesis is cheap, and it requires simpler downstream processing. This favors large-scale production of silver nanoparticles to explore its potential applications. Because of this, many studies were focused on extracellular methods for the synthesis of metal nanoparticles.

Biosynthesis of nanoparticles bv microorganisms is a green and eco-friendly technology. Diverse microorganisms, both prokarvotes and eukarvotes, are used for synthesis of metallic nanoparticles, viz., silver, gold, platinum, zirconium, palladium, iron, cadmium and metal oxides such as titanium oxide, zinc oxide, etc. (Hasan et al, 2015). These microorganisms include bacteria, actinomycetes, fungi, and algae. The synthesis of nanoparticles may be intracellular or extracellular according to the location of nanoparticles (Hulkoti et al, 2014). Biosynthesis of metal nanoparticles by bacteria is due to their defense mechanism (resistance mechanism), the resistance caused by the bacterial cell on metal ions in the environment is responsible for its nanoparticle synthesis (Saklani et al, 2012) and the cell wall being negatively charged interacts electrostatically with the positively charged metal ions. The enzymes present within the cell wall bio reduce the metal ions to nanoparticles, and finally the smallersized nanoparticles get Diffused of through the cell wall, and the nanoparticles are produced (Mukherjee et al, 2001).

Application of Nanoparticles

been Nanomaterials have in fact used unknowingly for thousands of years; for example, gold nanoparticles that were used to stain drinking glasses also cured certain diseases. Scientists have been progressively able to observe the shape- and size-dependent physiochemical properties of nanoparticles by using advanced techniques. Recently the diverse applications of metal nanoparticles have been explored in biomedical, environmental, and physiochemical areas (Rai et al. 2016, Abbasi et al, 2016). For instance, gold nanoparticles have been applied for the specific delivery of drugs, such as paclitaxel, methotrexate, and doxorubicin. Gold nanoparticles have been also used for tumor detection, angiogenesis, genetic disease and genetic disorder and photo thermal therapy. Iron oxide nanoparticles have been applied for cancer therapy, hyperthermia, drug delivery, tissue cell labeling, repair. targeting and immunoassays, detoxification of biological fluids, responsive drug delivery therapy (Huang et al, 2007). Silver nanoparticles have been used for many antimicrobial purposes, as well as in anticancer, anti-inflammatory, and wound treatment applications.

Copper and palladium nanoparticles have been applied in batteries, polymers, plastic plasmonic wave guides and optical limiting devices. Moreover, they were found to be antimicrobial in nature against many pathogenic microorganisms. Additionally, metal nanoparticles have been used in the spatial analysis of various biomolecules, including several metabolites, peptides, nucleic acids, lipids, fatty acids, glycosphingolipids and drug molecules, to visualize these molecules with higher sensitivity and spatial resolution. In addition, the unique properties of nanoparticles make them well suited for designing



electrochemical sensors and biosensors. For example, nanosensors have been developed for the detection of algal toxins, mycobacteria and mercury present in drinking water (Selid et al, 2009). Researchers also developed nanosensors by utilizing nanomaterials for hormonal regulation and for detecting crop pests, viruses, soil nutrient levels and stress factors.

Biosynthesis of NPs by Microorganisms

production of highly stable, well characterized and safer NPs than the chemical methods, which are usually not environmental friendly, less stable and not easy to scale up. (Figure 2)

Bacterial Mediated synthesis of NPs

Microorganisms like fungi, bacteria, and yeast are of huge interest for NP synthesis; however, the process is threatened by culture contamination, lengthy procedures and less control over NP size. NPs formed by microorganisms can be classified into distinct categories, depending upon the location where they are synthesized.

Yeast and Fungi Mediated synthesis of NPs Extracellular synthesis of Silver NPs using fungi is also a viable alternative, because of their economical large-scale production. Fungal strains are chosen over bacterial species, because of their better tolerance and metalbioaccumulation property. Silver nitrate was transformed into Silver oxide, forming well dispersed NPs, by the action of F. oxysporum metabolically. The introduction of Silver particles to F. oxysporum, brought about the release of nitrate reductase ensuing development of exceedingly stable Silver NPs in solution (Ahmed A-A et al, 2018). Nano-platinum has been incorporated by the culture filtrate of Alternaria alternata. Extracellular biosynthesis of Silver NPs from Silver nitrate solution is reported by the fungus Trichoderma viride (Fayaz M, et al, 2010). Algae Mediated Synthesis of NPs

A diverse group of aquatic microorganisms, algae have been used substantially and reported to synthesize Silver NPs. They vary in size, from microscopic (picoplankton) to macroscopic (Rhodophyta). Silver NPs were synthesized using microalgae *Chaetoceros calcitrans*, *C. salina*, *Isochrysis galbana and Tetraselmis gracilis* (Merin DD et al, 2010). Cyanobacteria and eukaryotic green development genera, for instance, *L. majuscule*, *S. subsalsa*, *R. hieroglyphics*, *C. vulgaris*, *C. prolifera*, *P. pavonica*, *S. Platensis and S. fluitans* can be used as cost effective materials for bio recovery of metal out of the liquid courses of action (Bakir EM et al, 2018).

Methods for NPs synthesis by Microorganisms Extracellular Synthesis

The culture to be tested is grown in suitable media and incubated on orbital shaker at 150 rpm at 37 °C. After incubation the broth is centrifuged, and the supernatant is used for synthesis of nanoparticles. The supernatant is added to separate reaction vessels containing the metal ions in suitable concentrations and incubated for a period of 72 h. The color change of the reaction mixture suggests the presence of nanoparticles in the solution and bioreduction of silver ions in the solution is monitored by sampling the aqueous solution and measuring the absorption spectrum using a UV-visible spectrophotometer. The morphology and uniformity of silver nanoparticles are investigated by X-ray diffraction (XRD) and scanning electron microscopy (SEM), while the interaction between protein and AgNPs is analyzed using Fourier transform infrared spectroscopy (FTIR) (Klaus et al. 1999).

Intracellular Synthesis

The extracellular synthesis of nanoparticles involves trapping the metal ions on the surface of the cells and reducing them in the presence of enzymes, while in intracellular synthesis ions are transported into the microbial cell to form nanoparticles in the presence of enzymes (Kalabegishvili et al, 2012). The culture is grown in liquid media incubated on shaker at optimal temperature. After incubation the flask is kept at



static condition to allow the biomass to settle following which the supernatant is discarded and sterile distilled water is added for washing the cells. The flask is kept steady for 30 min to settle the biomass post which the supernatant is again discarded. This step is repeated for three times. The biomass is then separated from the sterile distilled water by centrifugation for 10 min. The wet biomass is exposed to 50 ml of sterilized aqueous solution of metals at various dilutions and incubated on shaker at suitable temperature till visual color change is observed. The change in color from pale yellow to brownish color indicates the formation of silver nanoparticles, pale yellow to pinkish color indicates the formation of gold nanoparticles, and the formation of whitish vellow to vellow color indicates the formation of manganese and zinc nanoparticles (Waghmare et al, 2011). The biosynthesized nanoparticles have been used in a variety of applications including drug carriers for targeted delivery, cancer treatment, gene therapy and DNA analysis, antibacterial agents, biosensors, separation science, and magnetic resonance imaging (Li et al, 2011).

Types of Bacterial Synthesized NPs

Different bacteria have different mechanisms for the synthesis of NPs. Following are the different types of metallic NPs synthesized from the bacteria. (Table -1)

Silver NPs

The development of rapid and reliable processes for the synthesis of Ag nanomaterials is an important aspect of current nanotechnology research. Reports on the cell-associated biosynthesis of Ag NPs using bacteria have been published, but these methods of synthesis are rather slow. Actually, The formation of Ag NPs by bacteria such as Lactobacillus sp., Corynebacterium strain SH09, Plectonema boryanum UTEX 485 and several other species (Nair B et al, 2002) continued until a rapid method for synthesizing metallic NPs of Ag using the reduction of aqueous Ag (I) ion by the culture Klebsiella supernatants of pneumonia. Escherichia coli and Enterobacter cloacae (Enterobacteriacae) was described by Shahverdi et al. (Shahverdi AR et al, 2007). Lactobacillus spp. can be used for more rapid formation of Ag NPs. After adding the AgNO₃ solution to the biomass at pH 11.5, the suspension started to turn brown within a minute. Jain et al, reported Ag NP production by spore crystal mixture of Bacillus thuringiensis at Room temperature. The spore crystal mixture of Bacillus thuringiensis has been used because it is easily available throughout the year. This was for the first time that any bacterial spore crystal mixture was used for the synthesis of NPs. The development of silver nanostructures synthesized by the bacterial spores was verified by TEM.

Palladium NPs

The ability of a Pd (II) - resistant mixed bacterial culture (enriched from a sludge sample from a municipal waste water treatment plant), mainly composed of Clostridium species, to recover Pd from an aqueous medium, leading to the formation of Pd (0) NPs. (Martins et al, 2013). The bacteria like *Desulfovibrio desulfuricans* and *Shewanella oneidensis* have shown to be the able source for the preparation of Pd NPs. (Bunge et al, 2010). Bioreduction of Pd (II) by S. oneidensis cells resulted in recoveries higher than 90 % of Pd associated with biomass when organic electron donors lactate or formate were used, regardless of the presence of O_2 or NO_3 .

Gold NPs

An economic method was developed to synthesize Ag NPs of smaller then 140nm sizes by using two different microorganisms *Bacilus subtilus* 10833 and *Bacilus amylococus* 1853, problem with this method was lake of reproducibility, time consuming process (48 h) and impurity issue at some extent (Creamer NJ et al, 2007). The first interaction between bacteria and Au has been documented since 1986 and continues to this



day. In general, Au NPs were precipitated intracellularly and/or extracellularly depending on the species using Au (I) or Au (III) Complex. Korobushkina et al reported the extracellular synthesis of Au NPs (100-500 nm) from Au (III) by Bacillus nitrificans at an extremely slow reaction rate for a period of 360 days.

Metal Oxide NPs

Metal oxide nanomaterials have attracted tremendous interest due to their potential applications in catalysis, sensors, energy storage, magnetic data storage and biomedical application. The first report of biosynthesis of ZnO NPs by bacteria was using Aeromonas hydrophila as eco-friendly reducing and capping agent (Javaseelan C et al, 2012). Bharde et al. showed that the bacterium Actinobacter sp. is capable of extracellular synthesis of maghemite (y- Fe2O3) and greigite (Fe3S4) magnetic NPs after 48 h under aerobic and ambient conditions depending on the nature of precursors used. Microbial systems, especially bacteria like Pseudomonas putida strain MnB1, Bacillus sp. SG-1, Leptothrix discophora SP-6 and Leptothrix discophora SS-1 have been used as attractive model systems for the microbial Mn oxidation. However, these strains have rarely been exploited for the synthesis of Mn oxide NPs.

CONCLUSION :

The need of alternate approaches to develop NPs is "green synthesis because the conventional methods of developing NPs are costly and produce very toxic product, so the need of hour is to reduce the risk of toxicity in the environment from the different chemicals used in the physical and chemical methods. In this review we have focused on the biological methods to synthesize NPs in which the microorganisms like bacteria, algae and fungi. Bacteria, due to high growth rate, are useful tools in the production of NPs and their application in nanobiotechnology is expanding. The large-scale synthesis of NPs using bacteria is interesting because it does not need any hazardous, toxic, and expensive chemical materials for synthesis and stabilization processes.

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Figure 1- Different approaches for the synthesis of Nanoparticles





Figure 2 – Different source for the biosynthesis of nanoparticles

Source Bacteria	Type of NPs	Synthesis	Reference
S. oneidensis	Pd	Intracellular	Castro et al. (2014)
Rhodopseudomonas capsulate	Au	Extracellular	Castro et al. (2014)
Cupriavidus necator	Pd	Intracellular	Castro et al. (2014)
Morganella sp.	Ag	Intracellular	Castro et al. (2014)
Bacillus licheniformis	Ag	Intracellular	Castro et al. (2014)
Pseudomonas deceptionensis	Ag	Extracellular	Singh et al. (2016)
Weissella oryzae	Ag	Intracellular	Singh et al. (2016)
Bacillus methylotrophicus	Ag	Extracellular	Singh et al. (2016)
Bacillus subtilis	Co_3O_4	Extracellular	Shim et al. (2010)
Bacillus subtilis	TiO ₂	-	Kirthi et al. (2011)
Bacillus licheniformis	Au	-	Kalishwaralal etal. (2009)
Bacillus licheniformis	Ag	Extracellular	Kalishwaralal etal. (2009)
Escherichia coli	CdS	Intracellular	Sweeney et al. (2004)
Escherichia coli	CdTe	Extracellular	Bao et al. (2010)
Escherichia coli	Au	Intracellular	Du et al. (2007)
Escherichia coli	Pt	-	Attard et al. (2012)
Acinetobacter sp.	Fe3O4	Extracellular	Bharde et al. (2005)
Acinetobacter sp.	Si/SiO2	Extracellular	Singh et al. (2008)
Geobacter sulfurreducens	Pd	Extracellular	Yates et al. (2013)
Klebsiella pneumoniae	Ag	Extracellular	Mokhtari et al. (2009)
Klebsiella pneumoniae	Se	Intracellular	Fesharaki et al. (2010)
Thermoanaerobacter sp.	Cu	Extracellular	Jang et al. (2015)
Thiobacillus thioparus	Fe2O3	-	Elcey et al. (2014)
Azoarcus sp.	Se	Intracellular	Fernández et al. (2016)
Geobacillus sp.	Au	Intracellular	Correa et al. (2013)
Marinobacter Pelagius	Au	-	Sharma et al. (2012)
Myxococcus virescens	Ag	-	Wrótniak et al. (2014)
Streptomyces spp.	Ag	-	Tsibakhashvil (2010)

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Bacillus subtilis	Ag	Extracellular	Saifuddin et al. (2009)
Lactobacillus strains	Ag	Intracellular	Nair and Pradeep (2002)
Plectonema boryanum	Ag	Intracellular	Lengke et al. (2007)
Escherichia coli	CdS	Intracellular	Sweeney et al. (2004)
Clostridium thermoaceticum	CdS	Intracellular	Cunningham (1993)
Acinetobacter spp.	Fe ₂ O ₃	Extracellular	Thakkar et al. (2010)
Pseudomonas aeruginosa	Au	Extracellular	Shah et al. (2015)
Pseudomonas stutzeri	Ag	Intracellular	Shah et al. (2015)
Streptomyces sp.	Ag	Extracellular	Zarina and Nanda (2014)
Bacillus cereus	Ag	Extracellular	Prakash et al. (2011)
Escherichia coli	Ag	-	Kushwaha et al. (2015)
Bacillus flexus	Ag	Extracellular	Priyadarshini (2013)
Lactobacillus spp.	Ag	Extracellular	Ranganath et al. (2012)
Klebsiella pneumoniae	Se	Intracellular	Fesharaki et al. (2010)
Streptomyces sp.	Mn, Zn	Intracellular	Waghmare et al. (2011)