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Nano zinc oxide synthesized using *Lactobacillus sp.* to study the antibacterial activity against pathogenic microorganisms

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Abstract
The pharmaceutical industry is becoming more interested in *Lactobacillus*-capped ZnONPs (Ls-ZnOPs) as a way to improve the production of nanomedicines. In the current investigation, *Lactobacillus* species were isolated and characterized from curd samples. Next, ZnONPs capped by *Lactobacillus* (Ls-ZnONPs) were created. According to the findings, LsZnONPs have the potential to be exploited in the pharmaceutical industry for the production of sustainable nanomedicines. Furthermore, it exhibits strong antibacterial properties against a variety of harmful microbes.

Keywords: *Lactobacillus*-capped ZnO NPs (Ls-ZnO NPs), *Lactobacillus sp*., antibacterial activity

Introduction
The creation of various nanomaterials with potential uses in various fields is known as nanotechnology. Nanoparticles typically have a size range of 1 to 100 nanometres and are employed in a variety of fields, including pharmaceuticals, cosmetics, and biological sensors. Both chemical and biological methods can be used to create nanoparticles. The biological manufacturing of nanoparticles often involves the use of bacteria, fungi, and plants. Using microorganisms to synthesize nanoparticles is an environmentally benign process. Microorganisms such as bacteria, actinomycetes, fungi, and algae are frequently used to produce nanoparticles [1].
Salmonella typhi, Klebsiella pneumoniae, and Pseudomonas aeruginosa are just a few of the dangerous bacteria that *Lactobacillus*, a helpful bacterium, exhibits antibacterial effectiveness against. It helps maintain the function of our digestive system and can stop intestinal damage brought on by these bacteria [2].

Materials & Methods
Isolation of *Lactobacillus spp.*: The best source of *Lactobacillus* species is curd. Other dairy products include buttermilk, milk, and so forth. A sterile flask is used to hold the curd. Curd is serially diluted from 10^{-2} to 10^{-5} under aseptic conditions; dilutions 10^{-4} are chosen from this range. On MRS medium, the spread plate technique is combined with the streak plate technique. The ideal temperature for *Lactobacillus* broth is 37^0C, which is what they are incubated at for 24 hours at 37^0C. After 24 to 48 hours, the broth displayed the growth of *Lactobacillus* species for 24 hours. The particular isolated colonies were cultivated following the incubation period. Characterization of the colonies belonging to the *Lactobacillus* species is carried out. One of the colonies is exactly like *Lactobacillus acidophilus*. Gram stain biochemical assays were used to identify the isolated colonies that developed on the MRS agar plates. For long-term storage, the culture was maintained in MRS agar slant and stored at 4^0C [3].

Bacterial strain and culture condition: For the antibacterial test, two gram-positive and two gram-negative bacteria-*Pseudomonas aeruginosa*, *Bacillus subtilis*, *Salmonella typhi*, and *Klebsiella pneumoniae*-were supplied by the National Cholera Institute in Kolkata. These test organisms, both gram positive and gram negative, were stored at 4^0C in screw-capped tubes in butt-slants of Brain Heart Infusion Agar.

Gram Staining: After adding the crystal violet, the slides were quickly cleaned with tap water to get rid of any remaining stains. After that, tap water was used to wash the gram iodine mordent. Following a minute of gram safranin staining, the slides were dehydrated with alcohol.

Biochemical characterization of the isolated bacterial stain: The isolated bacteria were identified as *Lactobacillus spp*. based on their morphological, cultural, physiological, and biochemical traits [4]. Methyl Red (MR) testing, Citrate Utilization testing, Urease testing, Oxidase testing, and Catalase testing were all performed.

Biochemical tests:

Methyl Red test: *Lactobacillus sp*. isolates were added to the tube containing MR broth, and the mixture was incubated for 48 hours at 37^0C. The addition of five drops of methyl red solution came next, and the tubes' red hue was verified as a positive outcome. The tube that remained yellow was regarded as negative.

Citrate Utilization test: One of the metabolites in the TCA cycle is sodium citrate. During the fermentation of carbohydrates, several bacteria used citrate as a carbon source to generate energy. The tubes were filled with prepared Simmon's citrate agar medium, which was sterilized at 121 degrees Celsius for 20 minutes at 15 lbs. Slants were also made. From the medium's surface, a well-isolated colony was selected, streaked on the citrate agar slant, and incubated for 48 hours at 37 degrees Celsius. The colour shift from royal blue was seen as a positive sign.

Urease test: Christensen's urea broth was made and sterilized for 20 minutes at 121 degrees Celsius and 15 lbs. After being injected in the sterile urea broth tubes, the isolates were cultured for 48 hours at 37 degrees Celsius. The tube's reddish-violet colour signified a successful outcome. The unfavourable outcome was shown by the yellow-orange.

Oxidase test: A pure culture of *Lactobacillus* was created. The glass slide was cleaned and sanitized before the oxidizing disk was put on it. Next, two to three drops of pure culture were applied to the oxidized disk, and within ten seconds, the colour change was monitored. The oxidase disk's blue colour suggested a favourable outcome. No colour shift was indicated as the negative result.

Catalase test: Using a sterile loop, a loopful of *Lactobacillus* was aseptically removed, and a smear was formed on a pristine glass slide. After adding two to three drops of H_2O_2, the outcome was noted. The bad outcome was suggested by the effervescence.

Synthesis of zinc oxide nano particle using the broth culture of *Lactobacillus spp.*

In short, 50 millilitres of distilled water were combined with 20 millilitres of zinc acetate dihydrate (0.02 M) and agitated. Next, 250 millilitres of *Lactobacillus* broth culture and NaOH were added to a different flask. At pH 12, a white precipitate was produced. Ethanol and distilled water were used to wash the white precipitate. After that, they were dried at 60 degrees Celsius to produce powdered ZnO nanoparticles.

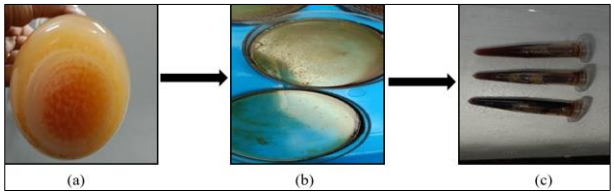

Fig 1: Preparation of nanoparticles (a) Accumulation of ZnO, (b) Evaporates the Ls ZnO solution, (c) Collection of Ls ZnONPs)

Zn functions as a respiratory enzyme cofactor. It is a multipurpose trace element for the human body since it is essential for many physical functions, including metabolism, immune system function, cognitive function, cell growth and development, and reproduction. It is thought that ZnONPs kill cells by breaking down the proteins and lipids in bacterial cell membranes and by generating reactive oxygen species, two important processes that give ZnONPs their antibacterial properties [5].

Antibacterial test of Ls-ZnONPs

Culture plate preparation

One hundred millilitres of water are used to dissolve 3.2 grams of nutritional agar. The media should next be autoclaved for 15 minutes at 121°C and 15 psi to sterilize it. After pouring into petri dishes, let them harden. Using a spreader, distribute the harmful bacteria around the agar plate. To place the Ls-ZnO NPs, bore the agar. After 24 hours of incubation at 37°C, the zone of inhibition was observed.

Results

Identification of *Lactobacillus spp.*

Using a phase contrast microscope, the isolated bacteria were examined. It is evident that the bacteria were rod-shaped, gram-positive, and could exist alone or in chains. The isolated bacteria were identified as *Lactobacillus acidophilus* based on the results of the gram staining. The isolated bacteria were not motile, as demonstrated by the hanging drop wet method. One of *L. acidophilus's* traits is its non-motile behaviour. Consequently, the bacteria found in the curd sample share traits with *Lactobacillus acidophilus*. Because of its ease of use, the catalase test is among the most effective diagnostic procedures for identifying bacteria. The absence of a bubble during the catalase test indicates that the isolated bacterium is catalase negative and unable to mediate the breakdown of H_2O_2 to create O_2. *Lactobacillus* is known to be catalase negative. As a result, the outcome matched the traits of the *L. acidophilus* strain. Table 1 and Figure 1 display their unique characteristics. Additionally, their molecular characteristics are presented in Table 2.

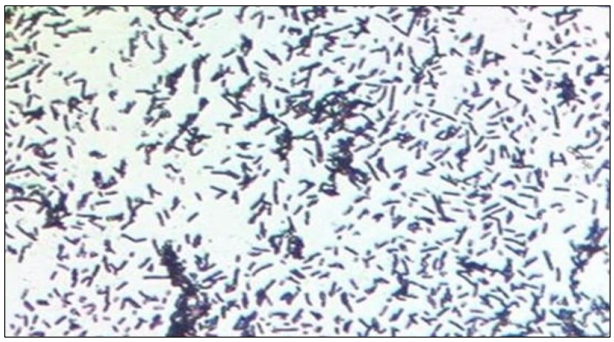

Fig 2: Phase contrast microscopy images of *Lactobacillus sp.*

Table 1: Morphological &physiological characterization of the isolated bacterial strain

Configuration	Round
Margin	Wavy
Elevation	Flat
Surface	Mucoid
Texture	Dry
Gram's reaction	+Ve
Spores	-Ve
Capsule	-Ve
Motility	Non-motile

Table 2: Biochemical Characterization of the isolates bacterial strain

Tests	Result
Methyl red test	-Ve
Citrate utilization	-Ve
Urease test	-Ve
Oxidase test	-Ve
Catalase test	-Ve

Table 3: Inhibition zone of Ls-ZnONPs against pathogenic bacterial culture

Test organism	Zone of inhibition(mm)
Pseudomonas aeruginosa	39
Bacillus subtilis	17
Salmonella typhi	39
Klebsiella pneumoniae	18

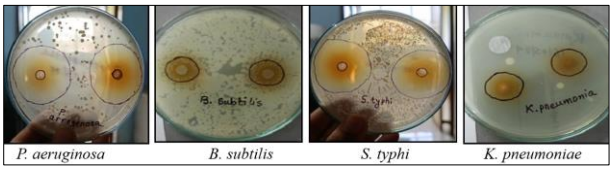

Fig 3: Inhibition zone of Ls-ZnONPs against pathogenic bacterial culture

Conclusion

Nanoparticles are viable options for treating microbial diseases because of their encouraging antimicrobial qualities. Their efficacy is demonstrated by their capacity to interfere with microbial cell membranes, interfere with cellular functions, and stop growth. Nanoparticles are viable options for treating microbial illnesses because of their encouraging antibacterial qualities. Their efficacy is demonstrated by their capacity to interfere with microbial cell membranes, interfere with cellular functions, and impede growth. Furthermore, as compared to conventional antibacterial treatments, nanoparticles have benefits including tailored distribution and a lower chance of resistance development. However, issues including environmental impact, long-term toxicity, and biocompatibility call for careful evaluation and control. Before broad clinical use, more research is required to improve the characteristics of nanoparticles, increase their effectiveness, and solve safety issues. In conclusion, even though nanoparticles have a lot of potential for antimicrobial applications, further testing and improvement are necessary to realize all of their potential while maintaining sustainability and safety.

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