Mycosynthesis of Inorganic Nanoparticles and their Evaluation in Catalysis and Nano-fertilization

SYNOPSIS

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The impact of nanotechnology is increasing at tremendous pace due to its remarkable potential. Nanoparticles are considered as the protagonist of the nanotechnology revolution. Amid nanoparticles, inorganic nanoparticles are of special interest due to their unique and tuneable properties. In recent years, the surging demand of nanoparticles has boosted unprecedented expansion of research for the development of high yielding and sustainable synthesis methods which can deliver nanomaterials with desired characteristics. Unlike the well-established physico-chemical methods which have various limitations, biological methods inspired by mimicking natural biomineralization processes have great potential for nanoparticle synthesis. Of special interest, microorganisms isolated from metal rich regions which develop resistance mechanism for their survival, can be exploited for synthesis of metal or metal oxide nanoparticles. Among various metal tolerant microorganisms, fungi represents a favourable natural machinery due to its large and most diverse secretome making it highly anticipated for on-cell/ extracellular synthesis of nanoparticles. Following this rationale and the hypothesis that "well adapted fungi isolated from native metal rich soil conditions can be a better source for bio-inspired synthesis of metal nanoparticles", the present work serves as the initial step in development of an eco-friendly method for synthesis of inorganic nanoparticles by selecting a promising fungal isolate(s).

The potential applications of nanoparticles have revolutionized various commercially important sectors. However, there is a clear possibility to utilize nanoparticles in the less explored areas of catalysis and agriculture. Moreover, studies comparing the application potential of myco-synthesized nanoparticles to that of chemically synthesized nanopartciles are still unexplored.

The current thesis entitled "**Mycosynthesis of Inorganic Nanoparticles and their Evaluation in Catalysis and Nano-fertilization**" deals with the synthesis of two important inorganic nanoparticles viz. iron oxide nanoparticles (IONPs) and gold nanoparticles (Au NPs). Moreover, the efficacy of as-synthesized Au NPs and IONPs has been explored in the field of catalysis and nano-fertilization. The thus obtained nanotechnology derived food has been tested for its potential toxicity, if any. The thesis has been systematically separated in two halves. Initial part (Chapters II-IV) deals with synthesis of nanoparticles and remaining part (Chapters V- VII) discusses the applications of these nanoparticles. The details of individual chapter are as follows:

Chapter I: General Introduction

The first chapter is an introduction to the research work presented in this thesis and gives a brief overview of nanotechnology. A brief discussion on nanotechnology and nanoparticles is provided. Briefly, unique and interesting physico-chemical properties observed in nanometer regime are discussed particularly with respect to IONPs and Au NPs. Further, an account of potential applications of nanoparticles has been described with special emphasis on catalysis and agricultural scenarios. The gap in existing research is discussed and the thesis objectives are presented.

Chapter II. Isolation, Identification and Metal Tolerance Profile of Fungi Isolated From Metal Rich Region

The second chapter discusses the steps followed for selection of metal tolerant fungi having potential for nanoparticle synthesis. The selection for efficient fungal isolate(s) for the nanoparticle synthesis was accomplished by isolating fungi from rhizosperic soil samples collected from metal rich regions of Rajasthan and Goa. A total of 11 morphologically distinct fungi were isolated and identified at molecular level. All the fungal isolates were screened for their metal tolerance ability against iron and gold ions. *Aspergillus japonicus* AJP01 was found to be the most tolerant fungus against iron while *Cladosporium oxysporum* AJP03 and *Lichtheimia ramosa* AJP11 exhibited highest metal tolerance against gold ions. In addition to iron, *Aspergillus japonicus* AJP01 also showed reasonably high gold metal tolerance. Hence, these fungal isolates were further utilized as potential candidates for respective nanoparticle synthesis.

Chapter III. Extracellular Synthesis, Characterization and Mechanistic Insights of Mycogenic Iron Oxide Nanoparticles

The third chapter of the thesis describes the extracellular synthesis of IONPs using *Aspergillus japonicus* AJP01. The fungal isolate demonstrated its ability to hydrolyze the precursor salt solution, a mixture of iron cyanide complexes, under ambient conditions. Hydrolysis of these complexes released ferric and ferrous ions, which underwent protein mediated coprecipitation and controlled nucleation resulting in the formation of IONPs. TEM, SAED, EDS and XRD analysis confirmed the mycosynthesis of IONPs. The synthesized particles were cubic in shape with a size range of 60-70 nm with crystal structure corresponding to magnetite. SEM analysis revealed the absence of IONPs on fungal biomass surface, indicating extracellular nature of synthesis. FTIR spectroscopy confirmed the presence of proteins on as-synthesised IONPs, which may confer their stability. On the basis of present findings, a probable mechanism for synthesis of IONPs is proposed. The simplicity and versatility of the present approach can be utilized for the synthesis of other nanomaterials.

Chapter IV. Biosynthesis of Gold Nanoparticles

The fourth chapter of the thesis deals with the potential of gold metal tolerant fungal isolates towards on-cell and extracellular synthesis of Au NPs. Among the various fungal isolates screened to test their ability of Au NPs synthesis, *Aspergillus japonicus* AJP01 was found to synthesize spherical Au NPs on its surface. Extracellular synthesis of Au NPs was achieved using two indigenous fungi, *Cladosporium oxysporum* AJP03 and *Lichtheimia ramosa* AJP11. The as-synthesized nanoparticles were characterized by standard techniques to confirm their size, shape, distribution and crystallinity. A series of experiments were conducted to study the effect of various process parameters on particle size and yield during extracellular synthesis. Investigations were also performed to elucidate the mechanism involved in extracellular synthesis of Au NPs by *Lichtheimia ramosa* AJP11. The obtained results provide appreciable evidence supporting the role of extracellular proteins in the reduction of gold ions followed by their nucleation and stabilization. The present work serves as the initial step in development of "nano-factories" for efficient nanoparticle synthesis.

Chapter V. Catalytic Applications of Gold Nanoparticles

The fifth chapter of thesis briefly describes the catalytic applications of Au NPs. Supported and unsupported Au NPs synthesized using fungi (discussed in chapter IV) were used as heterogeneous and homogenous catalyst respectively. The catalytic efficiency of fungal biomass supported Au NPs was tested by catalysing the A^3 coupling reactions to synthesize propargylamine. The as-synthesized nanoparticle-fungal hybrid was also found to catalyse sodium borohydride mediated reduction reaction of 4-nitrophenol and hexacyanoferrate (III). Free and unsupported protein capped Au NPs were employed for the reduction of environmentally hazardous organic dyes viz., rhodamine B, bismark brown, methyl orange and methylene blue. Owing to the presence of surface proteins, mycosynthesized Au NPs exhibited excellent dye degradation efficiency.

Chapter VI. Assessment of Iron Oxide Nanoparticle as Potential Nano-fertilizer

The sixth chapter of thesis discusses the assessment of IONPs as a source of micronutrient in plants. For this, a systematic comparison of various iron forms viz., ionic, bulk and nanoparticles (chemically and biologically synthesised) at different metal concentrations was made using wheat and spinach as test plants. Seed germination and a sub pilot experimental field study using foliar spray approach was conducted to determine the effect of various iron forms on wheat plant growth and grain yield. In addition, pot study was carried out to determine the effect of foliar spray of various iron forms on growth and biomass production of spinach plants. The results suggested that IONPs are not toxic to the tested plant species and instead if used at lower optimum concentration, can be beneficial.

Chapter VII. Toxicological Assessment of Nanotechnology Derived Food

This seventh chapter discusses assessment of toxicological potential of nanotechnology derived food in rodents. In a 90-day sub-acute repeated dose oral toxicity experiment, the effect of magnetite nanoparticles sprayed wheat on female Wistar rats was evaluated. The toxicity of test substance was compared with other experimental variables. Essential checkpoints like monitoring of health status, food and water intake by animals were carried out throughout the feeding trial followed by measurement of haematological and clinical biochemistry parameters. Examination of gross necroscopy and histopathology of selected tissues at terminal sacrifice were also performed. The obtained results show no toxicity of nanotechnology derived food under experimental conditions.

Conclusions

Overall, the present research work revolves around mycosynthesis and application of inorganic nanoparticles. Commercially important metals (iron and gold) were chosen for synthesis of nanoparticles using biological approaches, particularly by exploiting the potential of metal tolerant soil fungi. Attempts were made to increase the efficiency of the synthesis protocol by optimizing various reaction parameters and understanding the involved molecular mechanism. Application of Au NPs was explored in the field of catalysis for fine chemical synthesis and dye remediation. IONPs were assessed to test their potential as nano-fertilizer by conducting sub-pilot field and pot scale experiments using wheat and spinach plants, respectively. The as-obtained nanotechnology derive food (wheat) was evaluated for its toxicological potential in rodent model on the basis of recommended guidelines.

Future scope of work

The simplicity and versatility of present “biosynthesis” approach for nanoparticle synthesis allows its utilization for production of various other metal and metal oxide nanoparticles. However, biological approaches are still practised at laboratory level and have not been profitably scaled-up to the industrial level. Therefore, attempts are required to make the process more efficient and cost effective. These objectives can be fulfilled not only by selecting suitable organisms but also by understanding the factors that affect yield and properties of nanoparticles. In present study, the preliminary knowledge obtained for the mechanism involved in the synthesis of IONPs and Au NPs has just opened the door for a whole new domain of sophisticated molecular (genetic and proteomic) alterations which if successful will help in achieving mass scale production of nanoparticles with desired properties.

The excellent efficiency of mycosynthesized Au NPs in catalysis as observed in the present work has marked the importance of protein cap on nanoparticle surface. The efficacy of these protein capped nanoparticles can also be utilized for various other applications like molecular and chemical sensing, remediation and biomedical therapies. Further, these nanoparticles can be immobilized in suitable matrices for their repeated use in applications involving harsh conditions.

In the present work, use of IONPs as potential nano-fertilizers has been shown with experimental evidences. However, much more work at molecular level is still required to prove this notion. Experiments involving similar plants (wheat and spinach) at different geographical locations as well as using other plant species shall be carried out to ascertain the beneficial role of IONPs as well as other particles as nano-fertilizers.

The risk assessment of nanotechnology/ nano-fertilizers in agriculture is significantly important as it directly affects humans. Nano-fertilizers enable nanoparticles to enter in the food chain allowing their distribution in every organism related to the food chain. Also, it is most likely that different types of nanoparticles may exert varying toxicological response. Thus, it is very essential to execute further *in vivo* studies on oral exposure of nanotechnology derived food and check for any consequent toxicity as risk assessment of nanoparticles has to be performed on a case-by-case basis.

LIST OF PUBLICATIONS

From the thesis

Research Papers

- **Bhargava, A.**, Jain, N., Barathi L., M., Akhtar, M.S., Yun, Y.S. and Panwar, J. 2014. Synthesis, characterization and mechanistic insights of mycogenic iron oxide nanoparticles. *Journal of Nanoparticle Research*, 15, 2031-2042.
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From additional work during Ph.D. tenure

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Patent

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