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Antifungal activity of yogurt-derived *Lactobacillus* spp. against *Cryptococcus neoformans*

SUHAIL JAWDAT FADHIL[1,♥], ROAA MUHMMED YAHYA[2], MOHAMMED MAHMOOD HAMID[2]

[1]Department of Emergency Medicine and First Aid Techniques, Mosul Medical Technical Institute. Rashidiya, Mosul 41002, Iraq next to Ibn Sina Hospital. Tel./fax.: +96-4770-2021952, ♥email: Suhail.gawdat@ntu.edu.iq

[2]Northern Technical University. Al-Manassa Street Nineveh Governorate, Mosul 44001, Iraq

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Abstract. *Fadhil SJ, Yahya RM, Hamid MM. 2025. Antifungal activity of yogurt-derived* Lactobacillus *spp. against* Cryptococcus neoformans*. Biodiversitas 26: 3694-3700.* As antifungal drug resistance continues to escalate, and Lactic Acid Bacteria (LAB) find increasing application as a probiotic in food systems and public health, this study explored the potential of LAB as a bio-protective agent. This study assessed the antifungal activity of (LAB) against fungal isolates of *Cryptococcus neoformans*, a significant fungal agent known for causing life-threatening infections, especially for immunocompromised patients. Sixteen LAB isolates were identified from ten sheep's milk yogurt samples, cultured on MRS agar, and incubated anaerobically at the optimum temperature for the isolation. Some probiotic characteristics, such as pH, bile salt, and phenol tolerance were confirmed. Ten of the isolates were bacilli others were cocci; most of them showed a high level of resistance to pH and high tolerance to 0.3% concentration of bile salt. All identified LAB isolates exhibited great resistance to 0.3% phenol. 25/100 (25%) isolates from the pigeon droppings sample were identified as *C. neoformans*; these isolates were biochemically identified after being cultured on Sabouraud Dextrose Agar (SDA) and Cryptococcus Differential Agar, exposed to standard antifungal agents, and then challenged with LAB prepared in three concentrations (10^4,10^6,10^8) on Mueller-Hinton agar overlaid with SDA. The antifungal activity of LAB against *C. neoformans* was investigated using the agar well diffusion method. The strains of *C. neoformans* exhibited 8%, and 4% resistance to fluconazole and itraconazole, respectively, whereas the isolates were all susceptible to amphotericin B and flucytosine. Among twenty *C. neoformans* strains challenged with LAB isolated from yogurt, 80% of them were susceptible by obvious inhibition at (10^8), whereas only five *C. neoformans* isolates were resistant to LAB. The observed antifungal activity of LAB against *C. neoformans* presents a promising opportunity for the development of bio-protective and therapeutic measures against *C. neoformans* and other fungi with related features.

Keywords: Agar well diffusion method, antifungal, *Cryptococcus neoformans*, *Lactobacillus* species, yogurt

INTRODUCTION

The encapsulated opportunistic yeast-like fungus *Cryptococcus neoformans* is a common cause of meningoencephalitis in immunocompromised patients, and it can also sporadically infect healthy people (Latifi et al. 2024). *C. neoformans* is primarily found in soil that pigeon droppings have polluted, and it enters the body through the respiratory system (Panebianco and Caridi 2021). Furthermore, the treatment of cryptococcosis become difficult when resistance emerges in clinically isolated strains (Shi and Maktabdar 2022). *Lactobacillus* species comprises a genus of Lactic Acid Bacteria (LAB) found in a variety of environments, as the gastrointestinal tract (Abdulhussien and Isa 2024), dairy products, and plants. These LABs are non-spore forming, rod-shaped, Gram-positive bacteria (Vanitha et al. 2023). Given their fermentative activity and nutritional benefits, *Lactobacillus* spp. represents an important genus within LAB. The *Lactobacillus* species commonly used as probiotics include: *L. reuteri, L. acidophilus, L. fermentum, L. brevis, L. pentosus, L. plantarum, L. rhamnosus, L. paracasei, L. gasseri*, *L. casei,* and *L. mucosae* (Karami et al. 2017; Shori et al. 2018). The *Lactobacillus* species demonstrates favorable qualities as probiotics (Abdulhussien and Isa 2024).

Numerous studies have examined the antifungal properties of *Lactobacillus* strains (Arasu et al. 2015; Xie et al. 2022). Lactic Acid Bacteria (LABs) promote a healthy microbiome by competing with pathogens, producing antimicrobial substances like organic acids and hydrogen peroxide, and stimulating the immune system. They also inhibit key fungal virulence factors such as biofilm formation (Ferreira et al. 2024). Due to their ability to produce antimicrobial agents, probiotics are effective at controlling and treating infections. This property, combined with advances in biotechnology, has led to the widespread use of *Lactobacillus* strains in industries like medicine, food, and biofuel production (El-Ashmony et al. 2023). Lactic Acid Bacteria (LABs) function as probiotics and biopreservatives by boosting immune responses and acting as antioxidant, anti-inflammatory, and anti-obesity agents. Characterized by their low virulence (e.g., inability to produce hemolysin), LABs include thirteen genera, such as *Lactobacillus* and *Lactococcus*, which possess antibacterial properties that help protect food (Levorato-Vinche 2022; Abdulhussien and Isa 2024). Certain beneficial bacteria effectively fight pathogenic fungi by releasing antimicrobial molecules, such as hydrogen peroxide and organic acids, into their environment (Kaddouri et al. 2025). Probiotics provide numerous health benefits, including supporting a

healthy gut microbiome, enhancing the immune system, and aiding in the digestion of lactose, along with preventing allergies and lowering cholesterol (Tropcheva et al. 2014; Baie et al. 2020). The probiotic microorganisms found in probiotic food products are beneficial for the host's health and gut microbiota. The most widely consumed probiotic-fermented dairy product globally is yogurt. A bacterial starter culture consisting of lactic acid bacteria is used to ferment milk to produce yogurt (Aboh et al. 2021).

The yogurt starting culture's microbes generate bioactive substances that have been shown to have antifungal and antibacterial properties (Shori et al. 2018). Yoghurt has been shown to have antimicrobial properties against a number of pathogenic bacteria and fungi, including *Aspergillus flavus*, *Fusarium graminearum*, and *Candida albicans*, as well as *Salmonella*, *Shigella*, *Escherichia coli*, *Listeria monocytogenes, Enterobacter aerogenes, Proteus vulgaris, Streptococcus pyogenes,* and *Micrococcus luteus* (Afzali et al. 2020). The intriguing diversity of *Lactobacillus* species in food products is a key area of this study. The use of it as a biocontrol agent offers ecological benefits by reducing reliance on synthetic fungicides. Consequently, environmental contamination is reduced, and negative impacts on non-target creatures are lessened (Vasundaradevi et al. 2025). The emergence of drug resistance in *Cryptococcus* has led to the failure of traditional treatments for cryptococcosis (Ishtiaq and Ahmed 2013; Kamel et al. 2021). This study therefore explores a natural alternative: the antifungal properties of *Lactobacillus* species isolated from sheep milk yogurt against *C. neoformans* from pigeon droppings.

MATERIALS AND METHODS

Ethical approval

The study was reviewed and approved by Mosul Technical Medical Institute, Northern Technical University, Mosul, Iraq (MMTRC-2025-014).

Sample collection

Bacterial isolates

The study was conducted from January to June, 2024. Ten Fresh samples of locally produced sheep milk yogurt were purchased from commercial vendors from many areas in Mosul City, Iraq, and transported immediately in an ice box to the laboratories of Northern Technical University Research Center, Mosul, Iraq, within 3 hours.

Fungal isolates

A total of one hundred samples of pigeon droppings were collected in sterilized saline and brought to the laboratory in sterile conditions.

Identification of LAB

Isolation of lactic acid bacteria

Bacterial isolates were performed by preparing Fourfold serial dilutions by dispensing 1 mL of the yoghurt samples in 9 mL of sterile distilled water. From each prepared dilutions, 0.2 mL of the diluted sample was introduced to a sterile Petri-dishes, and already prepared MRS was aseptically poured and incubated at optimum temperature anaerobically by the Oxoid anaerobic gas-pack system for 48 hours at 37°C to produce individual, distinct colonies. The isolated colonies were further purified by streaking on MRS agar and maintained on nutrient agar slant at 4°C (Taye et al. 2021). The isolates that exhibited LAB traits were selected, and their antifungal activity was checked. Molecular analyses were used to define better of LAB strains that demonstrated suppression of fungal growth.

Phenotypic characterization

According to Bergey's manual of systematic bacteriology, the isolated bacterial colonies were identified by examining their motility test, biochemical responses (catalase and oxidase), and macroscopic and microscopic appearance, They were tested for sugar fermentation, catalase, and Gram staining. Gram-positive, bacilli, cocci, and catalase-negative bacteria were selected for additional biochemical analysis (Wu et al. 2020; Rahman et al. 2024).

Probiotic characteristics of the isolated LAB

pH tolerance test-1. The pH of the M17 and MRS broths was adjusted to 2.5 using 3 N HCl and 1 N NaOH. The corresponding MRS and M17 broths were then mixed with fresh bacterial cultures in test tubes, and the mixture was incubated for 48 hours at 37°C. A spectrophotometer was used to measure the Optical Density (OD) 620 nm after 24 and 48 hours. The results were documented after optical density examination of the cultured media (Bin Masalam et al. 2018; Xie et al. 2022).

Bile salt tolerance test-2. A sterilized M17 and MRS broth medium containing 0.3% bile salt was inoculated with 0.1 mL of overnight-grown colonies. A spectrophotometer was used to detect the OD 620 nm after 24 and 48 hours of incubation at 37°C. To ascertain the results, the optical density of the culture media was analyzed (Bin Masalam et al. 2018).

Phenol tolerance test-3. Phenol tolerance was assessed using MRS and M17 broth that had phenol concentrations of 0.1%, 0.2%, and 0.3%. A spectrophotometer was used to measure the OD 620 nm after 24 and 48 hours of incubation at 37°C. The optical density of the culture media was examined in order to attain the results, according to Rahman et al. (2024).

Molecular identification of LAB

Total genomic DNA of all isolates was extracted from overnight cultures on MRS agar plates. The genomic DNA of LAB was extracted according to the protocol of FavorPrep Genomic DNA extraction Kit. The isolates were identified based on the 16SrRNA gene using the primer pair: Sequence (5'-3'): (F) `GAAGTATCCAGAGCAAGCGGA`, (R) `CTCGCAATTTCGCTTACGGG`, the designed primer depending on NCBI- GenBank database was supported from (Macrogen, Korea) company with number of accessions to the Genbank (AJ438156) The Maxime PCR Premix Kiti-Taq methodology was used to prepare the PCR reaction. The master mix was made in accordance with the product size (550 bp) and the company's requirements (Al-Abedi et al. 2024).

Identification of fungal strains

Isolation of Cryptococcus neoformans

Samples of pigeon droppings were collected in sterilized saline and transported to the laboratory under sterile conditions during the period. All samples were incubated at 37°C for 24 hours then inoculated into SDA plates with chloramphenicol for 2-5 days at 37°C. The samples were cultured on Sabouraud's Dextrose Agar (SDA) then culture on Cryptococcus Differential Agar for further diagnosis (Hassanpour et al. 2024).

Conventional identification

Several traditional mycological identification methods were applied to the isolates, including morphological analysis based on the morphology of the colonies, using bright field microscopic examination with Indian ink (Su et al. 2024). *C. neoformans* species was confirmed with the Vitek 2 system (BioMerieux, France), a completely automated device for the identification (Al-Abedi et al. 2024).

Antifungal activity of Cryptococcus neoformans *to conventional antifungal agents (Disc diffusion Assay)*

The disk diffusion method was used to test for some antifungal agents. Mueller-Hinton agar containing 2% glucose was inoculated with the fungal cell suspension after it had been adjusted to a turbidity of 0.5 McFarland. On the plates, drug disks containing 50 µg of Itraconazole (ITR), 10 µg of flucytosine (Mast Diagnostics, UK), 20 µg of Amphotericin B (AMB), and 25 µg of Fluconazole (FCN) were displayed. To evaluate the growth inhibition zone, the plates were incubated for 48 hours at 35°C (Ma et al. 2020; Su et al. 2024).

Evaluation of antifungal activity of isolated *Lactobacillus* spp.

The agar well diffusion method was used to predominantly evaluate five *Lactobacillus* strains for antifungal activity against *C. neoformans*.

Agar well diffusion assay

Five strains of *Lactobacillus* with the highest tolerance to bile salts, low pH and show tolerance to phenol were selected, in which antifungal activity was decided against *C. neoformans* using the overlay technique as defined by Bulgasem et al. (2016). MRS agar plates were injected with *Lactobacillus* isolates in different concentration (10^4, 10^6, 10^8). The plates were incubated under anaerobic conditions for 24 hours at 30°C. Next, 15 mL of SDA (0.75% soft SD agar) containing 10^4 CFU/mL of overnight *C. neoformans* culture which was prepared according to Sahu et al. (2023) were applied to these plates. The zone preventing *C. neoformans* growth above the corresponding *Lactobacillus* culture was assessed after the plates were maintained aerobically heated at 30°C for 24 hours. Anti-*C. neoformans* activity was indicated by inhibition zones above the *Lactobacillus* spot. *Lactobacillus* inhibition tests against *C. neoformans* was carried out in duplication (Eddin et al. 2018). The results of the inhibition zone were compared with the inhibition results of Amphotericin B.

Statistical analysis

Results from the current study were analyzed using SPSS program version 27 and presented as the percentages and mean±SEM. Chi-square test was used for evaluation of qualitative data. Quantitative data were analyzed using a One-Way Analysis of Variance (ANOVA) followed by LSD.

RESULTS AND DISCUSSION

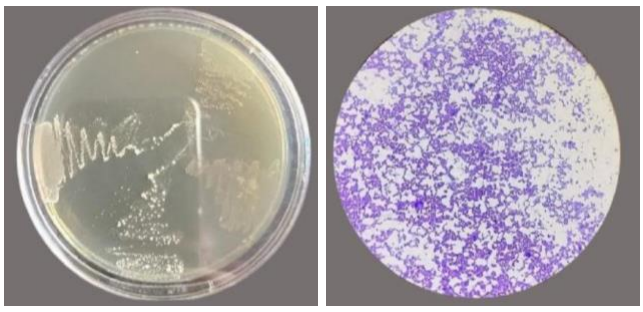
Phenotypic identification of LABs

Hence, sixteen LAB isolates were identified from ten sheep's milk yogurt samples. Macroscopically, the isolated LABs appeared as smooth colonies of various sizes with a white or creamy hue, as presented in Figure 1. Microscopically, the isolates were Gram-positive, facultative anaerobes, non-spore forming, coccus or bacillus shapes. They grew as single, paired, or chained specimens of different lengths and thicknesses.

Table 1 illustrates the probiotic and physiological traits of the bacteria that were separated from the local yogurt. Ten of the sixteen isolates were bacilli, and the other six were cocci. All isolates were catalase-negative and Gram-positive. Isolates 1, 4, 6, 8, 11, 12, 14, 15, and 16 in this investigation demonstrated a high level of resistance to pH 2.5, as did isolates 2, 3, 5, 7, 9, and 13 (Salari and Almani 2020).

Bile tolerance is one of the most crucial characteristics of probiotic lactic acid bacteria, This feature helps it survive and activate its therapeutic ability in the gastrointestinal tract, the evaluation of this prosperity of isolated LAB in this study showed high tolerance of the most isolates (2, 3, 5, 7, 11, 12, 14, 15 and 16) to 0.3% concentration of Bile Salt , also isolates (1, 4, 6, 8, 9, 10 and 13) were tolerant to the same concentration. With the exception of isolates 1, 4, 6, 8, 9, and 13, all of the isolates exhibited great resistance to 0.3% phenol. The probiotic qualities of each isolate were validated by these findings in which were conducted by (OD 620) nm after 24 and 48 hours of incubation using spectrometer.

Table 1 presents qualitative, categorical data (+, ++, ++++), not quantitative measurements (e.g., MICs). Therefore, a numerical cutoff for tolerance cannot be calculated from our data.



A B

Figure 1. A. Resembles the smooth, convex, and translucent colonies of LAB on MRS agar, B. Show positive Gram-stained rod-shaped *Lactobacillus* spp. under 1000× (Oil emersion)

Table 1. Physiological and probiotic characteristics of the isolated LABs

Isolates	Probiotic properties			
	Shape	pH (2.5%)	Bile salt (0.3%)	Phenol (0.3%)
1	Bacilli	+++	+	+
2	Bacilli	++++	+	+
3	Bacilli	+	++	++
4	Bacilli	++	++	+
5	Bacilli	+	++	+
6	Bacilli	++	+	+
7	Bacilli	+	++	++
8	Bacilli	++	+	+
9	Bacilli	++	++	+
10	Bacilli	++	++	++
11	Cocci	+++	+	++
12	Cocci	++	+	++
13	Cocci	+	+	+
14	Cocci	++	++	++
15	Cocci	++	++	++
16	Cocci	+++	++	++

Note: $+^2$: Indicate tolerance, $+^3$: Indicate high tolerance against the condition

Molecular identification of LABs

Using PCR (Polymerase Chain Reaction), isolates detected under a microscope were verified to the genus level, indicating that 16 isolates are members of the *Lactobacillus* genus. The PCR results revealed an amplicon of around 250 bp, as presented in Figure 2.

Identification of *Cryptococcus neoformans*

Based on identification tests, 25/100 (25%) isolates from a pigeon droppings sample were identified as *C. neoformans*.

Macroscopic identification

One hundred pigeon droppings samples plated onto Sabouraud′s Dextrose Agar, only twenty-five isolates presented *Cryptococcus* features: creamy to tan color, smooth, mucoid characteristic, and raised surface, while appeared as Light blue colored colonies on the *Cryptococcus* differential agar referring to *C. neoformans* species (Figure 3.B) and then were confirmed with Vitek 2 system (BioMerieux, France).

Microscopic characterization

Staining with Indian ink revealed oval or round cells, with approximately equal budding, the cells were unequal in size, and surrounded by a large unstained zone of clearance or "halo" as in Figure 3.A.

Antifungal susceptibility test

Using the disk diffusion test, 72% of the *C. neoformans* isolates were susceptible to fluconazole. Regarding other antibiotics, the susceptibility rates were 92% for Amphotericin B, 20% for flucytosine, and 32% for itraconszole (Table 2). The resistant patterns exhibited by the isolates of *C. neoformans* to the conventional antifungal agents are shown in Table 2.

Screening of antifungal activity

As shown in Table 3, the inhibitory effect of *Lactobacillus* on *C. neoformans* was concentration-dependent, with significantly ($P<0.05$) increasing inhibition zone diameters as *Lactobacillus* concentration increased. Specifically, 10^4 CFU of *Lactobacillus* yielded an inhibition zone of 12.84±0.92mm, while 10^6 CFU resulted of 15.02±1.12 mm, and 10^8 CFU produced the largest zone at 22.56±1.08 mm. All *Lactobacillus* concentrations demonstrated a highly significant inhibitory effect compared to the control (MRS broth), which showed no inhibition effect (0±0 mm), with all observed differences between groups being statistically ($P<0.05$) significant (Figure 4).

Discussion

Identification of LABs

The LAB isolates were Gram-positive and catalase-negative. The morphology and biochemical properties of the isolates studied corroborated previous findings on the morphological and biochemical properties of microorganisms (Salari and Almani 2020; Lionel et al. 2024).

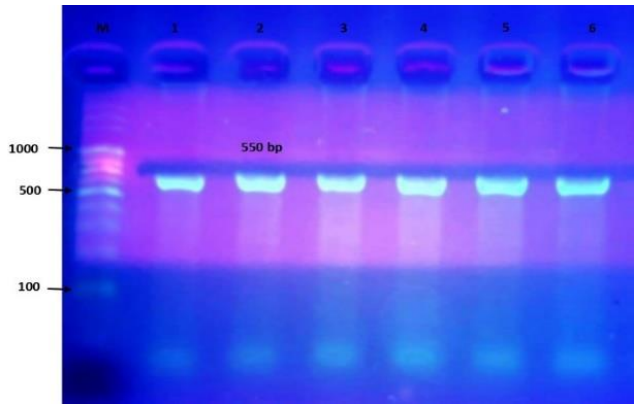
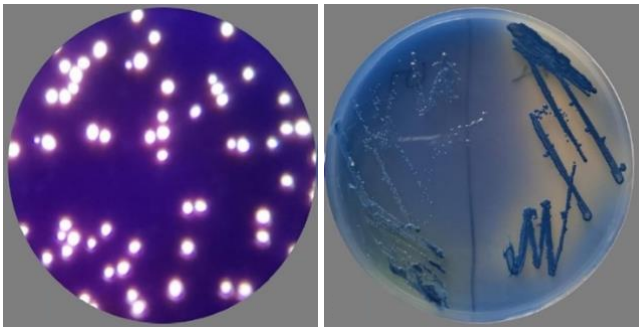


Figure 2. PCR product analysis for 16S rRNA gene in *Lactobacillus* spp. isolates on a 1.5% agarose gel electrophoresis. M (100-1500 bp). (1-6 represent +ve *Lactobacillus* isolates)



A **B**

Figure 3. A. Indian ink-stained *C. neoformans* reveals cells surrounded by a distinctive large polysaccharide capsule, a bright field light microscope slide, B. Featured colonies of *C. neoformans* on Cryptococcus Differential Agar after 5 days of incubation at 37°C

Table 2. The antifungal susceptibility results of *C. neoformans*

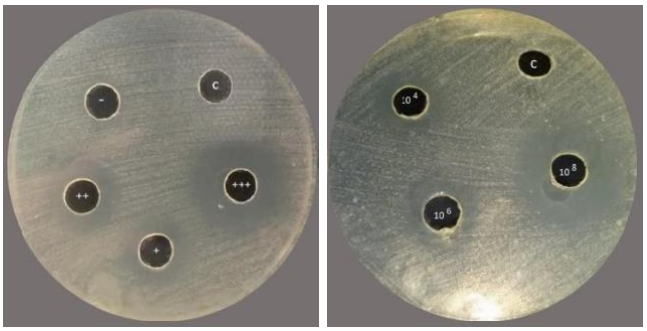
Fungi	Antifungal	Susceptible (N%)	Intermediate (N%)	Resistant (N%)
Cryptococcus neoformans	Fluconazole	18 (72%)	5 (20%)	2 (8%)
	Amphotericin B	23 (92%)	2 (8%)	0 (0)
	Flucytosine	2 (20%)	23 (92%)	0 (0)
	Itraconazole	8 (32%)	16 (64%)	1(4%)

Note: Calculated $X^2 = 45.68$; calculated P value = <0.0001 (Highly significant difference)

Table 3. Inhibition zone diameters of *C. neoformans* isolates by concentrations of *Lactobacillus*

Lactobacillus concentration	Inhibition zone (mm)
104 CFU	12.84±0.92a
106 CFU	15.02±1.12b
108 CFU	22.56±1.08c
Control (MRS broth)	0±0d

Note: Values represent mean±SEM; LSD value = 1.782, different letters between any two means denote to the significant difference at P<0.05



A B

Figure 4. Antifungal activities of *Lactobacillus* spp. against *C. neoformans* isolates (zones of growth inhibition from selected plates). A. C: Refer to the control (MRS Broth), B. (10^4, 10^6, 10^8): Refer to the concentration LABs

Significant evidence of the antifungal effectiveness of LABs has been achieved. The future vision of utilizing the antifungal properties of LABs as agricultural starters to improve the food industry has become clearer, especially after the possibility of using molecular biology in diagnosis and genetic understanding of the resistance mechanism for the different species of LABs bacteria (Izzo et al. 2020; Ghosh et al. 2021).

After applying the microbiological diagnosis and confirming the results using PCR, some properties of bacteria have been investigated. The most important factor in the probiotics selection is pH, as the strains must withstand low pH. In this study, 10 *Lactobacillus* isolates exhibited high tolerance to a pH of 2.5, whereas the remaining isolates exhibited moderate tolerance to pH.

According to the findings of this investigation and those of Bentahar et al. (2024), 7 of 13 *L. plantarum* isolates exhibited strong resistance to low pH (Bentahar et al. 2024). The results were also similar to those of Rahman et al. (2024), who confirmed the tolerance of *Lactobacillus* strains isolated from yogurt to low pH (Rahman et al. 2024). The bile tolerance of probiotic LABs enables them to survive, grow, and exert their therapeutic effects in the gastrointestinal system. Isolates 1, 2, 5, and 6 displayed high tolerance to 0.3% bile salt. Additionally, isolates 3, 4, 6, and 7 also tolerated this concentration. These results are consistent with those of Maragkoudakis et al. (2006), who found that 29 *Lactobacillus* strains had high tolerance to 0.3% bile salt. Conversely, only isolates 1, 3, 6, and 8 demonstrated high tolerance against 0.3% phenol, but all isolates could tolerate 0.1% and 0.2% phenol. These findings demonstrated the probiotic qualities of each isolate (Rahman et al. 2024). Isolates that had rod form, Gram (+) and catalase (-) were chosen for the realization of this study.

Identification of Cryptococcus neoformans

Different species of the *Cryptococcus* genus causes cryptococcosis which is a systemic infectious disease. *C. neoformans*, is primarily found in the secretions of birds such as pigeons or dust that has been exposed to bird faces (Aboh et al. 2021). Such excreta or contaminated dust could serve as reservoirs and facilities the spread of infections (Al-Abedi et al. 2024).

The isolation rate in this study was 25/100 (25%) from pigeon droppings, which is comparable to the work in Iraq by Hawrra et al. (in Al-Abedi et al. 2024), who isolated 15/50 (30%) from pet birds droppings samples and were companionable with study in Italy by Abbas et al. (2017). Birds can play an important role in the transmission and spread of *C. neoformans* and other potentially zoonotic yeasts that pose a threat to human and animal health. The isolation rate in this current study was higher than that reported in Baghdad (Vanitha et al. 2023), who described the proportion of *C. neoformans* (13%) isolated from bird droppings. Additionally, the results were similar to the research conducted in Malaysia, which reported 20 isolates of *C. neoformans* from feces of zoo birds (Mirpourian et al. 2021). Human infections are caused by a combination of direct contact and inhalation of fungal spores from communicable organisms that grow in the nutrient-rich deposits of bird droppings, as well as the dissemination of fungal spores by wind extracts (Soltani et al. 2013; Vernel-Pauillac et al. 2024). But higher from results from chicken droppings samples which show positive *C. neoformans* isolates 10/75 (13.3%) in Egypt (Zarrin et al. 2010). The researchers looked into the potential for *Cryptococcus* to be present in domestic chicken droppings and dust that which has been increased by droppings as a potential source of contamination to humans, mainly immunosuppressed persons (Elkady et al. 2016; Kwizera et al. 2024).

Antifungal activity assay

Some diseases cannot be adequately treated using standard antifungal medications. Consequently, several organic and natural substances have been investigated for their antifungal effects against harmful fungi (Leyva Salas et al. 2018). The isolates of *C. neoformans* from pigeon droppings exposed to conventional antifungal agents

including (Fluconazole, Amphotericin B, Flucytosine and Itraconazole), were susceptible to used antifungal with the exception of fluconazole with a resistance rate (8%) and against Itraconazole (4%) while were Intermediate to Flucytosine (92%) and Itraconazole (64%). Tested *C. neoformans* were challenged with the isolates of lactobacilli from yoghurt by agar diffusion technique (Spot overlay method). Twenty of the twenty-five isolates of *C. neoformans* challenged with sixteen lactobacilli were susceptible in high proportion, while only five *C. neoformans* strains were resistant to lactobacilli isolates. *Lactobacillus* species produce different antifungal compounds; these consist of volatile organic molecules, peptides, organic acids (such as lactic, acetic, and propionic acids), and 3-hydroxy fatty acids. By reducing pH, competing for resources, and directly interacting with fungal cells, these substances prevent fungal growth. It is also known that certain strains of *Lactobacillus* produce antibacterial peptides called bacteriocins, showing important effectiveness as a bio-preservative agent in many fields (Abouloifa et al. 2021). This may explain the sensitivity of fungi to probiotic bacteria and has also been reinforced by recent studies in this field, Like the study that showed antifungal activity of LABs at a rate of about 80% against some species of pathogenic fungi by inhibiting mycelial growth, destroying the hyphae membrane, leading to the leakage of nucleic acids and proteins, and collapsing of hyphae and shriveled which lead to changing of the spore's morphology, resulting in cell membrane damage, intracellular leakage, and organelles aggregation (Szczerbiec et al. 2022; Liu et al. 2023). Variation in the genetic and other characteristics of *C. neoformans* strains genetics could affect the anticryptococcal activity of LABs. In the present study, most *C. neoformans* isolates were susceptible to LABs isolated from yogurt samples. These findings corroborate the research of Liu et al. (2022). A separate study described the antifungal mechanisms and application of LABs as antifungal tools in bakery products (Mokoena et al. 2017). Recently, the antifungal activity several biological agents against bacterial species of *Lactobacillus* has been examined. According to Lionel et al. (2024), it has been discovered that lactobacilli are quite efficient against isolated pathogenic *Candida* from vaginitis. Many of the results obtained by studies in this field have confirmed LABs as a good alternative to chemical treatments that have side effects, in addition to the development of resistance with increased use. There was good evidence that organic acids produced by LABs played an important role in fungal inhibition, and to benefit from the medicinal properties as antifungal agents it was found that using the probiotic lactobacilli approximately doubled the shelf life and improved both the flavor and texture of grains and foodstuffs (Zhao et al. 2022).

In conclusion, despite the limited exploration of the antifungal activity of LABs against *C. neoformans*, several studies have hinted at the potential of certain probiotic bacteria species as alternative bioactive antifungal agents. The discovery that LABs could inhibit fungal growth not only offers significant health benefits but also instills confidence in a feasible prophylactic management option for serious infections caused by *C. neoformans*, especially in immunocompromised patients and animals. The need for further molecular and in vivo studies is a reassurance of our commitment to improving the field for using LABs in alternative industries for treatment and prevention. Further elucidation of the antifungal potential of LABs is not just recommended, but a potential game-changer in the fight against serious infections.

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