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# Antimicrobial activity and Probiotic Properties of Lactic Acid Bacteria Isolated From Traditional Fermented Dairy Products

**Ali F. S., Zayed G., Saad O.A.O., Salwa A. H. Gharib***

Department of Agricultural Microbiology, Faculty of Agriculture, Minia University, 61519 Minia, Egypt
* Correspondence: salwa.adelgharib@yahoo.com ; Tel: +201022875437; Fax: +20 862362182

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**Abstract**

One of the biggest problems for humans and animals worldwide is the harmful effects of the antibiotics, due to excessive use as a treatment for animal diseases. An alternative to overcome this problem is the use of certain growth promoters such as probiotics that have a good effect on host health and performance. Eight isolates included the following probiotic strains: *Lactobacillus plantarum, L. acidophilus, L.rhamnosus, L. salivarius, and L. paracasei, as well as Bifidobacterium longum, B. adolescentis, and B. breve* were investigated for low pH and bile salt tolerance, anti-bacterial and yeast activity using supernatant cell-free culture were assessed using agar-well diffusion method against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, , Escherichia coli MC1400 Listeria ivanovii and Candida albicans.* Co-culture has determined the antifungal activities with Aspergillus niger, As. flavous, As. fumigatus and Penicillium chrysogenum. The antibiotic sensitivity was tested using the agar disc diffusion method. Each of the strains examined had variable antibacterial activity. All the isolates showed a variable inhibition level, as well. All of the isolates were Ciprofloxacin resistant. Additionally, the lactobacilli strains were Vancomycin-resistant, and all of the strains show intermediate Clindamycin resistance. All isolates were Penicillin, Ampicillin, Tetracycline, Erythromycin, Gentamycin, Streptomycin, Florfenicol, Chloramphenicol, and Sulfamethoxazole & Trimethoprim susceptible. Collectively, the probiotic capacity of the strains tested and the antimicrobial activity without the transfer of antibiotic resistance suggested that these strains can be used as bio-preservatives in food products and medicinal preparations.

## 1. Introduction

Consumers no longer only consider food in terms of flavour and nutritional requirements but also in terms of their capability to deliver specific health benefits. [1]. The idea of probiotics emerged in the early 20th century from a theory first introduced by Eli Metchnikoff [2]. He proposed that the long and healthy life of Bulgarian farmers was because of the intake of fermented milk products [2]. Probiotics are described as "live micro-organisms that confer a health benefit on the host when administered in sufficient amounts" [3]. Effective doses of probiotics can improve the bowel function by enhancing the development of the healthy microbiota, the ability to increase the host's natural defences against entero-pathogens by delivering antimicrobials or preventing harmful pathogens from colonising the intestinal mucosa, improving digestive capacity, decreasing the pH, and stimulating mucosal immunity [4]. In addition, the use of beneficial microorganisms for food preservation has become increasingly important due to consumer needs for reduced use of chemical preservatives. Additionally, antibiotics in prophylactic dosages have been used in animals for several decades. However, there is growing concern about the risk that humans and livestock will expand cross-resistance and multiple antibiotic resistance in pathogenic bacteria [5], as well as the harmful effect of encountered antibiotic residues. An alternative to reducing such issues is the use of certain growth promoters such as probiotics that has a positive impact on host health and growth performance [6]. Lactic acid bacteria (LAB) produce variant antimicrobial compounds that are considered necessary for the food and feed bio-preservation. The antimicrobial activity of LAB is connected with the production of multiple products during lactic fermentation, such as organic acids, hydrogen peroxide (H2O2), and bacteriocins [7]. bacteriocin-producing strains are isolated from a wide variety of sources, including human, animal and animal products and fruits and vegetables. These strains have been known to produce potent antibacterial bacteriocins against a vast variety of pathogens [5]. Egyptian traditional fermented foods, especially in upper Egypt are expected to be a rich source of probiotic bacteria namely Mish (pickled ripened Karish cheese), Zabady (yoghurt), Karish cheese (skimmed milk cheese, Laban Rayeb (concentrated sour milk) and Kishk (wheat-based fermented milk) [8]. Eight probiotic isolates were previously isolated from traditional fermented products and molecularly identified [9] as *Lactobacillus plantarum, L. acidophilus, L. rhamnosus L salivarius, and L. paracasei, Bifidobacterium*

*longum, B. adolescentis, and B. breve.* The current work aimed to evaluate some probiotic properties, antimicrobial activity, and antibiotic susceptibility of these probiotic bacterial Isolates.

## 2. Material and Methods

### 2.1. Bacterial Isolates

The following probiotic strains: five Lactobacillus spp. and 3 Bifidobacterium spp. were obtained from the Department of Microbiology at the Faculty of Agriculture, Minia University in Minia, Egypt. Species confirmation was previously performed by 16S rDNA sequencing **(Table 1)**. [9]

### 2.2. Experimental Design

#### *2.2.1. Tolerance to Low pH.*

Acid tolerance was done according to [10] with some modifications by incubating in MRS broth, and the pH was modified to 2.5 with HCl and cultures were then incubated at 37 °C for two hr. Each of the eight strains of LAB and Bifidobacteria were sub-cultured at least three times before experimental use, inoculation (1 % vol/vol) in the broth, and growth was monitored using the plate count method. Serial dilutions were performed. A 1 ml was taken every 30 min for two h, and ten-fold serial dilutions were done using peptone water. Samples were plated onto MRS agar, and the cultures were incubated at 37 °C for 48h in an anaerobic chamber. Acid tolerance was detected by comparing the final plate count after two hr with the initial plate count at 0 h. counting was indicated in log colony-forming units per mL (log cfu/mL).

#### *2.2.2. Tolerance to Bile Salts*

Bile tolerance was conducted according [11], [12], where the eight isolates were grown overnight at 37 °C in MRS broth. Each culture was inoculated (1 % v/v) into MRS broth supplemented with 0.3 % (w/v) bile salt (Oxgall, USA). Then Samples were incubated at 37 °C for 2 hr, 4 hr, 6 hr and 8 hrs., and tubes without inoculation were left as a control. Spectrophotometer (O.D. at 660 nm) was used to detect the growth of the bacteria.

### 2.3. Antibacterial activity

The antibacterial effect was estimated by the agar well diffusion method as previously described [13] using cell-free culture supernatants (CFCS) of the isolated probiotic strains against pathogenic indicator bacteria *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans, Escherichia coli MC1400 and Listeria. ivanovii* Briefly, an initial inoculum of approximately 10^6 cfu /ml of the target pathogenic indicator bacteria was incorporated into 25ml nutrient soft agar, 25 ml LB soft agar and 25 ml of TSB soft agar 1 % inoculated with the indicator bacteria were plated in Petri dishes. Wells of 5mm diameter were prepared, and loaded with a volume of 80 µl of CFCS of the isolated probiotics and marked adequately with the isolates' names. The plates were kept for two hrs. at room temperature, then incubated for 24 hrs. at 37 °C. The zone diameter of inhibition (ZDI) values was measured. The tests were performed in triplicate and the data were represented with mean ± SD.

### 2.4. Antifungal activity

The eight strains were pre-activated and seeded until covering one-third of the surface of MRS agar plates and incubated in optimal conditions at 37 °C for 48 hr. PDA agar plug from freshly activated cultures with *Aspergillus flavus, As niger, As fumigatus and Penicillium chrysogenum* were placed on the center of the free surface of these MRS agar plates and incubated aerobically at 25 °C for 5 days in the dark. The zones of inhibition of the fungi were estimated using a semi-quantitative scale: (++) minimal inhibition, (+++) partial inhibition and (++++) total inhibition. Plates containing only the fungal plug inoculums (without probiotic strains) were used as a control. The tests were performed in triplicate. [14]

### 2.5. Antibiotic sensitivity

The pattern of resistance/sensitivity to the antibiotic of the isolated strains were tested using the disc diffusion method, as described previously. Antibiotic discs (Sigma) were employed to determine the pattern of the antibiotic resistance of the isolates. Twelve different antibiotic discs included the following mentioned in **(Table 2)**. The procedure included activation to each LAB and Bifidobacteria strains for 24 hr. A total of 100 µL of the diluted cultures (adjusting the optical density for each strain to 0.1 O.D.) was diffused in a Mueller-Hinton agar mixed very well with 5 % fresh horse blood and allowed to dry for 5-15 min. The different antibiotic discs were applied on the surface. The plates were incubated at 37 °C in anaerobic conditions and assessed after 24 h of inoculation. The inhibition zones were measured using a manual calliper. The results were expressed in terms of resistant, intermediate, sensitive and were compared with the interpretative zone diameters given by (CLSI M100) 2016: Clinical and Laboratory Standards Institute antimicrobial susceptibility testing standards.

### 2.6. Statistical analysis

All the measurements were carried out three times, and the results were expressed as the mean ± standard deviation (SD). Data were also statistically analyzed by adopting F- test, and Duncan's multiple range test (ANOVA) [16]

## 3. Results

**(Figure 1)** shows the results of the eight isolates rising and surviving at low pH (2.5) within two hours. At pH 2, 5 the rate of bacterial survival ranged from 97.6 % to 103 % for

*Lactobacillus* strains with the highest rate of growth was obtained by *L. rhamanosus* at (1.25 x 10^8 CFU/ml).

**Table 1:** Probiotic bacterial strains used in the present study and their accession numbers.

| Isolate | Identified as | Accession No. |
|---|---|---|
| **LAB 1** | *Lactobacillus plantarum* | MH544641.1 |
| **LAB 2** | *Lactobacillus acidophilus* | MF380369.1 |
| **LAB 3** | *Lactobacillus paracasei* | MH549144.1 |
| **LAB 4** | *Lactobacillus salivarius* | MG751346.1 |
| **LAB 5** | *Lactobacillus rhamnosus* | AB889723.1 |
| **Bifido.1** | *Bifidobacterium longum* | AP014658.1 |
| **Bifido.2** | *Bifidobacterium adolescentis* | MF380366.1 |
| **Bifido.3** | *Bifidobacterium breve* | M84776.1 |

**Table 2:** List of antibiotics used in the study.

| S. no | Name of drug & Concentration (µg) | Antibiotic group | Mode of action |
|---|---|---|---|
| **1** | Ampicillin 10 | β-Lactams | Inhibitors of the cell wall synthesis |
| **2** | Penicillin 10 | | |
| **3** | Vancomycin 30 | Glycopeptides | |
| **4** | Ciprofloxacin 5 | Quinolones | Inhibiting DNA replication and transcription |
| **5** | Gentamycin 10 | Aminoglycosides | Inhibitors of protein synthesis |
| **6** | Streptomycin 10 | | |
| **7** | Tetracycline 30 | Tetracyclines | |
| **8** | Erythromycin 15 | Macrolides | |
| **9** | Clindamycin 2 | Clinolamide | |
| **10** | Chloramphenicol 30 | Amphenicols | |
| **11** | Florfenicol 30 | | |
| **12** | Sulfamethoxazole &Trimethoprim 23.75 & 1.25 | Other | inhibit bacterial synthesis of dihydro-folic acid necessary for cell division |

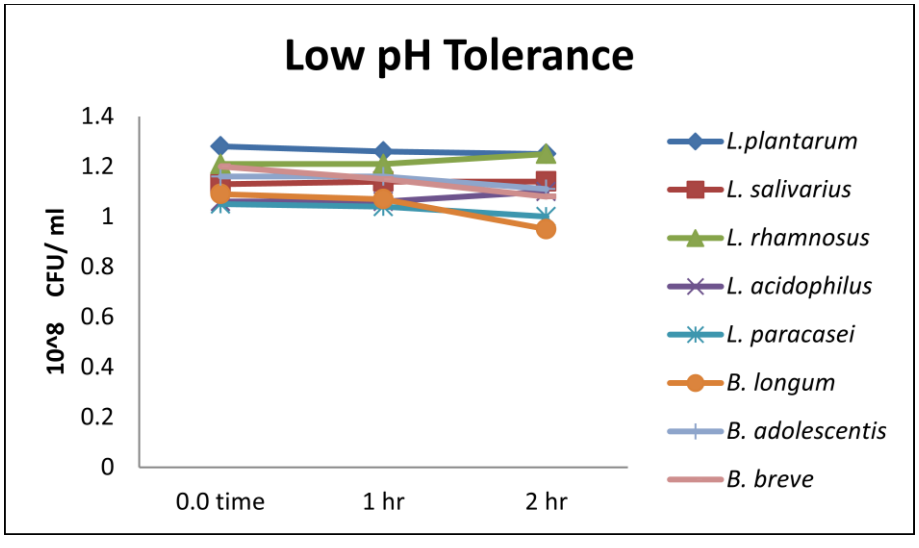


**Figure 1:** Growth and survival of eight probiotic strains at low pH (2.5) for 2 hrs.

Bifidobacteria strains were less tolerant to this low pH (2.5), where their rate of growth ranged from 87 % to 95.6 %, and B. adolescents was the most tolerant at (1.11x10^8 CFU/ml).

Results of 0.3 % bile salts tolerance are shown in **(Figure 2)**. The increase in bacterial growth reflected their high tolerance to bile salts when bile salt was exposed to these organisms for 8 hr, which equals to the period of food digestion in the human intestine. The findings also showed that the rate of bacterial growth for *Lactobacillus* strains ranged from 9.78 to 11.4 folds, and for *Bifidobacterium* strains ranged from 10.1 to 11.1 folds within 8 hrs.

The LAB and *Bifidobacterium* strains were screened for their antagonistic activity against certain bacterial pathogens, i.e. *E. coli, S. aureus, P. aeruginosa and C. albicans, E. coli MC1400 and L. ivanovii.* The results **(Table 3, Figure 3)** illustrate that the eight strains proved to have significant antibacterial activity against the aforementioned pathogens. For instance: P. aeruginosa was the most sensitive bacterial pathogen to the probiotic strains, where the ZDI ranged from (1.43±0.03 to 1.83±0.27), the highest effective strain was *L. plantarum from* LAB, and *B. adolescentis from Bifidobacteria. For the E. coli*, ranged between (1.53±0.03 to 1.77±0.13), and the highest efficient was *B. breve and L. rhamnosus. For St. aureus*, it was between (1.40±0.10 to 2.07±0.03), and the highest strains were B. adolescentis and L. rhamnosus. However, for *C. albicans* the zone was between (1.37±0.07 to 1.70±0.20), and the highest effective strains were *L. paracasei and B. adolescentis*. In case of the *E. coli MC1400* there was insignificant difference among the strains and ranged (1.40±0.04 to 1.50±0.10). Finally, for *L. ivanovii*, the zone was between (1.07±0.06 to 1.24±0.08), and the highest effective strain was *L. rhamnosus.*

Furthermore, the eight probiotic strains also showed inhibition of the growth of the pathogenic species of Aspergillus. **(Table 4)** shows the inhibition of the fungi growth compared to the control. All the strains were able to inhibit the growth of the fungi tested in variable levels compared with the control. L. rhamnosus from Lactobacillus spp. and B. adolescentis from Bifidobacteria spp. were the most efficient and showed the highest fungal growth inhibition. It was able to reduce the growth of the tested Aspergillus species as (+++ partial inhibition) and Penicillium chrysogenum (total inhibition ++++), meanwhile the other lactobacillus and Bifidobacterium strains inhibited the growth of the fungal strains from minimal inhibition (++) to partial inhibition (+++) **(Table 4, Figure 4)**.

The LAB isolates sensitivity results tested against 12 different types of common antimicrobials agents are shown in **(Table 5, Figure 5)**. All eight isolates were susceptible to the antibiotic group (ß-lactam), including penicillin and ampicillin. Moreover, they were susceptible to erythromycin and the protein synthesis antibiotics, which include chloramphenicol, florfenicol, and sulfamethoxazole & Trimethoprim, tetracycline in addition to aminoglycosides like streptomycin and gentamicin. Furthermore, all isolates were also intermediate to clindamycin, resistant to ciprofloxacin. Furthermore, the lactobacilli strains were resistant to vancomycin.

**Table 3:** Antibacterial activity of (CFCS) of 8 probiotic strains against certain pathogenic bcteria expressed as growth inhibition zone (cm) within 24 hrs.

| | **Indicator bacteria (Means of Inhibition zones SD cm)** | | | | | | |
|---|---|---|---|---|---|---|---|
| **Probiotic strains** | **Ps. aeruginosa** | **E. Coli** | **E. coli MC1400** | **St. aureus** | **C. albicans** | **L. ivanovii** | **pH** |
| *L. plantarum* | 1.83±0.27 a | 1.54±0.05 b | 1.45±0.07 a | 1.49±0.06 c | 1.40±0.12 ab | 1.13±0.06 ab | 3.88 |
| *L. salivarius* | 1.77±0.11 ab | 1.57±0.07 b | 1.50±0.10 a | 1.40±0.10 c | 1.37±0.07 b | 1.10±0.10 ab | 3.88 |
| *L. rhamnosus* | 1.56±0.04 abc | 1.69±0.03 ab | 1.55±0.05 a | 1.77±0.03 b | 1.43±0.12 ab | 1.24±0.08 a | 3.87 |
| *L. acidophilus* | 1.57±0.07 abc | 1.53±0.03 b | 1.45±0.15 a | 1.53±0.03 c | 1.47±0.09 ab | 1.13±0.06 ab | 3.89 |
| *L. paracasie* | 1.43± 0.03 c | 1.53±0.03 b | 1.40±0.04 a | 1.73±0.11 b | 1.70±0.20 a | 1.03±0.06 b | 3.88 |
| *B. longum* | 1.50±0.06 bc | 1.59±0.09 b | 1.45±0.12 a | 1.70±0.04 b | 1.50±0.10 ab | 1.07±0.06 ab | 3.9 |
| *B. adolescentis* | 1.83±0.06 a | 1.70±0.04 ab | 1.45±0.08 a | 2.07±0.03 a | 1.67±0.07 ab | 1.13±0.06 ab | 3.88 |
| *B. breve* | 1.83±0.03 a | 1.77±0.13 a | 1.45±0.11 a | 1.80±0.03 b | 1.48±0.08 ab | 1.07±0.06 ab | 3.9 |

Mean values in a column followed by a similar letter are insignificantly different at 1% level of Probability (Duncan's Multiple range test, Duncan [16]

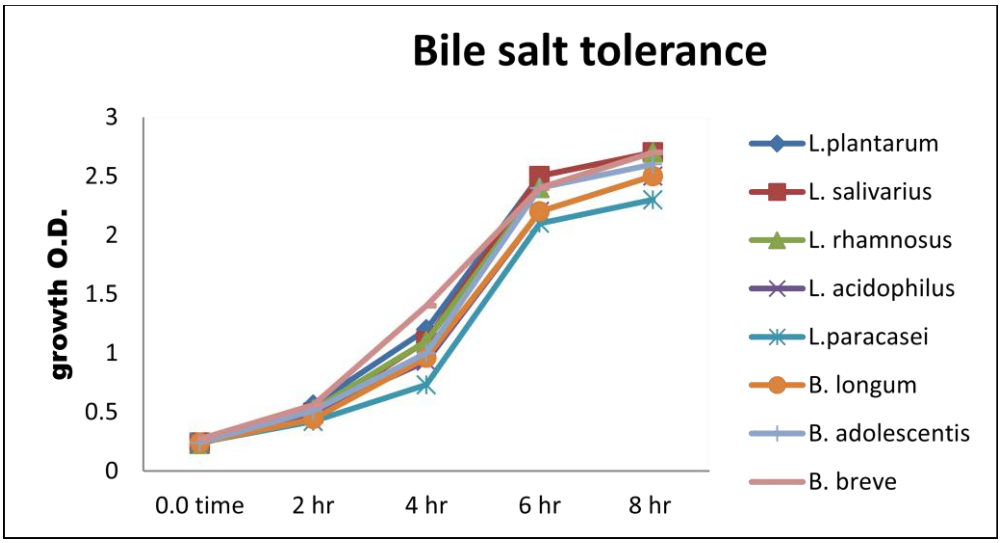


**Figure 2:** Tolerance to bile salts (0.3 %) of eight probiotic strains within 8 hrs.

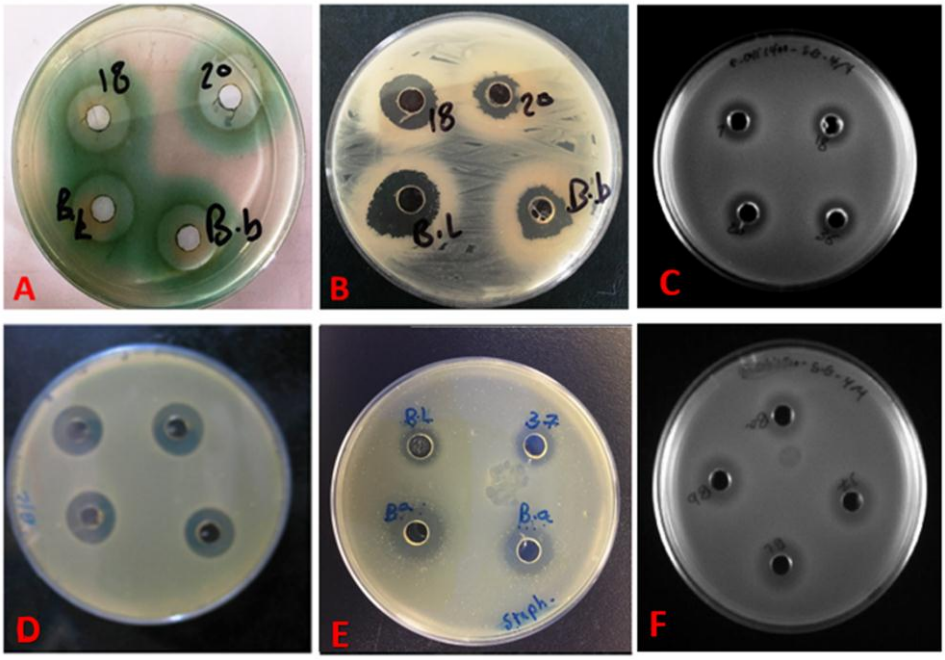


**Figure 3:** Growth inhibition by the probiotic strains against pathogenic bacteria: (A) *Pseudomonas aeruginosa,(B) Candida albicans*, (C) *Escherichia coli MC1400,* (D) *Escherichia coli, (E) Staphylococcus aureus,* and (F) *Listeria. ivanovii.*

**Table 4:** Anti-fungal Activity of 8 probiotic strains expressed as Inhibition Growth of fungi towards the edge of probiotic bacterial growth.

| Isolates \ Fungi | Antifungal (growth inhibition) | | | |
|---|---|---|---|---|
| | ***As .niger*** | ***As. flavus*** | ***As. fumigatus*** | ***P. chrysogenum*** |
| ***L. plantarum*** | ++ | +++ | ++ | ++ |
| ***L. salivarius*** | ++ | ++ | ++ | ++ |
| ***L. rhamnosus*** | +++ | +++ | ++++ | ++++ |
| ***L. acidophilus*** | +++ | ++ | ++ | ++ |
| ***L. paracasei*** | ++ | ++ | +++ | +++ |
| ***B. longum*** | ++ | ++ | ++ | ++ |
| ***B. adolescentis*** | +++ | +++ | +++ | +++ |
| ***B. bereve*** | ++ | +++ | +++ | ++ |

++ Minimal inhibition of fungi growth, +++ partial inhibition of fungi growth and
++++ total inhibition of fungi growth.

**Table 5:** Sensitivity of probiotic isolates to certain antibiotics expressed as growth:

| Antibiotics / Isolates | VA 30 | P 10 | S 10 | AM 10 | CC 2 | GM 10 | TE 30 | E 15 | C 30 | CIP 5 | FFC 30 | SXT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***L. plantarum*** | 0.0 $^{R}$ | 2.5 $^{S}$ | 1.3 $^{s}$ | 3.0 $^{S}$ | 1.5 $^{I}$ | 1.8 $^{S}$ | 3.0 $^{S}$ | 3.0 $^{S}$ | 3.9 $^{S}$ | 0.0 $^{R}$ | 3.2 $^{S}$ | 4.0 $^{S}$ |
| ***L. salivarius*** | 0.0 $^{R}$ | 2.0 $^{S}$ | 1.3 $^{S}$ | 2.3 $^{S}$ | 1.6 $^{I}$ | 2.0 $^{S}$ | 3.1 $^{S}$ | 3.8 $^{S}$ | 4.0 $^{S}$ | 1.0 $^{R}$ | 3.1 $^{S}$ | 4.0 $^{S}$ |
| ***L. rhamnosus*** | 0.0 $^{R}$ | 2.3 $^{S}$ | 1.3 $^{S}$ | 4.0 $^{S}$ | 1.8 $^{I}$ | 2.0 $^{S}$ | 3.5 $^{S}$ | 4.0 $^{S}$ | 4.2 $^{S}$ | 0.8 $^{R}$ | 3.0 $^{S}$ | 4.0 $^{S}$ |
| ***L. acidophilus*** | 0.0 $^{R}$ | 2.2 $^{S}$ | 1.4 $^{S}$ | 4.0 $^{S}$ | 1.5 $^{I}$ | 2.0 $^{S}$ | 4.0 $^{S}$ | 4.1 $^{S}$ | 4.0 $^{S}$ | 1.0 $^{R}$ | 3.1 $^{S}$ | 3.6 $^{S}$ |
| ***L. paracasei*** | 0.0 $^{R}$ | 2.6 $^{S}$ | 1.3 $^{s}$ | 4.1 $^{S}$ | 1.6 $^{I}$ | 1.6 $^{S}$ | 3.0 $^{S}$ | 3.6 $^{S}$ | 3.8 $^{S}$ | 0.0 $^{R}$ | 2.6 $^{S}$ | 4.0 $^{S}$ |
| ***B. longum*** | 2.2 $^{S}$ | 2.5 $^{S}$ | 1.3 $^{s}$ | 3.6 $^{S}$ | 1.6 $^{I}$ | 1.6 $^{S}$ | 3.8 $^{S}$ | 4.2 $^{S}$ | 3.6 $^{S}$ | 1.4 $^{R}$ | 2.6 $^{S}$ | 3.8 $^{S}$ |
| ***B. adolescentis*** | 2.4 $^{S}$ | 2.0 $^{S}$ | 1.3 $^{s}$ | 3.5 $^{S}$ | 1.6 $^{I}$ | 2.0 $^{S}$ | 3.0 $^{S}$ | 4.0 $^{S}$ | 3.6 $^{S}$ | 0.8 $^{R}$ | 2.7 $^{S}$ | 3.5 $^{S}$ |
| ***B. bereve*** | 2.3 $^{S}$ | 2.3 $^{S}$ | 1.3 $^{s}$ | 4.0 $^{S}$ | 1.6 $^{I}$ | 1.8 $^{S}$ | 2.8 $^{S}$ | 4.0 $^{S}$ | 3.5 $^{S}$ | 0.8 $^{R}$ | 2.3 $^{S}$ | 4.0 $^{S}$ |

(R) Resistant – (I) Intermediate – (S) Sensitive, florfenicol (FFC), gentamycin (GE), chloramphenicol (C), clindamycin (CC), erythromycin (ER), 23.75 , 1.25 µg of sulfamethoxazole Trimethoprim (SXT), vancomycin (V), tetracycline (TE), penicillin (P), ampicillin (AM), ciprofloxacin (CIP) and streptomycin (S).

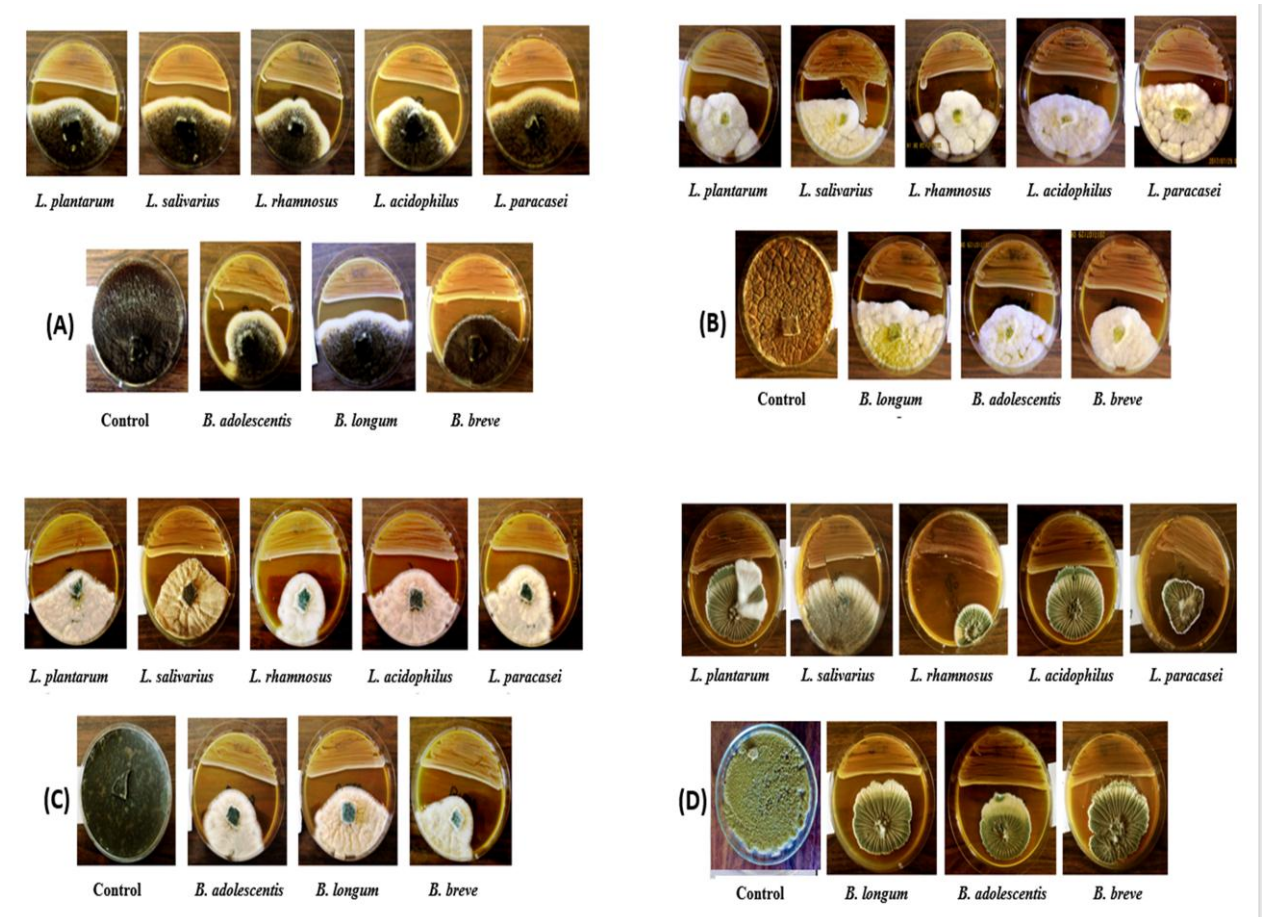


**Figure 4:** Growth inhibition effect of the eight probiotic strains against 4 different fungi (A) *Aspergillus niger,* (B) *As. flavous,* (C) *As. fumigatus,* and (D) *Pe. chrysogenum*

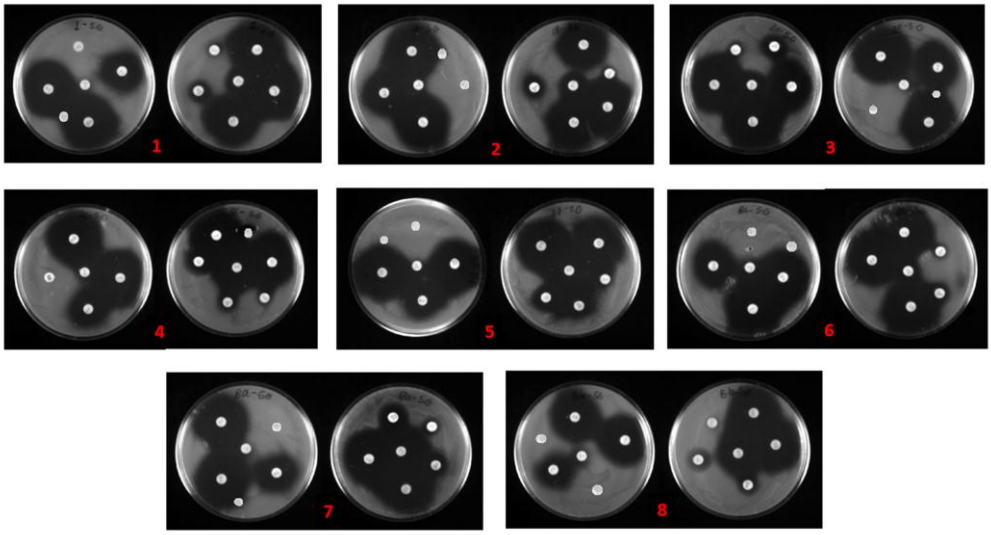


**Figure 5:** Antibiotic sensitivity of the eight probiotic strains: **(1)** *L. plantarum,* **(2)** *L. salivarius,* **(3)** *L. rhamnosus* **(4)** *L. acidophilus,* **(5)** *L. paracasei,* **(6)** *B. longum,* **(7)** *B. adolescentis,* **(8)** *B. breve.*

## 4. Discussion

Studying probiotic behaviors is of considerable interest in order to be used for food preservation and human health enhancement. Recently, interest in probiotic LAB's antagonistic features against foodborne pathogens has shown that they are likely to be alternatives to antibiotics [17]. Significant efforts have therefore been made to isolate LAB from Egyptian traditional fermented products based on the most relevant scientific, functional and health criteria to gain probiotic bacteria. All eight bacterial strains were classified as probiotics based on their morphological characteristics, and physiological and biochemical properties [18].

LAB has been used as a probiotic microorganism for humans and animals. Specific stress challenges must be addressed throughout the GIT to reach the large intestine in a viable state, for example, the highly acidic conditions of the stomach and the presence of bile salts in the duodenum [19]. For a minimum of 90 minutes, probiotic strains must tolerate harch environment (i.e. low pH [pH 2.0 to pH 3.0] and high bile salts [0.3% (w / v)]). [20] All eight strains in this study generally showed significant high survival rates under low pH conditions and high bile salt. Tolerance to the high HCL levels present in the stomach is an important property for defining a potential source of probiotics; for example, pH 1.5 was the lowest recorded value during fasting. A potent probiotic bacterium must, therefore, withstand low pH levels at least. [21]. In the current study, the eight isolates tested at pH 2.5, demonstrated tolerance to pH 2.5. Similar to the current work, Mourad et al. [22] Lactobacillus plantarum OL12, L. OL9 plantarum, L. OL15 plantarum, and L. Plantarum OL33 isolated from fermented olives proved to show a survival rate of 55 %, 49 %, 65 % and 57%, when exposed to pH 2.0 for two hours. These findings are dissimilar to that recorded by Akalu et al. [23] and Rajoka et al. [24] who demonstrated that most strains of L. plantarum isolated from variable sources exhibited a survival rate more than 80 % at pH 2 for three hr.

Also, the strong stomach acidity, the probiotic microorganisms in the GIT have to withstand the bile salt. Bile resistance is, therefore, one of the most crucial properties of probiotics because it determines their capability to survive as a probiotic and plays its functional role in the small intestine. In general, our results are in line with those reported by Hoque et al. [25] who observed that *Lactobacillus* spp. isolates were resistant to bile acid (0.05 – 0.3%). Moreover, Amer et al. [26] reported cocci isolate (LAB) survival, typically at 0.2, 0.3 and 0.4 % w / v bile salts. The highest concentration (0.4 %), however, demonstrated the suppression of all isolates relative to the control. Antibacterial activity is one of the most critical criteria of selection for probiotics. Probiotics achieve antimicrobial properties by processing other compounds, such as organic acids, HO, and bacteriocins. Probiotics are known to have an inhibitory action on the growth of a wide range of human pathogens. Moreover, some laboratory findings identified a protective action of probiotic bacteria against colon cancer [27].

*Lactobacillus* isolates have been subjected to antagonistic effects of indicator microorganisms, such as *S. aureus, E. faecalis, E. coli, S. typhii* and native isolated *Shigella spp* [28]. All *Lactobacillus* strains were antagonistic to all indicators tested. Gharaei-Fathabad and Eslamifar 2011 [29] Reports of the *Lactobacillus plantarum* strain isolated from the tea leaves showing potent inhibitory action against *S. typhii , E. coli , S. aureus , Citrobacter spp, and E. faecalis*. Isolates of the current work have shown similar antimicrobial actvity. In the current study, antagonistic activity *of Lactobacillus and bifidobacteria* isolates against six pathogens showed noticeable activity (**Figures 3- 4** and **Table 3-4**).

The spoilage and toxicity of fungi such as *Fusarium and Aspergillus* occur during food storage and maintenance of food products [30]. Moreover, fungi produce the allergen spores and mycotoxins that seriously threaten human health [31]. Currently, there is an increase in the use of microorganisms or their metabolites for biological protection and avoidance of food spoilage. LAB in the fermentation process produce bacteriocin-

like compounds and organic acids that can inhibit the growth of mould and further preventing aflatoxin B1 production. In the current work, our isolates showed potent antifungal activity against some fungi proposing their critical applications in food production technologies as bio-preservative agents pathogenic moulds. LAB are the microorganisms most widely used in fermented food. The advent of antibiotic resistance (AR) is a global threat because it restricts the efficacy of antibiotic therapy, which is exacerbated by the horizontal transfer of AR genes between bacteria [32]. Fermented foods could be crucial vehicles for vast amounts of living bacteria to enter the human body. Such bacteria may carry transferable AR, which could be transferred to commensal or pathogenic bacteria. While LAB for a long time have been widely used in the manufacture of fermented foods and were generally recognised as safe, some of them showed an acquired or intrinsic AR [33]. Therefore, The AR of LAB in various fermented foods needs to be assessed [32]. Our results of susceptibility to antibiotics are similar to previous studies that also reported the lack of acquired resistance in the LAB isolated from naturally fermented samples [34]. All the isolates were sensitive to Penicillin, Ampicillin, Tetracycline, Erythromycin, Gentamycin, Streptomycin, Florfenicol, Chloramphenicol, and Sulfamethoxazole &Trimethoprim. This is corroborated by data from other groups [35, 36]. All the isolated bacteria were resistant to Ciprofloxacin. Further, the lactobacilli strains were resistant to Vancomycin, and all the strains have shown intermediate resistance to Clindamycin. Our results agreed with that of Ammor et al. 2007 [35], who recorded that the resistance of some *Lactobacillus spp.* against vancomycin has been proposed as intrinsic. But, Lim et al. 1995 [37] mentioned that *Lactobacillus spp.* were susceptible to vancomycin and resistant to streptomycin and gentamicin.

## 5. Conclusion

In the current work, the isolated probiotics from traditional Egyptian fermented products have shown a wide range of antimicrobial properties and may be used as bio-preservatives in food production. We shed some light on screening probiotic bacteria from naturally fermented products. All eight isolates showed resistance to GIT conditions and exhibited potent antimicrobial activities. Collectively, the probiotic ability of the strains and the potent antimicrobial activity with no transfer of antibiotic resistance indicate that these strains can be used in the food and medicinal formulations as natural bio-preservatives because of their prophylactic and therapeutic potential. Moreover, this data supports the notion that traditional fermented foods are a promising source

## References

[1] Abdel-Salam A. Functional foods: Hopefulness to good health. *American Journal of Food Technology*. 2010. 5(2):86-99.
[2] Anukam KC, Reid GJCcr, topics e, microbiology tia. Probiotics: 100 years (1907–2007) after Elie Metchnikoff's observation. 2007; 1:466-74.
[3] Mack DR. Probiotics-mixed messages. Canadian family physician Medecin *de famille canadien*. 2005;51(11):1455-7, 62-4.
[4] Mathipa MG, Thantsha MS. Probiotic engineering: towards development of robust probiotic strains with enhanced functional properties and for targeted control of enteric pathogens. *Gut pathogens*. 2017; 9:28.
[5] Awaisheh SS, Ibrahim SA. Screening of antibacterial activity of lactic acid bacteria against different pathogens found in vacuum-packaged meat products. *Foodborne pathogens and disease*. 2009;6(9):1125-32.
[6] Dowarah R, Verma AK, Agarwal N. The use of Lactobacillus as an alternative of antibiotic growth promoters in pigs: A review. *Animal Nutrition*. 2017;3(1):1-6.
[7] Ocana VS, Elena Nader-Macias M. Production of antimicrobial substances by lactic acid bacteria II: screening bacteriocin-producing strains with probiotic purposes and characterization of a Lactobacillus bacteriocin. *Methods in molecular biology (Clifton, NJ)*. 2004; 268:347-53.
[8] Magdoub MNI, Hassan ZMR, Effat BAM, Sadek ZIM, Tawfik NF, Mabrouk AMM. Probiotic properties of some lactic acid bacteria isolated from Egyptian dairy products. *Int J Curr Microbiol App Sci*. 2015;4(12):758-66.
[9] Ali, F.S, G. Zayed , O. A. O.Saad, Salwa A. H. Gharib Molecular identification and properties of some probiotic bacteria isolated from fermented dairy products. *Minia J. Agri. Res.%Dev.* 2018; 36(3):447-457.
[10] Kaushik JK, Kumar A, Duary RK, Mohanty AK, Grover S, Batish VK. Functional and probiotic attributes of an indigenous isolate of Lactobacillus plantarum. *PloIS one*. 2009;4(12): e8099.
[11] Liong MT, Shah NP. Acid and bile tolerance and cholesterol removal ability of lactobacilli strains. *Journal of dairy science*. 2005;88(1):55-66.
[12] Westermann C, Gleinser M, Corr SC, Riedel CU. A Critical Evaluation of Bifidobacterial Adhesion to the Host Tissue. *Frontiers in microbiology*. 2016;7:1220-.
[13] Tejero-Sarinena S, Barlow J, Costabile A, Gibson GR, Rowland I. In vitro evaluation of the antimicrobial activity of a range of probiotics against pathogens: evidence for the effects of organic acids. *Anaerobe*. 2012;18(5):530-8.
[14] Gerbaldo GA, Barberis C, Pascual L, Dalcero A, Barberis L. Antifungal activity of two Lactobacillus strains with potential probiotic properties. *FEMS microbiology letters*. 2012;332(1):27-33.
[15] Sharma C, Gulati S, Thakur N, Singh BP, Gupta S, Kaur S, et al. Antibiotic sensitivity pattern of indigenous lactobacilli isolated from curd and human milk samples. *3 Biotech*. 2017;7(1):53.
[16] Duncan DB. Multiple Range and Multiple F Tests. *Biometrics*. 1955;11(1):1-42.
[17] Wan MLY, Forsythe SJ, El-Nezami H. Probiotics interaction with foodborne pathogens: a potential alternative to antibiotics and future challenges. *Critical reviews in food science and nutrition*. 2018:1-14.
[18] Azat R, Liu Y, Li W, Kayir A, Lin D-B, Zhou W-W, et al. Probiotic properties of lactic acid bacteria isolated from traditionally fermented Xinjiang cheese. *J Zhejiang Univ Sci B*. 2016;17(8):597-609.
[19] Ruiz L, Margolles A, Sánchez B. Bile resistance mechanisms in Lactobacillus and Bifidobacterium. *Front Microbiol.* 2013; 4:396-.
[20] Ben Salah R, Trabelsi I, Ben Mansour R, Lassoued S, Chouayekh H, Bejar S. A new Lactobacillus plantarum strain, TN8, from the gastro intestinal tract of poultry induces high cytokine production. *Anaerobe*. 2012;18(4):436-44.
[21] Lin WH, Hwang CF, Chen LW, Tsen HY. Viable counts, characteristic evaluation for commercial lactic acid bacteria products. *Food microbiology*. 2006;23(1):74-81.
[22] Mulaw G, Sisay Tessema T, Muleta D, Tesfaye A. In Vitro Evaluation of Probiotic Properties of Lactic Acid Bacteria Isolated from Some Traditionally Fermented Ethiopian Food Products. *Int J Microbiol.* 2019; 2019:7179514-.
[23] Mulaw G, Sisay Tessema T, Muleta D, Tesfaye A. In Vitro Evaluation of Probiotic Properties of Lactic Acid Bacteria Isolated from Some Traditionally Fermented Ethiopian Food Products %J *International Journal of Microbiology*. 2019; 2019:11.
[24] Riaz Rajoka MS, Mehwish HM, Siddiq M, Haobin Z, Zhu J, Yan L, et al. Identification, characterization, and probiotic potential of Lactobacillus rhamnosus isolated from human milk. *LWT*. 2017; 84:271-80.
[25] Hoque M, Akter F, Hossain K, Rahman M, Billah M, Islam KJWJoD, et al. Isolation, identification and analysis of probiotic properties of Lactobacillus spp. from *selective regional yoghurts*. 2010;5(1):39-46.
[26] Karami S, Roayaei M, Hamzavi H, Bahmani M, Hassanzad-Azar H, Leila M, et al. Isolation and identification of probiotic Lactobacillus from local dairy and evaluating their antagonistic effect on pathogens. *Int J Pharm Investig*. 2017;7(3):137-41.
[27] Drago L. Probiotics and Colon Cancer. Microorganisms. 2019;7(3):66.
[28] Prabhurajeshwar C, Chandrakanth K. Evaluation of antimicrobial properties and their substances against pathogenic bacteria in-vitro by probiotic Lactobacilli strains isolated from commercial yoghurt. *Clinical Nutrition Experimental*. 2019; 23:97-115.

[29] Gharaei-Fathabad E, Eslamifar M. Isolation and applications of one strain of Lactobacillus paraplantarum from tea leaves (Camellia sinensis). *American Journal of Food Technology*. 2011;6(5):429-34.
[30] Snyder AB, Worobo RWJJofp. *Fungal spoilage in food processing*. 2018;81(6):1035-40.
[31] Amin M, Jorfi M, Khosravi A, Samarbafzadeh A, Sheikh AFJJBS. Isolation and identification of Lactobacillus casei and Lactobacillus plantarum from *plants by PCR and detection of their antibacterial activity*. 2009;9(8):810-4.
[32] Clementi F, Aquilanti L. Recent investigations and updated criteria for the assessment of antibiotic resistance in food lactic acid bacteria. *Anaerobe*. 2011;17(6):394-8.
[33] Pan L, Hu X, Wang XJFC. Assessment of antibiotic resistance of lactic acid bacteria in Chinese fermented foods. *Food Control*. 2011;22(8):1316-21.
[34] Erginkaya Z, Turhan EU, Tatlı D. Determination of antibiotic resistance of lactic acid bacteria isolated from traditional Turkish fermented dairy products. *Iran J Vet Res*. 2018;19(1):53-6.
[35] Ammor MS, Florez AB, Mayo B. Antibiotic resistance in non-enterococcal lactic acid bacteria and bifidobacteria. Food microbiology. 2007;24(6):559-70.
[36] Gad GFM, Abdel-Hamid AM, Farag ZSH. Antibiotic resistance in lactic acid bacteria isolated from some pharmaceutical and dairy products. *Braz J Microbiol.* 2014;45(1):25-33.
[37] Nawaz M, Wang J, Zhou A, Ma C, Wu X, Moore JE, et al. Characterization and transfer of antibiotic resistance in lactic acid bacteria from fermented food products. *Current microbiology*. 2011;62(3):1081-9.