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Role of salt precursors for the synthesis of zinc oxide nanoparticles and in imparting variable antimicrobial activity

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Abstract: Synthesis of nanoparticles (NPs) having unique potentials and properties is of great importance in nanotechnology. The NP synthesis techniques may include the wet chemistry to microbial incubation reduction methods. This work reports generation of ZnO NPs by identical preparation including incubation of different zinc salts i.e. zinc acetate, zinc chloride and zinc sulphate as precursors with cell free extracts of *Bacillus circulans* MTCC 7906 (Bc7906) and *Pleurotus florida* (Pf). The synthesized NPs exhibited variation in their absorption peaks in UV-Vis spectra which appeared at 275 nm, 325 nm and 375 nm with *P. florida* for the three salt precursors respectively while the Bc7906 generated ZnO NPs showed peaks between 300-350 nm. A variation in ZnO NP morphology ranged from 50 to 120 nm in size and spherical, oval, cylindrical to trigonal anisotropic in shape by transmission EM. Further, the rough and corrugated surface topography of ZnO NPs was observed in Scanning EM. The % weight for Zn element surface composition as recorded by SEM-EDS was observed to be highest for zinc acetate (2.34%) and zinc sulphate (7.54 %) on microbial synthesis from Bc7906 and Pf respectively. The antimicrobial potential of the synthesized ZnO NPs on human pathogenic and plant beneficial bacteria was tested and it was observed to be highest for microbially synthesized ZnO NPs using zinc acetate (15 mm) and zinc sulphate (14 mm) as salt precursors @ 10 ppm. This is the first report on differential antimicrobial behavior of ZnO NPs on human pathogenic and plant beneficial microbes.

Keywords: Microscopy, Nanoparticles, UV-Vis spectroscopy, Zinc oxide

INTRODUCTION

The metal/ metal oxide NPs exhibit novel and improved physical, chemical and biological properties due to their larger surface area to volume ratios, nanoscale dimensions and random motion. These NPs may also exhibit potential antimicrobial properties particularly the ZnO NPs which hold considerable attention due to their unique catalytic antibacterial, antifungal and antiviral activities (Shrivastava *et al*., 2015). The NP synthesis protocols are as varied as the synthesized NPs spanning over physical, chemical and microbial methods. However, the microbial approach is most preferred due to involvement of less amount and types of chemicals and hence being more eco-friendly. The reports of synthesis of ZnO NPs using microbes like bacteria *Aeromonas hydrophila* (Jayaseelan *et al.*, 2012), fungal filtrates of *Aspergillus terreus* (Baskar *et al.*, 2013) and yeast *Candida diversa* strain JA1 (Chauhan *et al.*, 2014) are known. The incubation with cell free or cell based extracts of microbes are especially recommended over higher eukaryotes as it renders ease of handling and easy genetic manipulation. The morphology and size of NPs can be controlled by altering the reaction time and by using different biotemplates (extracellular biomacromolecules from bacteria, fungi and yeast) and metal salts. The synthesis of spherical shaped ZnO NPs with size ranges between 20 -40 nm using zinc acetate and spherobacterium *Streptococcus thermophilus* has been reported (Zhou *et al*., 2007). Similarly, another report on the use of probiotic bacteria *Lactobacillus plantarum* VITESO7 with zinc sulphate as metal salt precursor resulted in synthesis of spherical 7-17 nm ZnO NPs (Selvarajan and Mohanasrinivasan, 2013). Fungal filtrates of *Alternaria alternata* synthesized ZnO NPs of average size of 75 nm by using zinc sulphate solution (Sarkar *et al*., 2014). These NPs have a wide range of applications in pharmaceutics, paint and cosmetics industry. Moreover, ZnO is approved by FDA (Food and drug administration) to be used in sunscreens due to its ability to reflect the skin deteriorating UV radiation (Jin *et al*., 2009). The ZnO NPs have also been used in various textile, food and packaging industries owing to their antimicrobial properties probably because of production of reactive oxygen species on the surface of NPs ZnO possesses antibacterial activity both in microscale as well as nanoscale formulations (Nair *et al*., 2009).

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It also exhibits antisporicidal activity against high temperature and pressure resistant spores (Sawai, 2003). The concentrations may vary from 3 mmol/l to 12 mmol/l or higher concentrations to partially and completely inhibit the spore germination (Liu *et al*., 2009). The antimicrobial activity of ZnO NPs is higher particularly against water-borne pathogens namely *Shigella dysenteriae*, *Salmonella typhi*, *Vibrio cholera* and *Escherichia coli* on doping with other semiconductor elements like cobalt (Oves *et al.*, 2015). The ZnO NPs also possess antifungal activity against *Aspergillus niger*, *A. fumigatus and A. aculeatus* (Baskar *et al.*, 2013).The present study aims to compare the use of different salt precursors and two microbial extracellular extracts for the synthesis of ZnO NPs. The antimicrobial activity of the synthesized NPs was then assessed against both pathogenic and soil beneficial microorganisms.

MATERIALS AND METHODS

Chemicals: Analytical grade zinc acetate dihydrate, zinc chloride and zinc sulphate of Hi-Media Laboratory chemicals Pvt. Ltd, Mumbai, India and the commercial zinc oxide nanoparticles were from Sisco Research Laboratory Pvt. Ltd., Mumbai, India were used.

Microbial cultures: Microbial cultures, *Bacillus circulans* MTCC 7906, *Escherichia coli, Pleurotus florida, Pseudomonas fluorescence, Rhizobium and Staphylococcus aurens* were obtained from Department of Microbiology, Punjab Agricultural University, Ludhiana, Punjab, India. *Enterobacter cloacae* MTCC 509 was procured from IMTECH, Chandigarh, India.

Microbial synthesis of zinc oxide nanoparticles: The Bc7906 and Pf cultures were inoculated in nutrient broth and potato dextrose broth respectively and were incubated at $28^0C\pm2^0C$ for 48 hours in BOD incubator at 120 rpm. For the synthesis of ZnO NPs, 200 ml of cell free extract was incubated with 0.01 M zinc acetate, zinc chloride and zinc sulphate respectively at $28^0C\pm2^0C$ for 24 hours in shaking and static conditions till complete conversion of zinc acetate, zinc chloride and zinc sulphate into ZnO NPs. Simultaneously, the culture (cell free extracts of *Bacillus* and *Pleurotus* alone) and chemical (zinc salt(s) in distill water) controls were also run along with the reaction flasks.

Characterization of zinc oxide nanoparticles

UV-Vis Spectroscopy: The samples were subjected to absorption spectroscopy with wavelength ranging from 200 to 800 nm using Elico SL 218 Double Beam UV-Vis Spectrophotometer. The absorbance was plotted against the wavelength to observe the characteristic peaks for synthesized nanoparticles.

Scanning electron microscopy (SEM) and Energy dispersive spectroscopy (EDS): Morphology of synthesized nanoparticles were characterized using SEM (Hitachi S-3400N) at 15 kV accelerating voltage in secondary electron imaging mode. Sample was prepared by placing 10µl of the sample on the stub which was vacuum dried overnight and later sputter coated with gold Ion sputter coater (model Hitachi E-1010). The surface elemental composition in percentage of atom and weight present on the sample was analyzed by EDS (Thermo Noran) attached to SEM by using higher probe current (50 to 60 microamperes and aperture 2).

Transmission electron microscopy (TEM): The nanoparticle size and structure measurements were performed on TEM (Hitachi H-7650) at 80 kV acceleration voltage in high contrast imaging mode. All the samples were sonicated for 20 minutes in a bath sonicator (Model- Equitron) to obtain even dispersion of the particles. Samples were drop casted by placing 10 µl of the sample suspension on carbon/ formvar coated copper grid. The drop casted grids were air dried before imaging.

Antibacterial assay

Agar well assay (Modified Kirby-Bauer's method, 1966): Microbially synthesized nanoparticles were tested against three human pathogenic bacteria namely *E. coli*, *E. cloacae* MTCC 509 and *S. aureus* and three plant beneficial soil bacteria namely, *Rhizobium sp.*, *Azotobacter* and *Ps. fluorescens*. Modified agar well diffusion method was used to check the antibacterial activity of the nanoparticles. Lawn cultures of the test microbes were prepared by spreading 100 µl of freshly prepared log phase bacterial culture on the petriplates containing nutrient agar. Uniform wells of 0.5 mm diameter were made using a gel borer. Concentrations of 2, 5, 8 and 10 ppm of the synthesized nanoparticles were added in these wells. Zinc acetate (0.01M), zinc chloride (0.01 M), zinc sulphate (0.01 M), bulk zinc oxide (0.01 M), cell free extract of Bc7906 and Pf were used as control. The commercially available antibiotics were used as control i.e. Ampicillin, Chloroamphenicol, Penicillin G, Streptomycin, Sulphatriad and Tetracycline for Gram-positive microorganism and Ampicillin, Gentamicin, Nalidixic acid, Cefalexin and Co-Trimoxazol for Gram-negative microorganisms. The plates were incubated at 37 ^0C for 24 hours for human pathogenic cultures and at 25 ^0C for soil beneficial microorganism. After 24 hours, zone of inhibition (mm) were measured for each sample.

Viable cell count of cultures using dilution spread plate technique (Throndsen, 1978): All the bacterial cultures were grown in nutrient broth medium for 24-48 hours under shaking conditions (160 rpm on a rotary shaker) for attaining log phase growth measured at 600 nm on spectrophotometer using filter 3. Three concentrations of NPs i.e. 5 ppm, 8 ppm and 10 ppm were mixed in 50 ml nutrient broth in culture flasks and were ultra-sonicated for 15 minutes each. After sonication, 1 ml of bacterial culture aliquot was inoculated in all the flasks and was mixed properly. Viable cell count on nutrient agar was enumerated at 0, 2, 4, 6 and 8 hours after application respectively.

Statistical analysis: All the experiments were performed in triplicates. The mean values were calculated and reported as the mean ± standard error represented in graphs. One way ANOVA using SPSS 20 software was conducted in agar well assay and two way ANOVA was done to determine the interaction among treatments and hours of incubation.

RESULTS AND DISCUSSION

UV-Vis spectroscopy: The microbially synthesized NPs under shaking as well as static conditions showed characteristic absorption maxima between 300-400 nm and 250-350 nm for Bc7906 and Pf extracts respectively (Figs. 1 and 2). As ZnO NPs generally have

Table 1. Antimicrobial activity of Zinc oxide nanoparticles microbially synthesized against human pathogenic and soil beneficial microorganisms.

			Zone of inhibition (mm)					
Type of synthesis	**Type of salt precursor used**	**Conc. of NPs used**	***S. aureus***	***E. coli***	***E. cloacae* MTCC 509**	***Rhizobium***	***Ps. fluorecens***	***Azotobacter***
Bacterial synthesis	Zinc acetate	2 ppm	11	-	-	-	-	-
		5 ppm	11	-	-	-	-	-
		8 ppm	14	-	12	-	-	12
		10 ppm	15	-	14	11	-	13
	$ZnCl_2$	2 ppm	9	-	-	-	-	-
		5 ppm	9	-	-	-	-	-
		8 ppm	10	-	-	-	-	-
		10 ppm	11	-	8	10	-	-
	$ZnSO_4$	2 ppm	-	-	-	-	-	-
		5 ppm	-	-	-	-	-	-
		8 ppm	10	-	-	-	-	-
		10 ppm	14	-	-	-	-	-
Fungal synthesis	Zinc acetate	2 ppm	-	-	-	-	-	-
		5 ppm	-	-	-	-	-	-
		8 ppm	-	-	-	-	-	-
		10 ppm	13	-	-	-	-	12
	$ZnCl_2$	2 ppm	-	-	-	-	-	-
		5 ppm	-	-	-	-	-	-
		8 ppm	-	-	-	-	-	-
		10 ppm	-	-	-	-	-	-
	$ZnSO_4$	2 ppm	-	-	-	-	-	-
		5 ppm	-	-	-	-	-	-
		8 ppm	10	-	10	9	-	18
		10 ppm	13	-	12	10	-	21
Commercial ZnO NP		2 ppm	16	8	9	10	11	22
		5 ppm	20	9	11	14	12	24
		8 ppm	22	9	13	15	13	25
		10 ppm	24	11	14	17	15	28
C.D @5 %			2.06	NS	2.56	3.44	NS	4.34

Table 2. Zone of inhibition against test microorganisms for specific antibiotics.

Antibiotics (concentration in mcg)	**Zone of inhibition (mm)**					
Hexa G-minus	***S. aureus***	***E. cloacae* MTCC 509**	***E. coli***	***Rhizobium***	***Ps. fluorescens***	***Azotobacter***
Ampicillin (10 mcg)	-	-	-	-	32	35
Gentamicin (10 mcg)	21	12	19	25	30	36
Nalidixic acid (30 mcg)	-	27	-	-	14	35
Chloroamphenicol (30 mcg)	24	26	30	12	-	32
Cefalexin (30 mcg)	-	-	-	-	-	35
Co-Trimoxazole (25 mcg)	-	20	25	-	11	36
Hexa G-plus						
Ampicillin (10 mcg)	-	-	-	10	-	32
Penicillin G (0.6 mcg)	-	-	-	-	-	35
Streptomycin (10 mcg)	-	-	-	-	23	31
Sulphatriad (30 mcg)	-	-	14	22	12	35
Tetracycline (25 mcg)	20	23	29	33	17	36
C.D @ 5 %	2.31	2.91	2.90	3.70	2.89	NS

Table 3. Antimicrobial activity of chemical controls used against pathogenic microorganisms and soil beneficial microorganisms.

	Zone of inhibition (mm)		
Controls	***E.coli***	***E. cloacae* MTCC 509**	***Ps. fluorescens***
ZnO Bulk 2ppm	-	-	-
5ppm	-	-	-
8 ppm	-	-	-
10 ppm	-	-	-
$ZnCl_2$ 2ppm	-	-	-
5ppm	-	-	-
8 ppm	-	-	-
10 ppm	-	-	-
Zinc acetate 2 ppm	-	10	-
5 ppm	-	11	14
8 ppm	13	15	14
10 ppm	13	15	14
$ZnSO_4$ 2 ppm	-	-	-
5 ppm	-	-	-
8 ppm	22	-	-
10 ppm	22	-	-
C.D @ 5 %	2.31	2.31	NS

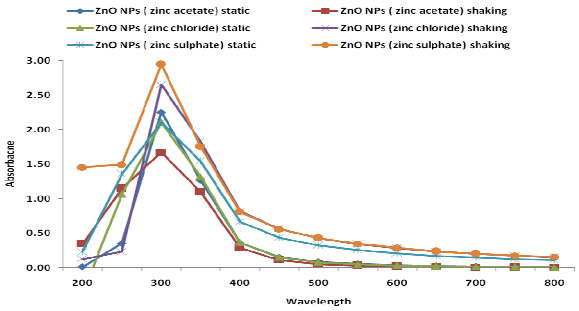


Fig. 1. *UV-vis spectra of ZnO NPs synthesized from cell free extract of Bc7906.*

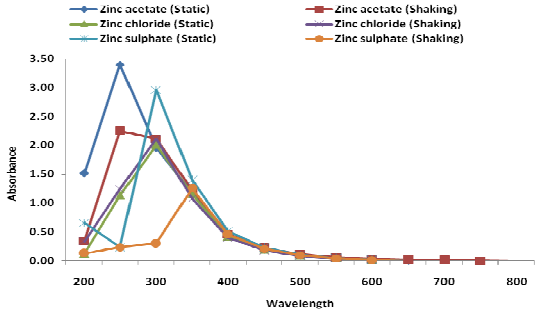


Fig. 2. *UV-vis spectra of ZnO NPs synthesized from cell free extract of Pf.*

peaks ranging from 200-300 nm wavelength (Kumar and Rani, 2013), the absorption maxima for our NPs have shown a red shift. Similar peaks at about 340 nm and 374 nm have been reported for *A. terrus* (Baskar *et al.*, 2013) and *A. hydrophila* synthesized ZnO NPs (Jayaseelan *et al.*, 2012).

Scanning electron microscopy (SEM): The ZnO NPs obtained by keeping microbial cell extracts with precursor salts under shaking conditions showed uniform size and shapes. Hence, these NPs were furthered used for the later characterization and antimicrobial activity tests. The SEM studies revealed synthesis of small size ZnO NPs existing in both homogenously monodispersed as well as in aggregated form (Fig.3). The SEM -EDS analysis of the different ZnO NPs in X-ray spectrum mode showed characteristic peaks for Zinc (L (alpha) = 1.012 *keV*, *Kα = 8.630 keV and Kβ = 9.570 keV*). It also gave % atom and % weight for the presence of zinc element which was highest (2.34 %) in zinc acetate precursor derived ZnO NPs as compared to other two salt precursors incubated with Bc7906 culture (Fig.4). While the Pf derived ZnO NPs from zinc sulphate as precursor had highest percent weight for Zn element (7.54 %) on their surface. Similar observation of signal peak for the presence of Zn element on the surface of ZnO NPs has been reported by Jayaseelan *et al*. (2012).

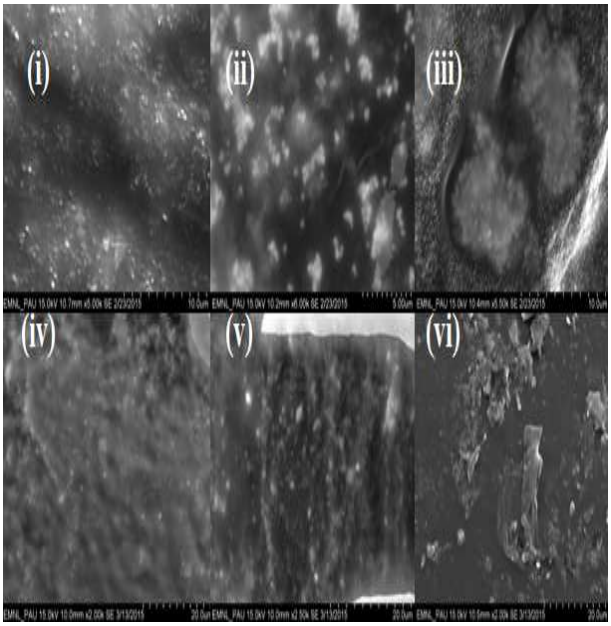


Fig. 3. *SEM images of ZnO nanoparticles synthesized from cell free extract of Bc7906 using (i) zinc acetate (ii) zinc chloride (iii) zinc sulphate as salt precursors. ZnO nanoparticles synthesized from cell free extract of Pf using (iv) Zinc acetate (v) zinc chloride (vi) zinc sulphate as salt precursors.*

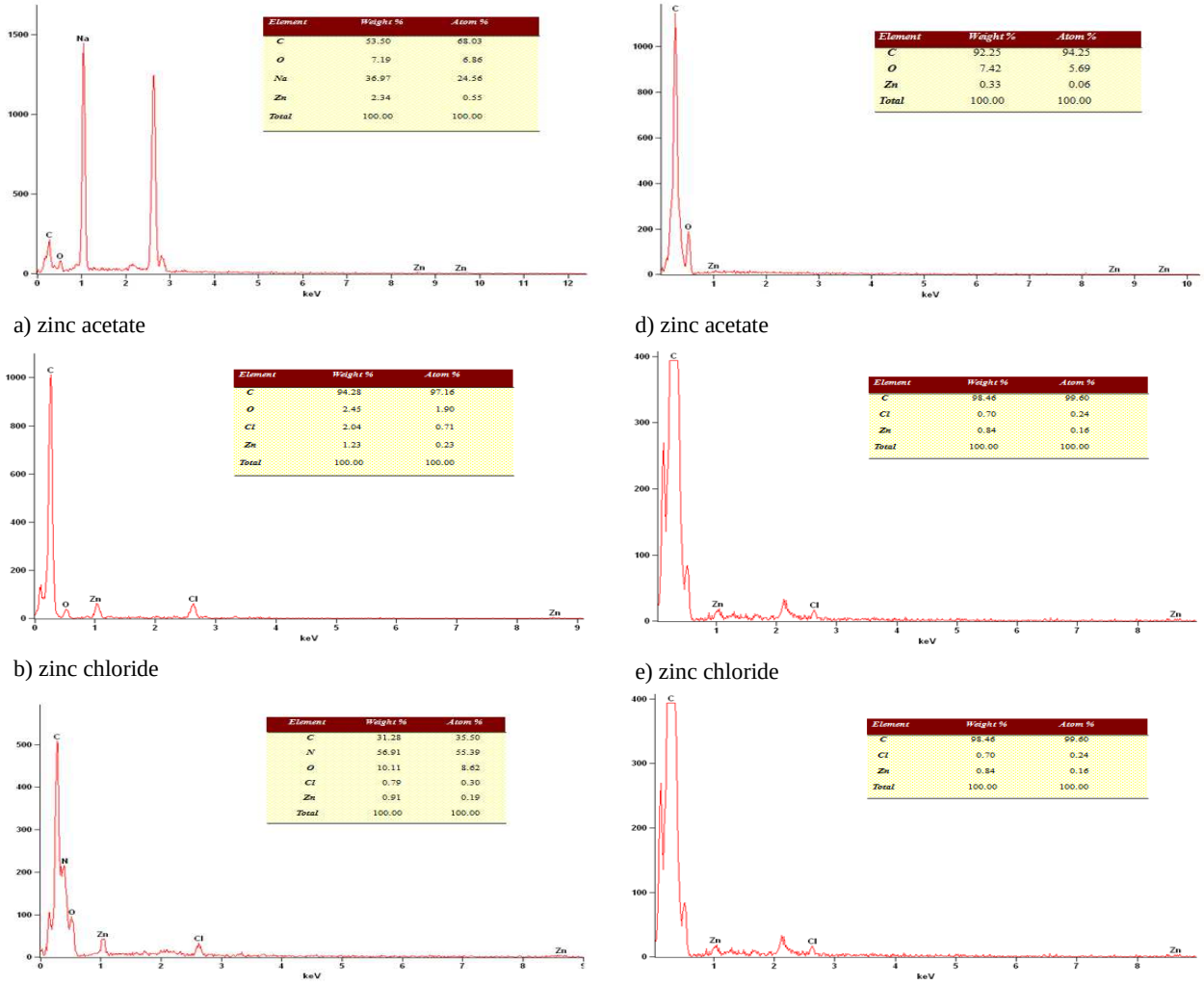


Fig. 4. *SEM-EDS spectral peaks of ZnO NPs synthesized from cell free extract of Bc7906 using (a) zinc acetate (b) zinc chloride (c) zinc sulphate as salt precursors. ZnO nanoparticles synthesized from cell free extract of Pf using (d) Zinc acetate (e) zinc chloride (f) zinc sulphate as salt precursors.*

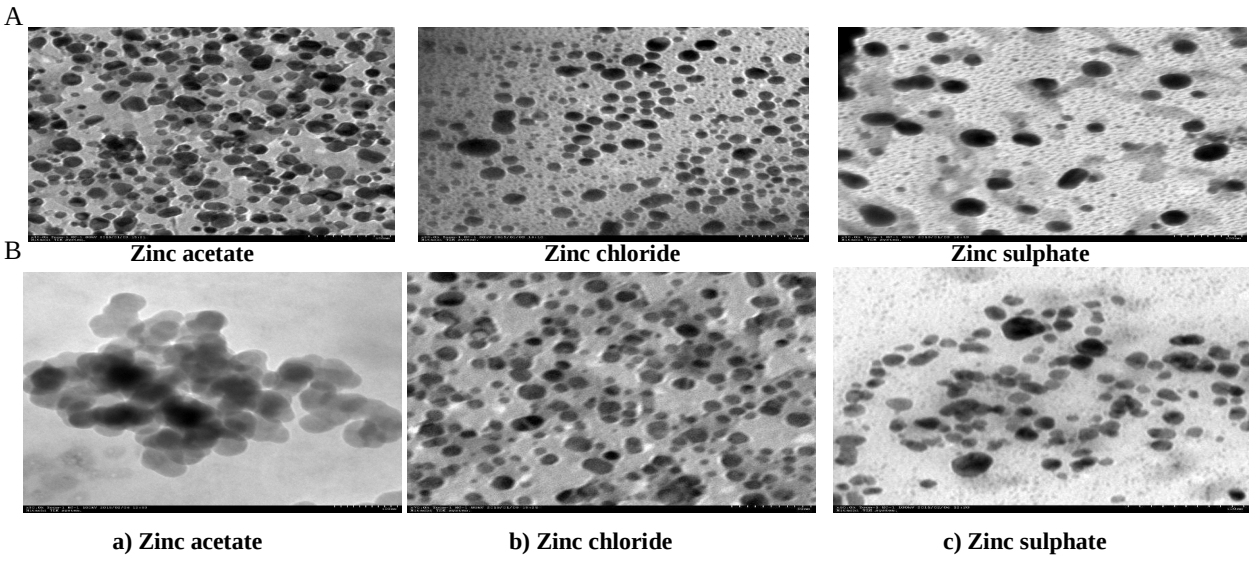
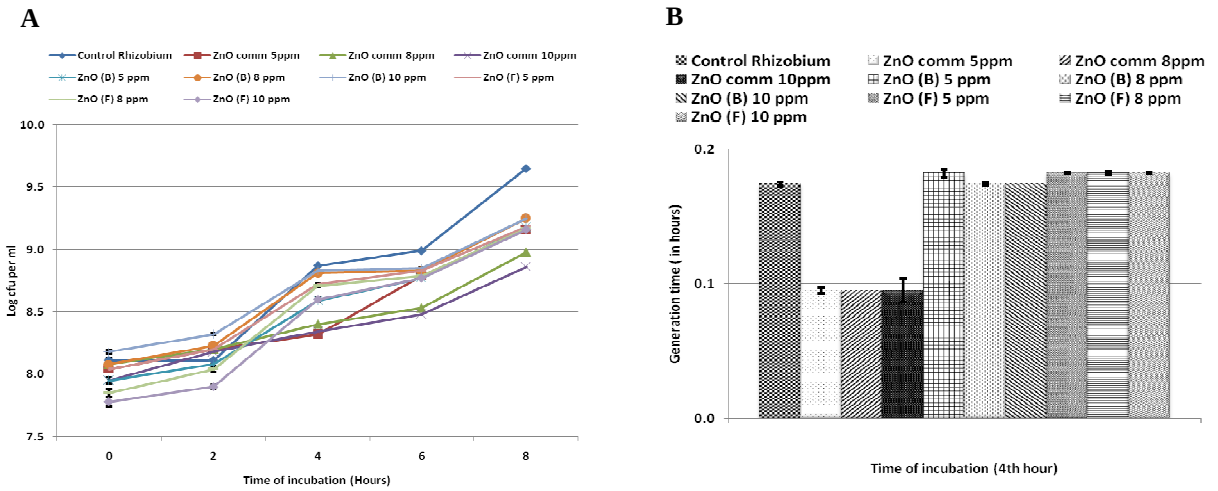
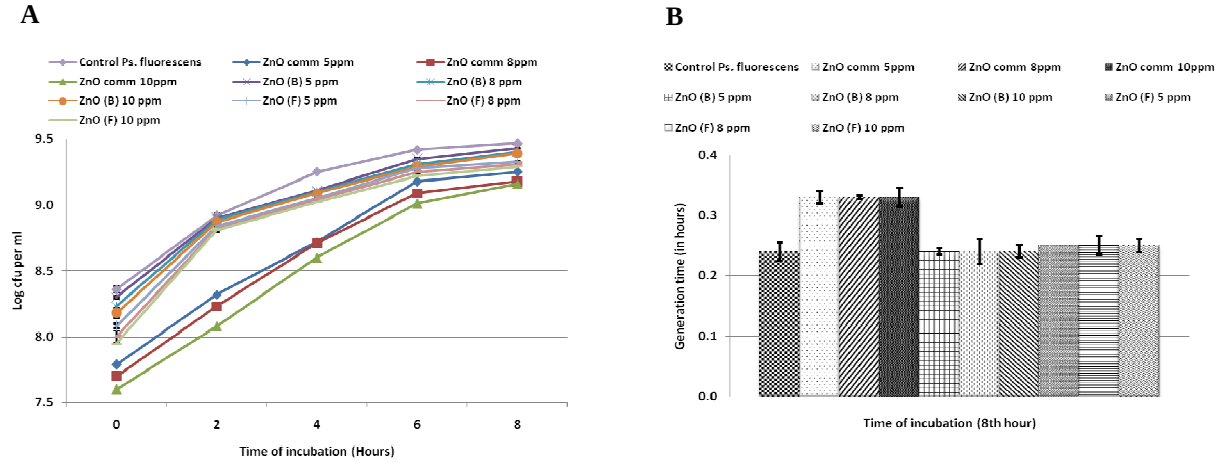


Fig.5. *Transmission electron micrographs of zinc oxide nanoparticles A) synthesized from Bc7906 and B) Pf*



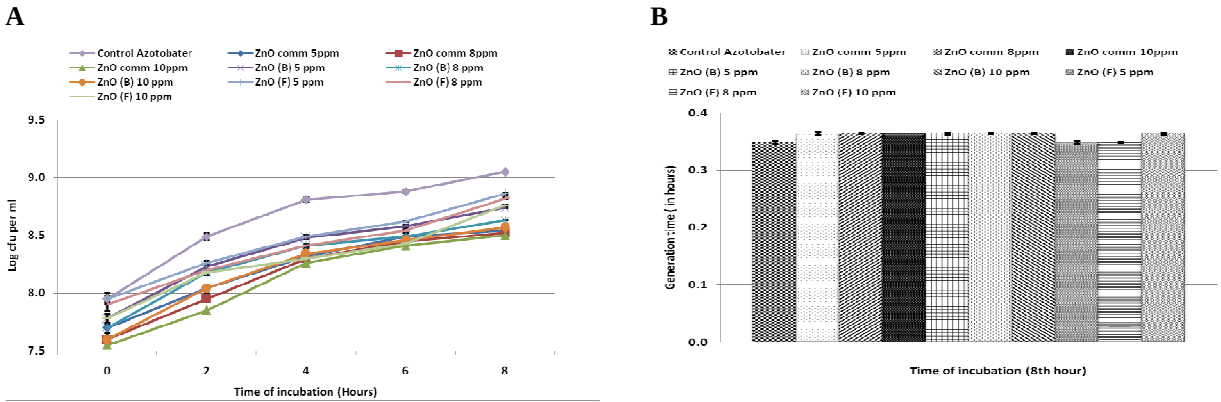
C.D @ 5 % A (Hours of incubation)= 0.017 B (Treatments)= 0.024 A×B= 0.053

Fig. 6. *Growth measurements for Rhizobium treated with zinc oxide nanoparticles A) Growth curve (Log cfu per ml) of Rhizobium cultures B) Generation time growth curve against hours of incubation.*



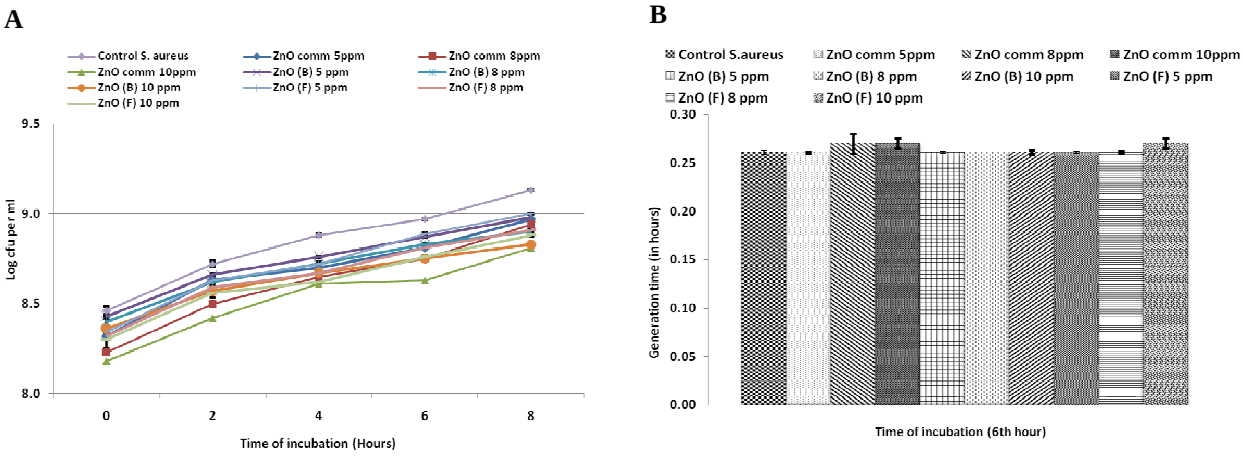
C.D @ 5 % A (Hours of incubation) = 0.019 B (Treatments) = 0.027 A×B = 0.06

Fig. 7. *Growth measurements for Ps. fluorescens treated with zinc oxide nanoparticles A) Growth curve (Log cfu per ml) of Ps. fluorescens cultures B) Generation time growth curve against hours of incubation.*



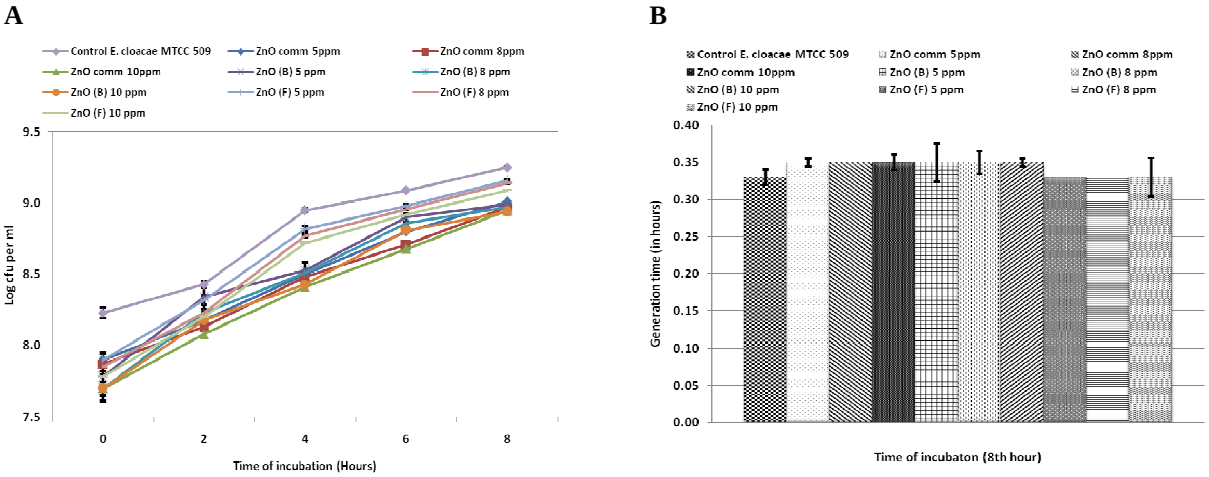
C.D @ 5 % A (Hours of incubation)= 0.0241 B (Treatments)= 0.0341 A×B= 0.076

Fig. 8. *Growth measurements for Azotobacter treated with zinc oxide nanoparticles A) Growth curve (Log cfu per ml) of Azotobacter cultures B) Generation time growth curve against hours of incubation.*



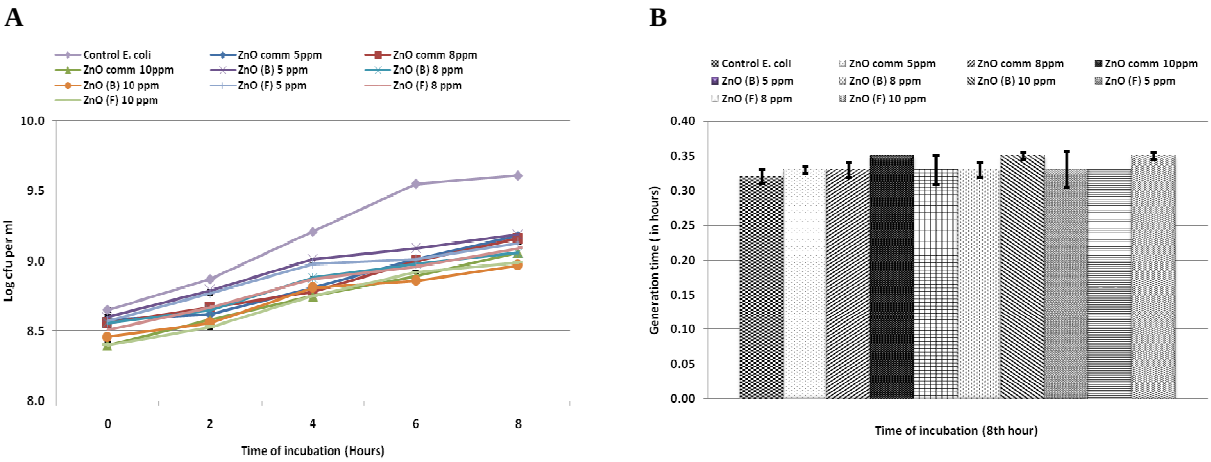
C.D @ 5 % A (Hours of incubation)= 0.016 B (Treatments)= 0.023 A×B= 0.051

Fig. 9. *Growth measurements for S. aureus treated with zinc oxide nanoparticles A) Growth curve (Log cfu per ml) of staphylococcal cultures B) Generation time against hours of incubation.*



C.D @ 5 % A (Hours of incubation)= 0.034 B (Treatments)= 0.047 A×B= 0.106

Fig. 10. *Growth measurements for E. cloacae MTCC 509 treated with zinc oxide nanoparticles A) Growth curve (Log cfu per ml) of E. cloacae MTCC 509 B) Generation time against hours of incubation.*



C.D @ 5 % A (Hours of incubation)= 0.031 B (Treatments)= 0.044 A×B= 0.098

Fig. 11. *Growth measurements for E. coli treated with zinc oxide nanoparticles A) Growth curve (Log cfu per ml) of E. coli B) Generation time against hours of incubation.*

Transmission electron microscopy (TEM): The reduction of all the three precursor zinc salts with the microbial cell free extracts under shaking condition generated oval to nearly spherical shaped NPs. However, the Pf extracts had the smallest size NPs (10-20 nm) synthesized using zinc sulphate precursor (Fig.5). Other precursors on incubation with the microbial cultures also yielded spherical shaped NPs. Thus the type of salt precursor has negligible effect on the morphology of the microbial synthesized ZnO NPs unlike the wet chemistry synthesis where altered morphologies were obtained on basis of precursor salt used (Mayekar *et al*., 2014). Similar synthesis of spherical shaped NPs with size range between 30-63 nm has been reported by Meruvu *et al*. (2011). Kundu *et al*. (2014) have also reported synthesis of 100-200 nm ZnO NPs by actinobacteria *Rhodococcus pyridinivorans* NT2 in presence of $ZnSO_4$ salt.

Agar well assay: The commercial ZnO NPs @ 10 ppm showed significantly higher antimicrobial activity (AMA) against all the test microorganisms. They showed maximum AMA against gram positive *S. aureus* with 24 mm followed by 14 mm zone of inhibition against *E. cloacae* MTCC 509. Lesser inhibition was observed against other gram negative pathogenic cultures like 11 mm only for *E. coli*. A higher AMA was observed as zone of inhibition against plant beneficial microorganism with highest against *Azotobacter* (28 mm) and lowest against *Ps. fluorescens* (15 mm) followed by *Rhizobium* (18 mm). Zinc acetate derived Bc7906 incubated ZnO NPs formed under shaking conditions showed maximum AMA @ 10 ppm concentration against *E. cloacae* MTCC 509 (17 mm) followed by *S. aureus* with 15 mm zone of inhibition (Table 1). Similar report of AMA of ZnO NPs at a higher concentration of 100 µg/ml against *S. aureus* with 14.52 mm zone of inhibition has been documented by Vani *et al.* (2011). Interestingly, these NPs exhibited lesser inhibition against gram negative plant beneficial rhizobacteria *Rhizobium* and *Azotobacter*, while no inhibition was observed against enterobacteriaceae member human pathogenic *E. coli* and plant probiotic pseudomonadaceae family member *Ps. fluorescens*. Contrary to our results, ZnO NPs synthesized from *A. hydrophila* have been reported to inhibit *E. coli* (Jayaseelan *et al.,* 2012) as well as had shown highest AMA against *Ps. aeruginosa*. The AMA of ZnO NPs synthesized from Pf using zinc sulphate as precursor showed maximum zone of clearance of 21 mm against plant beneficial gram negative *Azotobacter* and 17 mm each against gram positive human pathogenic *S. aureus* and gram negative *E. cloacae* MTCC 509. These NPs had smaller inhibition zones against *Ps. fluorescens* and *Rhizobium* and exhibited no inhibition against *E. coli*. Hence, *E. coli* seems to be resistant against both types of microbially synthesized ZnO NPs. This may probably due to presence of some capping protein(s) non-homologous to *E. coli* cell surface ligands or other proteins. The chemical controls (Bulk ZnO and Zinc chloride) showed no inhibitory effect on any of the test pathogens and soil beneficial microorganisms. Thus it could be ruled out that the lower concentrations of the ionic zinc (Zn^{2+}) released by these compounds were lethal. Chemical control (Zinc acetate) exhibited slight inhibitory activity against *E. coli*, *E. cloacae* MTCC 509 and *Ps. fluorescens* while zinc sulphate inhibited the growth of *E. coli.* Therefore, the chemical composition of the zinc salt utilized affects the AMA it renders as zinc acetate and zinc sulphate exhibited some inhibitory effect on the microbial growth. Some antibiotics showed better inhibition than the NPs such as Nalidixic acid and Chloroamphenicol with maximum zone of inhibition against *E. cloacae* MTCC 509. However, the concentrations being used were more in comparison to the nanoparticle concentrations used in this report. Strikingly, Cefalexin and ampicillin does not showed any inhibition against any of the pathogenic microorganisms. Diameter of zone of inhibitions by antibiotics against *E. cloacae* MTCC 509, *E. coli, S. aureus, Rhizobium, Ps. fluorescens* and *Azotobacter* are presented in Table 3. Taking in account the size of the inhibition zone formed by these NPs, commercial ZnO NPs @ 10 ppm are more effective in terms of their potential as antimicrobial agents compared to microbially synthesized ZnO NPs at the same concentration probably due to faster ionization yielding Zn^{2+} ions leading to higher toxicity and eventually delay in cell growth followed by death.

Growth of microbial cultures in broth supplemented with NPs. Various ZnO NPs synthesized using different salt precursors showed significant difference among their antimicrobial activity. Zinc acetate derived ZnO NPs generated from the cell free extract of Bc7906 and zinc sulphate derived ZnO NPs generated from cell free extract of Pf under shaking conditions were selected further for broth-NPs interaction study to determine the extent of cell morphological alteration on incubation with ZnO NPs as well to determine the variation in the generation time of the test microbes. The growth of *Rhizobium* in the presence of different ZnO NP concentration varied significantly. The rhizobial growth in control treatment without NP supplementation increased with the increase in hours of incubation. *Rhizobium* culture treated with commercial ZnO NPs exhibited a concentration dependent decrease i.e. lowest log cfu per ml @ 10 ppm followed by 8 ppm and 5 ppm concentrations. The zinc sulphate derived ZnO NPs using Pf cell free extract @ 10 ppm showed the lower log cfu per ml as compared to other microbially synthesized ZnO NPs (Fig.6). Other NP inoculated cultures showed significantly lesser growth in broth media than the control *Rhizobium* culture. However, in all the ZnO treatments, growth was retarded with increase in concentration of NPs. Generation time was observed to be highest by the *Rhizobium* culture inoculated with fungal derived ZnO NPs using

zinc sulphate @ 5, 8 and 10 ppm as compared to control as well as other treatments. In the absence of any NPs, *Ps. fluorescens* growth increased with increase in hours of incubation (Fig. 7). Incubation of *Ps. fluorescens* cells in the presence of all the ZnO NPs synthesized using different salt precursors enhanced their growth in a time dependent manner with growth equivalent to control culture treatment. Thus, ZnO NPs even at higher concentration (10 ppm) have enhancing effect on *Pseudomonas* growth. But contrary to that, commercial ZnO NPs @ 5, 8 and 10 ppm have retarded the growth of *Ps. fluorescens* with lowest log cfu per ml. The generation time determined after 8 hours of incubation showed variation among the treatments giving the evidence for results reported by the enumeration of viable cell count. The results indicated that the highest generation time was reported by commercial ZnO NPs @ 5, 8 and 10 ppm treated *Ps. fluorescens* cultures. Similarly, growth of *Azotobacter* treated and untreated with NPs increased with time (Fig. 8). However, on incubation with commercial ZnO NPs @ 10 ppm maximum growth was curbed compared to other treatments. This may be attributed to higher rate of dissolution and formation of Zn^{2+} ions and hence greater toxicity of commercial ZnO NPs. The growth reported by the determination of generation time after 8 hours of incubation also showed the greater toxicity of commercial ZnO NPs @ 10 ppm giving the highest generation time as compared to control culture of *Azotobacter*. ZnO NPs have an inhibitory effect on *S. aureus* growth. Both microbially synthesized and commercial ZnO NPs at 10 ppm concentration have retarded the bacterial growth in comparison to other lower concentrations used. These treatments @ 10 ppm were also reported to give highest generation time confirming the above stated results of growth inhibition.

As depicted in Fig. 10, a significant decrease in growth of the control and ZnO NPs treated *E. cloacae* MTCC 509 culture was observed. Highest growth inhibition was observed by both microbially generated ZnO NPs and commercial ZnO NPs @ 10 ppm giving lowest log cfu per ml and as well as highest generation time after 8 hours of incubation in comparison to control treatment. The decrease in growth exhibited NP concentration dependence. Chitra *et al.* (2013) have reported similar decrease in growth of *E. coli* and *P. aeruginosa* with increase in concentration of ZnO nanoparticles and maximum inhibition was obtained at 100 µg per ml concentration. The log cfu per ml of *E. coli* cells in presence of ZnO NPs was decreased with increasing concentration of NPs (Fig.11). However, this is not in consensus to the formation of no zone of inhibition in the agar well study. This signifies the higher potential of same concentration of ZnO NPs under aqueous suspensions over agar gel embedded NPs. Here in this case, lowest log cfu per ml and highest generation time was reported by the *E. coli* culture treated with ZnO NPs synthesized from Bc7906 and commercial ZnO NPs @ 10 ppm in comparison to control culture after 8 hours of incubation. Similar report of inhibition of *E. coli* by ZnO NPs in broth at 15 mM concentration has been documented by Paul and Ban (2014). Likewise, inhibition of growth of *E. coli* O157:H7 by application of ZnO NPs (@ 3 mmol/l) and @ 12 mmol/l or higher concentrations to completely inhibit the growth have been reported by Liu *et al.* (2009).

Conclusion

The microbially synthesized ZnO NPs had spherical morphologies with size ranging from 50 to 100 nm. Both bacterial and fungal derived ZnO NPs showed antimicrobial activity against the test human pathogenic and plant beneficial microorganisms. The commercial ZnO NPs exhibited higher bacterial cytotoxicity showing 28 mm zone of inhibition @10 ppm against *Azotobacter* (a plant beneficial microorganism) as compared to microbially synthesized NPs. Hence, this differential toxicity of microbially generated ZnO NPs can be better utilized for developing novel agri-food commercial products keeping in view their lesser post application ecotoxicity issues observed as lesser or rather positive effect on plant beneficial rhizobacteria.

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