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Zinc solubilizing plant growth promoting microbes produce zinc

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Research Article

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Abstract

At ICAR-CRIDA in Hyderabad, India, strains of *Pseudomonas*, *Bacillus*, and *Azospirillum* that were capable of supporting plant development were tested for their capacity to solubilise zinc. The capacity of effective zinc solubilising microbes to generate nanoscale zinc particles was assessed. Particle size, Zeta potential, and functional groups were measured in the nanoparticles derived from the cell-free culture filtrates of these strains. Particle distribution and scanning electron microscopy (SEM) examination verified the presence of Zn nanoparticles in the bacterial culture filtrate. The majority of nanoparticles' characteristics rely on their size. Zinc nanoparticles with sizes ranging from 52.0 to 106.0 nm were found to be spherical in form. To determine the stability of the particles, the zeta potential of the Zn nanoparticles was calculated. The zinc nanoparticles' great stability and dispersion were shown by the observed zeta potentials, which ranged from -14.5 mV to +179.10 mV. The function of protein functional groups in the production of zinc nanoparticles was shown by FTIR peaks at various wave numbers. These findings show that microorganisms of *Azospirillum*, *Pseudomonas*, and *Bacillus* that promote plant development and solubilise zinc may produce zinc nanoparticles in an environmentally friendly manner.

Key words: *Pseudomonas*, *Bacillus*, *Azospirillum*, FTIR, Zeta Potential, ZnO, ZnCO₃, and zinc nanoparticles.

Introduction

One of the essential elements needed for the best possible development of plants and animals, including humans, is zinc (Zn). Immune system deficiencies, mental retardation, and general ill health are all consequences of Zn deficiency. The issue of zinc insufficiency has drawn more attention in recent years and, together with vitamin A deficiency, seems to be the most dangerous micronutrient shortfall, particularly in children. It is essential for biochemical interactions in plants, and a lack of it causes a noticeable decrease in plant height as well as the development of whitish-brown areas that eventually become necrotic (Singh 1999). More than half of India's agro-ecosystems lack zinc, particularly the Indo-Gangetic plains in the states of Punjab and Uttar Pradesh. Over the last forty years, the nation's overall zinc deficiency has risen from 42% to 49%, and by 2025, it is predicted to reach 63% (Singh 2009). Loss of membrane integrity, decreased metabolite production, and heat stress sensitivity are the ways in which plants react to zinc shortage (Bhupinder et al., 2005). Zinc is added to plants exogenously by chemical fertilisers such as zinc sulphate, which then changes (96–99%) into various insoluble forms based on soil types and physico-chemical processes. In addition to being costly, external micronutrient supplementation causes an undesired buildup of these minerals in the soil in permanent forms. Numerous studies have been conducted on the solubilisation of zinc by bacteria and fungi (Suseelendra Desai et al. 2012). A fertilizer's specific surface area rose and the number of particles per unit weight of applied zinc increased as a consequence of decreased particle size (Mortvedt 1992). Mg, Zn, Fe, and P nanoparticles are structural elements of polysaccharides, chlorophyll, and enzymes like phytase and phosphatases. In order to transport chemicals to plant systems and tissues in a regulated manner, these nanoparticles may be attached to agrochemicals or other compounds. It is anticipated that the best candidates for usage as a zinc fertiliser in plants would be nanoparticles with a high surface area

and a tiny size (Prasad et al. 2012). *Pseudomonas stutzeri* was used to provide the first evidence of nanoparticle production, and the nanoparticles were deposited on the cell membrane (Klaus et al. 1999). Nanoparticles have since been discovered to be produced by *Lactobacillus* strains (Ahmad et al. 2003), *Fusarium oxysporum* (Minaian et al. 2008), *Bacillus subtilis* (Nair et al. 2002), *Corynebacterium* sp. (Saifuddin et al. 2009), *Klebsiella pneumonia* (Zhang et al. 2005), and *Escherichia coli* (Gurunathan et al. 2009). However, it has been shown that *E. coli* contributes to both intracellular and extracellular nanoparticle formation (Gurunathan et al. 2009). However, relatively few papers propose that microorganisms that promote plant development can synthesise zinc nanoparticles. In this work, we reveal that rhizobacteria that solubilise zinc may create zinc nanoparticles.

Materials and Methods:

The ICAR-Central Research Institute for Dryland Agriculture's culture bank in Hyderabad provided the bacterial strains utilised in this investigation. Five effective zinc-solubilizing strains—*Bacillus* B116, *Pseudomonas* P29, *Pseudomonas* P33, and *Azospirillum* 20—were chosen for nanoparticle investigation based on prior qualitative and quantitative screening [6]. With an initial population of 2×10^8 CFU/ml, all isolates were cultivated in minimal salt medium supplemented with 0.1% ZnO and kept at room temperature in an orbital shaking incubator running at 180 rpm. After 72 hours of inoculation, the culture media was centrifuged, and the supernatant was collected. Using a nanoparticle analyser, the supernatant was examined for the presence of nanoparticles. A cuvette containing ten millilitres of the supernatant was inserted into the Horiba Nanoparticula 100 nanoparticle analyser. For every sample, three duplicates were kept. The mean of the three readings of each sample was used to determine the particle size. A particle size analyser (Horiba Nanoparticula 100, Japan) was used to record the zeta potentials. This device makes advantage of the Dynamic Light Scattering (DLS) phenomena. In order to ascertain the stability profile of tiny particles in solution, the sample holder temperature was kept at 25°C. Under a scanning electron microscope, nanoscale zinc particles were seen. Ten microlitres of the solution were applied to the metal slide in order to see the zinc nanoparticles. To totally eliminate moisture, the metal slide was kept in a desiccator for the whole night. A gold sputtering of about 1 nm was done over the suspension after the slide was taken out of the desiccator. The bio-transformed product found in the cell-free filtrate was examined for Fourier Transform Infra Red (FTIR) spectroscopic measurement. The mid-IR region of the FTIR spectrum (Bruker Tensor 27, USA) was obtained between 400 and 4000 wavenumber cm^{-1} . Using a micropipette, a drop of the sample solution was carefully applied to the potassium bromide crystals, and the transmittance (%) mode of the spectrum was recorded.

Results and Discussion;

Pseudomonas, *Bacillus*, and *Azospirillum* strains that were shown to solubilise ZnO and ZnCO_3 were developed and tested in the field. It was discovered that these strains produced Zn nanoparticles in vitro when grown in MS broth supplemented with ZnO or ZnCO_3 in an effort to comprehend their mechanism of Zn solubilisation. The existence of zinc nanoparticles is confirmed by the particle size distribution (PSD) dynamic light scattering technique findings. The Z-Average particle size in each treatment was 10.5 to 14.6 times lower than the control. The P29 treatment showed the smallest particle size. Nevertheless, there was no discernible difference between the treatments (Table 1).

Table 1. Particle Z-Average size and Poly dispersity index of Zn nanoparticles produced by different bacterial strains in ZnO incorporated broth

Strains	Z-Average particle size (nm)	Poly dispersity Index
B116	593 .0	0.60
P 29	428.8	0.51
P 33	482.0	0.37
Asp 20	539.0	0.48
Control	6279	1.00

Additional information on the size, shape, morphology, and distribution of nanoparticles was revealed by an electron microscopy (SEM) analysis picture. The spherical or approximately spherical nanoparticles were evenly scattered in the SEM micrographs. The Zn nanoparticles were between 106 and 52 nm in size. Under control, the size was

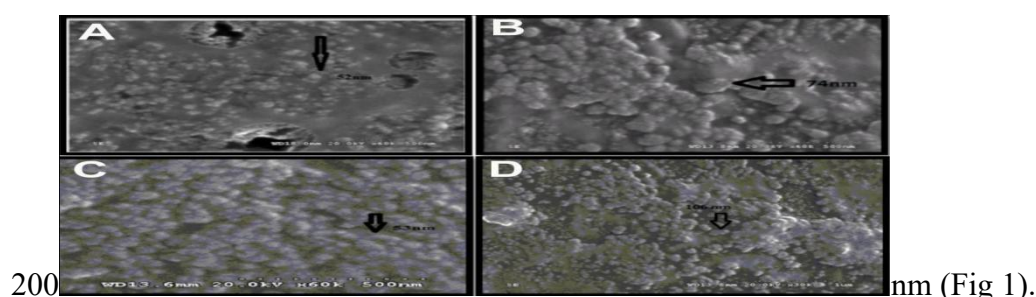


Fig 1.

SEM image of Nanoparticles of zn produced by a) P 29 (52nm) b) P 33 (74nm) c) Asp 20 (53nm) d) B 116 (106nm) in media amended with 1ppm ZnO.

The strains differed in their ability to produce zinc nanoparticles of various sizes. In contrast to the control, which generated particles with a size of 5200 nm, *Pseudomonas putida* P29 produced the smallest zinc nanoparticles, measuring 52 nm, followed by Asp20 and B116, which produced big particles, measuring 106 nm, according to particle size distribution and SEM examination. The fact that there was no discernible difference between any of the treatments and the control suggests that all organisms created nanoparticles within a certain range. The Zeta potential was evaluated using the nano-particle analyser because the Zeta Potential's magnitude (-200 mV to +200 mV) indicates the colloidal system's potential stability. Zn nanoparticles made by B116, P33, P29, and Asp20 had zeta potentials of +179.1 mV, -14.5 mV, +0.5 mV, and 0.5 mV, respectively, which amply

demonstrated their stability.

(Fig 2).

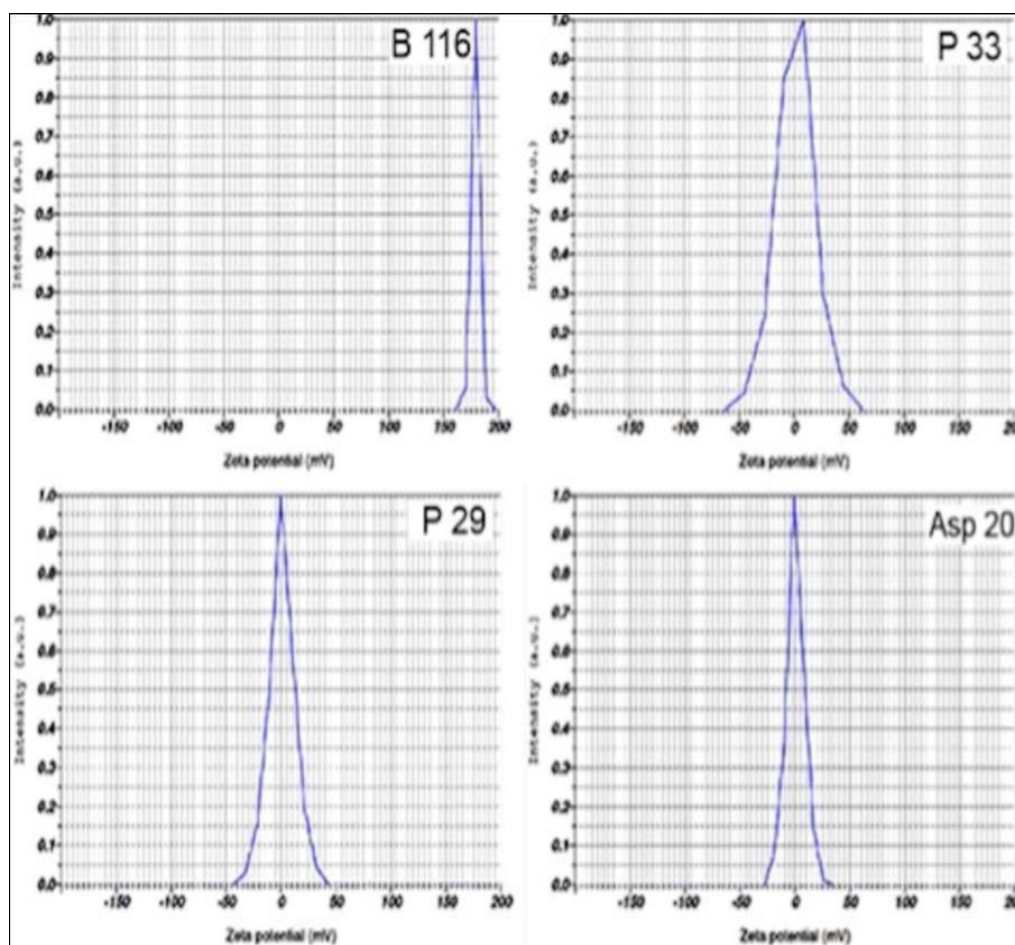


Fig 2. Zeta potential of Zn nanoparticles produced by B116 (+179.1 mV), P33 (-14.5 mV), P29 (+0.5 mV), Asp 20 (+0.8 mV), and in ZnO amended MS medium

The interactions between Zn and bioactive compounds that facilitate the creation and

stabilisation of Zn nanoparticles were identified using FTIR analysis of the cell-free culture filtrates of ZnO supplemented MS media (Fig 3).

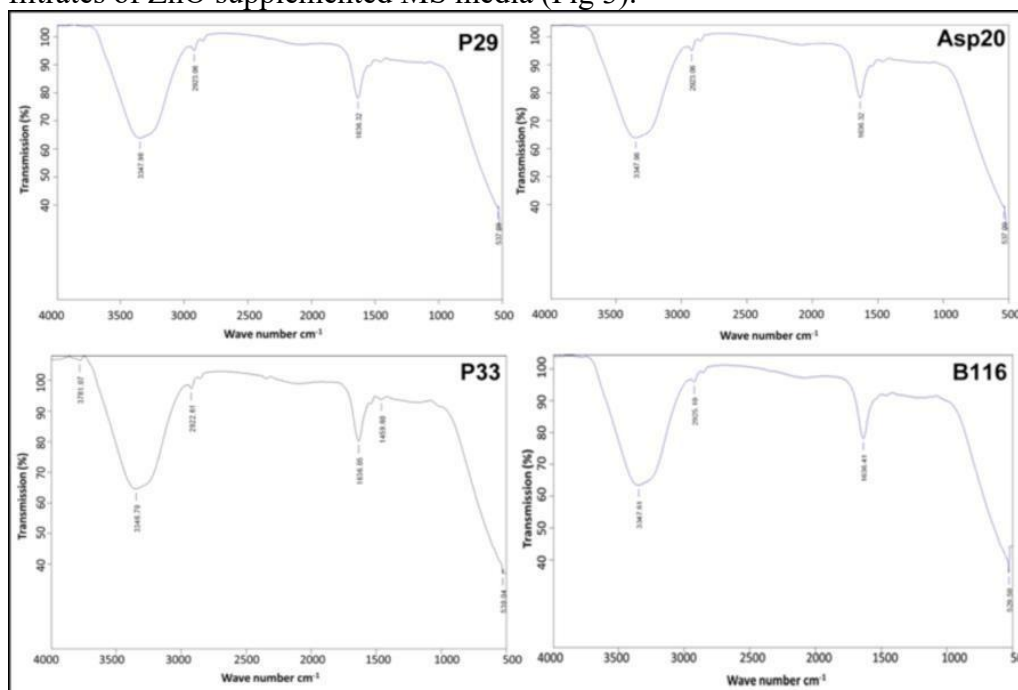


Fig 3. IR spectrum of cell-free culture filtrates of ZnO amended MS medium inoculated with different strains of bacteria

ZnO-amended MS medium cell-free extracts' FTIR spectra revealed notable peaks at wave numbers cm^{-1} of 3346, 2923, 1636, 1459, and 538. The NH stretch of amides is responsible for the strong wide wave at 3346 cm^{-1} . The stretch vibration of the protein's main and secondary amides is the wave seen at 2923 cm^{-1} . The band seen at 1636 cm^{-1} is attributed to amides' bending vibrational mode. The protein's amino and amino-methyl stretching groups are shown by the peaks at 1459. The stretching vibrations of Zn nanoparticles are represented by the peak seen at 529 cm^{-1} . Although the precise process by which biological agents create nanoparticles has not yet been determined, it has been proposed that several biomolecules are in charge of this process. According to Honary et al. (2012), the mechanism of extracellular production of nanoparticles is a reductase-mediated process that produces nanoparticles by bio-reducing metal ions. The FTIR spectrum of the nanoparticles used in this investigation revealed the presence of many chemical groups, including an amide. The bending vibrational mode of amides, an amide I band, was detected at 1636 wavecm^{-1} . This result was further supported by the stretching band of amide at 3346 wavecm^{-1} . Because of the carbonyl stretch in proteins, amide I is represented by the 1636 wave cm^{-1} . Functional groups including amide linkages and $-\text{COO}-$ were seen in the FTIR spectrum, perhaps between the synthesised Zn nanoparticles and protein amino acid residues. According to Honary et al. (2012), similar findings corroborated the connections between proteins and nanoparticles during biosynthesis. The stability and mobility of nanoparticles in colloidal solutions, as well as their adsorption, aggregation, and deposition, are known to be significantly influenced by their surface characteristics. The degree of

repulsion between neighbouring and similarly charged particles in a dispersion is indicated by zeta potential, which can be linked to the stability of colloidal dispersions. High zeta potential values indicate high stability, and stability also decreases as particle size decreases (Vielkind et al. 2013). The particle size of 106.0 nm generated by B116 had the highest zeta potential of +179.10 mV, suggesting optimal stability. However, according to Sonika et al. (2015), nanoparticles having zeta potential values higher than +25 mV and -25 mV are usually stable. Therefore, even if they are just 52 nm in size, it is possible that the nanoparticles made with lower zeta potentials, such +0.5mV, may not be stable. Additionally, poor stability nanoparticles often agglomerate in the medium due to a variety of causes. Ionic strength, pH, surface charge, and charge density all affect how metal oxide nanoparticles aggregate in aqueous solutions (Domingos et al., 2009; Guzman et al. 2006). Such aggregation was also seen in the current investigation when the zeta potential was low. The P33 treatment had the lowest polydispersity index, whereas B116 had the highest (Table 1). All of the treatments, however, did not substantially vary from the control. The generated zinc nanoparticles are both mono- and poly-dispersed in nature, as shown by the particle size distribution results, which varied from 0.37 to 0.60 across treatments. When researching the extracellular synthesis of ZnO nanoparticles, an average particle Z-Average size of 46.61 nm and a polydispersity index of 0.552 were also regarded as mono-dispersed in nature because samples with very broad distribution sizes typically have >0.70 polydispersity index (Nagarajan & Kuppusamy 2011).

Conclusion

The current work unequivocally demonstrates that strains of *Pseudomonas*, *Bacillus*, and *Azospirillum* spp. that solubilise zinc create zinc nanoparticles. These findings pave the way for further research into how plants absorb nutrients in relation to the function of rhizomicroorganisms that promote plant development. To better understand the dynamics of crop nutrition, similar trials might be developed for additional microbe-mediated plant nutrient supplementation research.

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