Microbial Synthesis of Nanoparticles and Its Application in Aquaculture

Surajkumar Irungbam[1], Wangkheimayum Malemnganbi Devi[1], Priyanka Irungbam[2] and Kenny Sarangthem[3]

[1]College of Fisheries. Lembucherra, CAU, Imphal

[2]College of Horticulture and Forestry, Pasighat, CAU, Imphal

[3]College of Agriculture, Iroisemba, CAU, Imphal

*Corresponding Author: surajkrirungbam@gmail.com

The science and technology of creating nanoparticles and manufacturing machines which have sizes within the range of nanometre is called Nanotechnology. Nanotechnology involves creating and manipulating organic and inorganic matter at the nanoscale. The use of NP-based antimicrobials, vaccines against many viral pathogens is a developing field in fish medicine research (Shaalan *et al.*, 2016). NPs have gained much interest as a specific and sensitive tool for diagnosis of bacterial, fungal and viral diseases in aquaculture. Nanotechnology has become an extensive field of research due to the unique properties of nanoparticles (NPs), which enable novel applications in the field of medicine, including antimicrobial effects, diagnostics, vaccination, drug and gene delivery (Aulenta *et al.*, 2003).

Table 1: Types of nanoparticles and its application.

Types of nanoparticles	Structure	Application
Nanospheres	Spherical shaped	Drug delivery, tissue regeneration
Nanocapsules	Shell and core combination	Controlled drug delivery
Carbon nanotubes	Cylindrical tubes	Drug delivery, anti-cancer
Liposome	Lipid bi-layer globules	Drug delivery for hydrophobic and hydrophilic drugs
Dendrimers	Highly branched ends and central core	Delivery system, tissue engineering, antimicrobials
Polymeric nanoparticles	Polymers as chitosan	Delivery system, tissue regeneration

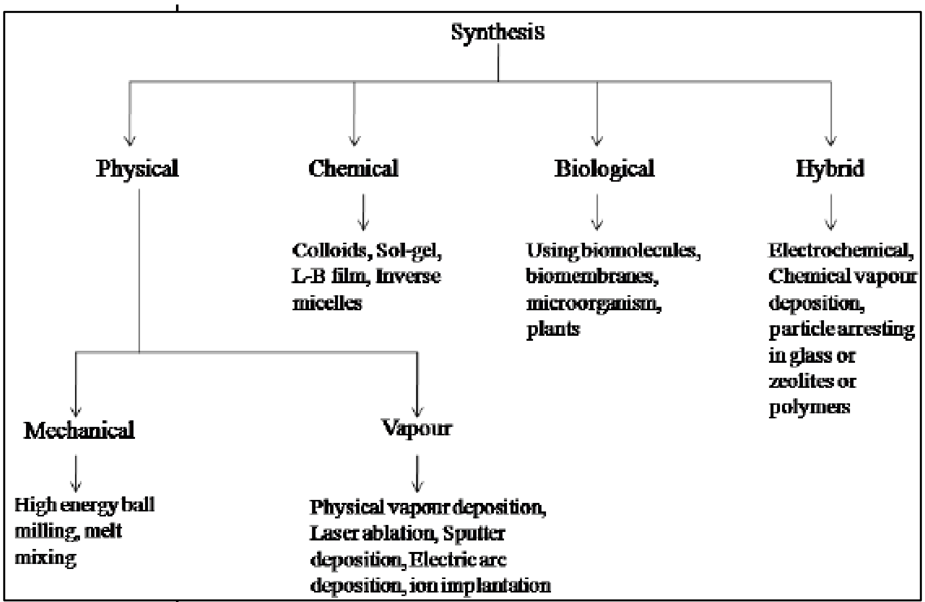

Fig 1: Different methods of synthesis of nanoparticles (Aher and Avinash, 2013)

Synthesis of nanoparticles

Synthesis of nanoparticles using microorganisms: Bacteria

There are two methods of synthesis of nanoparticles by bacteria:

i. Intracellular: inside the cell in cytoplasm
ii. Extracellular: outside the cell on the surface or between the cells inside a colony

Intracellular Synthesis of Nanoparticles

This method includes mainly the accumulation of the particles called as bioaccumulation. In order to release the intracellularly synthesized nanoparticles, additional processing steps such as ultrasound treatment or reaction with suitable detergents are required. *Bacillus subtillis* 168 reduced water-soluble Auric to Aurous producing octahedral morphology inside the cell walls in the dimension of 5-25 nm (Alsamhary, 2020). In Fe (III) reducing bacterium Geobacterferrireducens, gold was precipitated intracellularly in periplasmic space. Silver-based single crystals such as equilateral triangles and hexagons with particle sizes up to 200 nm in periplasmic space of the bacterium were produced by

Pseudomonas stutzeri AG259, a silver mine bacterium (Klaus *et al.*, 1999). It has been believed that the organic matrix contains silver-binding proteins that provide amino acid moieties, which serve as nucleation sites for the formation of silver nanoparticles.

Extracellular Synthesis of Nanoparticles

This method includes bio mineralization, bio sorption or precipitation. When the cell wall reductive enzymes or soluble secreted enzymes are involved in the reductive process of metal ions then the metal nanoparticles are extracellularly secreted (Narayanan and Sakthivel, 2010). With the change in pH of the solution, various shapes and sizes were formed. The culture supernatants of Enterobactericeae like *Klebsiella pneumonia*, *E.coli*, *Enterobacter cloaceae* rapidly synthesize silver nanoparticles by reducing Ag + to Ag. Titanium nanoparticles of spherical aggregates of 40–60 nm were produced extracellularly using *Lactobacillus sp.* at room temperature (Prakash *et al.*, 2013).

Synthesis of nanoparticles using microorganisms: Fungi

Synthesis of gold nanoparticles

Extracellularly gold nanoparticles are synthesised using the fungus *Fusarium oxysporum.* Incubation of the fungus mycelium with Auric chloride solution produces gold nanoparticles in 60 min (Thakker *et al.*, 2013). Gold nanoparticles were characterized by UV-Visible spectroscopy and particle size analysis. The particles synthesized were of 22 nm sized, capped by proteins and showed antimicrobial activity against *Pseudomonas sp.*

Synthesis of silver nanoparticles

AgNPs were synthesised using the fungus *Arthroderma fulvum* Strain HT77 which was cultivated in potato dextrose broth and had been inoculated with. The flasks were incubated at 28°C and 140 rpm for 7 days. After incubation, fungal biomass was separated by filtration, washed with sterile distilled water to remove the traces of culture media components, resuspended in 100 ml distilled water, incubated at 28°C for 24 hours, and then filtered. Silver nitrate ($AgNO_3$) was added to the filtrate to promote the formation of AgNPs. The ratio of cell filtrate to $AgNO_3$ was kept at 1:9 (v/v), and the reaction mixture was incubated at 28°C for 48 hours. Controls (without the addition of $AgNO_3$) were incubated under the same conditions. Color change in the reaction mixture was the initial indicator of the formation of AgNPs. When the color changed, 3 ml of the reaction mixture was removed to measure its absorbance using a UV-visible spectrophotometer. The presence of AgNPs was confirmed by X-ray diffraction and AgNPs were isolated by centrifugation cells (Narayanan and Sakthivel, 2010).

Mechanisms of antimicrobial activity of the metal NPs

Entering the cell

Attachment of the nanometer range metallic ions with the cell through trans-membrane protein is the initial step of the antibacterial mechanism. The initial interaction of ions with the cell surface of microbes starts with the attraction of positively charged silver with negatively charged microbial cells, thus leading to the development of several pores in the cell membrane and outflow of intracellular materials. The next step is to create structural changes in the cell membrane and obstructing its transport channels (Dutta *et al.*, 2012); this whole process depends on the size.

Reactive Oxidative Species Generation

For NPs antibacterial effectiveness, the formation of ROS plays a critical role. ROS contains ephemeral oxidants, like superoxide radicals ($O^{'2}$), hydrogen peroxide (H_20_2), hydroxyl radicals (OH^4) and singlet oxygen (O^{-2}) (Raffi *et al.*, 2008; Baek and An, 2011). ROS can result in damage to the peptidoglycans of cell membranes, DNA, mRNA, proteins, and ribosomes because of its high reactivity (Pelgrift *et al.*, 2013). Translation, transcription, the electron transport chain, and enzymatic activity are also inhibited by ROS (Raffi *et al.*, 2008).

Protein Inactivation and DNA Destruction:

To deactivate the function of enzymes, metal atoms attach with thiol groups of enzymes. It is observed that the bonding of hydrogen between two anti parallel strands of DNA is also disturbed by metal ion attachment, which ultimately destructs the molecule of DNA. The true metal ions also show a tendency to attach to DNA after they get inside the cell, but it is still under investigation (Jung *et al*., 2008).

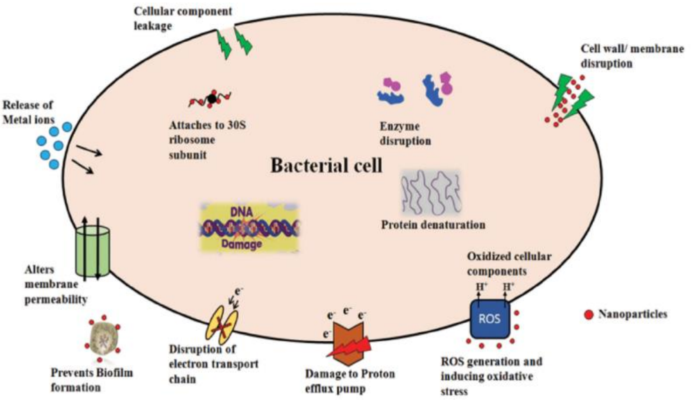

Fig 2: Antimicrobial activities of nanoparticles (Wang *et al*., 2017)

Nanoparticles as Antimicrobials in Fish Medicine

The utilization of NPs as alternative antimicrobials to combat emergence of microbial resistance to antibiotics in aquaculture has been investigated. Metal NPs have shown high antimicrobial activity against bacteria, fungi and viruses.

Silver nanoparticles (Ag-NPs): Ag-NPs demonstrate high antibacterial efficacy against multi-drug resistant bacteria isolates, antibacterial activity against *S. aureus* and *E. tarda*, and anti-cyanobacterial activity towards *Anabaena* and *Oscillatoria* species (Prakash *et al*., 2013). As an antifungal agent, Ag-NPs exhibited high inhibitory effects against *Candida* species, similar to the commercial antifungal Amphotericin B. Ag-NPs are active against influenza A virus.

Gold Nanoparticles (Au-NPs): Au-NPs can interact with biological proteins and non-proteins, e.g. LPS, and have biological functions. Au-NPs supported on zeolite exhibited bactericidal effects against *E. coli* and *S. typhi*. Functionalized Au-NPs inhibited the growth of MDR bacterial isolates. Au-NPs made by 'green' synthesis showed antibacterial activity against fish bacterial isolate. There are three pathways along which Au-NPs exert their antibacterial effects (Thakker *et al*., 2013).

The first pathway is via interfering with oxidative phosphorylation process with changing the potential of bacterial cell membrane; this leads to decrease in the activity of F-Type ATP synthase with a net decrease in ATP synthesis and metabolism.

The second pathway is interference with binding of tRNA to the two ribosome subunits.

The third pathway is achieved through enhancing chemotaxis.

Fungicidal activity against *Candida* species was reported for Au-NPs. Their efficacy was size-dependent, with smaller Au-NPs having higher antifungal effects (Gutiérrez, *et al*., 2018).

Zinc Oxide Nanoparticles (ZnO-NPs): ZnO-NPs have drawn much attention due to their antibacterial and antifungal effects. The antibacterial activity derives from the particles damage to the bacterial cell membrane, which makes cytoplasmic contents leak from the cell. In the field of fish medicine, ZnO-NPs can inhibit the growth of *A. hydrophila, E. tarda, Flavobacterium branchiophilum, Citrobacter spp*., *S. aureus, Vibrio, Bacillus cereus* and *Pseudomonas aeruginosa*. Ramamoorthy *et al* investigated the antibacterial effects of ZnO-NPs against the pathogenic *V. harveyi* and observed higher bactericidal effects of NPs compared to bulk ZnO (Ramamoorthy *et al*., 2013).

Conclusion and Future Directions

It has been summarized the current applications of NPs in aquaculture. There are, however, many research gaps in the field of nanotechnology applications in aquaculture. Different forms of NPs like nanocapsule, liposome, dendrimer and nanotubes could theoretically have applicability in fish diseases research. The antifungal and antiviral effects of NPs against fish diseases have yet to be explored. Given the demonstrated potential of NPs there are needs for more targeted investigations of their application in many fish medicine research topics, to promote more efficient fish disease diagnostics and therapy, to meet the ever-growing aquatic animal health demand.

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