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Antibacterial Activity of Zinc Oxide Nanoparticles-Poly-β hydroxybutyrate Bionanocomposites on Staphylococcus spp

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***Abstract*---**In the laboratories of the College of Medical Technologies/The Islamic University of Najaf, the biosynthetic zinc nanoparticles were synthesized by *Lactobacillus* bacteria. The structural and morphological properties of Zinc Oxide Nanoparticles were determined by Atomic force microscope and X–ray diffraction analysis, (hexagonal phase) shows that the synthesized nanoparticles were crystalline and pure in nature. The peaks at 2θ = 38.1, 44.18, 64.45, 77.12 & 81.03 were respection lines of spherical Zinc Oxide Nanoparticles respectively. Then, maximum diameter measured for particles is 8.4278 nm which determined by applying the Scherrer equation. After that, the samples of pathogenic bacteria (*Staphylococcus aureus*, *Staphylococcus Epidermidis*) diagnostic by Vitek2 compact system resistant to commercial antibiotics were collected and the antibacterial activity of these nanoparticles was tested. The results of inhibiting the growth of pathogenic bacteria were good. Then the ZnONps were combined with a polymeric compound (Poly-β hydroxybutyrate) to improve the properties of the nanocomposite as an antimicrobial, as the combined compound (ZnONPs/PHB) gave great inhibition results compared to the nanocomposite alone(ZnONPs). Four different concentrations of ZnO NPs /PHB (0.2, 0.1, 0.07 and 1.25 mg/ml) were used and the more effective inhibition zone against *Staph. aureus* 15 mm, *S. epidermidis* 34 mm While two concentrations of ZnO Nanoparticles (0.6 and 1.04 mg/ml) were used and the more effective inhibition zone against *S. aureus* 13 mm, *Staph.epidermidis* 29 mm, through disc diffusion agar

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method. It is clear that ZnO NPs/PHB bionanocomposite has stronger inhibitory effect on pathogenic bacteria compared with only Nanoparticles because the ZnO NPs/PHB exhibited reduced water uptake and superior gas as well as vapour barrier properties and causes increasing damage of pathogenic bacteria.

***Keywords*---**Nanoparticles, Poly-β-hydroxybutyrate, Antibacterial, Staphylococcus, Biosynthetic Zinc Oxide Nanoparticles.

Introduction

Lactobacillus bacteria is a major genera of the lactic acid bacteria group , well known for their extensive biological activities, characterized as Gram positive, non-motile, non-spore forming, facultative anaerobic conditions, fastidious, acid-tolerant & obligatory fermentation (Balakrishnan, 2011). Lactobacillus are existing in the flora of the mouth, reproductive organs of female and intestines (Yasodha, 2011). Due to its wide range of antibacterial action and biocompatibility, Zinc Oxide can be a safe and economical material for the treatment of different microorganisms. As broad-spectrum antibacterial agents, ZnO NPs have shown great promise and are effective material in compromising the stability of bacterial membranes that lead to increased cell permeability to nano products (Radhakrishnan, 2011). ZnO NPs are now extensively used in several consumer products (detergents, antibacterial products and protective creams) (Indumathy et al., 2011). Poly-β-hydroxybutyrate is a biodegradable thermoplastic polyester that can be used in medicine, agriculture, etc.. Because of its non-toxic, biodegradable and biocompatible nature, PHB is considered ideal drug carriers (Renuka, 2011).

Materials and Methods

ZnO Nps' biosynthesis by lactobacillus

The culture of Lactobacillus acidophilus 5ml was inoculated in the flask containing De man Ragosa Sharpe broth and incubated at 37° C for 24 h at 100rpm. After the incubation period, Centrifuged at 5000 rpm was done for 25 min. then the supernatant was taken. The pH of the supernatant was regulated by 0.4 M NaOH to delay the transformation process (the pH of the supernatant was acidic 4.7 to be neutral the NaOH was added to reach pH7 to eliminate the influence of organic acids). Aqueous zinc sulfate (ZnSO4.7H2O) 28.8 g. 0.1 M dissolved in 1000 ml of distilled water was added to 250 ml of the supernatant and then heated by a water bath of up to 85 ° C for 5-10 min. A white precipitate appears at the bottom of the flask indicates the process of transformation .Then the flask was incubated at 37 ° C for 12 hr. , all the particles are accumulated at the bottom of the flask. In order to separate the white precipitate , the product was centrifuged at 6000 rpm for 20 min. and washed with deionized water then the process was repeated 3 times to get pure products followed by drying at 60° C in a hot air oven for 4 hr (Ganesh, 2012).

Preparation of ZnO NPs Reinforced P (βHB)

0.3 g Poly-β hydroxybutyrate (manufactured by SIGMA Cop. / Germany) was put into the ZnO NPs mixed solution 0.18 g of ZnO NPs after that putting them into scuttle bottle containing 30 ml. chloroform then stirring by ultrasonic bath device for 30 min. continuing the process until achieving the homogeneous solution and verified the homogeneity by physical observation. Then, the films were poured into glass petri dishes (6 mm in diameter) covered with puncture aluminium sheets and left in a dark place for 24 h at 30°C room temperature for complete evaporation of chloroform (Salunkhe, 2012). In this study, we different weights of PHB (0.1, 0.2, 0.3 and 0.03g.) while using 0.18 and 0.3 g of ZnO NPs in preparation of ZnO NPs Reinforced P(βHB) Films.

Determination of Inhibition Zone for *Staphylococcus spp*

In discs, different amounts of ZnO NPs (0.6 and 1.04 mg/ml) and ZnO NPs/PHB bionanocomposite (0.2, 0.1, 0.07, and 1.25 mg/ml) were applied, and the results were analyzed. Following the inoculation and culture of various target bacteria on top of Muller Hinton agar, discs were put in specific areas on separate plates using a sterile technique. After a 24-hour incubation period, the zone of inhibition was assessed. ZnONPs and ZnONPs/PHB were used to compare the antibacterial activity of various concentrations of the compounds. An equal quantity of produced ZnONPs were dried at 70°C in order to get various nanoparticle sizes. The increase in temperature resulted in a larger size of particle (Wahab et al., 2010; Kanimozhi &Panneerselvam, 2011; Aljanaby & Aljanaby, 2018).

Disc diffusion method

The following steps were used to complete this technique on Muller Hinton media: 1.Bacterial isolate concentrations were collected and compared to McFarland solution to determine the appropriate concentration for each of the isolates. 2.The proper concentration of a substance One milliliter of each bacterial isolate was put to plates containing Muller Hinton agar, which was distributed on the surface of the dish using a wooden stick, and the dishes were left for one hour. 3.ZnO NPs/PHB were prepared in the manner indicated in the following Table 4.The Nanoparticles were dissolved in distilled water to get their final concentrations, and the ZnO NPs were dipped in filter paper to remove the excess water. 5. The ZnO NPs/PHB film and nanoparticles inhibition zones were measured using a ruler after incubation at 37°C for 24 hours (Aljanaby, 2018; Al-Labban et al., 2019).

Results

Zinc oxide nanoparticles biosynthesis by *Lactobacillus acidophilus*

The biosynthesis of nanoparticles from *Lactobacillus* has been confirmed by observing a change in solution color during the synthesis of ZnO NPs as shown in figure1 that explains the reduction of ZnO into ZnO NPs during exposure to bacterial extract followed by a change in color from brown to yellow during 24h.

Figure 1. Biosyntheisi steps of ZnONPs by *L.acidophilus*

Morphological &structural properties of ZnO NPs biosynthesized by *Lactobacillus acidophilus*
X-ray diffraction analysis (XRD)

Figure 2 shows X-ray diffraction patterns of the ZnONPs synthesized using *Lactobacillus acidophilus* . XRD analysis (hexagonal phase) shows that the synthesized nanoparticles were crystalline and pure in nature. The peaks at 2θ = 38.1 ,44.18 , 64.45 ,77.12 & 81.03 were respection lines of spherical Zn-ONPs respectively.

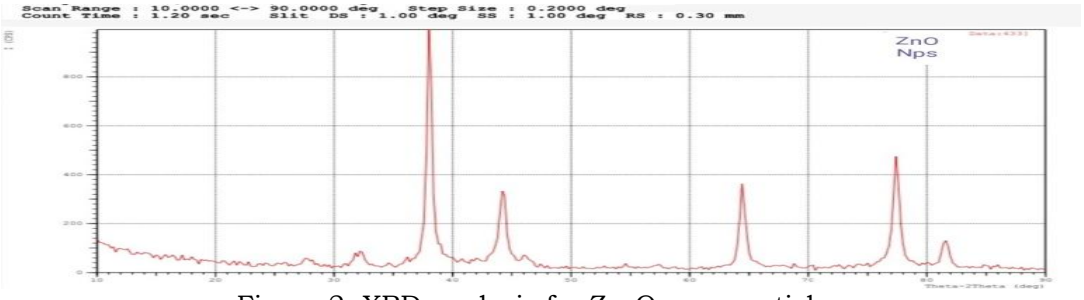

Figure 2. XRD analysis for Zn-O nanoparticles

Atomic force microscope

AFM analysis of synthesized ZnO NPs was carried out to assess their morphology (outer surface) and size range ,3-D images of AFM, figures 3 showed that most of the nanoparticles are spherical in shape and some of the agglomerations were present in the background of the nanoparticles.

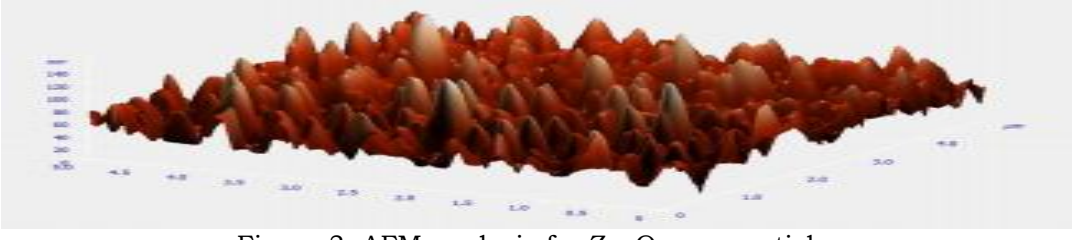

Figure 3. AFM analysis for Zn-O nanoparticles

Antibacterial Activity of ZnONPs and ZnONPs/ PHB against *Staph. Aureus*

It was observed in this study that the growth inhibition was also increased by increasing the concentration of ZnONPs or ZnONPs/PHB in the disc.

Table 1
Diameter of inhibition zone of ZnONPs and ZnO NPs/ PHB for *Staph. Aureus*

	ZnO NPs/ PHB wt.%	ZnO NPs/PHB Con.(mg/ml)	ZnO NPs/PHB I.Z.(mm)		ZnO NPs Con.(mg/ml)	ZnO NPs I.Z.(mm)
1	0.18:0.1	0.20	12	1	0.6	8
2	0.18:0.2	0.10	10			
3	0.18:0.3	0.07	9			
4	0.3:0.03	1.25	15	2	1.04	13

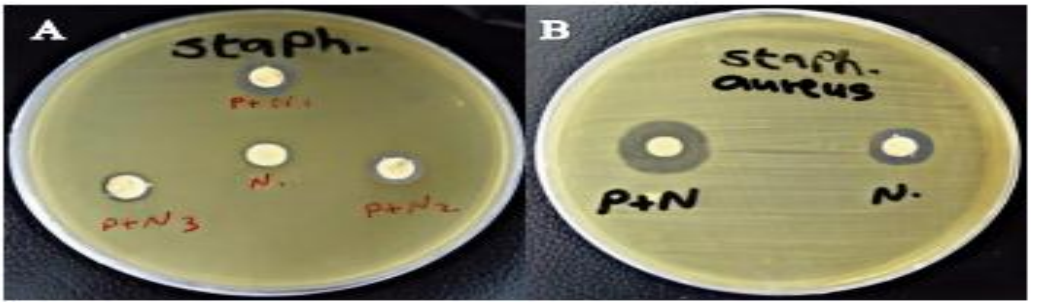

Figure 4. Antibacterial activity of ZnO NPs &ZnONPs /PHB against *Staph.aureus*.*P(PHB) *N(ZnO NPs)

figure 4 indicates by comparison of antibacterial activity of ZnONPs & ZnONPs/PHB that ZnONPs/ PHB shows more activity in the inhibitory effect as well as increases inhibitory zone by increasing of Inhibitors concentrations.

Antibacterial Activity of ZnONPs and ZnONPs/ PHB against *Staph. epidermidis*

The antibacterial activity of ZnONPs and ZnO NPs/PHB was tested by methods of the disc diffusion agar. Table 2 indicates that increasing concentrations of ZnO NPs and ZnO NPs/PHB leads to increase growth inhibition of the *Staph. epidermidis*, but ZnO NPs/PHB has more activity in the inhibitory effect for this bacteria (0.2 mg/ml reach to 31 mm in ZnO NPs while reach 34mm in ZnONPs/PHB bionanocomposites).

Table 2
Diameter of inhibition zone of ZnONPs and ZnO NPs/ PHB for *Staph.epidermidis*

	ZnO NPs/ PHB wt.%	ZnO NPs/PHB Con.(mg/ml)	ZnO NPs/PHB I.Z.(mm)		ZnO NPs Con.(mg/ml)	ZnO NPs I.Z.(mm)
1	0.18:0.1	0.20	31	1	0.6	24
2	0.18:0.2	0.10	29			
3	0.18:0.3	0.07	28			
4	0.3:0.03	1.25	34	2	1.04	29

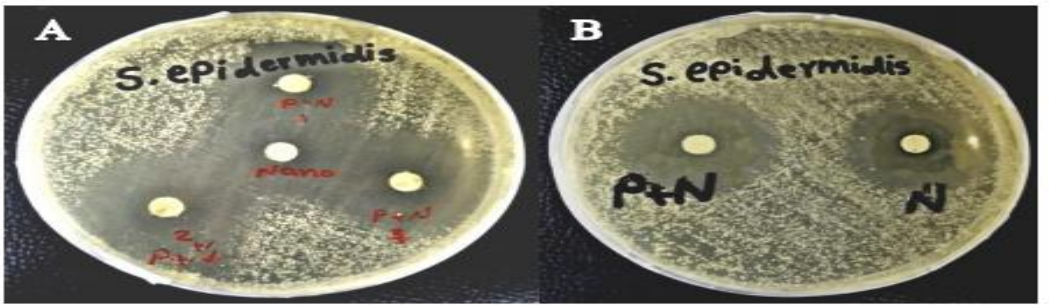

Figure 5. Antibacterial activity of ZnO NPs & ZnO NPs /PHB bionanocomposites against *Staph. epidermidis*. *P(PHB) *N(ZnO NPs)

Discussion

Zinc oxide nanoparticles biosynthesis by *Lactobacillus acidophilus*

The synthesis of ZnO NPs in the solution may have resulted from pHsensitive membrane bound oxide-reductase and carbon source based on rH2 (Wahab et al., 2010; Kanimozhi, 2011). Lactobacillus acidophilus has a major role in the production of ZnO NPs. It is possibly due to this bacteria possesses negative electro-kinetic potential which attracts the cations readily and triggers the synthesis of nanoparticles. Furthermore, Lactobacillus has the capacity to grow even in the presence of oxygen allowing more capability of metabolic growth. In addition, the presence of glucose in the MRS media used for ZnONPs synthesis tends to lower the potential for oxidation-reduction (Salunkhe, 2012).

Morphological & structural properties of ZnO NPs biosynthesized by *Lactobacillus acidophilus*

X-ray diffraction analysis (XRD)

The average particle size of Zinc Oxide nanoparticles was determined by applying the Scherrer equation, as shown in equation below for peak 1 reflection at two theta using the full width at half maximum was agreed with (Yasodha , 2011; Aljanaby & Alhasnawi, 2017). D=0.9λ/ $\Delta(2\theta).\cos(\theta)$ λ=0.154060 nm , 2θ=38 , k=0.9 D= 8 .4278 nm. The maximum diameter measured for particles is 8.42 nm. Line extension of diffraction peaks is an indicator that the synthesized materials

are within the range of the nanometer. The synthesis of the obtained nanoparticles is too small.

Atomic force microscope

AFM analyses revealed that obtained nanoparticles were in a hexagonal, polydispersed, nearly spherical in shape, these results were compatible with (Sabir et al., 2014; Shamsuzzaman et al., 2014).

Antibacterial Activity of ZnONPs and ZnONPs/ PHB against *Staph. aureus*

Antibacterial activity of ZnONPS/PHB on Staph. aureus is systematically greater than ordinary antibiotics (Yasodha , 2011). The antibacterial activity was due to cell membrane damage, leading to cell contents leakage and cell death. While the exact mechanism of action is still unclear, the generation of H2O2 (a potent oxidizing agent harmful to the cells of living organisms)from the surface of ZnO has been considered as the main factor of ZnO reinforced nanocomposites antibacterial activity (Ashokkumar et al., 2014). Interestingly, synthesized ZnONPs (Zhang et al., 2015). exhibits an excellent bactericidal effect against human bacterial pathogens, the result indicates the same result found by (Aljanaby et al., 2018) which showed that ZnONPs has antibacterial activity for pathogenic bacteria.

Antibacterial Activity of ZnONPs and ZnONPs/ PHB against *Staph. Epidermidis*

Bacterial cell death may be caused by two different factors. The most widely accepted toxicity mechanisms for ZnO NPs are believed to be a consequence of ROS production and Zn2+ release from the particles. If the reactive oxygen species produced, such as the superoxide anion, hydrogen peroxide, and hydroxide, enter the bacterial cell membrane, they may cause oxidative destruction of the lipids and proteins inside the cell membrane, resulting in DNA damage (Liu & Yang, 2003; Tam et al., 2008). One such method is to suppress bacterial cells via the interaction between positively charged ZnO and the negatively charged cell wall of the bacterium. The release of Zn2+ from ZnO NPs may also cause important metabolic pathways to be disrupted (Sathishkumar et al., 2017). These results compared to a commercial antibiotic were wonderful, biosynthesis ZnO NPs and ZnO NPs/PHB have high effect in inhibition the growth of S. epidermidis (Abinaya et al., 2017; Aljanaby & aljanaby, 2017; Al-Labban, 2017).The results reported that ZnONPs is effective against gram-positive bacteria, especially *Staph. epidermidis.*

Conclusions

- ZnO NPs/PHB bionanocomposites more antimicrobial activity than ZnO NPs
- Lactobacillus acidophilus have ability to biosynthesis of ZnO NPs
- ZnO NPs/PHB bionanocomposites and ZnONPs have inhibitory effect for staphylococcus spp. esepicilly aureus and epidirmidis

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