Published by :
https://www.ijert.org/
An International Peer-Reviewed Journal

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 01 , January - 2026

Biosynthesis of Zinc Oxide Nanoparticle (ZnO NPs) Using Bioactive Yeast Metabolite of Hilsa Fish Pickle and Assessing Their Antimicrobial Properties Against Biofilm Producing Bacteria

Rohit Kumar Pandey, Dr. Bipin Kumar Sharma, Bijaya Anandy Yumnam, Sribas Das,
Krishna Borgohain, Sanchayita Basu
Department of Microbiology, Tripura University, Suryamaninagar, Agartala- 799022

ABSTRACT - Nanotechnology become a hot research area over the past few years and metal nanoparticle synthesis using greener route has receive much attention as it is cost effective, eco-friendly alternative to physical and chemical methods. In the present study, zinc oxide nanoparticles (ZnO NPs) was biosynthesized using hilsa fish pickle as a precursor. The biosynthesized nanoparticles were characterized by performing UV-Vis spectrophotometer, SEM, EDX and FTIR. SEM observations reveal irregular needle and spherical morphology with particle size at the nanoscale mean size of 25nm to 42 nm. FT-IR analysis showed that the ZnO NPs' surfaces contained many functional groups. Antibacterial activity was shown by the ZnO-NPs on Gram-positive bacteria (*Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli*). A maximum inhibition zone of ~18.66 mm was observed for *Escherichia coli* at a concentration of 50 µg/mL for sample prepared at 24 h. Antibiofilm analysis also showed maximum inhibitory effects with the maximum concentration.

Keywords: Nanotechnology; zinc oxide nanoparticles; hilsa fish; Anti-biofilm; Antimicrobial; eco-friendly.

INTRODUCTION:

Nanotechnology has become one of the popular technologies in recent years due to its great impact on the electronics industry, electronics, and an aerospace industry, medicine, biomedicine, environment, and agriculture. In the past two decades, the biosynthesis of NPs has received considerable attention due to the growing need to develop environmentally friendly technologies in the field of material synthesis [1] e.g. biotic synthesis of metal NPs uses microorganisms such as fungi [2], yeasts [3], bacteria [4,5], plants [6] and algae [7]. Nanoparticles are rapidly attracted attention in areas such as health care, cosmetics, biomedicine, food and feed application, drug administration, environment and health [8]. Synthesis of metals and metal oxide nanoparticles by using green access considered to be an ecological friendly, a cost-effective, biocompatible, safe and valuable approach [9].

The potential use of yeast in the biosynthesis of NPs is promising due to the easy handling of yeast in laboratory conditions, their abundant enzyme synthesis and rapid growth without the need complex nutrients [10,11]. Yeast biomass is able to produce metal nanoparticles and nanostructures through the reduction of proteins in enzymes either by intracellular or extracellular. Zinc oxide nanoparticles (ZnO-NPs) are the most commonly used metal oxide nanoparticles because of their characteristic optical properties and chemical properties that can be easily modified by changing the morphology and width bandgap (3.37 eV) and high excitation binding energy (60 meV) for ZnO-NP simulation to be a strong photocatalytic and photo oxidative group against chemical and biological agents species [12,13] . ZnO-based structures have been exhibit to biodegradability both in the bulk phase and in the form of nanoparticles [14]. Zn ions also act as the principal mediators of intracellular bacterial toxicity, disrupting their cell membranes [15]. ZnO NPs have antimicrobial activity against many pathogenic organisms such as bacteria and fungi by penetrating the bacterial cell wall and cell membrane. Such different behaviour of ZnO NPs makes them effective for use as antibacterial agents against pathogenic microbes such as Gram-positive and Gram-negative bacteria and for the antibiofilm activities. The main cause is the emergence of microbes resistant to antibiotics nosocomial infections that are considered serious public health a problem that has led to increased morbidity and mortality worldwide [16,17]. Due to the worldwide escalation of bacterial resistance which has become a major problem nowadays is urgently needed improve and develop methods and strategies for solving this problem. Due to their antibacterial activities, they represent metal NPs an effective solution for overcoming bacterial resistance [18, 19]. In

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 01 , January - 2026

hospital, biofilm formation mainly on medical equipment, and a tactic used by microbes to resist antibiotics, allowing them to persist and easily spreads to patients, destroys immune defences and causes most chronic and recurrent infections [20,21]. In this context, it is essential to design original, novel, innovative and cost-effective strategies that can eradicate or prevent infections associated with biofilms. Some NPs such as Ag and ZnO have shown inhibitory effects on the formation bacterial biofilm [22,23]. The current study focussed on synthesizing ZnO NPs using green synthesis route using yeast metabolite of hilsa chilli fish pickle. As fish are very perishable, they start spoiling as soon as possible are harvested. Deterioration hilsa fish meat is caused by the action of enzymes, microorganism and chemical action. The biosynthesized NPs were characterized and confirmed by various instrumentation techniques such as UV–Vis, EDX, FE-SEM and FTIR. Finally, the biological activity of ZnO NPs including against antibacterial and antibiofilm activities.

Material and Methods:

Hilsa chilli fish pickles were collected from a local market of Manipur, North East India. Zinc acetate dihydrate ($Zn(Ac)_2.2H_2O$) as a precursor. Yeast extract peptone dextrose agar (YEPDA), yeast peptone dextrose broth, distilled water, aluminum foil, and Whatman No.1filter paper

Sample preparation and isolation of yeast:

5g of hilsa chilli fish pickle were washed with distilled water 2-3 times to remove oil and species. The hilsa chilli fish were kept in water at room temperature for 2-3 days. The fish biomass were separated by using Whatman filter paper No.1 and the filtrate were collected in a falcon tube for further yeast isolation. The isolation of yeast strain was done by standard serial dilution methods. Briefly, 1ml of filtrate (*Hilsa* pickle) was inoculated in 9ml YPD broth, 10^{-1} to 10^{-5} serial dilution was done and the samples were incubated for 24hrs at 37℃. In YPD agar plates, the samples were spread using a sterile spreader and incubated for 24hrs at 37℃. For pure culture isolation, three rounds of streaking were done and the colonies were marked properly for further characterization [24].

Morphological characteriza were studied on the basis of colour, texture, margin, elevation, shape, size. Methylene blue staining was performed to elucidate the morphology and arrangement of yeast cells and budding. Methylene blue penetrates into every cell. Living cells enzymatically reduce the dye to a colourless product and become unstained, whereas dead cells were stained blue.

Extracellular Synthesis of ZnO-NPs:

Yeast cells were inoculated into growth medium (Yeast extract, peptone, dextrose). 25 mL of yeast cell mixed with 2.5mL zinc acetate dihydrate (Zn(Ac)2·2H2O) solution (10 mmol/L) and incubated at 37◦C in the shaking incubator, agitated at 150 rpm for 24 to 36 h. After the colour of the solutions changed from light brown to pale and deep white, the biomass products were collected by using centrifugation at 10,000 rpm for 10 min and then dried the pellet at 150◦C for 6 h. The supernatant were kept for further test and the pellet converted into powder form for further analysis [24].

Characterization of synthesized zinc oxide nanoparticles:

Various techniques were utilized for nanoparticle characterization, including Fourier transform infrared technique (using a Bruker a-T , with wave number range of about 4000–400 $^{cm-1}$ and a resolution of about 4–8 cm), Field Emission scanning electron microscopy with EDX & sputter coated (model –sigma 300, carl Zeiss) UV–vis spectroscopy (UV-1800, Shimadzu). The optical characteristics of samples were determined using a UV–Visible spectrophotometer, which was operated in the range between 300–800 nm.

Antimicrobial Screening of ZnO-NPs:

Bacteria such as *E. coli,* and *B. subtilis* were used for the study. Antimicrobial activity of zinc oxide nanoparticles towards the gram positive and negative bacteria were studied by using well diffusion method. Muller Hinton Broth (2.1 g in 100 ml of distilled water) was prepared and each strains were inoculated separately into culture broth and incubated overnight at 37ºC. Muller Hinton Agar was prepared and autoclaved. Agar was then poured into sterile plates and allowed to solidify. 500µl fresh bacterial cultures were plated on an agar plate. Three wells with a diameter of 5mm each were created in the plates using a gel puncher. The wells were filled with 50µl each of crude extract, aqueous zinc salt, biosynthesis zinc oxide nanoparticles. Ciprofloxacin was used as positive control. After 24 hr zone of inhibition were measured [25].

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 01 , January - 2026

Anti-biofilm activity detection:

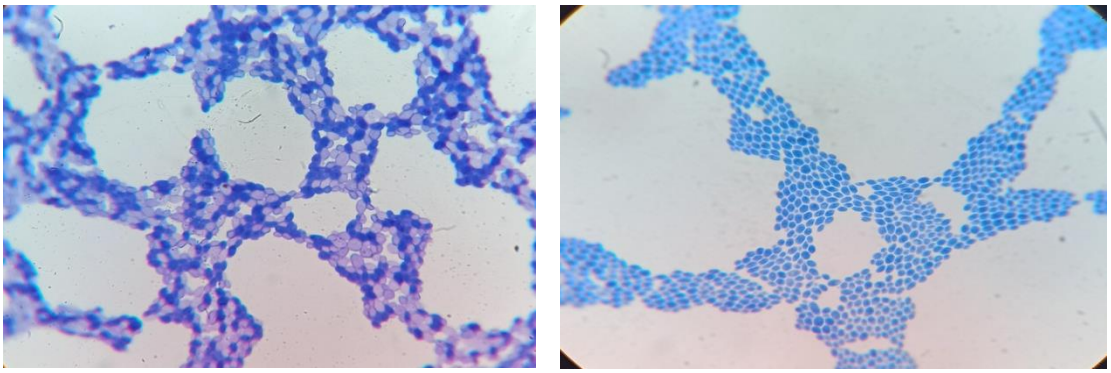
Anti-biofilm activity of ZnO NPs was determined against *E. coli,* and *B. subtilis* using 96-well microtiter plate method as described by Saising *et al*., 2012[26]. All biofilm experiments were repeated three times. In microplate wells, 100 µl of each bacterial suspension was mixed with the same volume of solution of biosynthesized ZnO nanoparticles having different concentrations (25, 50, 75 and 100 µg/ ml) and incubated at 37 °C for 24 h. The control test includes wells containing inoculum without ZnO NPs and wells containing only growth medium as positive control and negative control. After incubation, the culture supernatant was removed and the microplate wells were washed three times with phosphate buffered saline (PBS) to remove non-adherent cells. 200µl of 0.1% violet crystal solution were added to the adherent bacteria on the plate and kept it for 30 min. Then, the microplate was washed thrice with distilled water to remove excess crystal violet and make it dried at room temperature. 200 µl of ethanol-acetone mixture (75/25%) was added to each wells and kept it for 30 min. Biofilm inhibition was determined by optical density (OD) at 595 nm using a microplate reader (Bio Tek SN 200309B) and the biofilm inhibition rate was calculated using the equation below:

$$\text{Antibiofilm \%} = \frac{\text{Absorbance of control} - \text{Absorbance of ZnONPs}}{\text{Absorbance of control}} \text{ x } 100$$

RESULTS AND DISCUSSION

Isolation of yeast and ZnO NPs synthesis:

Morphologically distinct yeast strain HF1 and HF2 were selected to synthesize ZnO NP. This study describes the extracellular synthesis of ZnO-NPs by using two yeast strains HF1 and HF2. The formation of ZnO was visually observed through the color changes of the Zn biomass suspension from light brown to cream color after incubation for 24 to 36 hours. A deep white colour was observed after 24 h of incubation and remained stable up to 36 h (Figure 1). Biochemical mechanism generation and stabilization of nanoparticles remains largely unexplored, but some researcher groups have shown that the key biomolecules involved in the formation of metal/metal oxide nanoparticles are the ones that are present in the organisms [27, 28]. The most active functional groups for Zn ion complexation are the Hydroxyl groups of Amino acid. These molecules hydrolysis and finally synthesis of zinc nanoparticles by using thermal decomposition. The structure helps the zinc particles by stabilizing them and the ZnO-NPs prevent crystal growth [16].



(A) (B)

Figure: 100x methylene blue staining (A) HF1 (B) HF2

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 01 , January - 2026

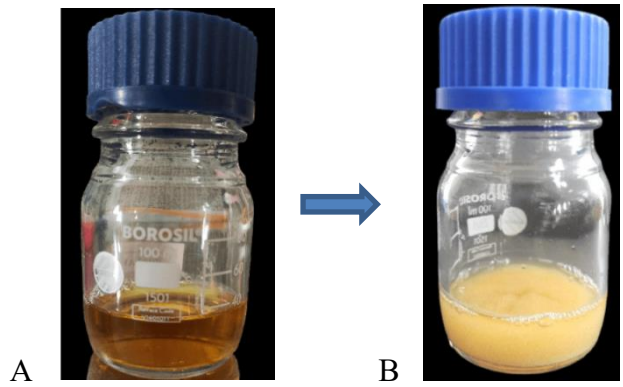


Figure 1. The changes in color of Zn (**A**) at 0 h; and (**B**) 24 h of incubation. .

Characterization of ZnO-NPs:

The UV-vis absorption spectra of the biosynthesized ZnO-NP samples are shown in Figure 1. The spectra demonstrate typical ZnO absorption peaks at wavelengths ranging from 320 to 340 nm which can be assigned to ZnO self-absorption in the band gap due to electronic transitions from the valence band to the conduction band (O2p→Zn3d) [29]. These changes can be attributed to differences in morphology, particle size and surface nanostructures of prepared nanoparticles. [29]

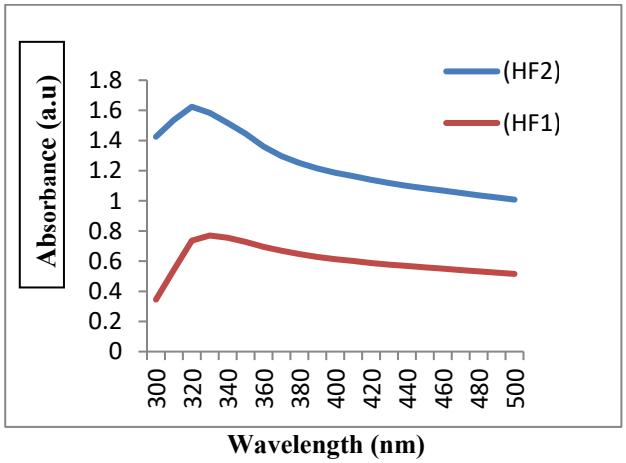
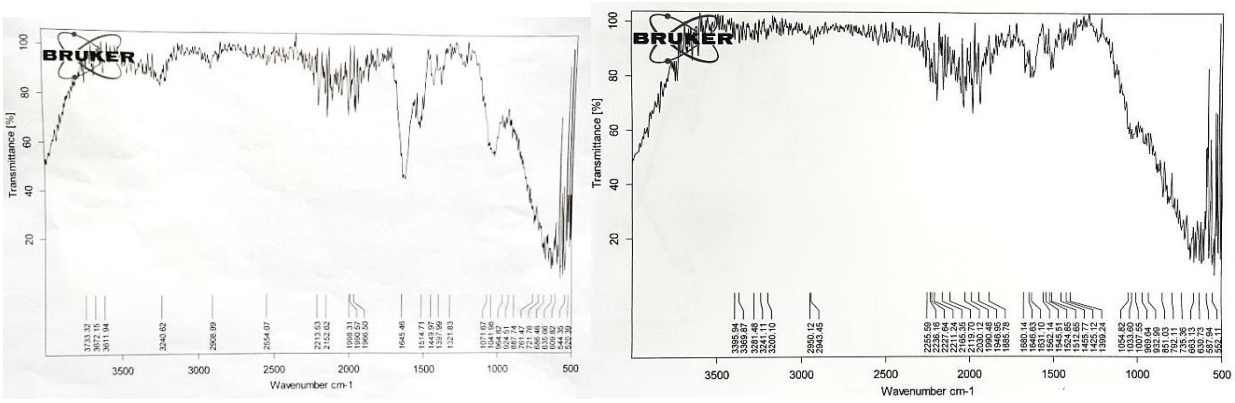


Figure 2: Absorption spectra of the ZnO-NPs

FTIR analysis of ZnO nanoparticles :

In the present study, FTIR was used to determine the functional groups on the surface of the synthetized ZnO NPs. The samples (HF1) have absorption peaks in the range of 3364, 1658, 1534, 1065, 609,541 cm^{-1} . The spectrum obtained clearly shows ZnO absorption band in the region 500 and 600 cm^{-1} [30]. The stretch for ZnO nanoparticles were found to be around 541 cm^{-1} -609 cm^{-1} **.** The C-O stretching alcohols, carboxylic acids, esters, ethers show the peak at 1065 cm^{-1} . The C-C stretch (in-ring) aromatics show the peak at 1534 cm^{-1} **.** The observed peak at 1658 cm^{-1} leads to N–H bend primary amines. The band showed at 3364 cm^{-1} relates to O–H stretching**.** The sample (HF2) exhibit absorption peaks in the region of 3240, 1245, 1514, 1041, 609, 544 cm $-^{1}$. The metal-oxygen (ZnO stretching vibrations) vibration pattern is correlated with the 544 cm^{-1} - 609cm^{-1} absorption peak. Here the C-O stretching alcohols, carboxylic acids, esters, ethers show the peak at 1041 cm^{-1} [31]. The C-C stretch (in-ring) aromatics show the absorption peak at 1514 cm^{-1}**.** The observed peak at 1645 cm^{-1} leads to N–H bend primary amines and 3240 cm^{-1} corresponds to strong stretching vibrations of hydroxyl functional groups. The properties of ZnO-NPs is predominantly depends on the surface structure of the Zn-O bonds [16].

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 01 , January - 2026

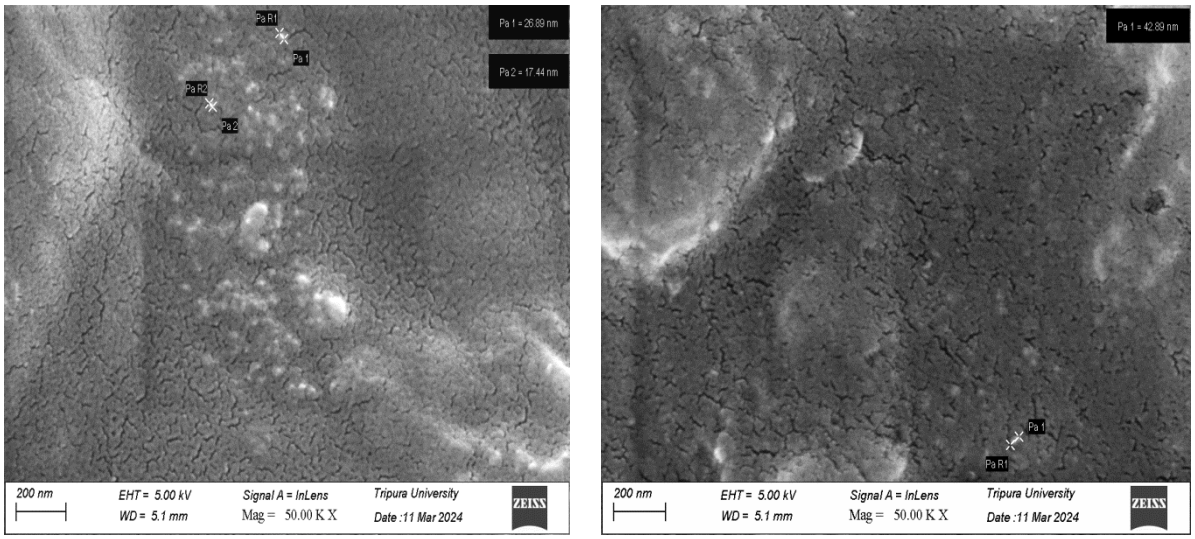


Hilsa chilli fish pickle (01) **Hilsa chilli fish pickle (02)**

Figure 3: FTIR spectra of the synthesized ZnO-NPs

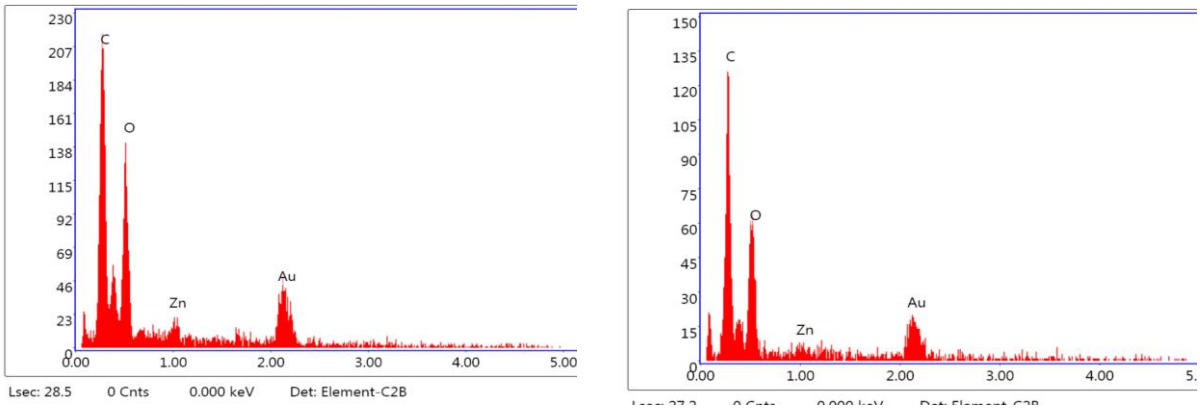
Structural characterization of zinc oxide nanoparticles using SEM:

The SEM results revealed the presence of the synthesized zinc oxide nanoparticles in HF1 and HF2 yeast strains as micro-sized ZnO with an average size range of 25nm to 42 nm. SEM photographs shows the presence of particles with almost spherical or triangular and needle-like shape, very adherent like cotton forming agglomerates. Magnification range from 5000X to 50,000X and resolution is from 10 to 200 nm. EDX with SEM is the other technique to determine the arrangement and composition of biologically synthesized nanoparticles. In this spectrum, it is observable that important elements of biologically synthesized nanoparticles are zinc and oxygen.



Hilsa chilli fish pickle (01) **Hilsa chilli fish pickle (02)**

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 01 , January - 2026



Hilsa chilli fish pickle (01) Hilsa chilli fish pickle (02)

Figure 4: SEM micrographs with EDX graphs of synthesized ZnO NPs

Antimicrobial activity:

The effect of zinc oxide nanoparticles on *E. coli,* and *B. subtilis* were studied using well diffusion method. ZnONPs exhibit good inhibitory activity against Gram-negative bacteria compared to Gram positive bacteria. A zone of maximum inhibition was recorded when the
concentration of ZnONPs (50 µg/ml) against *E.coli* for HF1 was (14mm) and HF2 was (18.6mm). *B. subtilis* for HF1 (14mm) and HF2 (11mm). The crude extract did not show significant zone of inhibition. A zone of inhibition was observed in all the plates from which it can be concluded that zinc oxide nanoparticles have antimicrobial activity. It was reported that the cell membranes of Gram-negative bacteria such as *E. coli* tested in this study, the peptidoglycan layer is protected by an external lipopolysaccharide (LPS) membrane in the cells of Gram-negative bacteria. In environments where exterior materials can damage it, it helps the bacteria to survive [32]. *B. subtilis* has some strategies to survive in harsh conditions. The formation of protective endospores is one of the important functions [33].
Antimicrobial activity of zinc oxide of nanoparticles is mainly caused by the formation
highly reactive species such as OH^-, H_2O_2, O^{2+}. H_2O_2, penetrates the cell membrane. OH^- and O^{2+} damage the cell membrane and cell wall from the outside. ZnO-NP acts as an effective bactericidal agent against both Gram-positive as well as Gram-negative bacteria and have been found to interact directly with the cell wall of bacteria leading to death. The values of zone of inhibition obtained from the study are presented in the Table.

Samples	**Antibiotic disc (mm) ciprofloxacin 5mcg**	**ZnO NPs (mm) (50µl)**
HF1 (*E. coli)*	**16.66**	**14**
HF2 (*E. coli)*	**15**	**18.66**
HF1 (*B. subtilis)*	**19.3**	**14**
HF2 (*B. subtilis)*	**12**	**11**

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 01 , January - 2026

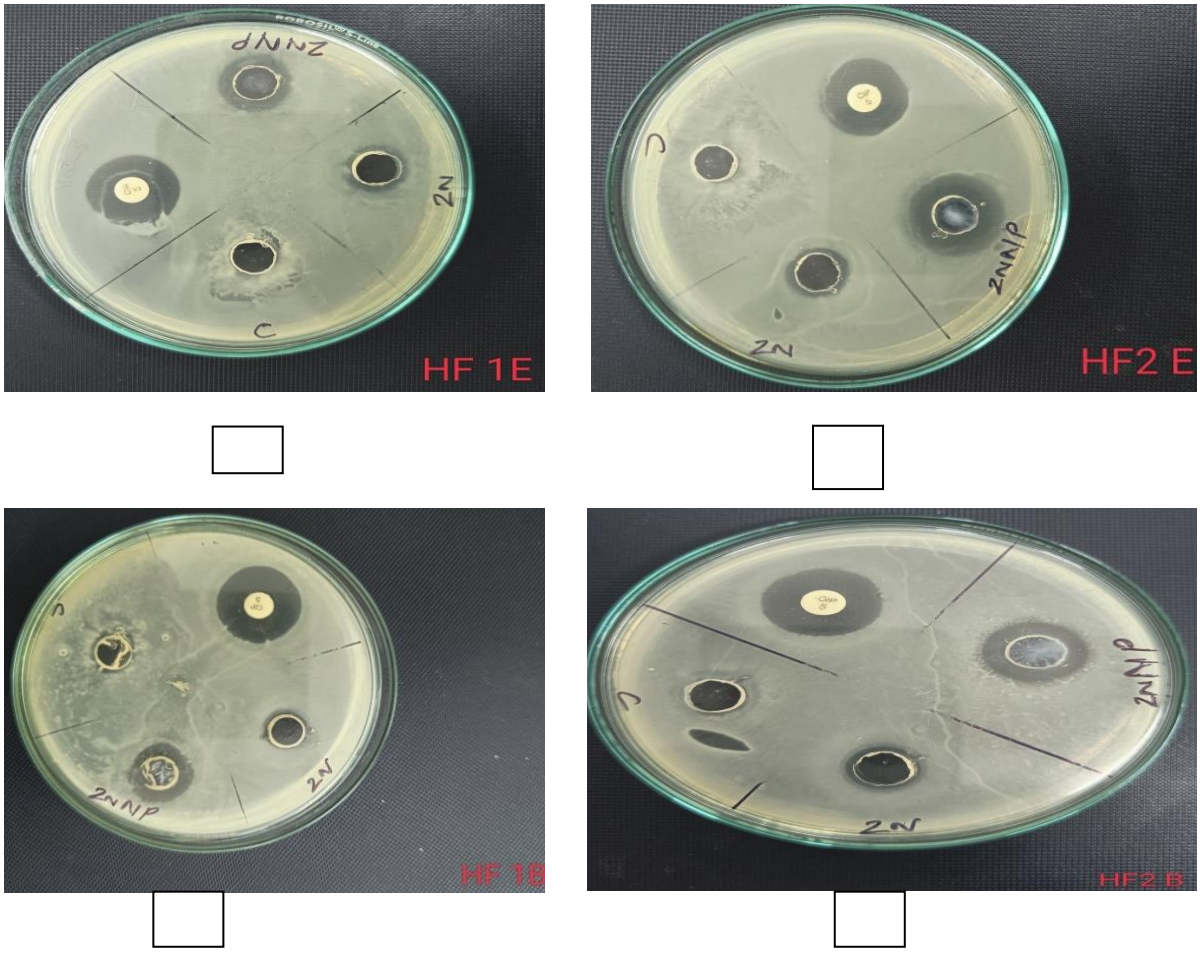


Figure 5: Plates showing inhibition zones containing ZnO NPs (A) HF1 (*E. coli)* **(B)** HF2 (*E. coli)* **(C)** HF1 (*B. subtilis)* **(D)** HF2 (*B. subtilis)*

Anti-biofilm activity of ZnO NPs:

The results indicate that the biofilm formed by the two bacterial strains is effectively inhibited by ZnO NPs in the two tested bacterial strains a significant inhibition rate was observed when treated with ZnO NPs in a concentration of 100 µg / ml. Biosynthesized ZnO NPs to inhibit biofilm formation confirms its potency and efficiency to eradicate biofilm layer that is considered like an impermeable barrier to antibiotics. ZnO NPs efficiently inhibited the formation of biofilm by both microorganisms in a dose-dependent manner (25, 50, 75, and 100 µg mL^{-1}). It was found that the rate of inhibition reached 87 and 83% in HF1 and HF2 for *B. subtilis.* For *E. coli the* ZnO-NPs reduced the biofilms development of by 89 and 86% in HF1 and HF2. The graph show that more the concentration of ZnO NPs less bacterial growth. The data revealed greater toxicity of NPs in concentration dependent manner with cell wall disruption and higher membrane permeability [34]. Green-synthesized ZnO NPs were able to cause biofilm degradation by weakening the structural components of biofilm-forming EPS [35].

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 01 , January - 2026

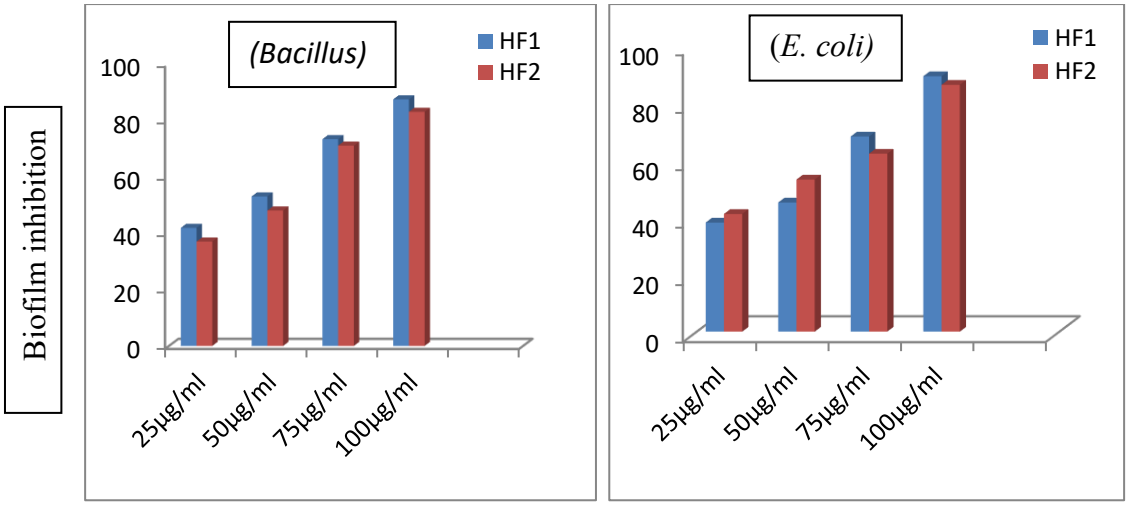


Figure 5 : Inhibition of biofilm formation of *B. subtilis* and *E. coli* by ZnO-NPs. The result are presented as mean of three replicates $\pm standard\ deviation$.

CONCLUSION:

For the first time, the potential use of isolated yeast strain from Hilsa chilli fish pickle for synthesis. In this study, ZnO-NPs are demonstrated using the green method. They were ZnO nanoparticles it was found to have a spherical or triangular structure with an average crystallite size of ~25nm–42 nm. The duration of the reaction was found to play a critical role in size, shape and distribution ZnO-NPs**.** Furthermore, ZnO NPs successfully inhibited biofilms formed by *E. coli,* and *B. subtilis*. The biosynthesized ZnO NPs exhibited good antimicrobial activity against both Gram-positive (*B. subtilis)* and Gram negative bacteria (*E. coli).* The ZnO-NPs were noticeably effective on *E. coli* . Moreover the synthesized ZnO nanoparticles can be used for combating biofilm and further analysis needs to be done on multi drug resistant pathogenic bacteria. This green synthesis approach appears to be a non-toxic, cost –effective and eco-friendly alternative to the physical and chemical methods, and would be suitable for developing a biological process for large scale production.

REFERENCES

[1] Mandal, D., Bolander, M. E., Mukhopadhyay, D., Sarkar, G., & Mukherjee, P. (2006). The use of microorganisms for the formation of metal nanoparticles and their application. Applied microbiology and biotechnology, 69, 485-492. https://doi.org/10.1007/s00253-005-0179-3

[2] Tarafdar, J. C., & Raliya, R. (2013). Rapid, low-cost, and ecofriendly approach for iron nanoparticle synthesis using Aspergillus oryzae TFR9. Journal of Nanoparticles, 2013(1), 141274..https://doi.org/10.1155/2013/141274

[3] Gericke, M., & Pinches, A. (2006). Biological synthesis of metal nanoparticles. *Hydrometallurgy*, *83*(1-4), 132-140.https://doi.org/10.1016/j.hydromet.2006.03.019

[4] Liu, L., Liu, T., Tade, M., Wang, S., Li, X., & Liu, S. (2014). Less is more, greener microbial synthesis of silver nanoparticles. Enzyme and microbial technology, 67, 53-58.https://doi.org/10.1016/j.enzmictec.2014.09.003 [CrossRef] [PubMed]

[5] Ahmad, A., Senapati, S., Khan, M. I., Kumar, R., & Sastry, M. (2003). Extracellular biosynthesis of monodisperse gold nanoparticles by a novel extremophilic actinomycete, Thermomonospora sp. Langmuir, 19(8), 3550-3553.https://doi.org/10.1021/la026772l [CrossRef]

[6] Sadeghi, B., & Gholamhoseinpoor, F. (2015). A study on the stability and green synthesis of silver nanoparticles using Ziziphora tenuior (Zt) extract at room temperature. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 134, 310-315. https://doi.org/10.1016/j.saa.2014.06.046 [CrossRef] [PubMed]

[7] Singaravelu, G., Arockiamary, J. S., Kumar, V. G., & Govindaraju, K. (2007). A novel extracellular synthesis of monodisperse gold nanoparticles using marine alga, Sargassum wightii Greville. Colloids and surfaces B: Biointerfaces, 57(1), 97-101.https://doi.org/10.1016/j.colsurfb.2007.01.010

[8] Kirthi, A. V., Rahuman, A. A., Rajakumar, G., Marimuthu, S., Santhoshkumar, T., Jayaseelan, C., … & Bagavan, A. (2011). Biosynthesis of titanium dioxide nanoparticles using bacterium Bacillus subtilis. Materials Letters, 65(17-18), 2745-2747.https://doi.org/10.1016/j.matlet.2011.05.077

[9] Abdul, H., Sivaraj, R., Venckatesh, R., 2014. Green synthesis and characterization of zinc oxide nanoparticles from *Ocimum basilicum* L. var. Purpurascens Benth- *lamiaceae* leaf extract. Mater. Lett. 131, 16–18 https://doi.org/10.1016/j.matlet.2014.05.033

[10] Kumar, D., Karthik, L., Kumar, G., & Roa, K. B. (2011). Biosynthesis of silver nanoparticles from marine yeast and their antimicrobial activity against multidrug resistant pathogens. Pharmacologyonline, 3, 1100-1111.

[11] Yan, S., He, W., Sun, C., Zhang, X., Zhao, H., Li, Z., ... & Han, X. (2009). The biomimetic synthesis of zinc phosphate nanoparticles. *Dyes and Pigments*, *80*(2), 254-258.https://doi.org/10.1016/j.dyepig.2008.06.010 [CrossRef]

[12] Rodnyi, P. A., & Khodyuk, I. V. (2011). Optical and luminescence properties of zinc oxide. Optics and spectroscopy, 111, 776-785. https://doi.org/10.1134/S0030400X11120216[CrossRef]

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 01 , January - 2026

[13] Shaba, E. Y., Jacob, J. O., Tijani, J. O., & Suleiman, M. A. T. (2021). A critical review of synthesis parameters affecting the properties of zinc oxide nanoparticle and its application in wastewater treatment. Applied Water Science, 11(2), 48.https://doi.org/10.1007/s13201-021-01370-z. [CrossRef]
[14] Kielbik, P., Kaszewski, J., Rosowska, J., Wolska, E., Witkowski, B. S., Gralak, M. A., … & Godlewski, M. M. (2017). Biodegradation of the ZnO: Eu nanoparticles in the tissues of adult mouse after alimentary application. Nanomedicine: Nanotechnology, Biology and Medicine, 13(3), 843-852.https://doi.org/10.1016/j.nano.2016.11.002 [CrossRef]
[15] Mandal, B. K. (2016). Scopes of green synthesized metal and metal oxide nanomaterials in antimicrobial therapy. In Nanobiomaterials in Antimicrobial Therapy (pp. 313-341). William Andrew Publishinghttps://doi.org/10.1016/B978-0-323-42864-4.00009-9 [CrossRef]
[16] 16. Samuel, S. O., Kayode, O. O., Musa, O. I., Nwigwe, G. C., Aboderin, A. O., Salami, T. A. T., & Taiwo, S. S. (2010). Nosocomial infections and the challenges of control in developing countries. African journal of clinical and experimental microbiology, 11(2).
[17] https://doi.org/ 10.4314/ajcem.v11i2.53916.
[18] 17. Liu, W. P., Tian, Y. Q., Hai, Y. T., Zheng, Z. N., & Cao, Q. L. (2015). Prevalence survey of nosocomial infections in the Inner Mongolia Autonomous Region of China [2012-2014]. Journal of Thoracic Disease, 7(9), 1650.https://doi.org/10.3978/j.issn.2072-1439. 2015.09.41.
[19] 18.Geffers, C., & Gastmeier, P. (2011). Nosocomial infections and multidrug-resistant organisms in Germany: epidemiological data from KISS (the Hospital Infection Surveillance System). Deutsches Ärzteblatt International, 108(6), 87. https://doi.org/10.3238/ arztebl.2011.0087.
[20] 19. Allahverdiyev, A. M., Kon, K. V., Abamor, E. S., Bagirova, M., & Rafailovich, M. (2011). Coping with antibiotic resistance: combining nanoparticles with antibiotics and other antimicrobial agents. Expert review of anti-infective therapy, 9(11), 1035-1052.
[21] https:// doi.org/10.1586/eri.11.121.
[22] 20. Stewart, P. S., & Costerton, J. W. (2001). Antibiotic resistance of bacteria in biofilms. The lancet, 358(9276), 135-138.https://doi.org/10.1016/s0140-6736(01) 05321-1.
[23] 21. Høiby, N., Ciofu, O., Johansen, H. K., Song, Z. J., Moser, C., Jensen, P. Ø., ... & Bjarnsholt, T. (2011). The clinical impact of bacterial biofilms. *International journal of oral science*, *3*(2), 55-65.https://doi.org/10.4248/ ijos11026.
[24] 22. Akhil, K., Jayakumar, J., Gayathri, G., & Khan, S. S. (2016). Effect of various capping agents on photocatalytic, antibacterial and antibiofilm activities of ZnO nanoparticles. Journal of Photochemistry and Photobiology B: Biology, 160, 32-42.
[25] https://doi.org/10.1016/j. jphotobiol.2016.03.015.
[26] 23. Lebeaux, D., Ghigo, J. M., & Beloin, C. (2014). Biofilm-related infections: bridging the gap between clinical management and fundamental aspects of recalcitrance toward antibiotics. Microbiology and Molecular Biology Reviews, 78(3), 510-543.
[27] https://doi.org/10.1128/MMBR.00013-14.
[28] 24.Boroumand Moghaddam, A., Moniri, M., Azizi, S., Abdul Rahim, R., Bin Ariff, A., Zuhainis Saad, W., … & Mohamad, R. (2017). Biosynthesis of ZnO nanoparticles by a new Pichia kudriavzevii yeast strain and evaluation of their antimicrobial and antioxidant activities. Molecules, 22(6), 872 https://doi.org/10.3390/molecules22060872
[29] 25. Rajan, A., Cherian, E., & Baskar, G. (2016). Biosynthesis of zinc oxide nanoparticles using Aspergillus fumigatus JCF and its antibacterial activity. Int. J. Mod. Sci. Technol, 1(2), 52-57.
[30] 26.Saising, J., Dube, L., Ziebandt, A. K., Voravuthikunchai, S. P., Nega, M., & Götz, F. (2012). Activity of gallidermin on Staphylococcus aureus and Staphylococcus epidermidis biofilms. Antimicrobial agents and chemotherapy, 56(11), 5804-5810. https://doi.org/10. 1128/AAC.01296-12.
[31] 27. Xie, J., Lee, J. Y., Wang, D. I., & Ting, Y. P. (2007). Silver nanoplates: from biological to biomimetic synthesis. ACS nano, 1(5), 429-439.https://doi.org/10.1021/nn7000883
[32] 28. Durán, N., Marcato, P. D., Alves, O. L., De Souza, G. I., & Esposito, E. (2005). Mechanistic aspects of biosynthesis of silver nanoparticles by several Fusarium oxysporum strains. Journal of nanobiotechnology, 3, 1-7.https://doi.org/10.1186/1477-3155-3-8
[33] 29.Zak, A. K., Abrishami, M. E., Majid, W. A., Yousefi, R., & Hosseini, S. M. (2011). Effects of annealing temperature on some structural and optical properties of ZnO nanoparticles prepared by a modified sol–gel combustion method. Ceramics International, 37(1), 393-398. https://doi.org/10.1016/j.ceramint.2010.08.017 [CrossRef]
[34] 30. Azizi, S., Ahmad, M., Mahdavi, M., & Abdolmohammadi, S. (2013). Preparation, Characterization, and Antimicrobial Activities of ZnO Nanoparticles/Cellulose Nanocrystal Nanocomposites. BioResources, 8(2).
[35] 31. Bandow, J. E., Brötz, H., & Hecker, M. (2002). Bacillus subtilis tolerance of moderate concentrations of rifampin involves the σB-dependent general and multiple stress response. Journal of bacteriology, 184(2), 459-467.https://doi.org/10.1128/jb.184.2.459-467.2002 [CrossRef] [PubMed]
[36] 32 Bandow, J. E., Brötz, H., & Hecker, M. (2002). Bacillus subtilis tolerance of moderate concentrations of rifampin involves the σB-dependent general and multiple stress response. Journal of bacteriology, 184(2), 459-467.https://doi.org/10.1166/jbn.2013.1652
[37] 33 Yuvakkumar, R., Suresh, J., Saravanakumar, B., Nathanael, A. J., Hong, S. I., & Rajendran, V. (2015). Rambutan peels promoted biomimetic synthesis of bioinspired zinc oxide nanochains for biomedical applications. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 137, 250-258. https://doi.org/10.1016/j.saa.2014.08. 022.
[38] 34. Nagajyothi, P. C., An, T. M., Sreekanth, T. V. M., Lee, J. I., Lee, D. J., & Lee, K. D. (2013). Green route biosynthesis: Characterization and catalytic activity of ZnO nanoparticles. *Materials Letters*, *108*, 160-163.https://doi.org/10.1016/j.matlet.2013.06.095
[39] 35. Hsueh, Y. H., Ke, W. J., Hsieh, C. T., Lin, K. S., Tzou, D. Y., & Chiang, C. L. (2015). ZnO nanoparticles affect Bacillus subtilis cell growth and biofilm formation. *PloS one*, *10*(6), e0128457.doi:10.1371/journal.pone.0128457