Microbe-mediated Synthesis of Selenium Nanoparticles by *Lactobacillus casei* and Its Inhibitory Potentials

[*1]Ogunleye, G.E., [2]Oyinlola, K.A., [1]Said-Ige, N., [3]Akintade, O.O., [1]Olatoye, M. J., [1]Balogun, A.I., and [3]Ayorinde, G.O.

[1]*Department of Biological Sciences, KolaDaisi University, Ibadan, Oyo State, Nigeria.*
[2]*Department of Microbiology, University of Ibadan, Nigeria.*
[3]*Department of Chemical Sciences, KolaDaisi University, Ibadan, Oyo State, Nigeria.*

***Corresponding Author:** Ogunleye Gbemisola E. *geegbemz@gmail.com*

Abstract

Lactic acid bacteria (LAB) are widely regarded as safe for use and have long been consumed in various fermented foods. Selenium offers substantial potential benefits, including immune system support, fertility enhancement, and antimicrobial and anticancer activity. This study investigated the inhibitory potential of biogenic selenium nanoparticles (SeNPs) prepared by using *Lactobacillus casei* supernatant. Selenium nanoparticles were biosynthesized using *L. casei* and subsequently characterized through visual observation, UV–visible spectrophotometry, Fourier Transform Infrared (FTIR) spectroscopy, Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Energy Dispersive X-ray (EDX) analysis, and X-ray Diffraction (XRD). The antimicrobial potential of the *Lactobacillus casei*-mediated selenium nanoparticles (LC-SeNPs) was evaluated using the agar well diffusion technique. A noticeable colour transition from light yellow to ruby red was observed, indicating nanoparticle formation. The absorption spectrum of LC-SeNPs extended between 250 and 450 nm, with a prominent surface plasmon resonance peak recorded at 350 nm. The FTIR absorption peaks were from 3626.00 cm^{-1} to 460.73 cm^{-1} indicating hydroxyl, ester aldehyde, amine, phenol, and ether groups, were responsible for effective bio-reduction and stabilization of the nanoparticles. The SeNPs were observed to be spherical in shape and occurred in aggregated forms, with particle sizes ranging from 20 to 100 nm. XRD confirmed their crystallographic nature. *E. coli* (25 mm) and *S. pneumoniae* (17 mm) showed susceptibility, demonstrating LC-SeNPs' effective antimicrobial activity. These *L. casei*-synthesized SeNPs are eco-friendly, non-toxic and has significant medical application.

Keywords: *Lactobacillus casei*-mediated nanoparticles**,** Biogenic selenium nanostructures**,** Microbial fabrication**,** Antimicrobial efficacy**,** Nanoparticle characterization.

DOI: 10.5281/zenodo.20190066

Introduction

Lactic acid bacteria (LAB) are Gram-positive bacteria that use carbohydrates as their only or primary carbon source (Adamu *et al.,* 2025). These non-sporulating, non-respiring bacteria exhibit high tolerance to low pH and appear as either rod-shaped bacilli or spherical cocci, sharing similar metabolic and physiological traits (Gizachew *et al*., 2023). The primary LAB genera include *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus*, and *Streptococcus* (Zheng *et al*., 2020).

Nanotechnology involves manipulating and producing materials or devices at the atomic or small-group scale, typically measured in nanometers (10^{-9}) (Amany *et al*., 2025). This field promises advances across medicine, consumer products, energy, materials, and manufacturing (Shahbaz *et al*., 2023). Selenium (Se) is an essential trace element with confirmed antioxidant effects in clinical and epidemiological studies (Genechi *et al*., 2023). Selenium nanoparticles (SeNPs) offer lower toxicity and higher biocompatibility than organic or inorganic Se compounds, attracting interest as therapeutic and theranostic agents (Sampath *et al*., 2021). Common SeNP synthesis methods include chemical reduction, biological agents (e.g., plants, fungi, or bacteria) for green biosynthesis, and physical techniques like pulsed laser ablation (PLA) (Hosnedlova *et al*., 2018; Au *et al*., 2023). However, chemically synthesized SeNPs are highly unstable, often aggregating and precipitating in aqueous solutions, which reduces their bioactivity (Gregircha *et al*., 2023). Surface modifications with amino acids (Feng et al., 2014), peptides (Fu *et al*., 2016), proteins (Zhang *et al*., 2018), chitosan, or polysaccharides (Bai *et al*., 2017) have been used to address this.

LAB biosynthesize SeNPs by reducing toxic selenite to less toxic elemental Se, often capped by bacterial proteins and polysaccharides. These SeNPs exhibit antibacterial and antioxidant properties with applications in food, medicine, and agriculture (Zhong *et al*., 2024). Amid rising antibiotic resistance, LAB-SeNP combinations offer promising antibacterial alternatives. While physical and chemical synthesis methods are effective, they are costly and hazardous to health. For instance, Adebayo-Tayo *et al*. (2021) reported SeNP production using *Lactobacillus pentosus* ADET MW861694, which showed antagonistic effects against food pathogens. Castañeda-Ovando *et al*. (2020) isolated *Lactobacillus* species from commercial fermented milk and evaluated their minimal inhibitory concentrations and growth kinetics. LAB are renowned for their potent antibacterial activity, and recent studies highlight SeNPs' anticancer, antioxidant, and antibacterial potential. This study investigates the synthesis, characterization, and antibacterial properties of SeNPs produced by *Lactobacillus casei*.

Materials and Methods

Culture Collection

Lactobacillus casei was sourced from fresh cow milk and retrieved from the culture repository of the Microbiology Laboratory, Department of Biological Sciences, KolaDaisi University, Ibadan, Nigeria. The bacterial strain was preserved in De Man, Rogosa, and Sharpe (MRS) broth (LabM, UK) containing 12% (v/v) glycerol. Stock cultures were kept at 4°C and routinely subcultured for subsequent experiments.

L. casei Supernatant Preparation

Sterilized MRS broth was freshly prepared and inoculated using a 24-hour-old culture of *L. casei.* The culture flask was incubated for 24 hr at 35°C. The culture was centrifuged (Himac CR21GII Hitachi, Japan) at 5000 rpm for 25 min at 37°C after the incubation period; the supernatant was obtained and subsequently used.

Microbial Synthesis of Selenium Nanoparticles (SeNPs)

50 mL of 2 mM sodium selenite was homogenized with 100 mL of *Lactobacillus casei* supernatant. The conditions were adjusted to obtain the best yield of SeNPs: pH 7.2, incubation temperature 30°C, and reaction time 24 hr under agitation at 150 rpm in a shaking incubator (El-Saadony *et al.,* 2021).

Analysis of the synthesized SeNPs

Visual detection and UV–Visual Spectroscopy Analysis

The appearance of a ruby red colour from the initial sample indicated successful selenium nanoparticle formation. UV–Visual spectroscopy (752 UV-Vis Spectrophotometer) was applied for analyzing the optical behavior of the synthesized SeNPs. 1 mL of each samples was taken and the absorbance was measured using a UV-visible spectrophotometer with wavelengths ranging from 250 to 750 nm (Bharathi *et al*., 2020).

Fourier Transform-Infrared (FT-IR) spectroscopy analysis of SeNPs

The functional groups in the SeNPs were determined using Fourier Transform Infrared spectroscopy. A nanoparticles sample weighing 2 mg, was mixed with 100 mg of KBr and pressed into translucent discs. Spectra were recorded using a Nicolet iS10 FT-IR spectrophotometer (China) over 400–4000 cm^{-1} (El-Saadony *et al*., 2021).

Morphological and Structural Analysis of SeNPs

Scanning electron microscopy (SEM) was employed to assess the shape and dimensions of SeNPs. The biosynthesized nanoparticles were freeze-dried, placed on a coverslip, and sputter-coated with gold prior to imaging. Images were captured using a JEOL JSM-7600F SEM (US) to assess shape and morphology (Adebayo-Tayo *et al.,* 2021).

To prepare the specimen, a drop of the sample was placed on a carbon-coated grid to obtain a thin film. The mixture was then centrifuged at 13,000 rpm for 10 min. The resulting pellet was rinsed to remove residual organic materials and dried through lyophilization. Shape and size were measured with a TEM-ARM200F Verios 460L (USA) at an accelerating voltage of 15 kV and 0.23 nm resolution (Akl *et al*., 2020).

Energy Dispersive X-ray (EDX) Analysis of SeNPs

The elemental constituents of the sample were analyzed using energy-dispersive X-ray spectroscopy (EDS) with a JEOL JSM-7600F instrument (US). The centrifuged sample was applied to a carbon film, and spot-profile analysis was performed by focusing the electron beam on the biosynthesized SeNPs surface at 40.0 kV and 350 mA (Adebayo-Tayo *et al*., 2021).

X-Ray Diffraction (XRD)

The crystallinity of the synthesized SeNPs with *L. casei* supernatant was assessed using X-ray diffraction (XRD). A suspension of dried SeNPs was loaded onto a quartz sample holder and analyzed with a Rigaku D/Max IIIc PW1800 X-ray diffractometer (China) employing Cu Kα\alphaα radiation (λ=1.541\lambda = 1.541λ=1.541 Å). Scans were performed over a 2θ\thetaθ range at 45 kV, 30 mA, and room temperature (Ghareib *et al*., 2016).

Antimicrobial Activities

The antimicrobial potential of the synthesized SeNPs was tested against selected pathogenic microorganisms, including Proteus vulgaris, Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella pneumoniae, and Salmonella typhi using the agar well diffusion technique. Mueller-Hinton agar was prepared and sterilized at 121 °C for 15 min, allowed to cool to approximately 45 °C, and then dispensed into sterile Petri plates. A calibrated inoculum of each microbial strain was distributed across the set agar surface using a sterile swab. Uniform 5 mm wells were created using a sterile cork borer, filled with SeNPs, and the plates were incubated at 37°C for 24 h. Zones of inhibition (excluding well diameter) were measured and recorded (Zhong *et al*., 2024).

Results

In this study, Selenium nanoparticle were biologically synthesized by *Lactobacillus casei.* The visual detection of synthesized SeNPs by *L. casei* is shown in Plate 1. A visible shift in colour from light yellow to ruby red was observed, confirming the formation of SeNPs.

Plate 1: Visual observation of the SeNPs synthesized by *L. casei* (LC-SeNPs)

Keywords: *L. casei - Lactobacillus casei* supernatant; Na_2SeO_3 - Sodium selenite; LC-SeNPs - Selenium nanoparticles synthesized by *Lactobacillus casei*

The UV-visible spectra of the synthesized SeNPs by *L. casei* are displayed in Figure 1. A distinct Surface Plasmon Resonance (SPR) peak was observed at 350 nm for the synthesized LC-SeNPs after 24 and 48 h of incubation, confirming nanoparticle formation. The absorption spectrum exhibited a broad band between 250 and 450 nm, with the sample incubated for 24 h showing the highest absorbance.

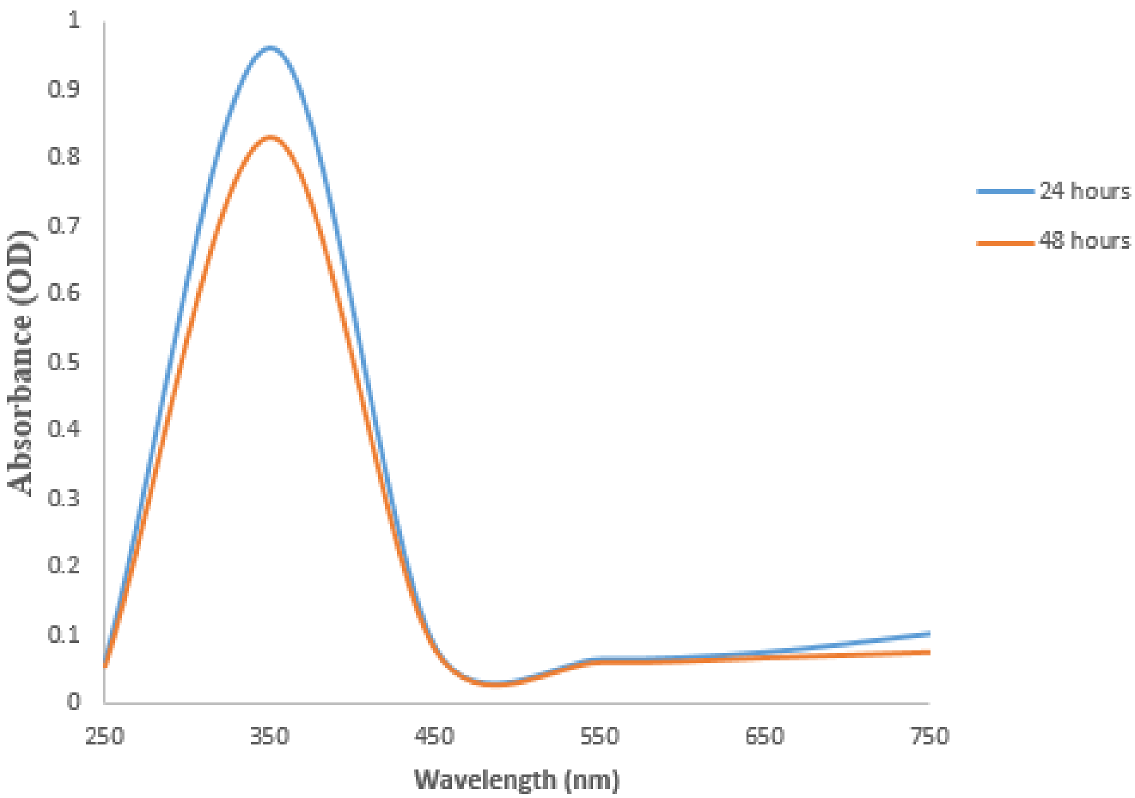

Fig. 1: UV-Visible absorption spectra of SeNPs synthesized by *L. casei.*

The FT-IR spectrum of the synthesized LC-SeNPs (Figure 2) revealed several characteristic absorption bands within the range of 3626.00–460.73 cm^{-1}. In total, ten distinct peaks were identified across this region. The strong bands at 3626.00 cm^{-1} and 3438.00 cm^{-1} are associated with broad O–H stretching vibrations typical of alcohol groups. A band appearing at 2380.72 cm^{-1} may be related to C–O stretching, suggesting the presence of carboxylic acid functionalities. The peak observed at 1648.53 cm^{-1} corresponds to C=C stretching vibrations within aromatic rings. An absorption band at 1028.00 cm^{-1} is characteristic of C–N stretching associated with aliphatic amines. The signal at 918.91 cm^{-1} indicates C–C bending vibrations attributed to aldehyde groups. Additionally, the band detected at 784.66 cm^{-1} is indicative of C–C bending in trisubstituted alkenes. The absorption peak at 684.09 cm^{-1} suggests the presence of halogenated compounds containing C–Br bonds, which may lead to the formation of organobromine derivatives. Another band at 531.00 cm^{-1} can be assigned to C–I stretching of halo compounds. Finally, a weak absorption band at 460.73 cm^{-1} may correspond to vibrations associated with alkane-related functional groups.

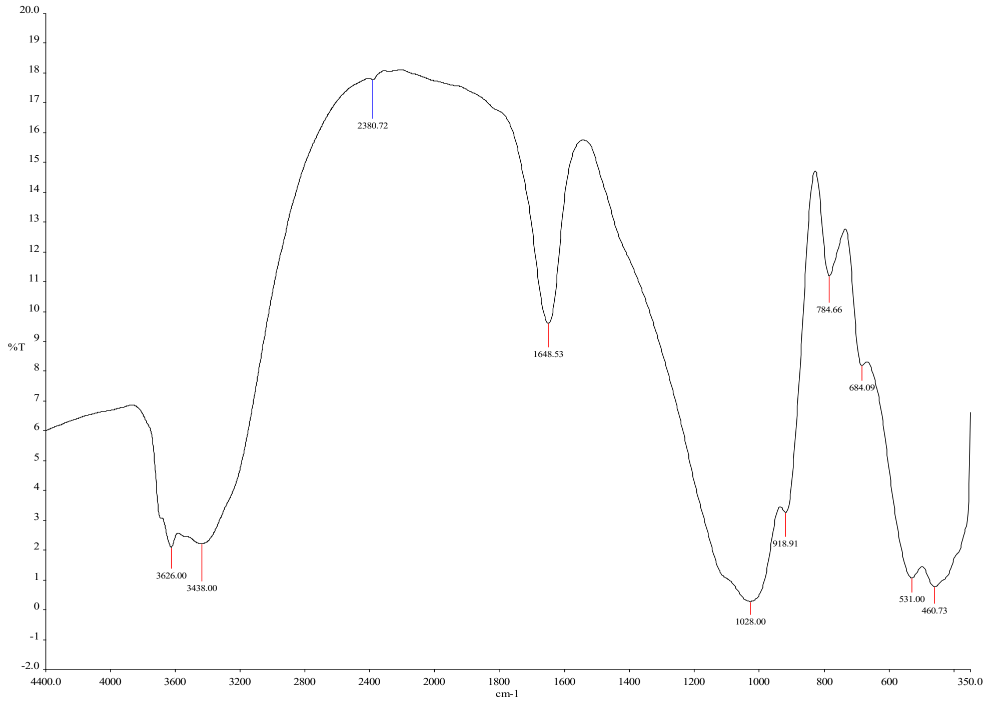

Fig. 2: Fourier transform infrared (FT-IR) Spectra of LC-SeNPs.

Scanning electron microscopy was utilized to investigate the morphology and structural characteristics of LC-SeNPs. SEM analysis of the nanoparticles produced by L. casei is shown in Plate 2. The SeNPs in this study exhibited polydispersed spherical shapes.

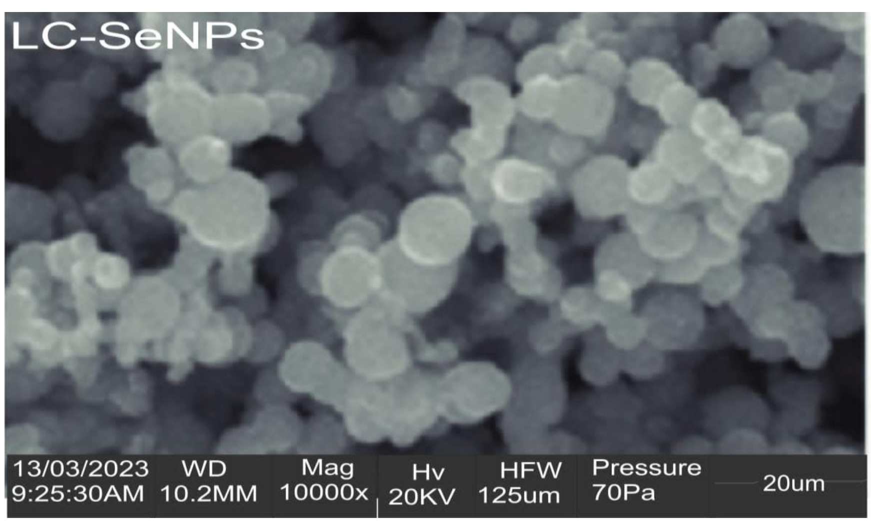

Plate 2: SEM micrograph of LC-SeNPs

The TEM was employed to measure the dimensions of the nanoparticles LC-SeNPs. The TEM micrograph of the biosynthesized SeNPs by *L. casei* is shown in Plate 3(a-c). In the micrograph of LC-SeNPs obtained, different size distributions of 20–100 nm was observed.

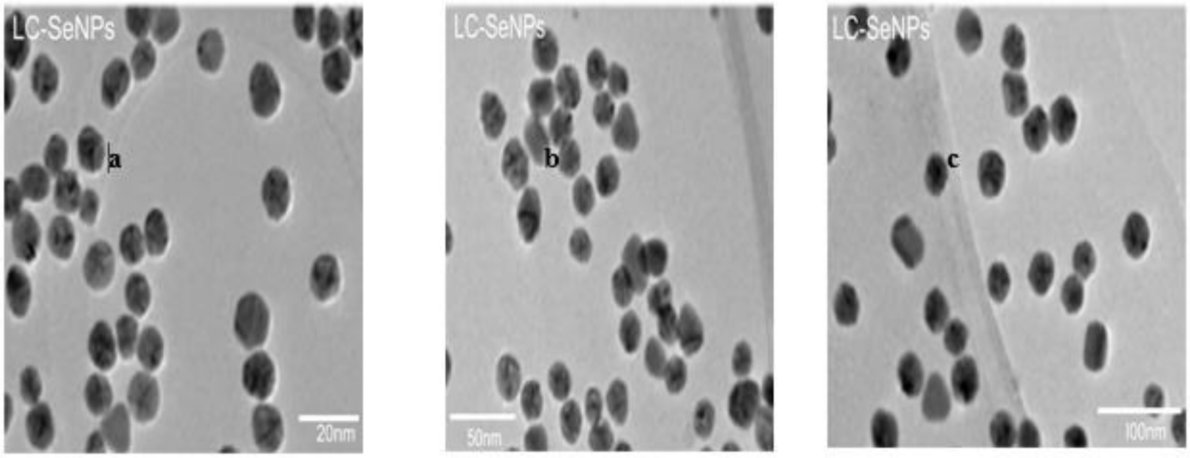

Plate 3(a-c): TEM micrograph of LC-SeNPs.

Energy dispersive X-ray was used for evaluating the sample's elemental composition. Figure 3 illustrates the EDX profile of the synthesized LC-SeNPs. The atom weight ranged from 4.20% to 80.57%. The EDX spectrum shows that selenium atoms (80.57%) were present with oxygen at 15.23% and carbon at 4.20%.

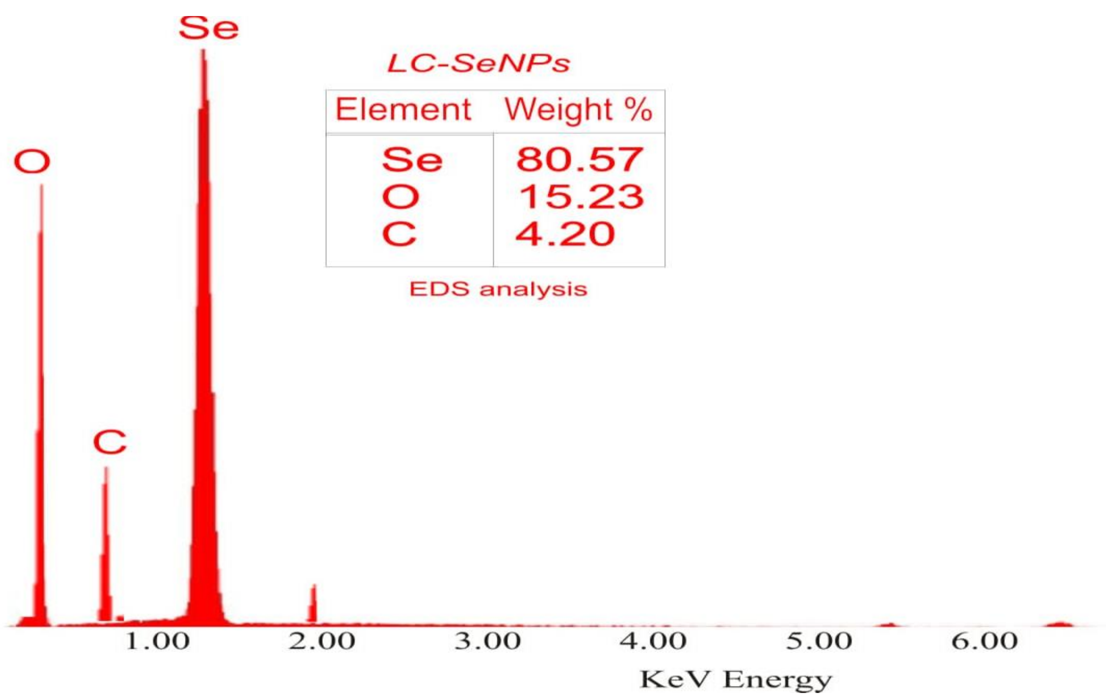

Fig. 3: EDX analysis of the synthesized LC-SeNPs.

The XRD analysis of the synthesized SeNPs by *L. casei* is shown in Figure 4. The XRD analysis indicated six peaks observed across the complete 2θ range of 10–70° in the diffraction pattern. The planes in the analysis are 101, 110, 111, 210, 211, and 220. Each diffraction peak was consistent with the characteristic FCC crystalline lattice.

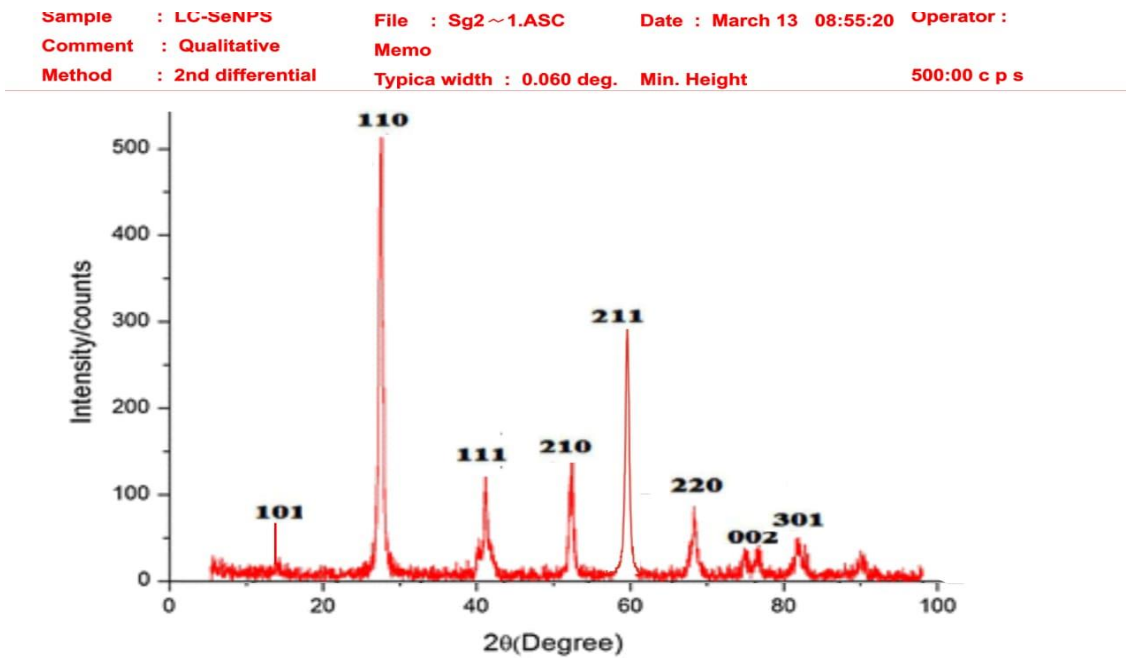

Fig. 4: XRD analysis of the synthesized LC-SeNPs.

Table 1 presents the antimicrobial effects of the synthesized LC-SeNPs on selected test microorganisms. The antimicrobial activity of LC-SeNPs ranged from 9–25 mm. The highest susceptibility was recorded against *E. coli* (25 mm) followed by *S. pneumoniae* (17 mm) and *K. pneumoniae* (15 mm).

Table 1: Antimicrobial Activity of SeNPs Synthesized by *L. casei* Compared to that of Antibiotics

Zone of Inhibition (mm)				
Test Organisms	**LC**	**LC-SeNPs**	**Na_2SeO_3**	**Ciprofloxacin**
P. vulgaris	10	16	NZ	24
E. coli	12	25	9	15
S. aureus	NZ	9	NZ	15
S. pneumoniae	9	17	NZ	14
K. pneumoniae	10	15	NZ	13
S. typhi	NZ	14	10	19

Keywords: Na_2SeO_3- Sodium selenite; LC-SeNPs- SeNPs synthesized by *L. casei;* LC- *L. casei* supernatant; NZ- No zone of inhibition

Discussion

Lactobacillus casei is a lactic acid bacteria with probiotic properties, allowing it to restore the normal balance of bacteria in a human stomach. Selenium, a trace element essential for biological systems, supports the health of humans, animals, and microorganisms. Its nanoparticulate form (SeNPs) is widely studied for its biocompatible nature, bioavailability, and low toxicity (Zhong *et al*., 2024). *Lactobacillus casei* generated selenium nanoparticles, as evidenced by the colour change from yellow to a distinct ruby-red appearance. The appearance of red within the mixture containing Na_2SeO_3 and the tested bacteria suggests that Na_2SeO_3 has been transformed into SeNPs by *L. casei*. Bharathi *et al*. (2020) reported a gradual change in colour from pale yellow to red when selenium was produced with *Bacillus* species. According to Mohamed *et al*. (2021), the ability of lactic acid bacteria to transform the brilliant yellow medium to red after a 32-hour incubation for the creation of selenium nanoparticles. The surface plasmon peak observed at 350 nm indicated the formation of selenium nanoparticles during the bioreduction of selenium ions. It's consistent with the findings of Adebayo-Tayo *et al.* (2021), of selenium nanoparticles by *Lactobacillus* who found an SPR peak at 300 nm for the creation *pentosus*. Ramachandran *et al*. (2023) reported the SPR peak of Selenium Nanoparticles–*Bacillus* sp. MKUST-01 exopolysaccharide (SeNPs–EPS) at 264 nm. Gregircha *et al.* (2023) reported SPR peak at 340 nm. Ogunleye *et al*. (2022) synthesized SeNPs using *O. gratissimum* extract which indicated the surface plasmon resonance peak at 300 nm.

These unique absorption peaks suggested that nanoparticles may be synthesized using hydroxyl, carboxyl, aldehyde, amine, or alkane. The presence of these functional groups indicates that biomolecules found in bacteria contribute to the stability of selenium nanoparticles. The vast stretch of alcohol or phenol group correlates to the findings of El-Saadony *et al*., (2021). The presence of C-H, C=C, and C-O functional groups is consistent with the findings of Ananth *et al*. (2019) in the characterization of nanoselenium. The typical C-C and C-N functional groups that suggest the presence of proteins are consistent with the findings of Jyoti *et al*. (2016).

The LC-SeNPs were spherical, with diameters ranging from 20 to 100 nm. This was due to the presence of capping agents in the extract, which act as stabilizers or binding molecules to ensure colloidal stability. Gregircha *et al*. (2023) found that most LABs create spherical selenium nanoparticles. Wang *et al*. (2023) described the spherical shape of selenium nanoparticles generated by *Pediococcus acidilactici* DSM2028. SeNPs size changes can be attributed to a variety of nucleation and growth mechanisms influenced by temperature and reaction conditions. This is consistent with the findings of Sampath *et al*. (2024), who found that the produced SeNPs were spherical and ranged in size from 20 to 70 nm. Ganapathy and Shanmugam (2021) also reported that the particle size ranged from 10 to 25 nm in the synthesis of selenium nanoparticles using *Brassica oleracea.* The average size of selenium nanoparticle synthesized by *Pediococcus acidilactici* DSM2028 was 239 nm (Wang *et al*., 2023).

Selenium atoms in the nanoparticles produced a strong signal, but oxygen and carbon signals were weaker. The existence of a prominent selenium peak was due to surface plasmon resonance, showing that the SeNPs were of great purity. Zhong *et al.* (2024) similarly found carbon, nitrogen, and oxygen peaks in selenium nanoparticles produced by *Lactiplantibacillus plantarum.* This is consistent with the findings of Alagesan and Venugopal (2019), who discovered three signals in the energy-dispersive X-ray analysis: a strong signal from the Se atom (50.79%), the O atom (35.55%), and the C atom (13.66%) in the synthesis of selenium nanoparticles.

Sharp and well-defined diffraction peaks obtained from the XRD pattern confirmed the crystalline nature of the synthesized SeNPs. The detected reflections were indexed to the trigonal phase of elemental selenium, demonstrating the structural purity of the nanoparticles. These observations are consistent with the findings of El-Deeba *et al*. (2018), who also reported that biologically synthesized selenium nanoparticles exhibited a distinct crystalline structure corresponding to elemental selenium during their structural characterization and antimicrobial evaluation.

In the review of Stabnikova *et al*. (2023), the crystallographic form of the synthesized selenium nanoparticles using lactic acid bacteria was observed.

The SeNPs of *L. casei* had varying antibacterial efficacy against the test pathogens. LC-SeNPs penetrate the bacterial membrane, causing damage and resulting in bacterial lysis (Sampath *et al*., 2021). SeNPs displayed stronger inhibitory effects against gram-positive bacteria species due to the nanoparticles' lower surface charges, which allow them to bind to the bacterial cell membrane (Zonaro *et al.,* 2015). Bharathi *et al*. (2020) found that *Bacillus* species' extracellular manufacture of nanoselenium inhibited *Pseudomonas aeruginosa* growth. According to the review of Stabnikova *et al*. (2023), selenium nanoparticles suppress harmful bacteria and fungus. This study supports the findings of Hernández-Díaz *et al.* (2021), who found selenium nanoparticles effective against *B. subtilis, E. coli, and S. aureus*. Zhong *et al.* (2024) found that LacSeNPs inhibited *Escherichia coli* and *Staphylococcus aureus*.

Conclusion

Reports from this study have shown that selenium nanoparticles were biologically synthesized by *L.*

casei. The molecules of LC-SeNPs acted as stabilizing as well as capping agents leading to the formation of selenium nanoparticles. There was a change in colour from light yellow to ruby red. The surface plasmon resonance peak was at 350 nm. The FTIR indicated the presence of hydroxyl, aldehyde, halo compound, amine, alkane, and phenol group respectively. The nanoparticles were spherical in shape with varying sizes. More importantly, the synthesized LC-SeNPs have potent inhibitory properties as they are effective against drug-resistant bacteria. The nanoparticles formed were stable, biocompatible and had applications in medicine.

Acknowledgments
The authors wish to appreciate the management of KolaDaisi University, Ibadan, for her augmentation in the provision of some equipment to carry out this research.

References

1. Adamu, B. B., Obioh, G. I. B., Ideh, R. R., Olukotun, G. B. (2025). The Role of Lactic Acid Bacteria in Food, Agriculture and Industry: A Review. History: *GSC Biological and Pharmaceutical Sciences*, 30(01), 099-106 *https://doi.org/10.30574/Gscbps.2025.30.1.0497*
2. Gizachew, S., Van Beeck, W., Spacova, I., Dekeukeleire, M., Alemu, A., Woldemedhin, W. M., Mariam, S. H., Lebeer, S. and Engidawork, E. (2023). "Antibacterial and Immunostimulatory Activity of Potential Probiotic Lactic Acid Bacteria Isolated from Ethiopian Fermented Dairy Products". *Fermentation*. 9(3), 258. *https://doi.org/10.3390/fermentation9030258*.
3. Zheng, J., Wittouck, S., Salvetti, E., Franz, C. M., Harris, H. M., Mattarelli, P., and Lebeer, S. (2020). "A taxonomic note on the genus Lactobacillus: Description of 23 novel genera, emended description of the genus Lactobacillus Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*". *International Journal of Systematic and Evolutionary Microbiology*. 70(4), 2782-2858. *doi: 10.1099/ijsem.0.004107*.
4. Amany, N., Mourad, A., Eman, Y. T., Elariny, A., Ahmed, S. A., Mahmoud, M., El-Saber, B., Abdualziz, A. C., Mohamed, S. D., Ahmed, G. E., Khalid, S., Alotaibi, F., Ali, O. (2025). Green synthesis of selenium nanoparticles, and its conjugation with antibacterial proteins for enhancing their antibacterial activity. *Bioorganic Chemistry*, 10(8), 443. *https://doi.org/10.1016/jbioorg.2025.108443*
5. Shahbaz, M., Akram, A., Raja, N. I., Mukhtar, T., Mehak, A., Fatima, N., *et al*. (2023). "Antifungal activity of green synthesized selenium nanoparticles and their effect on physiological, biochemical, and antioxidant defense system of mango under mango malformation disease". *PLoS ONE*. 18(2): e0274679. *https://doi.org/10.1371/journal.pone.0274679*.
6. Genchi, G., Lauria, G., Catalano, A., Sinicropi, M. S. and Carocci, A. (2023). "Biological Activity of Selenium and Its Impact on Human Health". *International Journal of Molecular Sciences*. 24(3), 2633. *https://doi: 10.3390/ijms24032633*.
7. Sampath, S., Sunderam, V., Manjusha, M., Dlamini, Z., Lawrance, A.V. (2024). Selenium Nanoparticles: A Comprehensive Examination of Synthesis Techniques and Their Diverse Applications in Medical Research and Toxicology Studies. *Molecules*, 29(4), 801. *https://doi: 10.3390/molecules29040801*
8. Hosnedlova, B., Kepinska, M., Skalickova, S., Fernandez, C., Ruttkay-Nedecky, B., Peng, Q., Baron, M., Melcova, M., Opatrilova, R., Zidkova, J., Bjørklund, G., Sochor, J., and Kizek, R. (2018). "Nano-selenium and its nanomedicine applications: a critical review". *International Journal of Nanomedicine,* 13, 2107-2128. *https://doi: 10.2147/IJN.S157541*.
9. Au, A., Mojadadi, A., Shao, J. Y., Ahmad, G. and Witting, P. K. (2023). "Physiological Benefits of Novel Selenium Delivery via Nanoparticles". *International Journal of Molecular Sciences.* 24(7): 6068. *https://doi: 10.3390/ijms24076068*
10. Gregircha, N., yniavska, D., Khonkiv, M., Stabnikov, V. (2023). Lactic acid bacteria for the synthesis of metals nanoparticles. *Ukrainian Food Journal*, 12. *http://dx.doi.org/10.24263/2304-974X-2023-12-4-8*
11. Feng, Y., Su, J., Zhao, Z., Zheng, W., Wu, H., Zhang, Y., and Chen, T. (2014). "Differential effects of amino acid surface decoration on the anticancer efficacy of selenium nanoparticles". *Dalton Transactions*. 43(4), 1854–1861. *https://doi: 10.1039/c3dt52468j.*
12. Fu, X., Yang, Y., Li, X., *et al*. (2016). "RGD peptide-conjugated selenium nanoparticles: Antiangiogenesis by suppressing VEGF-VEGFR2-ERK/AKT pathway". Nanomedicine*, Nano-technology, Biology, and Medicine.* 12(6), 1627-1639. *https://doi: 10.1016/j.nano.2016.01. 012*.
13. Zhang, J., Teng, Z., Yuan, Y., Zeng, Q. Z., Lou, Z., Lee, S. H., and Wang, Q. (2018). "Development, physicochemical characterization and cytotoxicity of selenium nanoparticles stabilized by beta-lactoglobulin". *International Journal of Biological Macromolecules*. 1406-1413. *https://doi: 10.1016/j.ijbiomac.2017.09.117*.

14. Bai, K., Hong, B., Hong, Z. *et al*. (2017). "Selenium nanoparticles-loaded chitosan/citrate complex and its protection against oxidative stress in D-galactose-induced aging mice". *Journal of Nanobiotechnolgy*. 15(1), 92. *https://doi.org/10.1186/s12951-017-0324-z*.
15. Zhong, B, Xu, W, Xie, H., Wu, Z. (2024). Biosynthesis and characterization of selenium nanoparticles by Se-tolerant *Lactiplantibacillus plantarum*. *Food Biosicence*, 59(10), 40-61. *https://doi.org/10.1016/j.fbio.2024.104061*
16. Adebayo-Tayo, B. C., Bola, O. Y. and Solomon O. A. (2021). "Antibacterial Activity of Intracellular Greenly Fabricated Selenium Nanoparticle of *Lactobacillus pentosus* ADET MW861694 against Selected Food Pathogens". *The International Journal of Biotechnology: Conscientia Beam*. 10(1), 39-51. *https://doi:10.18488/journal.57.2021.101.39.51.*
17. Castañeda-Ovando, A., Pérez-Escalante, E., Rodríguez-Serrano, G. M., Martínez-Ramírez, X., Contreras-López, E., Jaimez-Ordaz1, J., Añorve-Morga, J., Nieto-Velázquez, S., Ramírez-Godínez, J. and González-Olivares, L. G. (2020). "Selenium accumulation by Lactobacillus isolated from commercial fermented milk: Minimum inhibitory concentration and kinetic growth changes". *Revista Mexicana de Ingeniería Química*, 21(3), Bio2824.
18. El-Saadony, M. T., Saad, A. M., Najjar, A. A., Alzahrani, S. O., Alkhatib, F. M., Shafi, M. E., Selem, E., Desoky, E.-S. M., Fouda, S. E., and El-Tahan A. M. (2021). "The use of biological selenium nanoparticles to suppress *Triticum aestivum L.* crown and root rot diseases induced by Fusarium species and improve yield under drought and heat stress". *Saudi Journal of Biological Sciences*. 28(8), 4461-4471. *https://doi.org/10.1016/j.sjbs.2021.04.043.*
19. Bharathi, S., Kumaran, S., Suresh, G., Ramesh, M., Thangamani, V. and Pughazhventhan, S. R. (2020). "Extracellular synthesis of nanoselenium from fresh water bacteria *Bacillus sp*., and its validation of antibacterial and cytotoxic potential". *Biocatalysis and Agricultural Biotechnology*, *101655. doi:10.1016/j.bcab.2020.101655.*
20. Akl, B., Nader, M. and El-Saadony, M. (2020). "Biosynthesis of Silver Nanoparticles by *Serratia marcescens ssp sakuensis* and its Antibacterial Application against some Pathogenic Bacteria". *Journal of Agricultural Chemistry and Biotechnology*, 11(1), 1-8. *https://doi: 10.21608/jacb.2020.76656*.
21. Ghareib, M., Tahon, M. A., Saif, M. M. and Abdallah, W. E. S. (2016). "Rapid extracellular biosynthesis of silver nanoparticles by *Cunninghamella phaeospora* culture supernatant". *Iranian Journal of Pharmaceutical Research,* 15(4), 915-924.
22. Mohamed, T. E., Ahmed, M., Saad, B., Taha, F., Taha, B., Azhar, A., Najjar, C., Nidal, M., Zabermawi, C., Maha, M., Nader, A., Synan, F., AbuQamar, D., Khaled, A., El-Tarabily, D. E., Ali, S. (2021). Selenium nanoparticles from *Lactobacillus paracasei* HM1 capable of antagonizing animal pathogenic fungi as a new source from human breast milk. *Saudi Journal of Biological Sciences*, 07, 59. *https://doi.org/10.1016/j.sjbs.2021.07.059*
23. Ramachandran. T, Manoharan, T, Natesan S, Rajaram S.K, Karuppiah, P , Shaik M.R, Khan M, Shaik B, (2023). Synthesis and Structural Characterization of Selenium Nanoparticles–*Bacillus sp.* MKUST-01 Exopolysaccharide (SeNPs–EPS) Conjugate for Biomedical Applications. *Biomedcines,* 11(9):2520. *doi: 10.3390/biomedicines11092520*
24. Ogunleye, G. E., Oyinlola, K. A., Akintade, O., Fashogbon, R. and Adesina, T. (2022). "Green synthesis, Characterization and Antimicrobial potential of Selenium Nanoparticles from *Ocimum gratissimum*". *Turkish Journal of Agriculture - Food Science and Technology*, 10(sp2), 2903–2912. *doi: https://doi.org/10.24925/turjaf. v10isp2.2903-2912.5615*
25. Ananth, A., Keerthika, V. and Rajan, M. R. (2019). "Synthesis and characterization of nano selenium and its antibacterial response on some important human pathogens". *Current Science*, 116(2), 285-290. *https://doi:10.18520/cs/v116/i2/285-290*.
26. Jyoti, K., Baunthiyal, M. and Singh, A. (2016). "Characterization of silver nanoparticles synthesized using *Urtica dioica Linn*. Leaves and their synergistic effects with antibiotics". *Journal of Radiation Research and Applied Sciences*. 9(3), 217-227. *doi:10.1016/j.jrras.2015.10.002*.
27. Ganapathy, D. and Shanmugam R. (2021). "Anticariogenic Effect of Selenium Nanoparticles Synthesized Using *Brassica oleracea*". *Journal of Nanomaterials,* 9. *https://doi.org/10.1155/2021/8115585*
28. Wang, Q., Wang, C., Kuang, S., Wang, D., Shi, Y. (2023). Biological selenite reduction, characterization and bioactivities of selenium nanoparticles biosynthesized by *Pediococcus acidilactici* DSM20284. *Molecules*, 28: 3793. *https://doi.org/10.3390/molecules28093793*.
29. Alagesan, V. and Venugopal, S. (2019). "Green Synthesis of Selenium Nanoparticle Using Leaves Extract of *Withania somnifera* and Its Biological Applications and Photocatalytic Activities". *BioNanoScience*. 9, 105–116. *https://doi:10.1007/s12668-018-0566-8.*
30. El-Deeba, B., Al-Talhib, A., Mostafac, N. and Abou-assyd, R. (2018)." Biological synthesis and structural characterization of selenium

nanoparticles and assessment of their antimicrobial properties". *American Scientific Research Journal for Engineering, Technology and Sciences.* 45(1), 135-170.

31. Stabnikova, O., Khonkiv, M., Kovshar, I. *et al*. (2023). Biosynthesis of selenium nanoparticles by lactic acid bacteria and areas of their possible applications. *World J Microbiol Biotechnol,* 39, 230. *https://doi.org/10.1007/s11274-023-03673-6*
32. Zonaro, E., Lampis, S., Turner, R. J., Qazi, S. J. S. and Vallini, G. (2015). "Biogenic selenium and tellurium nanoparticles synthesized by environmental microbial isolates efficaciously inhibit bacterial planktonic cultures and biofilms". *Frontiers in Microbiology*. 6, 584. *https://doi: 10.3389/fmicb.2015.00584*.
33. Hernandez-Diaz, J., Garza-Garcia, J. J., Leon-Morales, J. M., Zamudio-Ojeda, A., Arratia-Quijada, J., Velazquez-Juarez, G., Lopez-Velazquez, J. C. and Garcia-Morales, S. (2021). "Activity of biosynthesized Selenium Nanoparticles using extracts of *Calendula officinalis* against Potentially Clinical Bacterial Strains". *Molecules*. 26, 5929. *https://doi: 10. 3390/molecules26195929*.