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REVIEW ARTICLE

Yeast-mediated Green Synthesis of Nanoparticles for Biological Applications

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ABSTRACT

Green synthesis of nanoparticles (NPs) using microorganisms and green plants is one of the latest trends in research that has gained momentum, since it is an ecofriendly approach, with lesser toxicity to the environment. Microbial synthesis of metal NPs is a function of the ability of resistance against heavy metal toxicity, carried out by redox enzymes that participate in converting toxic metal ions to inert forms, or by structural proteins that bind metals. Yeasts are non-pathogenic, eukaryotic microorganisms, and available in different strains which have economic importance in breweries and bakeries as well as in food industry as nutrient supplements and their ability to ferment sugar. A novel use of yeasts, explored in recent times, is their potentiality to produce metal (gold, silver, titanium, palladium, and selenium)-NPs, of specific dimensions, either extracellularly or intracellularly, through their reducing enzymes involving different mechanisms. Such synthesized NPs also have tremendous importance as antibacterial, antifungal, antioxidant, and anticancer agents. This review focuses on yeast as a major scaffold for synthesis of different metal NPs and their several biological applications including nanomedicines.

Keywords: Biological application, Green synthesis, Nanomedicine, Nanoparticles, Yeast.

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INTRODUCTION

Nanotechnology has emerged as one of the popular technologies in recent times because of its significant influence in electronics, energy and space industries, pharmaceuticals, biomedical, environmental, and agricultural sectors. This technology is dependent on the synthesis and regulation of nanoparticles (NPs), which are small-sized particles of the dimension range of 0.1–100 nm, exhibiting a range of desirable properties such as near identical strength (e.g., resistance to crushing), active surfaces with unusual catalytic properties, high surface-to-volume ratio and discrete energy levels, and yielding fascinating tailoring of electronic properties.[1] NPs are classified on the basis of their morphology, dimensionality, composition, uniformity, and agglomeration. Nanomaterials can be compared with the size of cellular organelles such as nano-size proteins. They can target the desired sites leaving other cellular machinery uninterfered. With regard to their morphology, they are available in different forms such as nanoprisms, nanobelts, nanorods, nanoplates, nanospheres, nanocubes, and nanotetrapods. The size and shape of NPs dictate their functions, namely, better microbial activity or efficiency of drug delivery in the target site is exhibited by smaller NPs.[2] In addition, the composition of NPs is another important aspect, namely, silver-NPs (Ag-NPs) can be applied in a wide range of areas such as photonics, electronic and optical appliances, information storage, photovoltaics, photonics, catalysts, diagnostics, therapeutics, and food storage.[3] NPs exist either naturally in biological systems such as bacteria, viruses, fungi, insect tentacles, spider silk, and human bones or in inorganic forms in nature such as cement, clay, opals, pigments, and so on.[4] They are quite regularly used, nowadays, in magnetic resonance imaging, tumor-therapy, gene therapy, and as antibacterial agents, biosensors, drug carriers in targeted delivery, nanopesticides, nanofertilizers, and DNA delivery in agriculture.[5] Nanomedicine aims to integrate modern nanotechnology with biotechnology and several molecular tools for curing diseases and repairing tissue damages for improving human health, generating novel drug delivery

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systems, and developing ultrasensitive diagnostic tools such as implantable biomaterials, surgical aids, biosensors, and biopharmaceutical objects.

SYNTHESIS OF NPS: CHEMICAL VIS-À-VIS BIOLOGICAL

A major part of nanoscience research involves synthesis of NPs of different chemical composition, size, morphology, and monodispersity. Several physical, chemical, biological, and hybrid methods are available for NP synthesis, of which physical and chemical methods are more common, since they offer higher production rate in a relatively short time and better control over NP size. However, they suffer from several limitations such as anaerobic conditions, use of toxic reagents for synthesis, high energy requirement and capital investment, lack of stability, being outdated, and production of hazardous wastes that leads to substantial pollution, and human health problems. All these factors together limit their biomedical applications in clinical fields. The chemically-synthesized NPs are less biocompatible.[6] Therefore, there is an increasing need in developing nontoxic, environmentally-safe, ecofriendly, economical, and biocompatible protocols for NP synthesis. The current focus is to go for green synthesis of NPs from natural sources such as micro-organisms, plants, microbial enzymes, polysaccharides, and biodegradable polymers. The biogenic process is not as energy-intensive as the chemical method, eliminates the use of expensive chemicals lowering the production cost and is environment-friendly. Moreover, particles generated by this process have greater specific surface area, higher catalytic reactivity and facilitates improved contact between the enzyme and metal salt.[7] NP catalysts, consisting of non-magnetic and magnetic materials, offer highly available active specific surface and foster microbiological reaction rates. The microorganisms, in particular, hold a special place in NP synthesis, since they can grab the target ions from the surrounding environment and convert them enzymatically into the element metal, either intracellularly (by transporting the ions within the cells and accumulating the NPs in the periplasmic space) or extracellularly (by trapping the metal ions on cell surface, and the reductive enzymes housed in the cell wall or soluble secreted enzymes are extruded out of the cell to cause reduction of metal ions).[8] Various categories of microbes such as magnetotactic bacteria, bacteria with S-layer, actinomycetes, and diatoms, fungi, including yeast and plants have high potentiality in generating nanostructured mineral crystals and metallic NPs with controlled shape, size, composition, and monodispersity of particles.[9] Microbes participate in biogeochemical cycles of metals through processes such as degradation (biocorrosion), decomposition (bioweathering), and precipitation (biomineralization), which are linked with distribution, mobilization, and chemical modification that control metal speciation and ultimate toxicity. Most metal ions are toxic to bacteria which trigger the bacterial defense machinery as a means of survival by undergoing ion bioreduction or the formation of water-insoluble complexes, thereby leading to the accumulation of NPs of silver, gold, platinum, palladium, titanium dioxide, magnetite, and cadmium sulfide. Thus, bacteria are regarded as tiny but active biofactories for NP production.[10] Therefore, NP biosynthesis by microbes is a function of resistance mechanisms against heavy metal stress, whereby toxic heavy metals are converted to non-toxic species which undergoes precipitation to metal clusters of definite shape and nanoscale dimension. Microbial synthesis of NPs has actually merged different disciplines such as microbiology, biotechnology, and nanotechnology into a new field of nanobiotechnology.

YEAST AS THE SOURCE OF NPS

Mycosynthesis of NPs is considered to be more straightforward and easy for stable production of NPs as compared to bacteria. Fungi have several advantages over bacteria showing (i) higher biomass and easy mode of culture, (ii) higher bioaccumulation of metabolites, (iii) higher tolerance to, and uptake capability of metals, and (iv) high wall binding capacity of metals. Some of the enzymes have been found to play significant role in NP synthesis, such as reductase from *Penicillium* sp., nitrate reductase and NADPH-reductase from *Fusarium oxysporum*, along with electron shuttle quinones.[11]

Yeasts according to invention are classified in the kingdom Fungi, phylum Ascomycota, in subphylum *Saccharomycotina*, in the class *Saccharomycetes*, in the order *Saccharomycetales*, in the family *Saccharomycetaceae*, and in the genus *Saccharomyces*,[12] with about 1500 species currently described. *Saccharomyces cerevisiae*, known as "baker's yeast", can reside in diverse environmental niches. *S. cerevisiae* is probably the best studied of all the yeast species in terms of physiology and genetics, and definitely of immense industrial significance because of its involvement in fermentation of bread, beer, or wine. It is an ideal source of different enzymes and vitamins, considered as nutrient supplement and can treat antibiotic-related diarrhea.[13] Rapid growth and easy control of mass production of yeasts using simple nutrient culture medium altogether make yeast as the preferred microorganism for NP synthesis, as compared to other microbes. Yeast cells can

act as a template that induces biomineralization, which is the major mechanism for NP formation.[14]

One of the advantages of using yeast cells as NP-carriers is that simple encapsulation mechanism is possible using only yeast cells, water, and reagents, with no requirement of stabilizers. Yeast cells are, on the one hand, biomacromolecular microparticles having envelope composed of chitin, glycoproteins, and β-glucans, while, on the other hand, they are microcapsules, with plasma membrane helping in encapsulation so that yeast cells can encapsulate polymer NPs.[15,16] Intracellular formation of metal NPs occurs by reduction of metal salts, which can occur in three steps, that is, passive diffusion of metal salts present in aqueous solution into cells, removal of extracellular salts, followed by reduction mediated by transport of reducing reagents into cells.[17] Activation of membrane-bound oxidoreductases, through increasing the pH of the interior of yeast cell, leads to the reduction of metal ions, so that NPs are generated. Because of their strong nucleophilic and redox properties, quinones can also reduce metal ions, converting them to NPs.[18] The main purpose of NP biosynthesis is to eliminate NP toxicity through cellular defense mechanisms through compounds such as phytochelatins (PCs) and glutathione, which can not only bind metal ions but also show unique redox and nucleophilic properties essential for bioreduction of metal ions.[19]

Ag-NPs

Jha *et al.*[20] synthesized Ag-NPs by growing yeast cells in the form of a suspension culture in the presence of 0.025 M $AgNO_3$ solution and finally filtering the sooty gray NPs that were formed extracellularly. It was suggested that such synthesis occurred in the presence of a sulfate or nitrate reductase system, operating in the presence of ATP and NADH or anthraquinone. Alternately, PC binding to Ag forms Ag-PC complex which is transported into vacuole, where the complex is degraded to release Ag-NPs. Moreover, there occurs an electrostatic interaction between Ag^+ and negatively charged COOH groups residing on specific enzymes, proteins, or polypeptides present on the cell wall. Mourato *et al*.[21] produced Ag-NPs with diameter smaller than 20 nm in extremophilic yeast strain from acid mine drainage, grown in the presence of $AgNO_3$ up to 1.5 mM. Zahran *et al.*[22] utilized three environment friendly strains of yeast fungi, namely, *S. cerevisiae*, *Rhodotorula glutinis*, and *Geotrichum candidum* for the extracellular biosynthesis of AgNPs (2.5–20 nm) by direct exposure to $AgNO_3$ solution. Niknejad *et al*.[23] also demonstrated extracellular synthesis of fairly monodispersed, spherical-shaped Ag-NPs, exhibiting high antifungal activity against some fluconazole-susceptible and fluconazole-resistant strains of *Candida albicans*. Ganbarov *et al*.[24] also showed the synthesis of Ag-NPs by yeast strain, BDU-XR1 isolated from spontaneous yogurt used in Azerbaijan. The Ag-NPs, in this case, were detected by absorption wavelength of 410–420 nm in UV–visible spectrophotometer. Li *et al.*[25] produced Ag-NPs both at the cell wall and inside cells, depending on the preparation methods. Glucans on the cell wall functioned as reductive reagents in Tollens' reaction (or silver-mirror reaction) to generate 9 nm Ag-NPs, while 4 nm NPs were produced inside the cells on UV illumination along with importing $AgNO_3$ solution in cytoplasm. Tollens' reaction usually occurs immediately upon addition of reducing reactants, and in this case formed thin films of Ag over the surfaces of yeast cells suspended in the reaction solution. Wilfred and Akin-Osanaiye[26] biosynthesized Ag-NPs which were characterized by UV-visible spectroscopy, showing a prominent absorption peak at 429 nm, attributed to plasmon resonance of Ag-NPs; scanning electron microscopy (SEM) which showed oval-shaped NPs; and Fourier-transform infrared, which revealed notable peaks at 3332.2, 2903.6, and 1636.3 cm, corresponding to the binding of the Ag-NPs to active biomolecules, namely, alcohols and phenols, carboxylic acids, and aromatic amines, respectively. The Ag-NPs, showing antibacterial activity, were found to be stable for 90 days. Very recently, Shu *et al.*[27] produced well-dispersed Ag-NPs of uniform spherical shape and average size of 13.8 nm, using yeast extract as reducing and capping agents. Yeast micelles were formed on mixing Ag^+ solution with yeast extract. The bioreducing biomolecules such as carbohydrates, reductive amino acids, aminobutyric acid, and α-linolenic acid, which are in abundance in yeast extract, have a significant role in the reduction of Ag^+ and of providing favorable stability, monodispersity, and controllable size distribution for the synthesized Ag-NPs. The amino acids located on the surface of Ag-NPs bear net negative charges which can maximize the electrostatically repulsive interactions in alkaline solution, and can, therefore, provide stability to the NPs without causing precipitation for more than a year.[27] Horstmann *et al.*[28] observed several differentially expressed genes through RNAseq-based transcriptome analyses in yeast cells, treated with 20 nm spherical citrate-coated Ag-NPs. Such genes were involved in the formation of cell wall, maintaining cell membrane integrity, biogenesis of ribosomes, processing of rRNA, and mitochondrial functions. The genes whose functions are associated with processing of ribosomal small and large subunits were up regulated, while genes for

maintaining the integrity of cell wall, plasma membrane, and mitochondria were downregulated.

Gold (Au)-NPs

Mourato *et al.*[21] produced Au-NPs with diameter ranging from 30 to 100 nm in extremophilic yeast strain from acid mine drainage, grown in thr presence of $HAuCl_4$ up to 0.09 mM, above which there was a strong inhibitory effect. Promising results of Au-NP production were obtained by Gericke and Pinches[29] with the yeast, *Pichia jadinii* through exposure to $HAuCl_4$. Another tropical marine yeast, *Yarrowia lipolytica* NCIM 3589 synthesized Au-NPs of 15 nm size at pH 7.0 and 9.0, when incubated with $HAuCl_4$ solution, either using constitutively produced NADH and NADH-dependent reductases, or an enzyme (protease) for reducing Au-salts to NPs, which were mostly associated with and organized on cell wall.

Titanium di Oxide (TiO_2)-NPs

Peiris *et al.*[30] synthesized anatase TiO_2-NPs of <12 nm by incubating yeast suspension in $TiCl_3$ using the surface of yeast cells as the site for Ti^{3+} nucleation. The yeast cells remain attached together during nucleation, and a porous lamellar structure containing TiO_2-NPs is formed during high temperature treatment, when yeast cells are removed, spilling over CO_2 gas and forming a unique lamellar mesophase structure. The redox reaction-mediated biosynthesis of TiO_2 NPs occurs by reducing agents such as urease, glucose, and α amylase in the medium. The NPs were characterized by several biophysical techniques such as X-ray diffraction, scanning electron microscopy, transmission electron microscopy, UV-visible spectroscopy, and energy dispersive X-ray analysis studies.

Selenium (Se)-NPs

Faramarzi *et al.*[31] have very recently produced Se-NPs in yeast by adding increasing amounts of sodium selenite in the culture medium, when the Se ions could be effectively reduced in selected amounts, leading to NP formation. However, minimum amount of sodium selenite led to more stable Se-NP formation with minimum particle, due to the higher amounts of reducing and stabilizing agents of the biomass. The fabricated Se-NPs, formed intracellularly, possessed particle size, polydispersity index, and zeta potential ranging from 75 to 709 nm, 0.189 to 0.989, and −7.06 to −10.3 mV, respectively. However, most uniform Se-NPs were formed using maximum amount of selenium salt (25 μg).

Palladium (Pd)-NPs

Li *et al.*[25] synthesized aggregated Pd-NPs of about 11 nm size which was distributed almost evenly in cell envelope and cytoplasm of yeast cells, since Pd ions do not have strong interactions with cellular materials. The first step involved the addition of saturated toluene solution of palladium acetate into the aqueous cell suspension, allowing oil droplets to diffuse into the cells. Palladium acetate external to yeast cells was washed away by filtration using ethanol. At the second step, hydrazine hydrate was added to the yeast cell suspension to reduce Pd ions.

EXTRACTION OF NPS

NPs produced intracellularly or extracellularly should be purified from the yeast strain. Centrifugation or dialysis from media enables extraction of extracellularly-produced NPs. In case of intracellularly-produced NPs, yeast strains should be lyzed chemically or physically.[32] Care should be taken so that there is no NP degradation or aggregation and yield is minimized. The extraction procedure should be carefully standardized to derive pure, stable, and highly effective NPs for various applications.

BIOLOGICAL APPLICATIONS OF NPS FROM YEAST

Metal NPs are considered as a promising and low-cost antimicrobial agent. Yeast cells carrying NPs can easily activate the phagocytosis process. Se-NPs, known as new generation drug, are important in medicines due to their antioxidant, anti-inflammatory, antimicrobial, and anticancer properties. The antioxidant activity of the Se-NPs was also established, being maximum at the lowest concentration of sodium selenite (5 μg) in the culture medium.[31] The patented sulfur-free Se-NP from yeast has been applied in medical field and fermentation industries. Both Se-NPs and Ag-NPs are considered as substitution agents for antibiotics. Shu *et al.*[27] observed that the yeast-synthesized Ag-NPs, when applied with ampicillin, exhibited significant antibacterial activity in a concentration-dependent manner by reversing the resistance property in ampicillin-resistant *Escherichia coli* cells. The surface coatings on Ag-NPs enhanced the affinity toward bacterial membrane by increasing the cell wall permeability. The configuration of the peptidoglycan was changed as a result of its interaction with Ag-NPs that finally led to the apoptosis of bacterial cell. Thus, it was predicted that these monodispersed Ag-NPs could have a promising role in the disinfection of multidrug-resistant bacterial strains. The Ag-NPs are also components of medical devices such as catheters, implants, and prostheses. The TiO_2-NPs were found to exhibit strong antimicrobial activity against Gram-positive bacteria (*Staphylococcus aureus*) and *C. albicans*, when compared

to Gram-negative bacteria (*Pseudomonas aeruginosa* and *E. coli*), both in the presence and absence of sunlight exposure.[30] Nowadays, the NPs also find application in the field of fermentation industry. Yeast strains which are used in fermentation processes could produce NPs as an effective defense against pathogenic microorganisms or contaminating bacterial strains which jeopardize the fermentation process, for example, Ag-NPs produced by yeast can control contaminating bacteria such as *Lactobacillus fermentum*, *Lactobacillus brevis*, and *Pediococcus pentosaceus* in a fuel ethanol fermentation production process. Pd-NPs are the catalysts for the Suzuki reactions. Yusop *et al.*[33] succeeded in importing Pd-NPs within HeLa cells for undergoing a Suzuki reaction. Au-NPs have been applied in angiogenesis and tumor detection, as well as for the specific delivery of drugs, such as paclitaxel, methotrexate, and doxorubicin.

CONCLUSION AND FUTURE PERSPECTIVES

Although yeasts have been shown to serve as ideal vehicles for production of NPs of different metals, the number of studies conducted in this field is quite inadequate. The biological production of NPs by yeast is still in its infant stage. Much works need to be done to improve the efficiency of synthesis, reduce the production time and optimize the control of particle size, morphology and monodispersity, by standardization of different parameters such as target NP, growth medium, synthesis conditions, pH, substrate concentrations, and temperature. The stability of the synthesized NPs for a longer duration is to be ensured. Understanding the synthesis mechanism at the cellular and molecular level, especially with regard to genome and proteome analyses is also necessary. The yet-uncharacterized functional reducing agents and enzymes released by yeasts to convert the harmful ions into non-harmful particles may have a definite role in NP synthesis. More focus is demanded with regard to extracellular synthesis of NPs over intracellular ones, since additional operations such as chemical reactions and ultrasound operations are eliminated in the former. Moreover, NP synthesis by yeasts or any microbes is still at the laboratory scale so that scale-up of NP production should be emphasized to meet the demand for practical applications. Another much-awaited usability of yeast-synthesized NPs which remain to be fully investigated is their potentiality in fermentation industry. This will actually mark the importance of nanotechnology in fermentation industry, in addition to their applications in medical sectors, which is mostly examined by research groups. Synthesizing NPs by biological means, which has the advantages of non-toxicity, reproducibility in production, easy scaling-up, and well-defined morphology, will continue to become a common trend in NP production in the forthcoming years.

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