Scanning Electron Microscopy and Energy-Dispersive X-Ray Spectroscopy of Postbiotics- Exopolysaccharides Zinc Oxide Nanoparticles

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Abstract- Postbiotics are defined as metabolites of a probiotic which gives beneficial effects to the host. It is derived both extracellular and intracellular. Exopolysaccharides are one of the most important secondary metabolites which are secreted externally to the cell surface. Exopolysaccharides have positive effect on health such as antitumor effects, antiulcer, antioxidant activities, immune stimulatory activity and also lowers blood cholesterol. Exopolysaccharides derived from lactic acid bacteria. Zinc nanoparticles have excellent property of anticancer and antibacterial and it is also low in cost and less in toxicity. Zinc oxide nanoparticles synthesized using microbial exopolysaccharides are used as antibacterial food packaging coating to improve the shelf life of the food products. The morphology, elemental analysis or chemical characterization of postbiotics-exopolysaccharides zinc oxide nanoparticles was analyzed by Scanning Electron Microscopy which also gives the Energy-dispersive X-ray spectroscopy.

Index Terms-Postbiotics, Exopolysaccharides, Zinc oxide nanoparticles, Scanning Electron Microscopy (SEM) and Energy-dispersive X-ray (EDX) spectroscopy

I. INTRODUCTION

Postbiotics refers to soluble factors, products or metabolic products i.e. Metabolites secreted by live bacteria or released after bacterial lysis i.e. cell free supernatants such as polysaccharides, cell surface proteins and organic acids. It is derived both extracellular and intracellular **[4].**

Exopolysaccharides are one of the most important secondary metabolites which are secreted externally to the cell surface. Exopolysaccharides have positive effect on health such as antitumor effects, antiulcer, antioxidant activities, immune-stimulatory activity and also lowers blood cholesterol. The exopolysaccharides is an important feature of some strains of dairy lactic acid bacteria **[3].**

Lactic Acid Bacteria especially *Lactobacillus fermentum* has double functional properties antimicrobial activity against intestinal pathogens and high Total Antioxidative Activity (TAA) and Total Anti-oxidative Status (TAS) of intact cells. It also plays an important role in improving Low Density Lipid (LDL) particles, i.e. the antianther genic effect **[5].**

Zinc is a nutritional supplement and food additive. Zinc oxide nanoparticles that affect the growth of many bacteria like Staphylococcus, Streptococcus and *E.coli*. Currently, there are widespread applications of zinc oxide nanoparticles in medicine because of their anti-bacterial effect. The food industry uses zinc oxide as a source of zinc, which is an essential micronutrient. Zinc nanoparticles have excellent property of anticancer and antibacterial and it is also low in cost and less in toxicity **[1].** Zinc oxide is currently listed as a Generally Recognized as Safe (GRAS) material by the Food and Drug Administration. Thus, zinc oxide in Nano scale has shown antimicrobial properties and potential applications in food preservation **[2].**

This study is shows the morphology, elemental analysis or chemical characterization of postbiotics-exopolysaccharides zinc oxide nanoparticles was analyzed by Scanning Electron Microscopy which also gives the Energy-dispersive X-ray spectroscopy.

II. MATERIALS AND METHODS

A. Isolation of probiotic *Lactobacillus*

Probiotic *Lactobacillus* species was isolated from raw cow's milk using MRS broth (DeMan, Rogosa and Sharpe). The raw milk was diluted using distilled water in different ratios 10^{-1}, 10^{-2}, $10^{-}3$ and 10^{-4}. 10^{-2} serial dilution was used and poured in the spread plate method. This method helps to identify the species present in the milk and was identified as *Lactobacillus fermentum.*

B. Isolation of postbiotics-exopolysaccharides

Postbiotics - exopolysaccharides from probiotic *Lactobacillus* culture medium was isolated using centrifugation method. The MRS broth containing probiotic *lactobacillus fermentum* cells were centrifuged at 8000 rpm for 20 min. The supernatant was collected to remove the *Lactobacillus fermentum* cells. The supernatant containing exopolysaccharides were precipitated using 3 volumes of chilled 95% ethanol and incubated at 44ºC for 24 hours. Allowed to stand overnight at 4ºC in a refrigerator. The precipitate formed was again centrifuged at 8000 rpm for 20 min to obtain cell free solution.

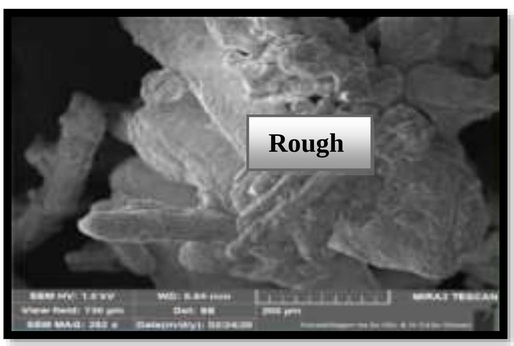
The precipitate was dissolved in 10% TCA (Tri Chloro Acetic acid) and stored at 4 ºC for 1 hour. The exopolysaccharides was then dissolved and dialyzed in distilled water for 4 days at 4ºC. The cell free extract precipitate filled bag was kept inside the beaker containing distilled water. The water acts as a buffer solution and helps to separate small molecules and protein present in the bag **[7].**

III. RESULTS AND DISCUSSION

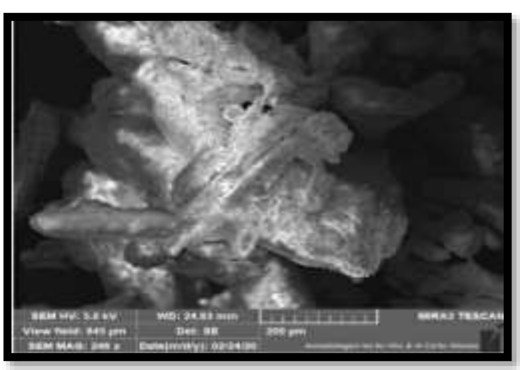
A. Scanning Electron Microscopic Analysis

The surface topography and composition of postbiotics - exopolysaccharides zinc oxide nanoparticles were determined using Scanning Electron Microscopic analysis. The SEM results illustrated using different precursor affects the size and shape of nanoparticles. The micrographs of zinc nanoparticles proved that they are Nano-sized range, shape and uniform distribution.

The scanning electron micrograph of the postbiotics-exopolysaccharides zinc oxide nanoparticles is given in Figure 1. Exopolysaccharides formed a rough and rigid surface structure upon 137X magnification. The postbiotics-exopolysaccharides zinc oxide nanoparticles from *Lactobacillus fermentum* were observed to have a compact structure. SEM scan showed the integral surface of postbiotics-exopolysaccharides zinc oxide nanoparticles from *Lactobacillus fermentum* which was an important feature required for making coating material. Exopolysaccharides are one of the promising polymers for developing food packaging coating material **[6].**

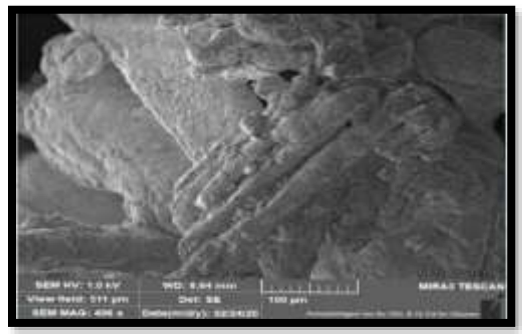


a. 200 µm, View Field at 736 µm

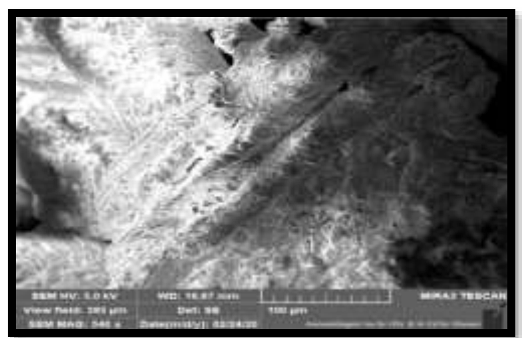


b. 200 µm, View Field at 845

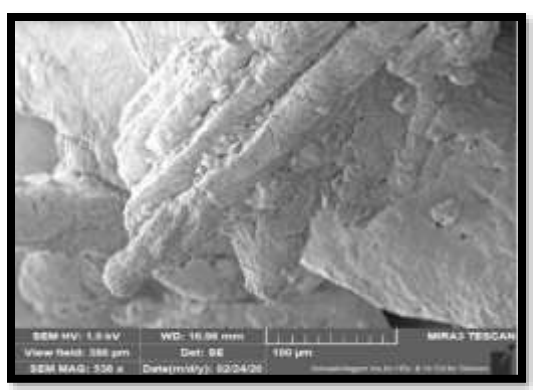
c. 200 µm, View Field at 922 µm



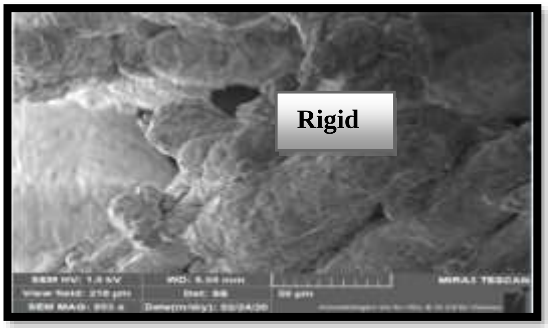
d. 100 µm, View Field at 511 µm



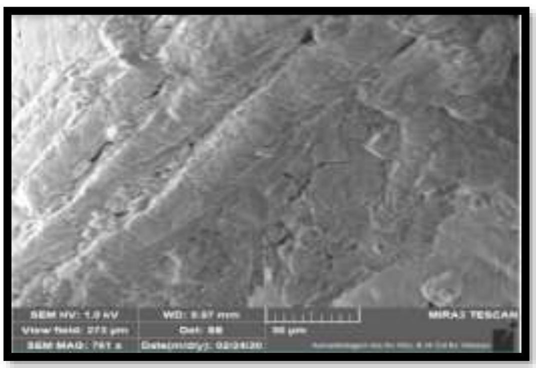
e. 100 µm, View Field at 385 µm



f. 100 µm, View Field at 386 µm



g. 50µm, View Field at 218 µm



f.50µm, View Field at 273 µm

Figure 1
Characteristic Features of Postbiotics-Exopolysaccharides Zinc Oxide Nanoparticle Formed by Lactic Acid Bacteria (L. *fermentum*) Using FESEM

B. Energy-dispersive X-ray spectroscopy

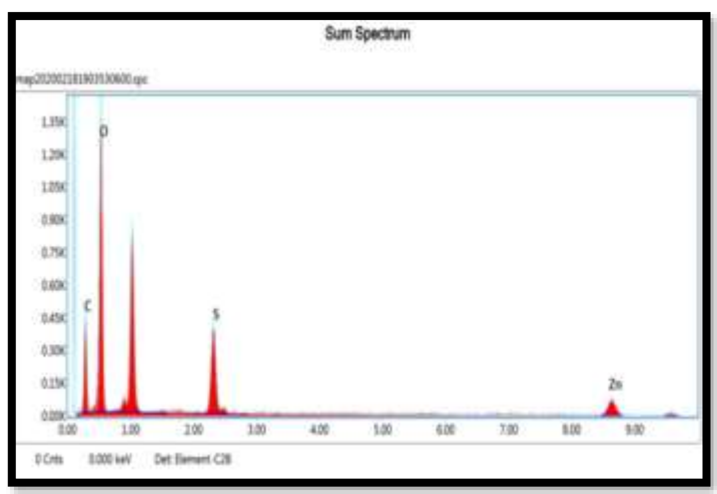
Energy Dispersive X-ray spectroscopy (EDS or EDX) is an analytical technique used for the elemental analysis or chemical characterization of a sample used in conjunction with Scanning Electron Microscopy. It depends on an interaction of some source of X-ray excitation and a sample. Its characterization shows that each element has a unique atomic structure permitting a distinctive set of peaks on its electromagnetic emission spectrum.

TABLE I
ELEMENTAL COMPOSITION OF POSTBIOTICS - EXOPOLYSACCHARIDES ZINC NANOPARTICLES (AREA 1)

eZAF Smart Quant Analysis			
Element	**Weight %**	**Atomic %**	**Error%**
C K	**25.61**	**40.6**	**11.31**
O K	**38.86**	**46.25**	**9.11**
Zn K	**26.32**	**7.67**	**4.98**
S K	**9.22**	**5.48**	**4.44**

The distribution of carbon, oxygen, zinc and sulphur present in the postbiotics - exopolysaccharides zinc oxide nanoparticles, their weight and atomic percentage is mentioned in Table I.

Figure 2
EDX Analysis of Postbiotics-Exopolysaccharides Zinc Oxide Nanoparticles (Area 1)



Y-axis depicts the number of counts and x-axis the energy of the X-rays. The position of the peaks leads to the identification of the elements and the peak height helps in the quantification of each element's concentration in the sample.

Figure 3
EDX Spectrum 1 of Postbiotics - Exopolysaccharides Zinc Oxide Nanoparticles (Area 1)

TABLE II
ELEMENTAL COMPOSITION OF POSTBIOTICS - EXOPOLYSACCHARIDES ZINC OXIDE NANOPARTICLES (AREA 2)

eZAF Smart Quant Analysis								
Element	Weight%	Atomic%	Net Int	Error%	K ratio	Z	A	F
C K	23.90	39.81	30.89	11.70	0.0547	1.1319	0.2022	1.0000
O K	35.92	44.91	108.21	9.25	0.1258	1.0804	0.3242	1.0000
S K	9.37	5.85	49.42	4.79	0.0778	0.9556	0.8648	1.0044
Zn K	30.81	9.43	15.21	8.66	0.2422	0.7653	1.0054	1.0218

The distribution of carbon, oxygen, zinc and sulphur present in the postbiotics - exopolysaccharides zinc oxide nanoparticles and their weight and atomic percentage is mentioned in Table II and figure 5.

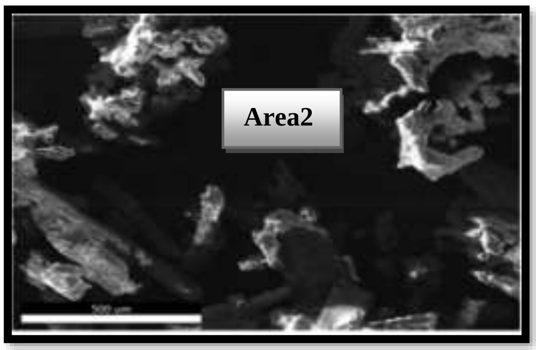
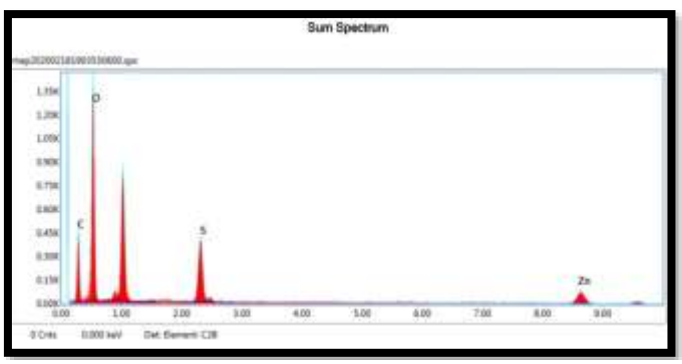


Figure 4
EDX Analysis of Postbiotics-Exopolysaccharides Zinc Nanoparticles (Area 2)



Y-axis depicts the number of counts and x-axis the energy of the X-rays. The position of the peaks leads to the identification of the elements and the peak height helps in the quantification of each element's concentration in the sample.

Figure 5
EDX Spectrum 2 for the Postbiotics-Exopolysaccharides Zinc Oxide Nanoparticles (Area 2)

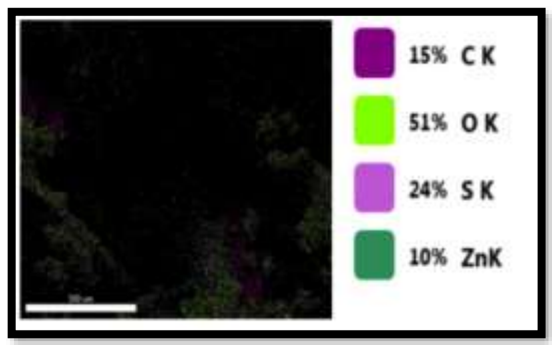
Figure 5 shows the distribution of carbon, oxygen, zinc and sulphur present in the postbiotics exopolysaccharides zinc oxide nanoparticles in spectrum graph.

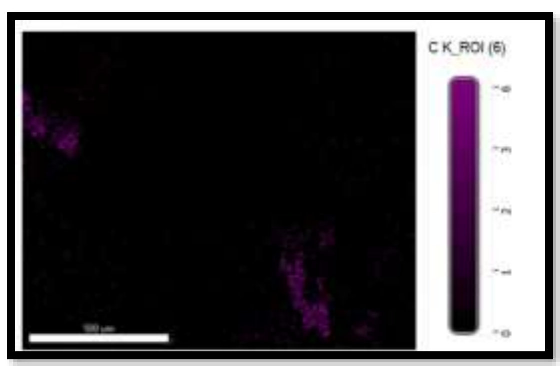
EDX analysis was performed confirms the formation of zinc (Zn K), oxygen (O K), carbon (C K) and sulphur (S K) in postbiotics-exopolysaccharides zinc oxide nanoparticles. During the EDX measurement different areas were focused and the corresponding peaks are shown in the Figures 2 and 4. The Zn K, O K, C K and S K can be seen in the synthesized postbiotics-exopolysaccharides zinc oxide nanoparticles in the EDX spectrum. In spectrum 1, the quantity of Zn K, O K, C K and S K were 7.67, 46.25, 40.6 and 5.48 respectively, while in spectrum 2, the values were 9.43, 44.91, 39.81 and 5.85 measured in atomic % for Zn K, O K, C K and S K respectively.

The comparison between area 1 and area 2 of smart quant result revealed that area 1 has 25.61 weight% of carbon (C K) and area 2 has 23.90 weight% of carbon (C K). Area 1 has 38.86 weight% of oxygen (O K) and area 2 has 23.90 weight% of oxygen (O K). Area 1 has 26.32 weight% of zinc (Zn K) and area 2 has 30.81 weight% of zinc (Zn K). Area 1 has 9.22 weight% of sulphur (S K) and area 2 has 9.37 weight% of sulphur (S K). While comparing area 1 and area 2, carbon (C K) and oxygen (O K) weight % is higher in area 1. Zinc (Zn K) and sulphur (S K) weight% is higher in area 2.

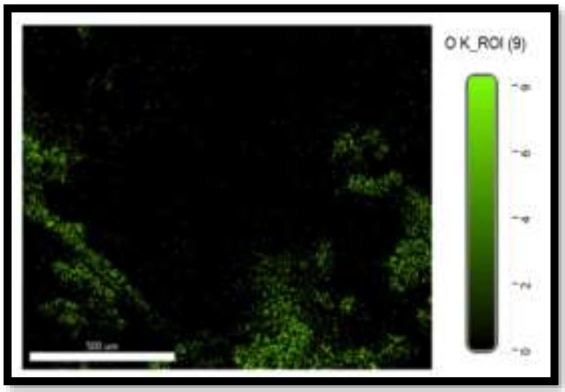
C. Smart Quant Results

Smart quant results use ZAF-corrected spectral analysis for quantitative compositional information. It simultaneously performs quantitative analysis during spectral data collection. The elemental composition analyses of the zinc oxide nanoparticles from the EDX plot of the SEM images are shown in Figure 6.





a. **Carbon Element**



b. **Oxygen Element**

c. **Sulphur Element**

d. **Zinc Element**

Figure 6
SEM (EDX) of Element Overlay for the Postbiotics-Exopolysaccharides Zinc Oxide Nanoparticles

The EDX spectra revealed that postbiotics-exopolysaccharides zinc oxide nanoparticles had the required phase of zinc, oxygen, carbon and sulphur at a concentration of 10%, 51%, 15% and 24% respectively.

IV. CONCLUSION

The scanning electron micrograph of the postbiotics-exopolysaccharides zinc oxide nanoparticles formed a rough and rigid surface structure upon 137X magnification. The postbiotics-exopolysaccharides zinc oxide nanoparticles from *Lactobacillus fermentum* were observed to have a compact structure. EDX analysis was performed confirms the formation of zinc (Zn K), oxygen (O K), carbon (C K) and sulphur (S K) in postbiotics-exopolysaccharides zinc oxide nanoparticles. In spectrum 1, the quantity of Zn K, O K, C K and S K were 7.67, 46.25, 40.6 and 5.48 respectively, while in spectrum 2, the values were 9.43, 44.91, 39.81 and 5.85 measured in atomic % for Zn K, O K, C K and S K respectively.

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REFERENCES

[1] Elshama, S.S., Metwally, E., Abdallah and Rehab, I., and Karim, A. (2018).Zinc Oxide Nanoparticles: Therapeutic Benefits and Toxicological Hazards. *The Open Nanomedicine Journal*, 5(1), 16-22.

[2] Espitia, P.J.P., Soares, N.D.F.F., Coimbra, J.S.D.R., Andrade, N.J.D., Cruz, R.S., & Medeiros, E.A.A. (2012). Zinc Oxide Nanoparticles: Synthesis, Antimicrobial Activity and Food Packaging Applications. *Food Bioprocess Technology*, 5(1), 1447–1464.

[3] Leo, F., Hashida, S., Kumagai, D., Uchida, K., Motoshima, H., Arai, L., Asakuma, S., Fukuda, K., and Urashima, T. (2007). Studies on a Neutral Exopolysaccharide of *Lactobacillus fermentum* TDS030603. *Journal of Applied Glycoscience*, 54(4), 223—229.

[4] Malashree, L., Angadi, K.V, Yadav, S., and Prabha, V. (2019). "Postbiotics" – One Step Ahead of Probiotics. *International Journal Current .Microbiology Applied Sciences*, 8(1), 2049-2053.

[5] Mikelsaar, M., & Zilmer, M. (2009). *Lactobacillus fermentum* ME-3an antimicrobial and anti-oxidative probiotic. *Microbial Ecology in Health and Disease*, 21(1), 1-27.

[6] Shukla, G., sharma, V., and Harjai, K. (2018). Purification, characterisation and antibiofilm potential

of postbiotics (bacteriocin and exopolysaccharides) isolated from *Lactobacillus fermentum pseudomonas aeruginosa* PA01. *British Journal of Pharmaceutical and Medical Research*, 3(6), 1510-1523.

[7] Krithika, R., Balasasirekha, R. (2021). FTIR Spectrum and XRD of Postbiotics-Exopolysaccharides Zinc Oxide Nanoparticles, 12(3), 292-300.

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