Role of nanomaterials in symbiotic fungus growth enhancement

Nanotechnology is an enabling technology dealing with nanometre-sized particles. A decade ago, nanoparticles were studied because of their size-dependent physical and chemical properties[1]. Now they have entered a commercial exploration period in the area of biotechnology, leading to the development of a new field of science – nanobiotechnology[2]. Understanding of biological processes at the nanoscale level is a strong driving force behind the development of nanobiotechnology[3]. As it is well known, living organisms are built of cells that are typically 10 μm across. However, the biomolecules are much smaller and are in the sub-micron size domain. Even smaller are the proteins with a typical size of just 5 nm, comparable with the smallest man-made nanoparticles. This simple size comparison gives an idea of using nanoparticles as nanoprobes that would allow us to spy at the cellular machinery without too much interference[4].

In order to enhance the utilization of nanomaterials in biological systems, it is important to understand the influence they have on the cellular health and function. Nanomaterials present a research challenge as little is known about how they behave in relation to microorganisms, particularly at the cellular level. Most of the nanomaterials reported earlier have been demonstrated to be efficient antimicrobial agents[5]. There are only a few or no reports on the growth-promoting role of the nanomaterials, especially with respect to microbes. It has been reported that nanoparticles possess more surface area than a microparticle, thus improving the physical and chemical characteristics of a particle, which may also influence the biological property of the material[1].

Titanium dioxide nanoparticles (TNPs) are reported to induce spinach seed germination and plant growth, particularly in aged seeds and their vigour[6]. Furthermore, the presence of TNPs was observed to increase the biomass, chlorophyll synthesis and metabolism in photosynthetic organisms. These positive effects are possibly due to antimicrobial properties of nanoparticles increasing strength and tolerance in plants[7]. The role of microorganisms such as bacteria and fungi, including yeast in remediation of toxic metals through reduction is well documented[8]. Recently, their application as nanofactories for the synthesis of nanomaterials has been reported[9]. In the present communication, the interaction of nanomaterials with the microorganisms and their effect on the growth processes at different stages of development have been studied. Viewing the plant growth-promoting effects in many economically and medicinally important plants by symbiotic fungus *Piriformospora indica*[10–12], the fungus was chosen as a representative model to observe the effect of nanoparticles on growth enhancement. This property has already been patented (Application No. 14/DEL/2009; Reference No. E-12/3/2009-DEL).

During the interaction between nanomaterials and *P. indica*, three types of nanomaterials were used: TNPs, carbon nanotubes (CNTs) and silver nanoparticles (AgNPs) with two negative controls: activated charcoal and control (without charcoal). Each of these was mixed (10 mg/100 ml) in the Hill and Kaefer[13] broth with 1% glucose, in separate Erlenmeyer flasks with initial pH adjusted to 6.4. Usually 4–5 fully grown fungal agar discs (4 mm in diameter) were inoculated into each 500 ml Erlenmeyer flask containing 100 ml of Hill and Kaefer broth medium. Flasks were incubated for 7–10 days at 28 ± 2°C with constant shaking at 100 rotations per min on a rotary shaker. The samples were stained with 0.05% (w/v) Trypan blue for morphological characterization and studied under a microscope.

Nanoparticles-inoculated fungal grown mycelia samples were washed 3–4 times with saline solution, fixed in 2.5% glutaraldehyde for 2 h at 4°C and again washed three times with buffer, each for 2 h. The samples were dehydrated in acetone serially 30–95% each for 30 min at 4°C and finally dehydrated using dry acetone (100%) with two changes each for 1 h duration, once at 4°C and another at room temperature. Samples were dried in liquid CO_2 in critical point drying apparatus and mounted on SEM stubs with silver paint and coated with metal (Au) approximately at 400 Å (40 nm) using the sputter coater (Balzers). Specimens were examined using a scanning electron microscope (SEM) (Philips SEM-501 B) at 15 kV. SEM screening was performed at the All India Institute of Medical Sciences, New Delhi.

The preliminary observation indicated that the fungal biomass was enhanced 2–3-fold in the presence of almost all the nanomaterials used compared to the negative control (Figure 1 *a*(i)). Fresh biomass of the fungus was maximum in case of TNP environment followed by CNT (Table 1). Further, the colony morphology of *P. indica* also differed with the addition of nanoparticles. The colonies were larger in size, more smooth and round in TNP-infused medium (Figure 1 *a*(ii)). In another experiment similar results were obtained in the CNT infused medium, but these were irregular with short and long protrusions depicting sea urchin-like radial growth (Figure 1 *a*(iii)). No influence of activated charcoal was recorded (Figure 1 *a*(iv)). It is a general practice to include activated charcoal in biological culture medium to absorb unwanted toxic complexes resulting in enhanced growth.

It was observed that the stage of nanomaterials inclusion is crucial. The antimicrobial property of nanomaterials was recorded when added at a late growth phase. However, the inclusion before wet sterilization showed a positive and stimulating effect on the fungus. Electron microscopic studies were conducted to see the effect of TNPs on the growth, morphology and sporulation of the test fungus. SEM clearly indicated the stimulating effect on the size of the chlamydospores. The increase in size of the spores was almost 50% in comparison to the control (Figure 2). The results also exhibited the increased spore density of the test fungus on interaction with TNPs.

The contradictory effect of nanomaterials at a specific stage of addition can be best explained by considering the mechanism of antimicrobial action. Antimicrobial behaviour of the nanoparticles is reported to be due to the presence of electronic effects brought about as a result of changes in the local electronic structure of the surfaces due to small sizes (<100 nm). Nanomaterials, especially nanosilver, strongly interact with the thiol groups of the vital enzymes and inactivate them. As a result, the DNA loses its ability to replicate[14]. It also destabilizes

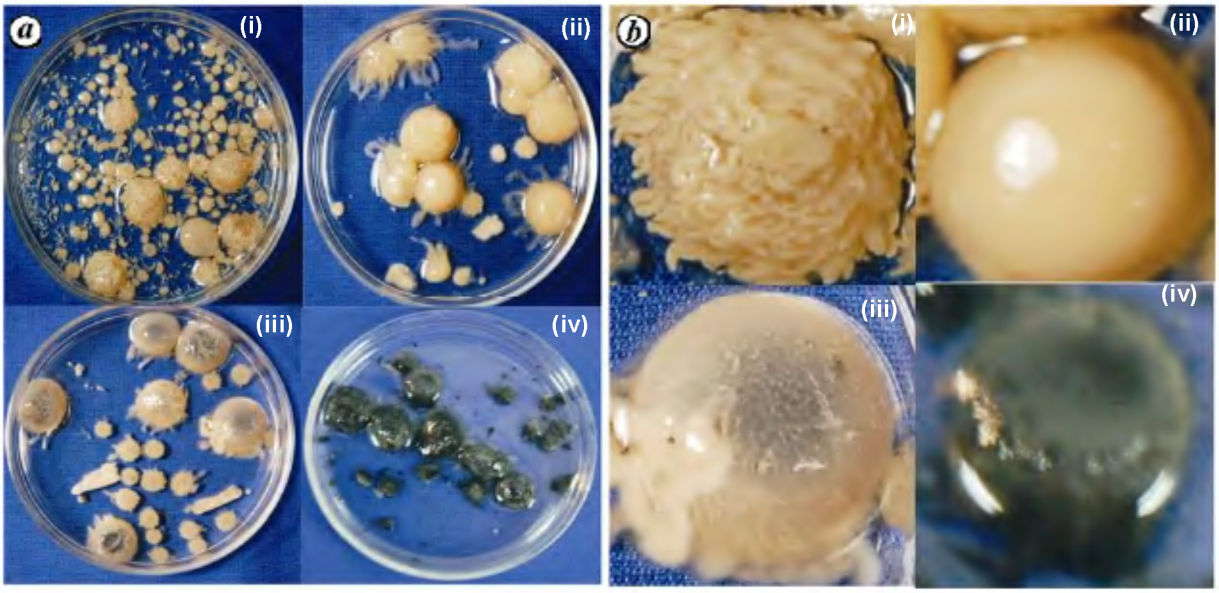

Figure 1. The fungus *Piriformospora indica* interacting with the nanomaterials; control, titanium dioxide nanoparticles (TNP), carbon nanotubes (CNT) and activated charcoal. These were added to the medium before autoclaving at a concentration of 10 mg/100 ml and imparted large variations in the morphology of the fungus. (i) Control without nanoparticles fungal surface is rough and overall size of the colonies is not very large. (ii) The colonies in case of TNP environment are larger in size, more smooth and spherical. (iii) In case of CNT also colony size is bigger, but morphologically these are not smooth but burging outwards. (iv) Fungus with charcoal as a negative control. ***b***, An enlarged view of (***a***).

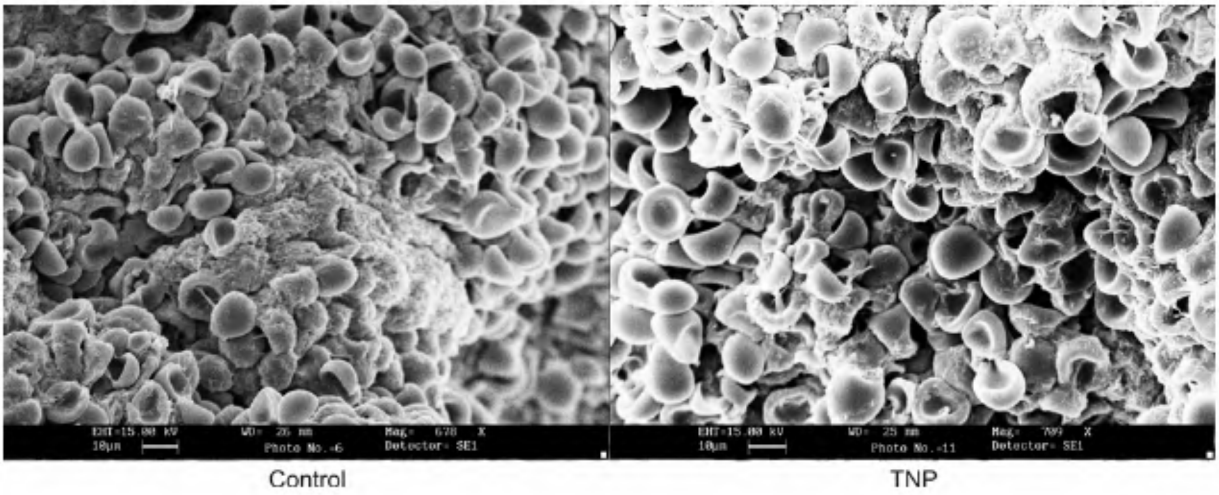

Figure 2. Scanning electron microscopic images of control and TNP-treated *P. indica*.

Table 1. Fresh biomass (g) of the fungus *Piriformospora indica* after interaction with nanoparticles

	Control	Carbon nanotubes (CNTs)	Titanium dioxide nanoparticles (TNPs)	Silver nanoparticles (AgNPs)
Mean ± standard deviation	2.984[a] ± 0.0864	3.929[a] ± 0.640	4.847 ± 0.340	3.483[a] ± 0.510
Critical difference (CD)	0.5973			

[a]In each column, averages followed by the same letters are not significantly different using analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) at 5% probability level.

the plasma membrane potential and results in the depletion of intracellular energy bond of ATP, thus resulting in cell death[15].

In the present set of experiments, growth promotion of the test fungus *P. indica* may be achieved due to the incorporation of the nanomaterials as media ingredients and acting as a carrier for the fast uptake of nutrients and gases due to their large surface area, small size and absorption capacity by the test fungus[16].

Table 2. Seedling length of the brocolli plant after interaction with culture filtrates of *P. indica* and nanoparticles

	Control	CNTs	TNPs	AgNPs
Mean ± standard deviation	5.25 ± 0.64	6.875[a] ± 0.18	8.575 ± 0.15	6.375[a] ± 0.66
CD	0.737			

[a]In each column, means followed by the same letters are not significantly different using ANOVA and DMRT at 5% probability level.

P. indica culture filtrate is known to enhance seed germination and growth of several plants[17]. An independent test was performed with the culture filtrate of the nanomaterials-treated fungal broth on the seeds of broccoli (*Brassica oleracea* var. *italica*). The results indicate stimulation of seed germination by TNP followed by CNT (Table 2), and thus have excellent potential for producing liquid biofertilizers.

From the present experimental results, it can be concluded that besides acting as an antimicrobial agent, the nanomaterials may also influence growth promotion of fungi depending upon the stage of inclusion. Further, the nanomaterials-treated *P. indica* culture filtrate has also shown to be helpful in seed germination and growth of seedlings in plants. Nanomaterials have already been reported to enhance plant growth directly[8]. In future, it can be utilized not only as a microbial growth enhancer, but also as a potential tool for early diagnosis of diseases.

Future research needs to address questions such as to what extent molecular and genetic mechanisms may mediate microbial responses to nanomaterials exposure and furthermore, how to control such responses for utilizing their maximum beneficial effects.

1. Murray, C. B., Kagan, C. R. and Bawendi, M. G., *Annu. Rev. Mater. Sci.*, 2000, **30**, 546–610.
2. Paull, R., Wolfe, J., Hebert, P. and Sinkula, M., *Nature Biotechnol.*, 2003, **21**, 1134–1147.
3. Whitesides, G. M., *Nature Biotechnol.*, 2003, **21**, 1161–1165.
4. Taton, T. A., *Trends Biotechnol.*, 2002, **20**, 277–279.
5. Raffi, M., Hussain, F., Bhatti, T. M., Akhter, J. J., Hameed, A. and Hasan, M. M., *J. Mater. Sci. Technol.*, 2008, **24**, 192–196.
6. Zheng, L., Hong, F. S., Lu, S. P. and Liu, C., *Biol. Trace Element Res.*, 2005, **104**, 83–91.
7. Navarro, E. *et al.*, *Ecotoxicology*, 2008, **17**, 372–386.
8. Mukherjee, P. *et al.*, *Nano Lett.*, 2001, **1**, 515.
9. Duran, N., Marcato, P. D., Alves, O. L., De Souza, G. I. H. and Esposito, E., *J. Nanobiotechnol.*, 2005, **3**, 8.
10. Verma, S. *et al.*, *Mycologia*, 1998, **90**, 896–903.
11. Shahollari, B., Vadassery, J., Varma, A. and Oelmüller, R., *Plant J.*, 2007, **50**, 1–13.
12. Deshmukh, S. *et al.*, *Proc. Natl. Acad. Sci. USA*, 2006, **103**, 18450–18457.
13. Hill, T. W. and Käfer, E., *Fungal Genet. Newsl.*, 2001, **48**, 20–21.
14. Morones, J. R., Elechiguerra, J. L., Camacho, A., Holt, K., Kouri, J. B., Ramirez, J. T. and Yacaman, M. J., *Nanotechnology*, 2005, **16**, 2346–2353.
15. Lok, C. N. *et al.*, *J. Proteome Res.*, 2006, **5**, 916–924.
16. Gleiter, H., *Acta Mater.*, 2000, **48**, 1.
17. Oelmüller, R., Sherameti, I., Tripathi, S. and Varma, A., *Symbiosis*, 2009, **49**, 1–18.

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Importance of upper ocean heat content in the intensification and translation speed of cyclones over the Bay of Bengal

It is a well-known fact that the frequency of cyclones is 3–4 times higher over the Bay of Bengal (BOB) compared to the Arabian Sea (AS). Most of these storms generally move in the west-northwesterly direction and some take a recurve and hit Bangladesh. In addition to atmospheric parameters, it is now realized that the upper ocean heat content (UOHC) plays a vital role in the intensification of storms rather than sea-surface temperature (SST). Studies on the intensification of hurricane *Opal* over warm core rings, and *Bret* over high heat potential (>90 kJ/cm^2) were reported earlier[1,2]. Rapid intensification of *Nargis* over the warm ocean region was reported[3] due to high enthalpy flux (300% higher than climatological) and UOHC (77–105 kJ/cm^2). A recent study[4] showed a negative relationship between the translation speed of a storm (U_h) with UOHC and the depth of 26°C isotherm (D26) in the western-north Pacific Ocean (PO). Hence UOHC and D26 gained importance in forecasting the intensification and movement of storms. Sadhuram *et al.*[5] showed that a threshold value of about 60 kJ/cm^2 may be necessary for the genesis and intensification of the storms in BOB during post-monsoon season.

In this study an attempt has been made to examine the relationship between