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Antimicrobial Activity of Silver Nanoparticles Biosynthesized by Metabolites of Lactic Acid Bacteria Isolated from Fermented *Cyperus esculentus* Milk

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ABSTRACT

Antimicrobial resistance is a growing global public health threat, with the rapid rise of multidrug-resistant microbes becoming alarming. The quest for natural antimicrobials has increased as consumers seek safer alternatives for ensuring food safety, nutrition, and sensory quality. This current study investigates the antimicrobial activities of biosynthesized silver nanoparticles (AgNPs) from metabolites of lactic acid bacteria (LAB) isolated from *Cyperus esculentus* milk. LAB were isolated and characterized using conventional and molecular methods. Metabolites from the LAB strains were used to synthesize AgNPs. The AgNPs were characterized using UV-Visible Spectroscopy (UV-Vis), Transmission Electron Microscopy (TEM), Energy Dispersive X-ray (EDX). Antimicrobial assay was done using agar well diffusion method. Results from the study showed that *Lactobacillus plantarum* has the highest occurrence (37.5%) and *Lactococcus acidophilus* (4.2%) occurred least. UV-Vis confirmed the formation of LAB metabolite-synthesized AgNPs with absorbance peaks at 450 and 500 nm. Transmission Electron Microscopy revealed spherical AgNPs ranging from 1.32 to 23.22 nm, with size variations attributed to different LAB strains. EDX results indicated a significant presence of silver, with weak signals of other elements. The biosynthesized AgNPs possess antibacterial and antifungal activities against selected food pathogens. *Lactobacillus plantarum* strain 218 had the strongest antibacterial activities (22±2.00 mm) against *Staphylococcus aureus*. *L. plantarum* strain a27 had the strongest antifungal activities (11.5±0.71 mm) against *Aspergillus flavus*. Silver nanoparticles synthesized from the metabolites of LAB isolated from *C. esculentus* milk therefore show great potential as natural antimicrobials offering an alternative to combat antimicrobial resistance and improve microbial food safety.

Keywords

Antimicrobial resistance, *Cyperus esculentus*, Food safety, Lactic acid bacteria

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Introduction

The crucial role antibiotics play in treating patients with infectious diseases and those undergoing surgical operations makes it the most vital antimicrobial accomplishment in medicine in the 20th century (Salomoni *et al.,* 2017). Antibiotics have been a major defense against bacterial infection since it has been discovered in 1945. However, the prolonged indiscriminate use of antibiotics and other antimicrobial agents has led to the advance of increasing resistant bacteria. This has resulted in administering larger doses of antibiotics, thus increasing the pool of multidrug-resistant microorganisms (Barabadi *et al.,* 2019). Antimicrobial resistance has posed a threat to public health globally, and a great challenge to medical and pharmaceutical professionals (Mortezaee *et al.,* 2019).

High death rates have been reported as a result of pandemic and epidemic infectious diseases caused by antimicrobial-resistant pathogens which have made the emergence and re-emergence of microorganisms resistant to antimicrobial agents a serious problem (Baptista *et al.,* 2018; Kailasa *et al.,* 2019). Therefore, there is a need for the modification and development of compounds with better efficiency. Silver possesses antimicrobial components and when the size is reduced to a nanometer, its antimicrobial activity becomes strongly potent so silver nanoparticles (AgNPs) have great potential for antimicrobial applications (Saravana *et al.,* 2018).

Silver nanoparticles are nanomaterials obtained by nanotechnology and owning to their antimicrobial belongings, they are used to control several fungal, bacterial, and viral infections. Silver nanoparticles are also applied in the medicine, food, and cosmetics industries (Bruna *et al.,* 2021).

Biosynthesized silver nanoparticles (AgNPs) have emerged and attracted much attention lately. The production of AgNPs employing lactic acid bacteria (LAB), especially probiotics and its metabolites is being explored as a novel approach for various applications (Awadelkareem *et al.,* 2023).

Lactic acid bacteria metabolites can be categorized as natural antimicrobials because they can prevent the growth of spoilage and foodborne pathogenic microbes (Moradi *et al.,* 2021).

LAB are facultative, fermentative, catalase-negative, non-spore forming, gram-positive anaerobic bacteria that are employed extensively in dairy, food, and agricultural applications (Moradi *et al.,* 2021). Several studies have been investigated to isolate and characterize lactic acid bacteria from numerous sources including fermented food products, raw biotic substrates, and the gastrointestinal tracts (Endo *et al.,* 2019; Sethi & Anurag, 2021; Verni *et al.,* 2019). Moreover, there is a growing demand to discover novel indigenous strains with broader applications because each species of fermented product offers a unique environment. *Cyperus esculentus* is an underutilized crop belonging to the family Cyperaceae, commonly called Tigernut (Ogunka-Nnoka *et al.,* 2020). Milk derived from *Cyperus esculentus* has antibacterial qualities with bacteriostatic and bactericidal effects and favors the dominance of LAB during fermentation (Naeem & Youssef, 2022). This study aims to investigate the antimicrobial activity of silver nanoparticles (AgNPs) biosynthesized from metabolites of lactic acid bacteria isolated from *Cyperus esculentus* milk.

Materials and Methods

Research location and design

The research was done at the Department of Microbiology Food Laboratory (Lat 7.44222, Long 3.89656), University of Ibadan Nigeria. The study explores an experimental design with laboratory testing and analyses.

Fresh and dried *Cyperus esculentus* tubers were purchased from Bodija Market (Lat 7.43501, Long 3.91057) Ibadan and transported to the laboratory in a clean polythene bag for processing and analysis.

Test organisms (*Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella typhi, Bacillus cereus*, *Aspergillus niger, Aspergillus flavus, Fusarium sp*, *Rhizopus sp* and *Penicillium sp*) were obtained from the Department of Microbiology Food Laboratory, University of Ibadan, Ibadan, Oyo State, Nigeria. All culture media used for the isolation were manufactured by Himedia Laboratories Pvt. Ltd., Mumbai, India. The bacterial isolates were resuscitated on nutrient agar while the fungal isolates were cultured on Potato Dextrose agar before use.

Preparation and Fermentation of *Cyperus esculentus*

The preparation and fermentation were done as described by Wakil *et al.,* (2014). *Cyperus esculentus* were rinsed in distilled water after sorting and soaked for 6hrs at 60˚C to soften the fibre. 500mls of warm distilled water was added to 200g of tigernut and blended several times with sterile kenwood blender. The mash was filtered through a clean sterile muslin cloth to separate the milk and further strained to obtain a fine consistency. The filtered tigernut milk was transferred into a clean container, pasteurized in a water bath at 90˚C for 15minutes, cooled to a temperature of 45˚C and later maintained for 12hrs. Spontaneous fermentation was carried out for 18 hrs by the natural flora of the milk

Isolation and Characterization of Lactic Acid Bacteria isolated from *Cyperus esculentus* Milk

Lactic acid bacteria were isolated from fermented *C. esculentus* milk by culturing 1ml of the 10^4 to 10^8 CFU/ml on De Mann Rogosa and Sharpe (MRS) agar prepared according to the manufacturer's specifications. After incubation at 37ºC for 48 hours under aerobic conditions, colonies on each plate were repeatedly streaked out to obtain a pure culture of each isolate, labelled appropriately, and stored on an agar slant at 4ºC. The pure LAB was characterized by microscopic examination and biochemical testing (Wakil *et al.,* 2014).

In vitro Assessment of LAB Probiotics Survival in the Gastrointestinal Tract

Acid Tolerance

The acid tolerance of the isolated LAB assay was tested according to Li (2020). Acidified MRS broth with hydrochloric acid adjusting the pH to 2, 3, 4, and 5 was inoculated with fresh culture of LAB isolates. The inoculated plates were aerobically incubated at 37ºC for 18 hours and the optical density was determined using a Spectrophotometer at 600nm to monitor the growth kinetics (Li, 2020). This test was carried out in triplicates for each strain.

Phenol Tolerance

Tolerance to Phenol by the LAB isolates was examined by transferring fresh-grown LAB cultures to a freshly prepared MRS broth with varied concentrations (0.1%, 0.2%, 10.3%, and 20.4%) of phenol at 37°C. The viable colonies of cultures were counted using plate counts after a 24-hour incubation to determine the viability of the LAB isolates (Zhang *et al.,* 2022).

Bile Tolerance

Ten milliliters (10 ml) of MRS broth enriched with bile salt concentrations of 0.5%, 1.0%, 1.5%, and 2.0% respectively were used to test the tolerance of the isolates to the bile salt. LAB isolates were inoculated into the enriched medium. Samples were taken after 24 hours of incubation LAB culture at 37°C and the optical density of the cells was measured at 600 nm using a spectrophotometer (Chen *et al.,* 2022).

Sodium Chloride (NaCl) Tolerance

The NaCl tolerance of the LAB isolates was analyzed using MRS broth with different NaCl concentrations of 2%, 4%, 6%, and 8%. Test tubes with 5mL of this composition were individually inoculated with 50µL of 1% of a 24-hour LAB culture and incubated for 48 hours at 37°C. Change in broth color was monitored and measured with the Spectrophotometer (Chen *et al.,* 2022).

Safety Assessment of Isolated LAB

DNase Activity of the Isolated LAB

Evaluation of the LAB isolates to produce deoxyribonuclease (DNase) enzymes was done by streaking each of the LAB isolates on DNase agar plates (HiMedia, Mumbai, India). The clear zone formation after incubation for 48h at 37°C indicates positive DNase activity (Boricha *et al.,* 2019).

Hemolytic activity of the LAB isolates

The hemolytic activity of isolated LAB from *C. esculentus* was determined by streaking overnight culture of LAB cultures on blood agar plates. After 48hours of incubation at 37°C, the hemolytic reaction was evaluated by observing both the partial hydrolysis of red blood cells and the production of a green zone (α-hemolysis), as well as the total hydrolysis of red blood cells producing a clear zone around the bacterial colony (β-hemolysis) or no reaction (γ-hemolysis) (Zhang *et al.,* 2022).

Gelatinase hydrolysis test of the LAB isolates

This was done by stabbing overnight culture of LAB isolates on sterile nutrient gelatin slants. After incubating for 48 hours, 37ºC, the slants were placed in a refrigerator at 4°C for 10 to 15 minutes and slants were observed (negative reaction for gelatin solidification and positive response for gelatin hydrolysis). Pathogenic *Staphylococcus aureus* was a positive control (Rajput & Dubey, 2020).

Molecular identification of the LAB strains using 16S rRNA

The molecular characterization of four selected probiotic LAB isolates with the best performance was carried out at Africa's Genomics Company (Inqaba Biotech) Ibadan, Oyo State, Nigeria using 16S rRNA as described by Altschul *et al.,* (1997).

Biosynthesis of AgNPs from the LAB Metabolites

With a few modifications, the biosynthesis of AgNPs was carried out using the methodology outlined by Naseer *et al.,* (2022). Ten milliliters each of the LAB (*Lactobacillus plantarum* strain 218 (TG1), *Lactobacillus plantarum strain* L2 (TG2), *Pediococcus acidilactici, Lactobacillus plantarum* strain a27) supernatant was mixed with 40mL of freshly prepared 1mM Silver nitrate ($AgNO_3$) solution in different 250mL Erlenmeyer flasks. After that, the suspension was incubated in the dark at 37^0C for 24 hours. The dark brown solution formed from the incubation period indicates the biosynthesized AgNPs.

Characterization of Biosynthesized AgNPs from LAB Metabolites

The gradual change in colour of the biosynthesized silver nanoparticles from yellowish to brown after adding silver nitrate ($AgNO_3$) was visually observed. The synthesized AgNPs were characterized using various analytical techniques including UV-visible spectrophotometry, transmission electron microscopy (TEM) and energy dispersive X-ray spectroscopy (EDX) using the standard protocol (Dakhil, 2017). The reduction of $Ag+$ ions to form Ag nanoparticles (AgNPs) in the aqueous solution was monitored and the optical properties of the LAB synthesized silver nanoparticles was determined using a UV–visible spectrophotometer (Uviline 9400, Secomam, Alès, France). Transmission electron microscopy (TEM) was used to determine the size and shape of the silver nanoparticles (AgNPs) synthesized by LAB metabolites. A drop of the nanoparticle solution was placed on a carbon-coated copper grid, and the water was allowed to evaporate before examination. TEM measurements were taken using a JEM-2100F (Jeol, Tokyo, Japan) instrument operated at an accelerating voltage of 15kV with resolution of 0.23 nm (Dong *et al.,* 2019). Energy Dispersive X-ray (EDX) was used to determine the elemental composition of silver nanoparticles. Biosynthesized AgNPs were prepared in carbon coated copper grid and analyzed using Rigaku instrument model NEXCG.

Antimicrobial Activity of AgNPs synthesized by LAB metabolites

Antibacterial Activity of AgNPs Synthesized by LAB metabolites

This was done using the agar well diffusion method against six selected pathogens (*Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia. coli, Klebsiella pneumoniae*, *Salmonella typhi*, *Bacillus cereus*). Sterile cotton swabs were used to streak the standardized inoculum of the selected pathogens on Mueller Hinton agar plates. A sterile borer (6 mm in diameter) was used to create wells in the agar, introducing approximately 200µL of biosynthesized AgNPs. The plates were incubated at 37°C for 24 hours, and the diameter of the zone of inhibition was measured and recorded. Positive controls were Streptomycin and $AgNO_3$ while Dimethyl sulfoxide (DMSO) was the negative control (Charannya *et al.,* 2018). This experiment was in triplicate.

Minimum inhibitory concentration (MIC) of AgNPs against bacterial pathogens

MIC was determined according to the method of Charannya *et al.,* (2018) using different concentrations of the biosynthesized AgNPs against tested bacterial pathogens. The standardized tested bacteria pathogens were inoculated on Mueller Hinton agar using the streaking techniques. A sterile cork borer (6 mm) was used to bore holes in the agar plates. The biosynthesized AgNPs were diluted to different concentrations (10, 5, 2.5, 1.25, and 0.625 mg/ml) and 100µl of each concentration was added to the wells. The MIC value was determined after incubation of the plates at 37°C for 24 hours by observing the presence of bacterial growth. The lowest dilution of the AgNPs at which inhibition was observed against the pathogens is regarded as the MIC for the AgNPs.

Antifungal activity of AgNPs synthesized by LAB metabolites

The antifungal properties of AgNPs synthesized by LAB metabolites were done against pathogenic fungi

(*Aspergillus niger, Aspergillus flavus, Fusarium sp.*, *Rhizopus sp.,* and *Penicillium sp.*) employing an agar well diffusion method. The fungal suspension concentration was adjusted to match the 0.5 McFarland standard in normal saline, corresponding to 1.5 × 10^6 CFU/ml. Using a cotton swab, this suspension was evenly spread on Sabouraud dextrose agar plates. Sterilized cork borers, 6 mm in diameter, were used to create wells in the agar plates, and 50µl of biogenic AgNPs was introduced into these wells. Positive controls were Miconazole and $AgNO_3$ while Dimethyl sulfoxide (DMSO) was the negative control. After a 3-day incubation period at 28°C, the diameter of inhibition was measured according to the method described by Buszewski *et al.,* (2018).

Minimum inhibitory concentration (MIC) of AgNPs against fungal test pathogens

This was done against selected fungal pathogens (*Aspergillus niger, Aspergillus flavus, Fusarium sp.*, *Rhizopus sp.,* and *Penicillium sp*.) as described for MIC of bacteria in this study (Charannya *et al.,* 2018).

Statistical Analysis

Each of the experiment was carried out in triplicates. Data was presented as mean ± Standard deviation where applicable.

Results and Discussion

Isolation and Screening of LAB Isolates

A total of 24 LAB was isolated and characterized from *C. esculentus* with *L. plantarum* having the highest occurrence (37.5%) and *L. acidophilus* (4.2%) occurring least (Figure 1). The *in vitro* assessment of LAB probiotic survival in the gastrointestinal tract was summarized in Table 1.

The result of acid tolerance of isolated LAB shows that the growth of all LAB isolates decreased as the pH decreased. All the LAB isolates could withstand the different phenol concentrations (0.1%, 0.2%, 10.3%, 20.4%). Also, as the concentration of bile salt increases the growth of all LAB isolates decreases. Results from the safety assessment showed that all the LAB were negative for hemolysis test, gelatinase activity, DNase, and lecithinase.

Antibacterial and Antifungal Activities of LAB Isolates against Bacterial Pathogens

Screening for the antibacterial and antifungal potential of the LAB metabolites is presented in Table 2. Isolates with high antimicrobial potential (in red) were selected and further analyzed and characterized for AgNPs activity. After characterization, four LABs were selected (Table 3).

Characterization of Biosynthesized AgNPs from LAB Metabolites

The metabolites of selected LAB strains were used to synthesize silver nanoparticles (AgNPs). The AgNPs produced by each LAB metabolite were characterized using UV-visible spectroscopy at 300 – 900 nm. The formation of AgNPs was confirmed by absorption peaks in the UV–visible spectrum. AgNPs synthesized by *Lactobacillus plantarum* strain 218 (TG1) metabolites had a surface plasmon resonance peak at 450 nm. In contrast, the formed AgNPs synthesized by metabolites of *Lactobacillus plantarum* strain L2 (TG2), *Pediococcus acidilactici* AV1 24 (TG3), and *Lactobacillus plantarum* a27 (TG4) gave a surface plasmon resonance peak at 500 nm. The surface plasmon resonance band for the AgNPs was obtained for each LAB strain at 450 nm and 500 nm due to the formation of AgNPs (Figure 2).

The result of the transmission electron microscopy (TEM) revealed detailed insight of the size and shape of the AgNPs biosynthesized by LAB metabolites (Figure 3). The TEM micrograph of *Lactobacillus plantarum* TG1 AgNPs revealed the formation of polydisperse spherical shape with size ranging from 1.32 – 5.32 nm.

The shape of *Lactobacillus plantarum* TG2 AgNPs appeared spherical with size in the range of 1.32 – 4.33 nm. *Pediococcus acidilactici* TG3 AgNPs appeared spherical in shape with some large particles having irregular edges and aggregated with size ranging from 1.32 - 9.39 nm. The shape of *Lactobacillus plantarum* TG4 AgNPs appeared near spherical and partially aggregated with polydisperse distribution in the size range of 1.32 – 23.22 nm.

Based on the EDX analysis, strong signals of AgNPs were confirmed in the nanoparticles synthesized using selected LAB strains (Figures 4 -7). EDX analysis for *Lactobacillus plantarum* TG1 AgNPs revealed a strong peak at 3 KeV which was attributed to SPR of silver crystals (65.20%) and weak signals of C (2.10%), O (20.20%), K (1.10%), Fe (9.20%) and Si (2.20%). For *Lactobacillus plantarum* TG2 AgNPs, strong speaks attributed to silver particles (65.20%) at 2 – 3 KeV and weak signals of C (6.10%), O (15.20%), K (2.10%), Fe (8.20%), and Si (3.20%).

The strong peaks observed at 3 KeV were attributed to silver particles (64.50) from *Pediococcus acidilactici* TG3 AgNPs, while the weak peaks were attributed to C (2.30%), O (15.40%), Na (2.10%), Fe (12.40%) and Si (3.20%).

Furthermore, for *Lactobacillus plantarum* TG4 AgNPs strong peak was observed at 3 KeV and this was attributed to the SPR of silver particles (65.60%) and weak peaks of C (2.30%), O (15.40%), Fe (12.40%) and S (4.20%) were observed.

The antibacterial and antifungal activities of the silver nanoparticles synthesized from LAB metabolites were reported in Table 4. The Minimum Inhibitory Concentration (MIC) of Biosynthesized AgNPs against Bacterial and Fungal Pathogens was recorded in Table 5.

Microbially synthesized silver nanoparticles possess tremendous benefits such as reduced toxicity, being environmentally friendly, ease of production, and being cost-effective. These have made the exploration of silver nanoparticles in the food, clinical, agricultural, and pharmaceutical industries of great importance. The present study harnesses the antimicrobial potential of biosynthesized silver nanoparticles by LAB isolated from *C. esculentus* L. in Ibadan Nigeria.

The current study showed that 24 lactic acid bacteria were isolated from *C. esculentus*. The report that *Lactobacillus plantarum* has the highest occurrence (38%) agrees with Ogunremi *et al.,* (2022) who recently reported the highest occurrence of *L. plantarum* from tigernuts in Southwest Nigeria. However, the report that *L. acidophilus* occurred the least (4%) contrasts the findings of Makut *et al.,* (2018) who reported a high percentage of *L. acidophilus* (92.7%) from *C. esculentus* in northern Nigeria.

All the lactic acid bacteria isolated, selected, and characterized in this study are all confirmed to be beneficial for technological use (Bourdichon *et al.,* 2012). This is consistent with the findings of other researchers who have demonstrated LAB for sustainable, affordable, and eco-friendly protection of food and agricultural products from toxigenic and spoilage microbes (Ogunremi *et al.,* 2022; Romanens *et al.,* 2019, 2020).

Four of the lactic acid bacteria strains (*Lactobacillus plantarum* strain 218, *Lactobacillus plantarum* strain L2, *Pediococcus acidilactici* strain AV1 24, *Lactobacillus plantarum* strain a27) showed strong antimicrobial potency and were used to biosynthesized AgNPs. In a similar pattern, findings from other studies reported broad-spectrum antimicrobial LAB isolated from traditional fermented foods; Abouloifa *et al.,* (2021) and Bartkiene *et al.,* (2019) reported strong antifungal activities from fermented green olives and sourdoughs respectively.

The synthesis of silver nanoparticles by the metabolites of LAB was confirmed by the colour change which signifies the reduction of Ag^+ to AgNPs after incubation. The colour change observed during the biosynthesis of AgNPs was due to the excitation of surface plasmon vibrations in AgNPs. Similar studies also reported this (Ajayi *et al.,* 2015; Kabo *et al.,* 2019; Jabbar & Hussein, 2021).

The UV-VIS absorption spectra of AgNPs reported in this study indicates the synthesis of silver nanoparticles by the metabolites of the selected LAB strains, which agrees with patterns from similar studies (Adebayo-Tayo *et al.,* 2017; Sarvamangala *et al.,* 2013; Prabhu *et al.,* 2014). TEM analysis revealed spherical AgNPs ranging from 1.32 to 23.22 nm, with size variations attributed to different LAB strains. Mohd Yusof *et al.,* (2020) reported the average particle size distribution of *Lactobacillus plantarum* TA4 biosynthesized AgNPs ranging from 4.7 to 24.3 nm. In this study, Energy Dispersive X-ray spectroscopy analysis was used to determine the total elemental composition in the AgNPs synthesized by the LAB metabolites.

The EDX results indicated a significant presence of silver nanoparticles (AgNPs), and in addition to AgNPs, other elements were also detected, likely originating from the culture media composition. These elements may act as capping or stabilizing agents attached to the surface of the biosynthesized silver nanoparticles. Previous studies have also utilized EDX for examining the synthesis and reduction of AgNPs (Loi *et al.,* 2023).

Table.1 Screening for the Probiotic Potential of LAB isolates

ISOLATES	pH Ranges			Phenol concentration			Bile Salts concentration			NaCl Concentration		
	3.0	**4.0**	**5.0**	**0.2%**	**10.3%**	**20.4%**	**1.0%**	**1.5%**	**2.0%**	**4%**	**6%**	**8%**
TG1$_1$	0.410±0.011	0.818±0.008	1.009±0.005	0.526±0.003	0.304±0.003	0.122±0.002	0.822±0.001	0.603±0.002	0.328±0.003	0.787±0.005	0.529±0.007	0.403±0.002
TG1$_2$	0.184±0.006	0.191±0.004	0.260±0.004	0.419±0.002	0.302±0.003	0.090±0.002	0.520±0.005	0.407±0.003	0.286±0.001	0.284±0.003	0.201±0.002	0.137±0.004
TG1$_3$	0.275±0.006	0.307±0.006	0.370±0.004	0.278±0.002	0.134±0.003	0.106±0.003	0.239±0.003	0.210±0.001	0.161±0.002	0.202±0.002	0.172±0.002	0.144±0.003
TG1$_4$	0.290±0.003	0.303±0.003	0.346±0.006	0.219±0.002	0.165±0.002	0.036±0.002	0.517±0.003	0.405±0.001	0.245±0.002	0.311±0.002	0.238±0.005	0.165±0.002
TG1$_5$	0.206±0.009	0.224±0.001	0.285±0.003	0.184±0.004	0.030±0.002	0.332±0.003	0.291±0.002	0.229±0.002	0.140±0.002	0.248±0.001	0.206±0.003	0.145±0.002
TG1$_6$	0.215±0.006	0.267±0.006	0.286±0.004	0.117±0.004	0.107±0.003	0.019±0.001	0.216±0.002	0.177±0.001	0.129±0.003	0.413±0.005	0.288±0.002	0.233±0.002
TG2$_1$	0.205±0.006	0.216±0.004	0.226±0.004	0.224±0.004	0.017±0.003	0.013±0.001	0.266±0.005	0.218±0.006	0.151±0.001	0.425±0.002	0.509±0.002	0.331±0.009
TG2$_2$	0.624±0.004	0.926±0.001	0.979±0.003	0.533±0.003	0.214±0.003	0.100±0.002	0.894±0.004	0.615±0.002	0.405±0.003	0.989±0.002	0.567±0.002	0.500±0.004
TG2$_3$	0.188±0.002	0.192±0.003	0.228±0.001	0.590±0.004	0.124±0.001	0.018±0.002	0.415±0.002	0.273±0.001	0.126±0.003	0.427±0.007	0.389±0.002	0.303±0.003
TG2$_4$	0.288±0.003	0.304±0.003	0.320±0.002	0.079±0.002	0.079±0.002	0.039±0.003	0.278±0.002	0.209±0.005	0.136±0.000	0.411±0.003	0.322±0.001	0.209±0.003
TG2$_5$	0.152±0.002	0.186±0.002	0.207±0.004	0.060±0.002	0.060±0.002	0.007±0.001	0.197±0.005	0.357±0.002	0.019±0.001	0.203±0.003	0.166±0.003	0.131±0.001
TG2$_6$	0.290±0.001	0.309±0.003	0.392±0.004	0.023±0.002	0.023±0.002	0.010±0.001	0.276±0.002	0.219±0.003	0.108±0.002	0.305±0.003	0.280±0.002	0.209±0.002
TG3$_1$	0.398±0.002	0.428±0.008	0.506±0.008	0.122±0.003	0.020±0.004	0.009±0.001	0.733±0.001	0.605±0.002	0.515±0.002	0.755±0.000	0.709±0.003	0.574±0.003
TG3$_2$	0.173±0.048	0.172±0.002	0.269±0.003	0.223±0.003	0.017±0.003	0.009±0.002	0.247±0.006	0.185±0.002	0.097±0.003	0.436±0.001	0.401±0.002	0.273±0.002
TG3$_3$	0.060±0.005	0.202±0.003	0.212±0.001	0.405±0.004	0.169±0.004	0.102±0.004	0.304±0.005	0.254±0.003	0.154±0.003	0.221±0.002	0.196±0.001	0.101±0.002

TG3$_4$	0.115±0.005	0.307±0.004	0.309±0.004	0.102±0.011	0.017±0.002	0.013±0.004	0.266±0.005	0.173±0.002	0.124±0.009	0.315±0.006	0.264±0.001	0.208±0.004
TG3$_5$	0.187±0.005	0.213±0.007	0.252±0.003	0.180±0.007	0.097±0.005	0.001±0.002	1.175±0.002	0.092±0.002	0.027±0.001	0.510±0.001	0.458±0.004	0.409±0.005
TG3$_6$	0.250±0.005	0.274±0.007	0.304±0.007	0.214±0.003	0.104±0.005	0.062±0.003	0.116±0.001	0.101±0.003	0.067±0.001	0.305±0.006	0.270±0.002	0.208±0.001
TG4$_1$`	0.188±0.005	0.212±0.002	0.273±0.009	0.284±0.006	0.033±0.004	0.170±0.004	0.525±0.001	0.329±0.002	0.226±0.002	0.460±0.004	0.372±0.002	0.323±0.002
TG4$_2$	0.216±0.005	0.296±0.004	0.299±0.002	0.109±0.002	0.114±0.005	0.041±0.003	0.212±0.009	0.168±0.002	0.117±0.001	0.207±0.002	0.186±0.004	0.165±0.002
TG4$_3$	0.299±0.013	0.294±0.009	0.346±0.005	0.236±0.005	0.224±0.004	0.026±0.005	0.273±0.003	0.217±0.003	0.113±0.002	0.513±0.002	0.464±0.002	0.346±0.004
TG4$_4$	0.390±0.004	0.676±0.005	0.694±0.007	0.357±0.008	0.183±0.005	0.052±0.005	0.955±0.002	0.726±0.004	0.616±0.003	0.858±0.004	0.685±0.002	0.501±0.003
TG4$_5$	0.247±0.005	0.253±0.004	0.277±0.005	0.060±0.002	0.021±0.004	0.009±0.002	0.069±0.003	0.022±0.003	0.010±0.003	0.217±0.004	0.191±0.002	0.147±0.002
TG4$_6$	0.543±0.010	0.175±0.003	0.201±0.005	0.157±0.003	0.142±0.002	0.037±0.003	0.213±0.005	0.168±0.002	0.095±0.001	0.477±0.005	0.400±0.001	0.255±0.002

Data are presented as Mean ± Standard Deviation

Table.2 Screening for Antibacterial and Antifungal Potential of Isolated LAB against selected Pathogens

	Antibacterial Activities (mm)						**Antifungal Activities (mm)**			
ISOLATES	***Pseudomonas sp.***	***S. aureus***	***E. coli***	***Klebsiella sp.***	***Salmonella typhi***	***B. cereus***	***A. niger***	***A. flavus***	***Rhizopus sp.***	***Penicillium sp.***
TG1$_1$	**17±1.41**	**14±0.00**	**17±1.41**	**8±0.00**	**11±1.41**	**10±0.00**	**11±1.41**	**8±0.00**	**6±0.00**	**11.5±0.71**
TG1$_2$	5±1.41	9±1.41	11±1.41	0.00	0.00	7±1.41	4.5±0.71	0.00	3±1.41	5±1.41
TG1$_3$	0.00	3±1.41	6.5±0.71	3±1.41	2±0.00	0.00	7.5±0.71	3±1.41	2.5±0.71	0.00
TG1$_4$	2±0.00	7±1.41	7±1.41	4±0.00	3.5±0.71	3±1.41	3±1.41	0.00	5±1.41	0.00
TG1$_5$	5±1.41	0.00	0.00	11±1.41	3±1.41	1±1.41	2±0.00	5±1.41	8±0.00	0.00
TG1$_6$	9±1.41	4±0.00	7.5±0.71	3±1.41	0.00	0.00	4±0.00	6±0.00	1±1.41	2±0.00
TG2$_1$	6±0.00	2±0.00	0.00	4±0.00	1±1.41	0.00	0.00	2±0.00	2±0.00	4±0.00
TG2$_2$	**11±1.41**	**10±0.00**	**13±1.41**	**10.5±0.71**	**13.5±0.71**	**6.5±0.71**	**9.5±0.71**	**5±1.41**	**8±0.00**	**7±1.41**
TG2$_3$	8±0.00	3±1.41	4±0.00	3±1.41	0.00	0.00	2±0.00	0.00	3±1.41	2±0.00
TG2$_4$	0.00	0.00	1±1.41	1±1.41	1±1.41	3±1.41	4±0.00	7±1.41	5.5±0.71	3±1.41
TG2$_5$	3±1.41	5.5±0.71	0.00	9±1.41	3±1.41	0.00	0.00	2±0.00	0.00	2±0.00
TG2$_6$	2±0.00	4±0.00	3±1.41	6±0.00	3.5±0.71	1±1.41	3.5±0.71	7±1.41	2.5±0.71	3±1.41
TG3$_1$	**13±1.41**	**9±1.41**	**11±1.41**	**11.5±0.71**	**11±1.41**	**12±0.00**	**9±1.41**	**11±1.41**	**7±1.41**	**10.5±0.71**
TG3$_2$	8.5±0.71	1±1.41	1±1.41	0.00	2.5±0.71	9±1.41	0.00	6±0.00	0.00	0.00
TG3$_3$	0.00	0.00	0.00	7±1.41	0.00	0.00	0.00	3±1.41	4±0.00	0.00
TG3$_4$	7±1.41	9±1.41	11±1.41	5±1.41	7±1.41	0.00	1.00±1.41	2±.00	1±1.41	2±0.00
TG3$_5$	0.00	2±0.00	0.00	1±1.41	3±1.41	7±1.41	0.00	7.5±.071	6±0.00	1±1.41
TG3$_6$	0.00	5±1.41	3±1.41	0.00	9±1.41	0.00	7.5±0.71	9.5±0.71	3±1.41	3.5±1.41
TG4$_1$	1±1.41	4±0.00	4±0.00	11±1.41	6±0.00	11±1.41	1.00±1.41	2±0.00	4±0.00	0.00
TG4$_2$	9±1.41	7±1.41	1±1.41	1±1.41	3±1.41	5±1.41	3±1.41	0.00	7±1.41	7±1.41
TG4$_3$	11±1.41	6±0.00	2±0.00	5±1.41	2±0.00	0.00	0.00	0.00	0.00	1±1.41
TG4$_4$	**9±1.41**	**11±1.41**	**13±1.41**	**13±1.41**	**11±1.41**	**13±1.41**	**9±1.41**	**11±1.41**	**7±1.41**	**10.5±0.71**
TG4$_5$	0.00	5±1.41	5±1.41	0.00	0.00	3±1.41	0.00	3±1.41	4±0.00	4±0.00
TG4$_6$	4±5.65	1±1.41	4±0.00	0.00	0.00	9±1.41	4±0.00	2±0.00	0.00	2.5±0.71

Data are presented as Mean ± Standard Deviation

Table.3 Molecular Characterization of the Selected Lactic Acid Bacteria Isolated from Fermented Tigernut Milk

S/N	Isolate Code	Probable identity
1	TG1	*Lactobacillus plantarum* strain 218
2	TG2	*Lactobacillus plantarum* strain L2
3	TG3	*Pediococcus acidilactici* strain AV1 24
4	TG4	*Lactobacillus plantarum* strain a27

Table.4 Antibacterial and Antifungal activities of the silver nanoparticles synthesized from LAB metabolites

Biosynthesized AgNPs	Antimicrobial Activity of Biosynthesized AgNPs										
	Antibacterial Activity(mm)						Antifungal Activity(mm)				
	S. aureus	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*S. typhi*	*B. cereus*	*A. niger*	*A. flavus*	*Fusarium sp.*	*Rhizopus sp.*	*Penicillium sp.*
TG1AgNPs	22±2.00	20±2.00	12±2.00	20±2.00	14±2.00	16±2.00	16±2.00	12±2.00	8±2.00	10±2.00	14±2.00
TG2AgNPs	18±2.00	16±4.00	14±0.00	16±2.00	14±2.00	12±2.00	12±2.00	10±2.00	10±2.00	12±2.00	10±1.00
TG3AgNPs	14±1.00	12±2.00	18±4.00	16±1.00	16±0.00	14±2.00	14±2.00	14±2.00	8±0.00	10±2.00	12±2.00
TG4AgNPs	20±1.00	18±1.00	16±2.00	14±2.00	14±1.00	16±2.00	12±2.00	18±2.00	12±2.00	10±2.00	11±1.00
Streptomycin	16±2.00	14±2.00	22±0.00	20±0.00	18±2.00	14±2.00	12±0.00	10±0.00	12±2.00	14±2.00	12±2.00
$AgNO_3$	8±1.00	6±0.00	4±0.00	4±2.00	8±0.00	6±0.00	2±0.00	4±2.00	2±2.00	6±2.00	4±2.00
DMSO	-	-	-	-	-	-	-	-	-	-	-

Data are presented as mean ± standard deviation

Table.5 Minimum Inhibitory Concentration (MIC) of Biosynthesized AgNPs against Bacterial and Fungal Pathogens

Biosynthesized AgNPs	Tested Pathogens										
	Bacterial MIC (µg/ml)						Fungal MIC (µg/ml)				
	S. aureus	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*S. typhi*	*B. cereus*	*A. niger*	*A. flavus*	*Fusarium sp.*	*Rhizopus sp.*	*Penicillium sp.*
TG1AgNPs	2.50	2.50	1.25	1.25	2.50	1.25	2.50	2.50	5.00	5.00	2.50
TG2AgNPs	5.00	5.00	5.00	2.50	2.50	1.20	1.25	2.50	2.50	1.25	5.00
TG3AgNPs	10.00	5.00	1.25	5.00	2.50	10.00	2.50	5.00	5.00	5.00	2.50
TG4AgNPs	1.25	2.50	5.00	1.25	5.00	5.00	5.00	1.25	5.00	2.50	5.00

Figure.1 Occurrence of LAB Isolates from *C. esculentus*

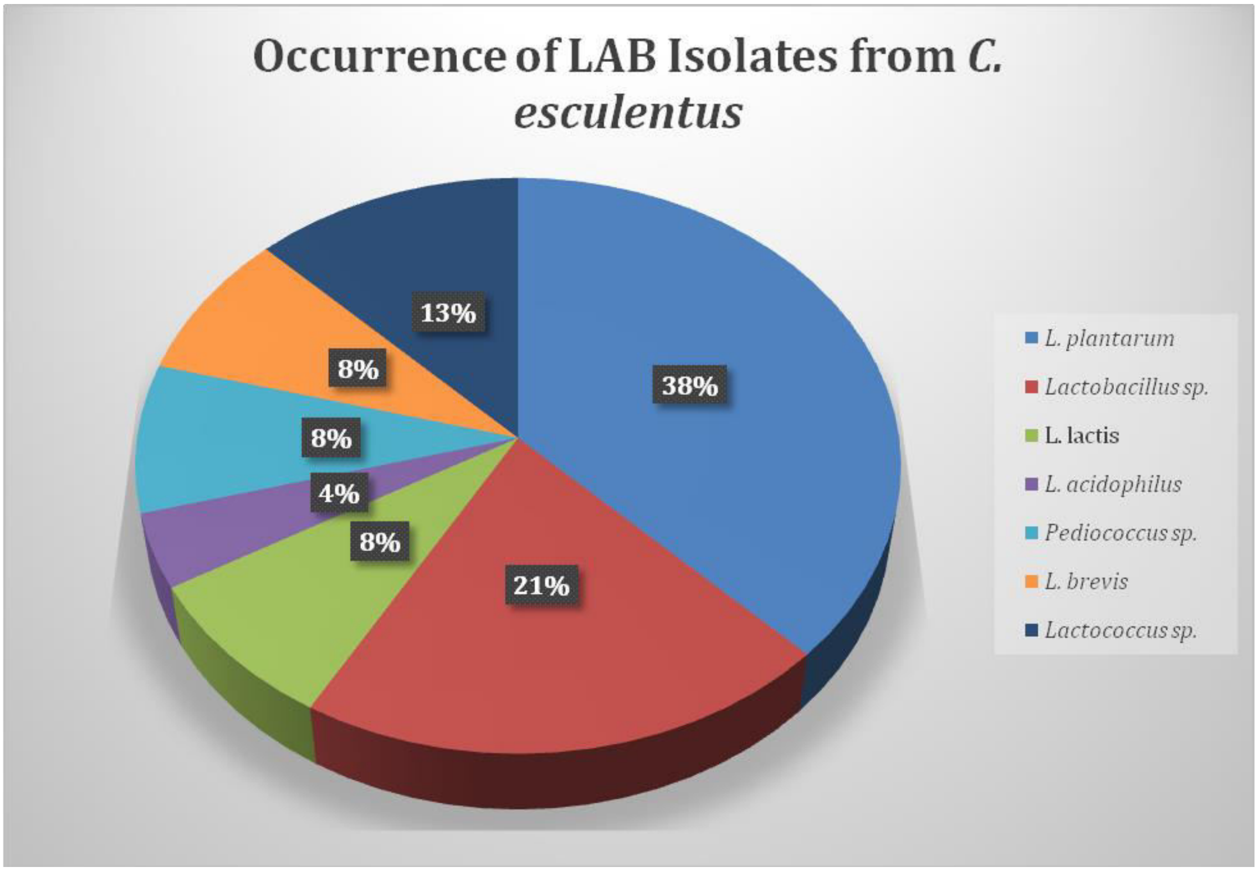


Figure.2

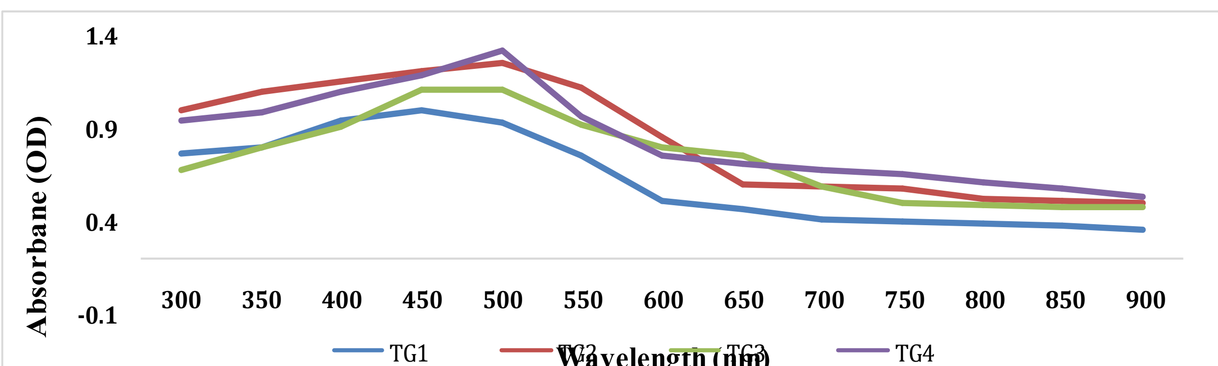


Figure 2: UV-visible Absorption Spectra of Biosynthesized AgNPs by LAB

Figure.3 Transmission Electron Micrograph (TEM) of biosynthesized AgNPs using different LAB metabolites: *Lactobacillus plantarum* TG1, *Lactobacillus Plantarum* TG2, *Pediococcus acidilactici* TG3, *Lactobacillus plantarum* TG4

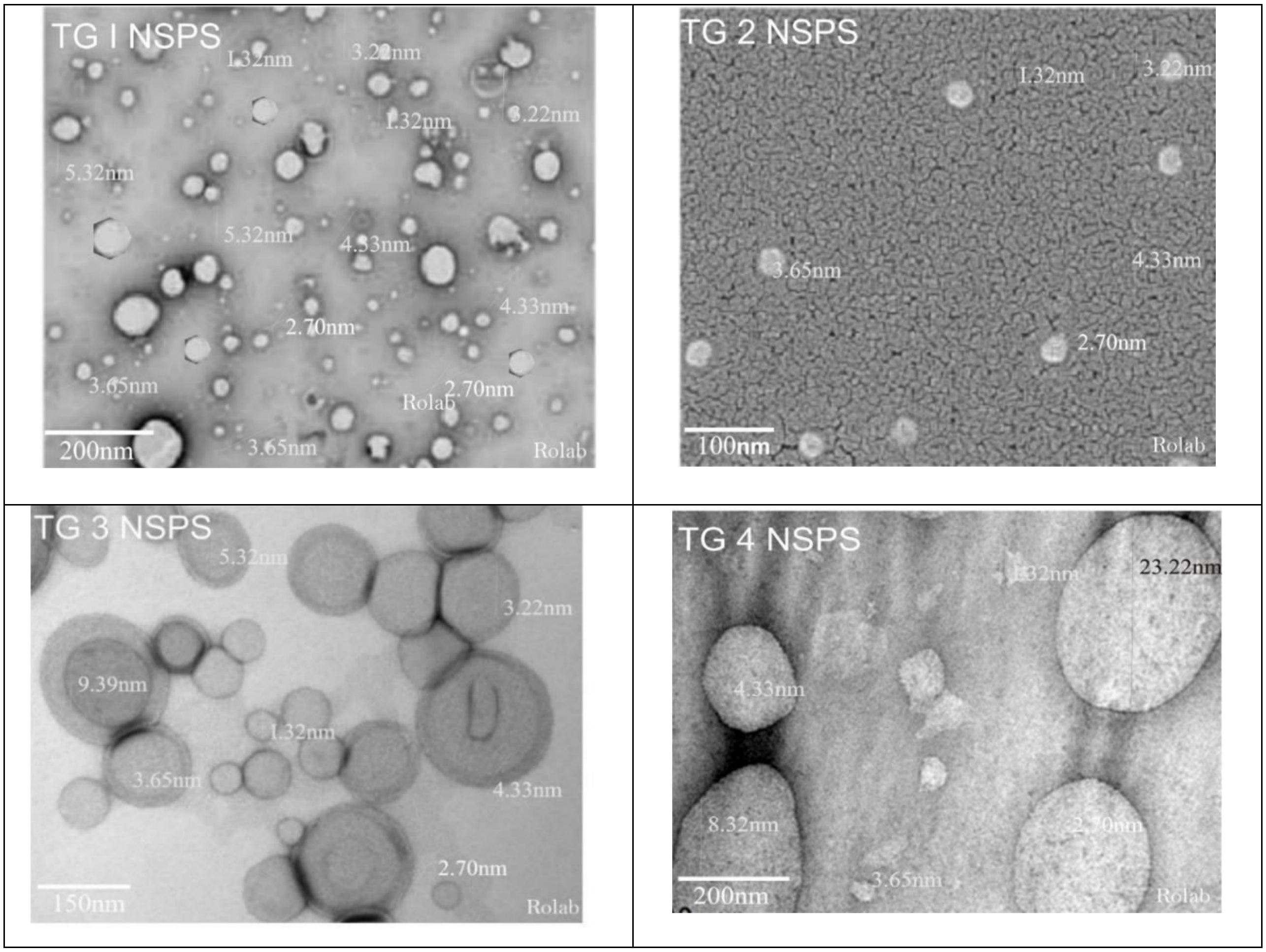


Figure.4 EDX Analysis of Biosynthesized AgNPs by *Lactobacillus plantarum* TG1

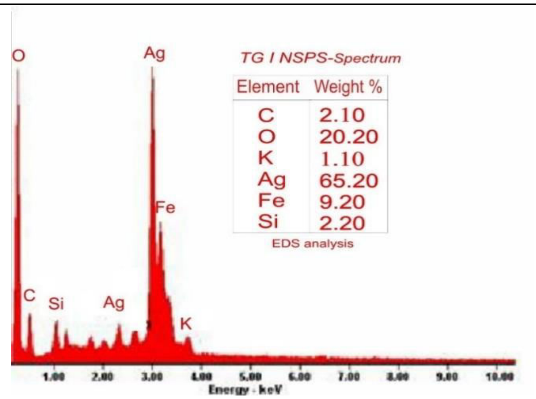


Figure.5 EDX Analysis of Biosynthesized AgNPs *by Lactobacillus plantarum* TG2

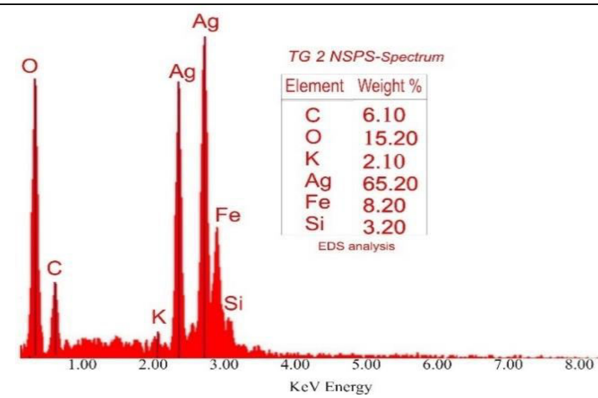


Figure.6 EDX Analysis of Biosynthesized AgNPs *by Pediococcus acidilactici* TG3

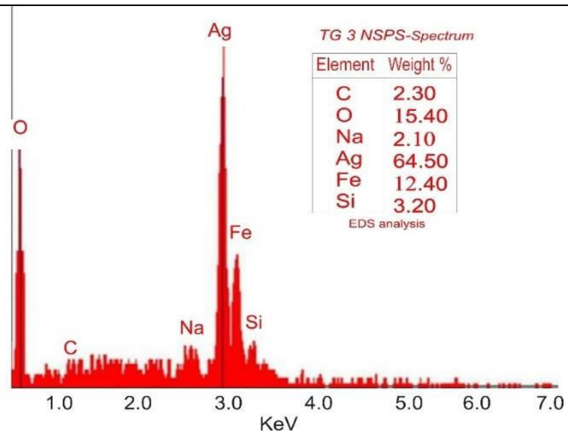
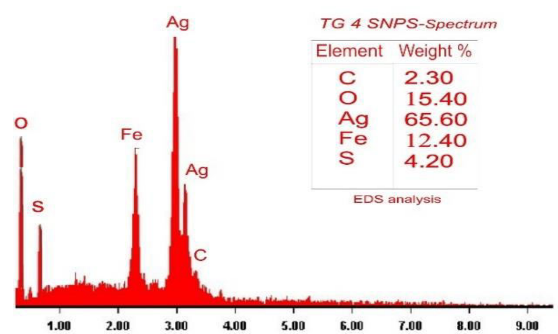


Figure.7 EDX Analysis of Biosynthesized *AgNPs by Lactobacillus plantarum* TG4



Observation from this study shows that the antimicrobial activity of the biosynthesized silver nanoparticles was not unvaryingly distributed. This correlates with Naseer *et al.,* (2022) who reported similar results.

Biologically synthesized AgNPs from *Lactobacillus plantarum* had the strongest antibacterial and antifungal activity, forming distinct zones of inhibition and low MIC against the tested pathogens. This result is similar to the work of Khodeer *et al.,* (2023) who reported low MIC of greenly synthesized AgNPs against microbial pathogens. The MIC of silver nanoparticles reported by Mortazavi-Derazkola *et al.,* (2021) were higher compared to the outcome reported in this study.

These differences may result from varied sources of AgNPs synthesis and the different strains of test pathogens used. Overall, the LAB metabolite-synthesized AgNPs were effective against wide varieties of pathogenic bacteria. This is consistent with the findings of Adebayo-Tayo *et al.,* (2019) who concluded that biosynthesized AgNPs are effective against a range of pathogens such as *Escherichia coli* and *Bacillus cereus*.

In Conclusion, the discoveries of the present study suggest that lactic acid bacteria are useful in the extracellular biosynthesis of silver nanoparticles. Lactic acid bacteria isolated from *Cyperus esculentus*, a traditional plant in Nigeria has the potential for the biosynthesis of AgNPs which possess antimicrobial activities against selected bacterial and fungal pathogens.

The biosynthesized AgNPs displayed excellent antibacterial and antifungal activities, they were heat-stable, nontoxic, and cost-effective in production. Thus, biosynthesized AgNPs from metabolites of lactic acid bacteria isolated from *C. esculentus* may provide a promising alternative to the conventional physical and chemical methods that are not cost-effective. Therefore, these silver nanoparticles should be implemented to control food-borne pathogens and prolong the shelf-life of foods.

Author Contributions

O. T. Mamora: Investigation, formal analysis, writing—original draft. D. A. Aina: Validation, methodology, writing—reviewing. S. G. Jonathan:—Formal analysis, writing—review and editing. S. Amodu: Investigation, writing—reviewing. K. O. Fagbemi: Resources, investigation writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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