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BIOGENIC SYNTHESIS OF SILVER NANOPARTICLES BY *LACTOBACILLUS PLANTARIUM* ISOLATED FROM FERMENTED *SORGHUM BICOLOR* AND *IN VITRO* ANTIMICROBIAL ACTIVITIES

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ABSTRACT
The need for novel antimicrobial agents has driven interest in the synthesis of nanoparticles with broad-spectrum antimicrobial potential. This study was undertaken to synthesize silver nanoparticle**s** **(**Ag-NPs) using *Lactobacillus plantarum* from fermented sorghum. The Ag-NPs were characterized using UV-vis spectrophotometer, X-ray diffractometer, and IR-470 spectrometer. The antimicrobial efficacies of Ag-NPs were determined by the agar well diffusion method. The UV-Vis spectroscopy analysis showed that the Ag-NPs had an absorption peak at 420 nm. Of the sixteen isolates tested, 25 µg/mL of biosynthesized AgNPs inhibited 81.3% of isolates, 100 µg/mL of biosynthesized AgNPs inhibited 93.8% of isolates, while all the isolates were sensitive to biosynthesized AgNPs at a concentration of 200 µg/mL. The lowest and highest mean zone of inhibition obtained was 9.2 ± 0.2 mm and 20.0 ± 1.0 mm, respectively. The minimum inhibitory concentration (MIC) ranged from 6.25 µg/mL for *S. aureus*, *C. freundii, P. aeruginosa*, *C. dubliniensis,* and *C. parapsilosis* to 100 µg/mL for *S. flexneri* and *C. glabrata*. The minimum bacteriocidal concentration (MBC) and minimum fungicidal concentration (MFC) values of biosynthesized AgNPs ranged between 12.5 to >200 µg/mL. The MBC/MIC biosynthesized AgNPs on bacterial isolates and MFC/MIC ratios of biosynthesized AgNPs on Candida isolates ranged from 1 to 4 and 2 to 4, respectively. The regression values of biosynthesized AgNPs, as exhibited by the bacterial and Candida isolates, ranged from 0.6049 to 0.9285 and 0.5750 to 0.8902, respectively. Biosynthesized AgNPs from the CFS of *L. plantarum* demonstrated broad-spectrum antimicrobial activity, with MBC and MFC values confirming their bactericidal and fungicidal effects.

Keywords: Synthesis, Bactericidal, Fungicidal, Nanoparticles, Inhibition, Isolates.

INTRODUCTION

Antimicrobial resistance (AMR) has become a severe global threat and serious worldwide health problem that frequently creates challenges in infection treatment, leads to increased mortality, prolonged hospital stays, and rising healthcare costs (Akinjogunla *et al*., 2022; Ajayi *et al*., 2024). The urgent need for novel antimicrobial agents has driven interest in nanoscience and nanotechnology, particularly for the synthesis of nanoparticles (NPs) with broad-spectrum antimicrobial potential utilizing chemical and biological processes (Ahmed *et al*., 2016; Gupta *et al*., 2017). Silver nanoparticles (AgNPs) have gained attention due to their multifunctional antimicrobial activity against bacteria, viruses, fungi, and parasites. The mechanisms of action of AgNPs include microbial cell membranes disruption, enzyme inhibition, and alteration of microbial cell wall and nucleic material pathways (Medda *et al*., 2015).

However, chemically synthesized NPs are expensive and generate toxic wastes that are hazardous to both the environment and human health (Zhang *et al*., 2016), and this has led to a shift toward the biogenic NPs synthesized using microorganisms or the byproducts of their metabolism, which is relatively sustainable, less toxic, environmentally non-hazardous, cost-effective, and provides greater biocompatibility in the use of nanoparticles (Gholami-Shabani *et al*., 2014).

The lactic acid bacteria (LAB) are Gram-positive, rod-shaped, non-spore-forming bacteria (Mokoena, 2017) that use carbohydrates as the only or main carbon source and are generally recognized as safe. The LAB include more than 60 genera and are frequently isolated from fermented foods such as *Sorghum bicolor*, *Hordeum vulgare,* etc. (Wang *et al*., 2021). The LAB, such as *Lactobacillus plantarum* and *Lactobacillus brevis*, are commercially available as probiotics with health benefits (Pandey *et al*., 2015). The probiotic-synthesized NPs have been found to possess antimicrobial effects due to their smaller size, enhanced surface area to volume ratio, high catalytic capabilities, and a tendency to generate reactive oxygen species (Ibrahim *et al*., 2021). The inclusion of a biological capping agent as a protective coating against oxidation, agglomeration, and aggregation on some biogenic NPs provides greater stability (Vaseghi *et al*., 2018). In view of the increasing ineffectiveness of many conventional antimicrobial agents, there is a need to the search for novel and safer antimicrobial agents to combat these "super bugs" of bacteria and fungi. Thus, this necessitated this study on the biogenic synthesis of AgNPs by *L. plantarium* isolated from fermented *S. bicolor* and *in vitro* antimicrobial activities.

MATERIALS AND METHODS

Source of Isolates

Clinical isolates consisted of *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae, Citrobacter freundii*, *Shigella flexneri*, *Pseudomonas aeruginosa, Escherichia coli*, *Enterococcus faecalis*, *Bacillus* spp., *Serratia marcescens*, *Salmonella typhi, Candida albicans*, *Candida dubliniensis*, *Candida tropicalis*, *Candida krusei*, *Candida glabrata*, and *Candida parapsilosis* were obtained from the department of Microbiology, University of Uyo. The bacterial strains were checked for purity, maintained on Nutrient Agar (NA), and re-identified using conventional biochemical tests and the VITEK 2 automated system (BioMerieux, Inc., France). The yeast isolates were re-identified

using the chromogenic medium CHROM Agar™ and the VITEK 2 automated system, and identifications were achieved by reading cards and comparing them with the database using the software version 8.01.

Isolation and Phenotypic Identification of *Lactobacillus plantarum*

Isolation of *L. plantarum* from the fermented sorghum samples was carried out using De Man, Rogosa, and Sharpe (MRS) agar (Alhaag *et al.*, 2016). Briefly, 1 mL of aliquots from serially diluted fermented sorghum (liquor) samples was inoculated on plates of MRS agar (Oxoid, Basingstoke, Hampshire, England) and incubated under anaerobic conditions using an anaerobic candle jar for 48 h at 37ºC (Mulaw *et al*., 2019). After incubation, colonies were subcultured on MRS agar plates and incubated for 48 h at 37ºC. Thereafter, pure cultures of isolates were streaked onto MRS agar slants, incubated for 48 h at 37°C, and stored at 4°C. The isolates were subjected to Gram staining and conventional biochemical tests, and were further phenotypically identified using the VITEK 2 automated system.

Preparation of Cell-Free Supernatant of *L. plantarum*

The cell-free supernatant (CFS) was prepared using MRS broth (1% *v*/*v*) following the method of Chaudhari *et al.* (2012). A loopful of *L. plantarum* culture was inoculated into MRS broth (200 mL), and the mixture was incubated without shaking at 37°C for 48 h. After incubation, the cultures were centrifuged at 10000 rpm for 10 min. The process was repeated thrice and the supernatant was filtered through a 0.2-micrometer filter. The filtered CFS of *L. plantarum* was stored at 4°C and used for nanoparticle synthesis.

Biogenic Synthesis of Silver Nanoparticles (AgNPs)

Biogenic synthesis of AgNPs by *L. plantarum* was determined using the method described by Matei *et al.* (2020). The 1 mM $AgNO_3$ solution was prepared by dissolving 0.17 g of $AgNO_3$ in 1000 mL of deionized water and covered with aluminum foil to prevent the photooxidation of the solution. Then, 50 mL of CFS from a 48-h liquid culture of *L. plantarum* were mixed with 50 mL of an aqueous solution of 1 mM $AgNO_3$. The mixture was incubated in 250 mL Erlenmeyer flasks on an orbital shaker (200 rpm) at 28 ± 2ºC in the dark for 72 h. A flask with CFS without $AgNO_3$, maintained at the same condition, was run along with the experimental flask and was utilized as a control. The colour change was observed and reported.

Characterization of Silver Nanoparticles

The reduction of silver ions from Ag^+ to Ag^0, as evinced by colour change were monitored using a UV-vis spectrophotometer (Shimadzu UV-1650, Japan). Briefly, 5 mL of the supernatant of the reaction solution was taken after 72 h, and absorbance was measured using a UV-vis spectrophotometer at a wavelength between 350 and 800 nm (Matei *et al*., 2020). The X-ray diffraction measurement of the biosynthesized AgNPs, so as to check the quality and crystallinity, was determined using an XRD-6100 X-ray Diffractometer (Shimadzu XD-3A, Japan) as described by Matei *et al.* (2020). The functional groups responsible for the synthesis of silver nanoparticles were determined using a Fourier transform infrared spectroscopy (Shimadzu IR-470 Spectrometer, Japan) in the range of 500 to 4000 cm^{-1} (Arland and Kumar, 2024).

Antibacterial Assay of Biosynthesized Silver Nanoparticle (AgNPs)

In vitro antimicrobial activity of the biosynthesized AgNPs was evaluated using the agar well diffusion method (CLSI, 2016). Bacterial isolates (*S. pneumoniae*, *H. influenzae, S. aureus*, *C. freundii*, *S. flexneri*, *P. aeruginosa, E. coli*, *Bacillus* spp., *S. marcescens*, and *S. typhi*) and yeasts (*C. albicans, C. dubliniensis, C. tropicalis, C. krusei, C. glabrata,* and *C. parapsilosis*) were used. Briefly, 10 µL of each overnight microbial suspension, adjusted to a 0.5 McFarland turbidity standard, was streaked homogenously onto each plate of Mueller Hilton Agar (MHA). The biosynthesized AgNPs was dissolved in sterilized deionized water to achieve graded concentrations of 2.5, 5.0, 10, and 20 mg/mL. Then, 6 wells were punched over the culture MHA plate using a sterilized cork borer (6 mm diameter) and 100 µL of 2.5, 5.0, 10, and 20 mg/mL concentrations of biosynthesized AgNPs to give 25, 50, 100 and 200 µg/mL, respectively, were dispensed into four (4) labelled wells; 200 µL of cell free supernatant without $AgNO_3$ was dispensed into the 5th well (positive control), and 200 µL of 1% (*v*/v) DMSO was dispensed into the 6th well (negative control) using micropipettes. The culture plates incubated for 18 h at 35 ± 2°C. The experiments were performed in triplicate, and the mean zones of inhibition diameter in millimeters were measured and considered as an indication for antimicrobial activity.

Determination of Minimum Inhibitory Concentration (MIC) of Biosynthesized AgNPs

The micro-broth dilution technique was used to determine the MIC values of biosynthesized AgNPs against bacteria / candida (Akinjogunla and Oluyege, 2016; CLSI, 2016). The concentrations of 20, 10, 5, 2.5, 1.25, 0.625 and 0.3125 mg/mL were obtained by serially diluting 100 µL of stock solution of biosynthesized AgNPs (20 mg/mL). The final concentrations of 200, 100, 50, 25, 12.5, 6.25 and 3.125 µg/mL were then obtained by adding 100 µL of each concentration to 9.9 mL of nutrient broth (NB)/sabourand dextrose broth (SDB) in each test tube. Thereafter, 100 µL of each isolate was added to each test tube. A test tube containing NB/SDB that was inoculated with isolate (positive control) and a test tube containing NB/SDB that was inoculated with biosynthesized AgNPs (negative control). All test tubes were incubated at 37ºC for 24 - 48 h, then examined for microbial growth. The MIC was the lowest concentration of the biosynthesized AgNPs that inhibited microbial growth, as measured by the observed turbidity, after 24–48 h of incubation.

Determination of Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) of Biosynthesized AgNPs

The MBC and MFC values of the biosynthesized AgNPs were determined by taking a loopful from each MIC broth tube exhibiting no visible growth and inoculating it onto plates of NA (for bacteria) and SDA (for candida). The inoculated plates were incubated at 37°C for 24–48 h, then examined for microbial growth (Akinjogunla *et al*., 2021). The MBC and MFC values was considered as the lowest concentrations that killed the bacterial and candida isolates, respectively

RESULTS

The results of the morphology (shape), biochemical tests (coagulase, catalase, starch, vogues Proskauer, methyl red,

nitrate, indole, urease, motility, citrate, hydrogen sulphide, and oxidase), enzymatic characteristics (D-cellobiose, arginine dehydrolase, ornithine decarboxylase, lipase, beta-galactosidase, and hyaluronidase), and sugar fermentation tests (fructose, raffinose, mannitol, maltose, galactose, lactose, glucose, and sucrose) of *L. plantarum* and other bacterial isolates as obtained using the conventional technique and VITEK 2 automated system are presented in Table 1. The results of the re-identification species of Candida species based on their growth at 35°C and 45°C, the germ tube test, the nature of hyphae, enzymatic characteristics (α-amylase, β-galactosidase, N-acetyl-β-glucosaminidase, and β-glucuronidase), and carbohydrate fermentation tests are presented in Table 2.

Biogenic synthesis of AgNPs from $AgNO_3$ by CFS of *L. plantarum* is shown in Fig. 1. The reaction started within 2 to 6 h, and the colour of the mixture changed after the incubation period of 72 h to yellowish-brown by optical observation, indicating the synthesis of AgNPs by CFS of *L. plantarum.* The UV-Vis spectroscopy analysis showed that the formed Ag-NPs had an absorption peak at 420 nm (Fig. 2).

The results of the antimicrobial activity of biosynthesized AgNPs from $AgNO_3$ by CFS of *L. plantarum* are presented in Table 3. Of the sixteen isolates tested, 25 µg/mL of biosynthesized AgNPs had antimicrobial activities on 81.3% of the isolates, and 50 µg/mL of biosynthesized AgNPs had antimicrobial activities on 87.5% of the isolates. The results showed that biosynthesized AgNPs inhibited 93.8% of the isolates (with the exception of *S. flexneri)* at a concentration of 100 µg/mL, while all the isolates were sensitive to the growth inhibition of biosynthesized AgNPs at a concentration of 200 µg/mL. *Shigella flexneri* was resistant to growth inhibition of biosynthesized AgNPs (≤ 100 µg/mL), and *C. glabrata* was resistant to growth inhibition of ≤ 50 µg/mL of biosynthesized AgNPs (Table 3). The lowest and highest mean zone of inhibition (x ± S.D) obtained was 9.2 ± 0.2 mm, as shown by the plate containing *H. influenzae,* and 20.0 ± 1.0 mm, as shown by the plate containing *E. coli,* respectively. The $AgNO_3$ did not reveal any antimicrobial activity against *S. typhi, S. flexneri, C. tropicalis,* and *C. glabrata.*

The minimum inhibitory concentration (MIC), minimum bacteriocidal concentration (MBC), and minimum fungicidal concentration (MFC) values for biosynthesized AgNPs against the bacterial and Candida isolates are shown in Table 4. The MIC values ranged from the lowest (6.25 µg/mL) for *S. aureus*, *E. coli, C. freundii, P. aeruginosa*, *C. dubliniensis,* and *C. parapsilosis* to the highest (100 µg/mL) for *S. flexneri* and *C. glabrata*. The MBC values of biosynthesized AgNPs ranged between 12.5 to >200 µg/mL for bacterial isolates, and the MFC values of biosynthesized AgNPs ranged between 12.5 to >200 µg/mL for Candida isolates. The MBC/MIC biosynthesized AgNPs on bacterial isolates and MFC/MIC ratios of biosynthesized AgNPs on Candida isolates ranged from 1 to 4 and 2 to 4, respectively.

The regression values of biosynthesized AgNPs, as exhibited by the bacterial isolates, ranged from 0.6049 to 0.9285, while the regression values of biosynthesized AgNPs, as exhibited by the Candida isolates, ranged from 0.5750 to 0.8902 (Table 5). The relationships between concentrations of biosynthesized AgNPs and zones of inhibition**,** as exhibited by *S. aureus*, *E. coli, S. flexneri, Bacillus* spp., C. *albicans*, and *C. krusei,* are shown in Fig. 3.

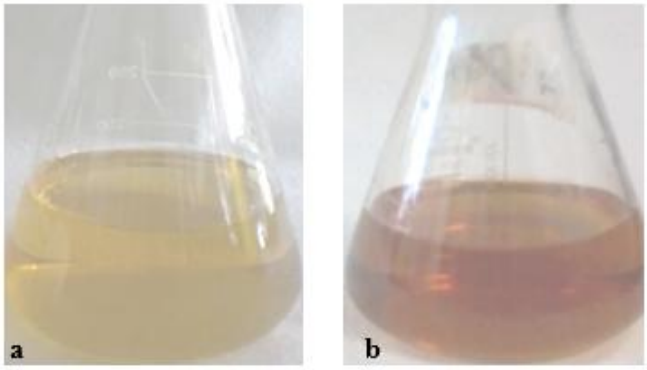

Fig. 1: a. Mixture of CFS of *L. plantarum* and $AgNO_3$ before Colour Change; b. Mixture of CFS of *L. plantarum* and $AgNO_3$ after Colour Change.

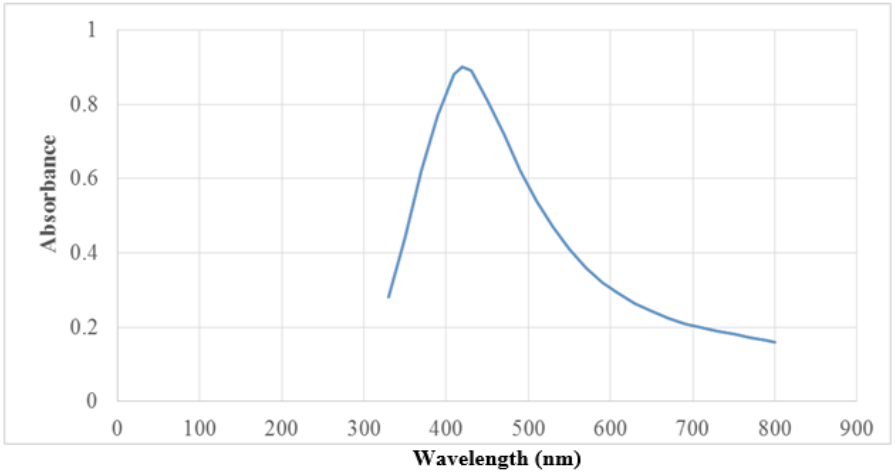

Fig. 2: UV-visible absorption spectra of Ag-NPs synthesized by CFS of *L. plantarum*

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Table 1: Morphological and Biochemical Characteristics and Enzymatic Reactions of Bacterial Isolates

Gram reaction																											
Gram	Shape	COA	CAT	STA	VP	MR	NIT	IND	URE	MOT	CIT	H_2S	OXI	OPT	DCE	ARD	ODX	LIP	ONP	HYA	FRU	RAF	MAN	MAL	GAL	LAC	GLU	SUC	Probable Bacteria
+	rod	-	-	+	-	+	-	-	-	-	-	+	-	nd	+	+	-	-	+	-	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
+	cocci	+	+	-	+	+	+	-	+	-	+	-	+	nd	-	+	+	+	-	+	-	-	+	+	+	+	+	+	*Staphylococcus aureus*
+	cocci	-	-	+	-	+	+	-	-	-	+	-	-	+	+	+	-	+	-	+	+	+	-	+	+	+	+	+	*Streptococcus pneumoniae*
-	rod	-	+	-	-	+	+	-	-	+	-	+	-	nd	-	-	-	+	-	-	-	-	+	+	+	-	+	-	*Salmonella typhi*
-	rod	-	+	-	-	+	+	+	-	+	-	-	-	nd	-	-	-	-	+	-	-	-	+	-	+	+	+	+	*Escherichia coli*
-	rod	-	+	+	+	-	+	-	+	+	+	-	-	nd	-	-	+	-	+	-	+	-	+	+	+	-	+	+	*Serratia marcescens*
-	rod	-	+	-	-	+	+	-	+	+	+	+	-	nd	+	-	-	-	+	-	+	+	+	+	+	+	+	+	*Citrobacter freundii*
-	rod	-	+	-	-	+	+	+	-	-	-	-	-	nd	-	-	-	-	-	-	-	-	+	+	+	-	+	-	*Shigella flexneri*
-	C-rod	-	+	-	-	-	+	+	+	-	+	-	+	nd	-	-	+	+	-	+	-	-	-	+	+	-	+	-	*Haemophilus influenzae*
-	rod	-	+	-	-	-	+	-	-	+	+	-	+	nd	-	+	-	+	-	+	-	-	+	-	-	-	-	-	*Pseudomonas aeruginosa*
+	rod	-	+	+	+	-	+	-	-	+	+	-	+	nd	-	+	-	-	+	-	-	+	+	+	-	-	+	+	*Bacillus* spp.

Keys: COA: Coagulase; CAT: Catalase; STA: Starch; VP: Vogues Proskauer; MR: Methyl red; NIT: Nitrate; IND: Indole; URE: Urease; MOT: Motility; CIT: Citrate; H_2S: Hydrogen sulphide; OXI: Oxidase; OPT: Optochin; DCE: D-Cellobiose; ARD; Arginine Dehydrolase; ODX: Ornithine Decarboxylase; LIP: Lipase; ONPG: Beta-galactosidase; HYA: Hyaluronidase. FRU: Fructose; RAF: Raffinose; MAN: Mannitol; MAL: Maltose; GAL: Galactose; LAC: Lactose; GLU: Glucose; SUC: Sucrose; nd: Not determined; C-rod: Cocco-bacillus; +: Positive; -: Negative

Table 2: Morphological Characteristics, Carbohydrate Fermentation and Enzymatic Tests of *Candida* spp

		Growth at										Carbohydrate Fermentation Tests									
CHROM Agar ™	Shape	35°C	45°C	Germ Tube	Nature of hyphae	Pseudo-hyphae	α-amylase	β-galactosidase	β-NAG	β-Glucuronidase	Urease	Inositol	Cellobiose	Glucose	Raffinose	Xylose	Trehalose	Maltose	Galactose	Sucrose	Lactose	Probable *Candida* spp.
Blue-green	Oval	+	+	+	Septate	+	+	-	+	-	-	-	-	+	-	+	+	+	+	+	-	*Candida albicans*
Dark-green	Oval	+	-	+	Septate	+	-	-	-	-	-	-	-	+	-	-	+	+	+	+	-	*Candida dubliniensis*
Dark-blue	Oval	+	-	-	Septate	+	+	-	-	-	-	-	+	+	-	+	+	+	+	+	-	*Candida tropicalis*
Purple	Oval	+	-	-	Septate	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	*Candida krusei*
White	Oval	+	-	-	Septate	-	-	-	-	-	-	-	-	+	-	+	+	+	+	+	-	*Candida glabrata*
Pink	Oval	+	-	-	Septate	+	-	-	-	-	-	-	-	+	-	+	+	+	+	+	-	*Candida parapsilosis*

Keys: +: Positive; - : Negative; β-NAG: N-acetyl-β-glucosaminidase

Table 3: Antimicrobial Efficacies of Biosynthesized Silver Nanoparticle

	Mean Zone of Inhibition (mm) / $\bar{x}$ ± S.D					
Isolates	25 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL	$AgNO_3$	DMSO
S. aureus	14.5 ± 0.0^b	15.7 ± 0.3^b	17.0 ± 1.0^c	18.3 ± 1.1^c	11.0 ± 0.0^b	NZ
S. pneumoniae	10.5 ± 0.0^a	13.1 ± 0.1^a	14.5 ± 0.5^b	16.0 ± 0.0^b	13.2 ± 0.2^c	NZ
S. typhi	9.7 ± 0.1^a	12.2 ± 0.1^a	14.0 ± 0.0^b	14.5 ± 0.5^b	NZ	NZ
E. coli	13.6 ± 0.2^a	15.5 ± 0.5^b	18.0 ± 1.0^c	20.0 ± 1.0^c	12.0 ± 0.0^b	NZ
S. marcescens	10.2 ± 0.1^a	12.0 ± 0.0^b	15.6 ± 0.2^b	17.5 ± 0.5^b	9.5 ± 0.5^a	NZ
C. freundii	12.5 ± 0.0^a	14.6 ± 0.3^b	17.5 ± 0.5^c	18.2 ± 1.2^c	12.2 ± 0.2^b	NZ
S. flexneri	NZ	NZ	NZ	8.5 ± 0.5^a	NZ	NZ
H. influenzae	9.2 ± 0.2^a	11.0 ± 0.0^b	14.0 ± 1.0^b	14.6 ± 0.4^b	11.0 ± 0.0^b	NZ
P. aeruginosa	12.6 ± 0.4^a	15.1 ± 0.2^b	17.4 ± 0.2^c	18.5 ± 0.5^c	9.5 ± 0.5^b	NZ
Bacillus spp.	10.5 ± 0.0^a	13.6 ± 0.3^a	15.0 ± 0.0^b	15.2 ± 0.2^b	12.8 ± 0.2^b	NZ
C. albicans	NZ	9.8 ± 0.1^a	13.5 ± 0.5^a	14.0 ± 1.0^b	8.0 ± 0.0^a	NZ
C. dubliniensis	13.0 ± 0.0^a	16.5 ± 0.5^b	19.0 ± 1.0^c	19.5 ± 0.5^c	11.5 ± 0.5^b	NZ
C. tropicalis	11.5 ± 0.5^a	14.5 ± 0.5^b	15.8 ± 0.2^b	17.5 ± 0.5^c	NZ	NZ
C. krusei	10.6 ± 0.1^a	12.0 ± 0.0^a	14.0 ± 1.0^b	15.2 ± 0.2^b	13.0 ± 1.0^c	NZ
C. glabrata	NZ	NZ	11.5 ± 0.0^a	13.4 ± 0.3^a	NZ	NZ
C. parapsilosis	13.0 ± 0.0^a	15.5 ± 0.5^a	17.0 ± 1.0^c	18.5 ± 1.5^c	11.5 ± 0.5^b	NZ

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Keys: mm: Millimetre; *x*: Mean; S.D.: Standard Deviation; NZ: No zone of Inhibition; DMSO: Dimethyl Sulphoxide; $AgNO_3$: Silver Nitrate; Mean within the column followed by the different superscript letters are significant as determined by Duncan's multiple range test ($P < 0.05$).

Table 4: Minimum Inhibitory Concentration, Minimum Bactericidal Concentration and Minimum Fungicidal Concentration of Biosynthesized *Silver Nanoparticle*

Bacteria	Concentrations of Biosynthesized AgNPs (µg/mL)							Conc. (%)		MBC / MIC
	3.125	6.25	12.5	25	50	100	200	MIC	MBC	Ratio
S. aureus	+	-	-	-	-	-	-	6.25	12.5	2
S. pneumoniae	+	+	-	-	-	-	-	12.5	50	4
S. typhi	+	+	-	-	-	-	-	12.5	25	2
E. coli	+	-	-	-	-	-	-	6.25	12.5	2
S. marcescens	+	+	-	-	-	-	-	12.5	50	4
C. freundii	+	-	-	-	-	-	-	6.25	25	4
S. flexneri	+	+	+	+	+	-	-	100	>200	>2
H. influenzae	+	+	-	-	-	-	-	12.5	12.5	1
P. aeruginosa	+	-	-	-	-	-	-	6.25	12.5	2
Bacillus spp.	+	+	-	-	-	-	-	12.5	50	4
Fungi	3.125	6.25	12.5	25	50	100	200	MIC	MFC	Ratio
C. albicans	+	+	+	+	-	-	-	50	>200	4
C. dubliniensis	+	-	-	-	-	-	-	6.25	12.5	2
C. tropicalis	+	+	-	-	-	-	-	12.5	50	4
C. krusei	+	+	-	-	-	-	-	12.5	25	2
C. glabrata	+	+	+	+	+	-	-	100	>200	2
C. parapsilosis	+	-	-	-	-	-	-	6.25	12.5	2

Keys: MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration; MFC: Minimum Fungicidal Concentration- : No growth; +: Growth; AgNPs**:** Silver Nanoparticle

Table 5: Regression Coefficients between Different Concentrations of Biosynthesized Silver Nanoparticles and Diameters of Zone of Inhibition Exhibited by Isolates

Isolates	Regression (R^2)
S. aureus	0.9285
S. pneumoniae	0.8332
S. typhi	0.7193
E. coli	0.9190
S. marcescens	0.8968
C. freundii	0.7898
S. flexneri	0.8377
H. influenzae	0.7890
P. aeruginosa	0.8122
Bacillus spp.	0.6049
C. albicans	0.5750
C. dubliniensis	0.6943
C. tropicalis	0.8209
C. krusei	0.8902
C. glabrata	0.7939
C. parapsilosis	0.8432

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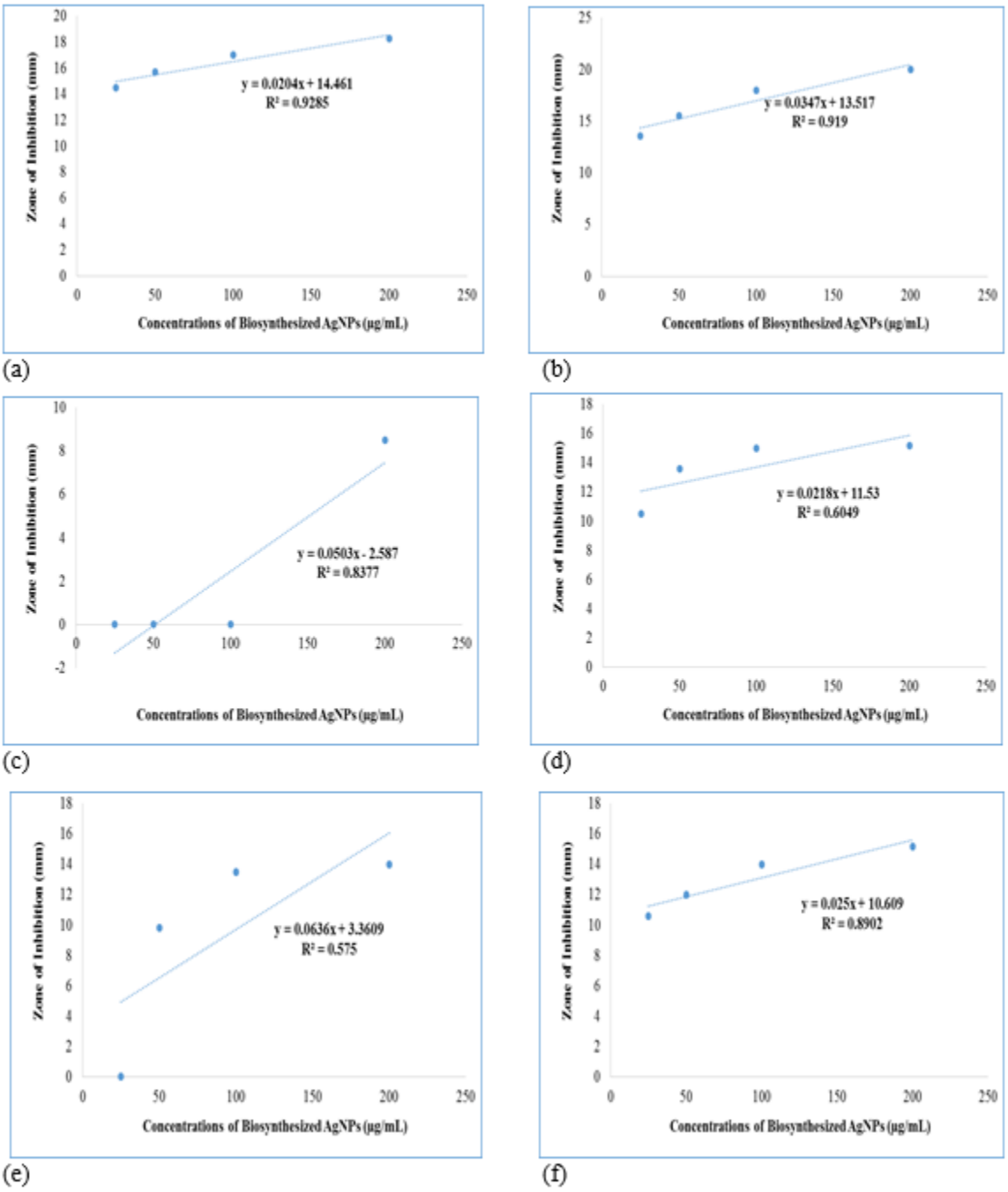

Fig 3: Regression Coefficients between Different Concentrations of Biosynthesized AgNPs and Diameters of Zone of Inhibition as Exhibited by (a) *S. aureus*, (b) *E. coli,* (c) *S. flexneri,* (d) *Bacillus* spp, (e) *C. albicans*, (f) *C. krusei*

DISCUSSION

The *L. plantarum* obtained in this study was a Gram-positive, rod-shaped, non-motile bacterium, and this was consistent with the reports of Papadimitriou *et al*. (2015). The *L. plantarum* was negative for catalase, oxidase, citrate utilization, nitrate reduction, hydrogen sulphide, and indole production tests. The negative results for citrate utilization and indole production in this study align with the reports by Tannock (2004) in their study on fermentation and metabolic profiling of *L. plantarum* strains isolated from dairy products, reinforcing the metabolic profiles of the species. Also, the results of nitrate reduction and hydrogen sulphide production tests of *L. plantarum* agree with the finding of Tannock (2004) in a study on a special fondness for lactobacilli. The enzymatic characteristics showed that *L. plantarum* obtained was positive for beta-galactosidase and arginine dehydrolase activity. These results corroborates the findings that *L. plantarum* are positive for beta-

galactosidase, supporting its ability to ferment lactose (Vasudha, and Gayathri, 2024). The presence of arginine dehydrolase activity has been reported as a distinguishing feature of *L. plantarum* from other lactic acid bacteria (Zhang *et al*., 2020).

Biosynthesized AgNPs have shown promising antimicrobial activity against a wide range of pathogens. In our study, biosynthesized AgNPs from $AgNO_3$ using the CFS of *L. plantarum* exhibited significant antimicrobial effects against *S. pneumoniae*, *H. influenzae, S. aureus*, *C. freundii*, *S. flexneri*, *P. aeruginosa, Bacillus* spp., *E. coli*, *S. marcescens*, and *S. typhi.* The antimicrobial efficacy of the biosynthesized AgNPs against *Bacillus* spp. and *E. coli* agrees with the findings of Manivasagan *et al.* (2013), who reported similar effects in their study on the biosynthesis, antimicrobial, and cytotoxic effect of silver nanoparticles using a novel *Nocardiopsis* sp. MBRC-1. The biosynthesized AgNPs had antimicrobial effect on both Gram positive bacteria and Gram negative bacteria, indicating their broad-spectrum activity. This broad-spectrum activity of biosynthesized AgNPs may be attributed to their ability to penetrate cell membranes, disrupt metabolic pathways, and generate reactive oxygen species, leading to cell structural damage, leakage of intracellular components, and cell death (Manivasagan *et al*., 2013; Gurunathan *et al.,* 2014). However, in this study, *C. glabrata* exhibited resistance to AgNPs at concentrations of ≤ 50 µg/mL, suggesting intrinsic or adaptive tolerance and species-specific differences in susceptibility. These findings are consistent with reports of Feng *et al.* (2000) and Kuhn *et al*. (2002) that some fungal species, especially *C. glabrata*, possess resistance mechanisms, such as efflux pump activity, biofilm formation, and modifications in membrane permeability that enable them to resist the antimicrobial efficacy of silver-based compounds. In this study, the antimicrobial activity of AgNPs varied across different bacterial and candida species, with the lowest mean inhibition zone (9.2 ± 0.2 mm) was observed against *H. influenzae* and the highest (20.0 ± 1.0 mm) was observed against *E. coli*. The variations in inhibition zones may be attributed to differences in bacterial cell wall structures. Gram-negative bacteria like *E. coli* have an outer membrane composed of lipopolysaccharides, which may facilitate the penetration of AgNPs, leading to increased susceptibility (Morones *et al*., 2005).

The MIC values ranged from the lowest (6.25 µg/mL) for *S. aureus*, *E. coli, C. freundii, P. aeruginosa*, *C. dubliniensis,* and *C. parapsilosis* to the highest (100 µg/mL) for *S. flexneri* and *C. glabrata*. The highest MIC value of 100 µg/mL obtained for *S. flexneri* and *C. glabrata* indicates greater resistance. The MBC values of biosynthesized AgNPs for bacterial isolates and the MFC values for *Candida* isolates ranged from 12.5 to >200 µg/mL, indicating variations in microbial susceptibility. These variations in microbial susceptibility to AgNPs may be attributed to factors such as cell wall composition, metabolic activity, and resistance mechanisms (Feng *et al*., 2000; Rai *et al*., 2012). The MBC/MIC ratio of biosynthesized AgNPs for bacterial isolates and MFC/MIC ratios of biosynthesized AgNPs for Candida isolates ranged from 1 to 4 and 2 to 4, respectively. An MBC/MIC or MFC/MIC ratio ≤ 4 suggests a bactericidal or fungicidal effect rather than microbial growth inhibition (CLSI, 2016). This suggests that biosynthesized AgNPs possess strong antimicrobial properties, making them promising antimicrobial agents to combat resistant pathogens (Morones *et al*., 2005; Gurunathan *et al*., 2014).

The regression values of biosynthesized AgNPs, as exhibited by the bacterial isolates, ranged from 0.6049 to 0.9285, while the regression values of biosynthesized AgNPs, as exhibited by the Candida isolates, ranged from 0.5750 to 0.8902. These values indicate a varying degree of correlation between concentrations of AgNPs and antimicrobial activity, with *S. aureus* and *E. coli* exhibiting a stronger dose-dependent response than other organisms tested. A higher regression value suggests a stronger relationship between increasing AgNP concentrations and larger inhibition zones, while lower values may indicate variability in microbial susceptibility (Gurunathan *et al*., 2014).

Conclusion

Biosynthesized AgNPs from CFS of *L. plantarum* exhibited broad-spectrum antimicrobial activity, with varying microbial susceptibility. The MIC, MBC, and MFC values confirmed the bactericidal and fungicidal effects of biosynthesized AgNPs, highlighting their antimicrobial potential. However, species-specific resistance, especially in *C. glabrata*, shows the need for further research to optimize their efficacy.

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