Biosynthesis and Characterization of Zinc Nanoparticles Using Strains of Pseudomonas andActinobacteria

ABSTRACT

Research was carried out at the Green Nanotechnology Laboratory, University of Agricultural Sciences, Dharwad, Karnataka, with a specific emphasis on the biosynthesis of zinc nanoparticles using Pseudomonas and Actinobacteria. Collection and screening of Pseudomonas and Actinobacterial isolates were done at Microbial Genetics Laboratory, Department of Agricultural Microbiology, UAS, Dharwad. The ZnNPswere biosynthesized and characterized through UV-Visible spectroscopy, Particle Size Analyzer (PSA), Scanning Electron Microscope (SEM), Energy-dispersive X-ray spectroscopy (EDS), X-Ray Diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR). The ZnNPs synthesized through Pseudomonas(AUDP209) and Actinobacteria(AUDT636)isolates exhibited peak values at 365 and 374 nm, with average sizes of 48.7 nm and 54.9 nm, respectively.SEM images revealed the spherical shape of the biosynthesized ZnNPs, while EDX confirmed the zinc ions presence in the biosynthesized samples and the crystalline structure of the ZnNPswas confirmed by XRD.FTIR spectroscopy confirmed the organic compounds present in the microbial extracts responsible for the capping and stabilizing agents for biosynthesis of ZnNPs.

Key Words:Biosynthesis, Zn-Nanoparticles, Pseudomonas, Actinobacteria, Characterization

1. INTRODUCTION

"Nanotechnologies can contribute to a new technological revolution in agriculture. Several problems with conventional biofortification could potentially resolved by nanotechnology" (Shakiba*et al*. 2020). "It is possible to produce nanofertilizers using nanomaterials because of their high surface-to-volume ratio, gradual and controlled release at target places, and other characteristics"(Feregrino-Perez *et al.,* 2018). "The encapsulation of nutrients with nanomaterials results in efficient nutrient absorption by plants, due to the gradual or controlled release of NPs and simple passage through biological barriers by NPs entering the plant vascular system" (De La Torre-Roche *et al*., 2020)."In comparison to conventional fertilisers, long-term delivery of plants *via*nanofertilizers enables enhanced crop growth. As nanofertilizers are added in small amounts, these also prevent soil from becoming burdened with the by-products of chemical fertilisers and reduce the environmental hazards "(Silva *et al.*, 2018). "Unlike chemical fertilisers, nanofertilizers can be synthesized and applied based on the crop's nutritional needs and the status of the soil's nutrient levels using biosensors" (Kah *et al.,* 2018). "Additionally, nanofertilizers, as opposed to chemical fertilisers, allow for high mineral bioavailability to plants due to their smaller size, greater reactivity, and higher surface area" (Liu and Lal, 2015).

Zinc is essential for the synthesis and activation of several hormones (auxin and gibberellin) and enzymes that enhance seed germination and seedling growth. Additionally, Zn plays a important role in biosynthesis of proteins, carbohydrates, lipids, and nucleic acids in plants (Sturikova*et al.*, 2018). The ZnNPs are among the top three most manufactured and used engineered NPs (Zhang *et al.*, 2015). The ZnNPs, one of the best source for preventing Zn deficiency and enhancing crop quality and productivity (Kisan*et al.*, 2015). Zn can serve as a cofactor for P-solubilizing enzymes like phosphatase and phytase, and nano-ZnO boosted their activity in the soil (Raliya*et al.,* 2015). Zn NPs have an impact on plant metabolism at the molecular level by activating antioxidants and reductases, as well as influencing the synthesis of plant hormones (Timilsina and Chen, 2021).

Synthesis of nanoparticles involves a variety of biological, physical and chemical methods; however, only the biological methods are safe and environment friendly. Traditional chemical methods, which could be hazardous to the environment, are being replaced with biological systems, which are preferred for NP synthesis (Bogas *et al.,* 2022). Microorganisms (fungi, viruses, bacteria, yeast, and actinomycetes) can be thought of as biological nanofactories since they can take in metal ions from the environment and change them into elemental compounds. These processes can take place as either intracellular or extracellular events, and as a result, NPs produced by bacteria are categorised as either intracellular or extracellular NPs. Additionally, bacteria are great producers of NPs it can create a variety of secondary metabolites (Rani *et al.,* 2022).Keeping the above facts in view, the present investigation is planned to study the biosynthesis of zinc nanoparticles using two different microbial strains and characterized through UV-Vis Spectroscopy, PSA, SEM, EDS, XRD and FTIR.

2. MATERIAL AND METHODS

2.1 Biosynthesis and characterization of ZnNPs

The Pseudomonas and Actinobacterial isolates were collected from culture collection maintained at Microbial Genetics Laboratory, Department of Microbiology, UAS, Dharwad. The collected isolates were spotted and sub cultured in King's B and Starch casein agar media (pH 7) and incubated for 2 and 7 days, respectively, in incubator at 30 ºC.Pseudomonas isolates: AUDP204, AUDP207, AUDP209, AUDP218, AUDP219, AUDP223, AUDP264 and AUDP265.Actinobacteria isolates: AUDT504, AUDT535, AUDT574, AUDT592, AUDT606, AUDT608, AUDT620 and AUDT636.Out of eight Pseudomonas and

Actinobacterialstrains AUDP209 and AUDT636 performed very well in terms of both germination percentage and seedling vigour index and was selected for synthesis of zinc nanoparticles and used in further experiments.

2.1.1 Biosynthesis of ZnNPsusing Pseudomonas strain AUDP209

Pseudomonas strain AUDP 209 bacterial cells were inoculated into Erlenmeyer flasks, each containing 100 ml of King's B broth. The flasks were then placed in an incubator-shaker, set at 30°Cand 150 rpm for two days to allow bacterial growth. At the end of each incubation period, the liquid medium was centrifuged at 10,000 rpm for 15 minutes to remove cell debris. Subsequently, 60 ml of a zinc acetate dihydrate stock solution was introduced into the 20 ml supernatant solution. The flasks were then placed in an incubator-shaker at 150 rpm for 48 hours at 30 ºC. Then, the pH of the culture broth was adjusted to 10-11 by adding NaOH 1%. After the addition of NaOH, the flasks were kept in autoclave for 30 minutes.After that, the pellet was collected by centrifugation process at 10000 rpm for 5 minutes, which was subsequently washed three times with deionized water. The collected pellet was then dried in a hot air oven, and the dried powder was utilized for characterization.

2.1.2 Biosynthesis of ZnNPsusing actinobacterial strain AUDT636

Actinobacterial strain AUDT 636 bacterial cells were inoculated into Erlenmeyer flasks, each containing 100 ml of starch casein broth. The flasks were then placed in an incubator-shaker, set at 30°Cand 150 rpm for seven days to allow bacterial growth. At the end of each incubation period, the liquid medium was centrifuged at 10,000 rpm for 15 minutes to remove cell debris.Subsequently, 85 ml of a zinc acetate dihydrate stock solution (1500 ppm) was introduced into the 15 ml supernatant solution. The flasks were then placed in an incubator-shaker at 150 rpm for 72 hours at 30 ºC. Then, the pH of the culture broth was adjusted to 10-11 by adding NaOH 1%. After the addition of NaOH, the flasks were kept in autoclave for 30 minutes.After that, the pellet was collected by centrifugation process at 10000 rpm for 5 minutes, which was subsequently washed three times with deionized water. The collected pellet was then dried in a hot air oven, and the dried powder was utilized for characterization.

2.2 Characterization of biosynthesized ZnNPs

2.2.1 UV-Visible spectroscopy

The confirmation of nanoparticle reduction was achieved through UV-Visible spectrophotometer analysis at scanning wavelengths ranging from 200 to 700 nm, based on their optical absorbance peak. As the nanoparticles' size decreases, the band gap increases, leading to an elevation in optical absorbance compared to