Probiotic Lactic acid bacteria: A Promising alternative approach to control human threats

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ABSTRACT

Dairy and fermented products have been utilized by human residents since early ages and have been a familiar source of Lactic acid bacteria (LAB). The valuable effects of viable probiotic bacteria as dietetic supplements have gained massive research note, *Lactobacillus spp.* with probiotic properties are extensively used to prepare dairy products such as yoghurts, Cheese etc. The target of the present study is to assess LAB species with prospective activities, 22 samples of fermented food samples were collected from local markets of Giza for isolation of probiotic microbes. From the samples, 10 LAB strains were isolated, promising isolates were selected by primary screening as potential probiotics with antimicrobial activity against bacterial pathogens. Selected LAB isolates were *in vitro* characterized for their probiotic pattern and antimicrobial activities against pathogens. Isolates were found to be resistant to NaCl% (6%), bile salt% (0.5%) and exhibited respectable growth in the acidic condition, while highest growth was noted at pH around 6.0. The most potent LAB isolate was exhibited antimicrobial activity against 4 different test pathogens with inhibition zone diameter (24-32) mm. Additionally, the MIC of the same isolate was (1.5-3 mg/ml) which is considerable result when compared to studies of the same field. Organic acids production with quantity of 0.76% are found to be responsible for antimicrobial activity. Also, the isolates showed remarkable aggregation and hydrophobicity properties. According to the results, selected LAB isolates (Y2c) were believed to be potential probiotic bacteria. Subsequently, identification of the Y2c isolate conventionally and genetically revealed that it is found to be *Limosilactobacillus fermentum* and has been registered in GenBank under *Limosilactobacillus fermentum* strain HwOs.

Keywords: *Probiotics; LAB; Lactobacilli; Dairy products; Fermented products; Antimicrobial activity*

INTRODUCTION

The correlation between foodstuffs and health benefits has been being investigated for decades. Recently, probiotics and non-pathogenic microbes that have positive influences on their host's health have gathered great attention due to raising commercial profit (Thorakkattu et al., 2022). Accumulated research studies have helped in the description of probiotic microorganisms and their health support.

Probiotics are widely used in fermented food manufacture and are considered harmless with implementation in veterinary and medical activities. In food fabrication, probiotics are commonly employed as starter cultures and have been announced as part of human microbiota. Cheese, yoghurt and fermented dairy products are the primary sources of probiotics. Lactic acid generated by fermentation of lactose sugar causes the sour taste of yogurt by reducing its pH and allows the formation of the typical texture by affecting milk proteins (de Souza, de Oliveira, & de Oliveira, 2022).

Probiotics are viable, health-promoting microorganisms that are integrated into various forms of foods. Many studies have obviously shown that yogurt containing alive bacteria (*Lactobacillus delbrueckii spp. Bulgaricus* and *Streptococcus thermophilus*) that enhances lactose digestion and reduces symptoms of lactose intolerance. Bacterial probiotics, a constituent of "thermophilic" starter cultures and employed in commercial products nowadays, are mostly members of the genera Bifidobacterium and Lactobacillus (Coelho, Malcata, & Silva, 2022). Moreover, the capability of probiotics to resist the acidic conditions of stomach juices and bactericidal nature of bile salts, as well as the formation of lactic acid that obstructs the growing of other microorganisms, let them to establish themselves in the intestine (Pahumunto, Teanpaisan, & Proteins, 2023). Probiotics generate a wide range of antimicrobial metabolites, i.e., acetoin, diacetyl, organic acids, bacteriocins and hydrogen peroxide. These actions enhance the microbiological safety by controlling the development of other microorganisms, and inhibition of pathogens (Fidan et al., 2022).

Lactobacilli are significant part of microflora of the intestine and their effect in the general condition of human condition is

being seriously explored (Wu et al., 2022). Lactobacillus is one of the main groups of LAB used in food fermentation and is subsequently of great economic value. Strains of *L. acidophilus* or neighbored species, *L. paracasei subsp. paracasei and subsp. tolerans*, *Lactobacillus rhamnosus* and *Lactobacillus casei* are being progressively used in new yogurts (Torres-Miranda, Melis-Arcos, Garrido, & Proteins, 2022).

Taxonomy of Lactobacilli has experienced significant alterations in latest years causing a little confusion (Whitman et al., 2022). Numerous studies have been managed for the identification and classification of lactic acid bacteria containing classical biochemical tests, as fermentation of different carbohydrate patterns using available kits, physiological tests as well as DNA-based methods (Jeyagowri et al., 2023).

Generally, Lactobacilli have not been correlated with diseases and have been considered as non- pathogenic portions of gastrointestinal and urogenital flora (Saha & Saroj, 2022). Lactobacilli, through antagonistic effect against pathogenic bacteria, maintains the GIT ecosystem in a healthy state. Accordingly, the aim of the study is to isolate a high potential probiotic strain to combat Human pathogens.

MATERIAL AND METHODS

Isolation of lactic acid bacteria (LAB)

Twenty-two samples of fermented foods, including different types of cheese, yoghurt, milk and pickles were placed in sterile bags and were taken to the lab for analysis in a way that kept bacteria away from proliferating. For sample preparation, 10 g of each sample was blended for 30 seconds in a lab-blender with 90 ml of sterile sodium citrate solution (2% w/v) for solid samples and 90 ml of sterile physiological saline (0.85% NaCl w/v) for liquid samples. On de Man, Rogosa, and Sharpe (MRS) (Meat extract, 8.0; Peptone, 10; Yeast extract, 5.0; Ammonium citrate, 2.0; Sodium acetate, 5.0; Magnesium sulphate, heptahydrate, 0.2; Manganese sulphate, tetrahydrate, 0.05; Dipotassium phosphate, 2.0; Glucose, anhydrous, 20; Polysorbate 80, 1.0; Agar, 12 (g/L)) (Oxoid, England) agar plates, a loopful from each sample was spread and then all plates were kept at 37°C for 48–72 hours under anaerobic conditions using anaerobic jar (Śliżewska & Chlebicz-Wójcik, 2020). To get pure culture, a single colony was picked up and re-streaked on an agar plate, According to (Abushiba et al., 2019), with a few minor modifications.

For Morphological characterization, cultural identification and microscopic observation were performed. Cultural characterization of LAB isolates was done on different agar media plates including colony colour; margin; form; surface; elevation; optical density. Microscopic observation was investigated by Gram's staining, slides were examined under oil immersion objective for Gram's reaction, cell shape and arrangements (Mishra, Shukla, David, & Raisagar, 2023). In addition, catalase test was also performed, and bacterial isolates showing gram-positive and catalase-negative results were selected for further screening (Mohamad, Manan, Sallehhuddin, Musa, & Ikhwanuddin, 2020). The selected bacterial isolates were stored in MRS broth supplemented with 20% glycerol at -20 °C until further analysis.

Characterization of the selected LAB isolates

Viability at Different pH

By cultivating the bacteria in MRS broth with multiple pH levels, the bacterial isolate's capability to withstand acid was investigated. As food persists in the stomach for a duration of three hours, the resistance to stomach pH is frequently assessed using *in vitro* assays (Afrin, Akter, Begum, & Hossain, 2021). A 100 μL of overnight cultures of the isolates was administered into10 ml MRS broth with a variety of pHs from 2.0 to 8.0, adjusted with 1 M HCl by a pH meter, to measure the growth at different pHs. Growth was detected using spectrophotometer at 600 nm after 18 hours of anaerobic incubation at 37 °C in the injected broths (Liu et al., 2022).

Bile salt tolerance

The strain's tolerance to bile salts was tested using a modified method developed by Gilliland and coworkers in 1984 (Prete et al., 2020). This test determines optimal growth after inoculating the isolate into MRS broth tubes containing 0.3, 0.4 and 0.5 % bile salts (Himedia). Bacterial growth was measured at 600 nm after 18–24 hours of culture at 37 °C. For this experiment, bile-salt-free MRS broth was used as a control.

NaCl Tolerance

Using MRS broth supplemented with different levels of NaCl, from 2 to 8%, the tolerance of isolates to NaCl was investigated. The NaCl broths were inoculated with 1% inoculum of each isolate. For confirmation of

growth after a 24-hour incubation period at 37 °C, optical density at 600 nm was determined (Jobby et al., 2020).

Determination of anti-bacterial activity of the LAB

To evaluate the activity of selected LABs against different tested bacteria, they were assessed via agar well diffusion methods on Muller Hinton Agar (Acharjee et al., 2022a). According to the recommended method by Clinical and Standared Laboratory Institute (CSLI), a loopful culture of the tested bacteria (*B. subtilis*, *Bacillus cereus* (*B. cereus*), *Escherichia coli* (*E. coli*) and *S. typhi*) were inoculated into the appropriately labeled sterile tubes containing Mueller Hinton (MH) broth to make the bacterial suspension followed by forming the bacterial lawn onto the surface of the MHA media. Subsequently, wells (8 mm) were made on the inoculated MHA media and concentrated filtrate of probiotics, prepared by was added into each well. After incubation at 37°C for 24 h, the presence of clear zone around the sample solution (if any) was analyzed for the existence of the antibacterial activity. (Acharjee et al., 2022a).

Determination of MIC of the most potent isolate

In this well-known procedure, the agar plate surface was inoculated by streaking the standardized inoculum of the test microorganism (0.5 McFarland) over the entire agar surface. Then, several holes with a diameter of 8 mm were punched aseptically with a sterile cork borer, and a volume of 100 µL of double-fold dilution of concentrated most potent isolate filtrate (12 mg/ml) was introduced into the wells. Then, agar plates were incubated under suitable conditions, depending on the test microorganism. The lowest concentration of the filtrate inhibits the growth of the microbial strain, is considered to be the MIC (Bassyouni et al., 2022).

Characterization of antimicrobial substances

The well-selected LAB isolate was examined for the production of antimicrobial substances like, bacteriocins, organic acids and hydrogen peroxide using agar well-diffusion technique with slight modification of the method (Awadallah, Emara, & Nasr, 2023). A 20-ml grown culture on MRS broth was divided into equal fractions for different assays. For bacteriocins assay, 5 ml of supernatant was treated with 1 mg/ml trypsin then treated supernatant was filtered with 0.22 mm pore-size filters (Axiva Pvt Ltd). Organic acids assay was performed using 5 ml of supernatant adjusted to pH 6.5 ± 0.1 using 1 N NaOH. Finally, hydrogen peroxide investigated by 5 ml of supernatant treated with 0.5 mg/ml of catalase (Hi-Media Pvt Ltd), and the last fraction was not treated as control. A volume of 100 µl of each supernatant was placed in 8-mm diameter wells and the plates were swabbed with 1% (v/v) overnight culture of each test pathogen. Inhibitory characteristics were observed, and the zone of inhibition was noted after 24 h of incubation at 37 °C.

Quantification of organic acid and determination of pH value

One percent (v/v) of 24 h freshly prepared culture of the most potent isolate was inoculated in 10% sterilized skim milk (Hi-Media Pvt Ltd, Bangalore) and the initial pH was adjusted at 6.76 using HCl using digital electrode pH meter. The inoculated skim milk was incubated at 37 °C for 72 h, samples assembled every 12, 24, 48 and 72 h and liquids of coagulated milk were separated by centrifugation (4000 rpm for 10 min.). The pH of the separated liquid was recorded and total acidity was determined by end point equation using phenolphthalein as color indicator through titration with 0.1 N NaOH (Guo et al., 2022). The following equation was used:

TA % = [volume of NaOH used] x [0.1 N NaOH] x [Acid milliequivalent factor] x dilution factor x [100] Volume of sample (ml)

Autoaggregation Assay

According to the autoaggregation percentage, the capacity of autoaggregation was evaluated, as reported by (Khromova et al., 2022). After 24h of incubation, the cells were removed by centrifugation (4500 g for 20 min at 4 °C), twice-washed with phosphate-salt buffer (PBS: 130 mM sodium chloride, 10 mM sodium phosphate, pH 7.2), resuspended in the same buffer under sterile conditions, and incubated at 37°C. At varied intervals of time (0, 4, 12, and 18 hours), OD was noted. According to the equation:

*A*utoaggregation %=1-(A time/A initial) ×100

2.8. Coaggregation of most potent LAB with different pathogenic cells

This study investigated the coaggregation between the most potent LAB isolate and various test pathogens according to (Prabhurajeshwar & Chandrakanth, 2019). Separate bacterial culture was grown in MRS and NB media for 24 h at 37 °C. Equal volumes

of cells from the LAB isolate and the test pathogenic strains (1:1 v/v) were mixed to form the bacterial suspension, which was then incubated at 37 °C without agitation as recommended in the autoaggregation procedure above. At 4, 12, 18, and 24 h during the incubation process, the mixture's absorbance (A_{600}) was examined. The following formula was used to determine the coaggregation percentage:

Coaggregation (%) = [(A pathogen + A lactobacillus)/2 – A mix (A pathogen + A lactobacillus)/2×100

Cell surface hydrophobicity

Cell-surface hydrophobicity studies the potential association between physicochemical features and its efficient adherence to the intestinal mucus. *In vitro* cell surface hydrophobicity was assessed using the method published by (dos Santos Leandro et al., 2021) through the measurement of microbial adherence to xylene hydrocarbon. Culture was propagated for 14 hours at 37 °C in 10 mL of MRS broth, then separated at 4500 x g for 20 minutes at 4 °C. The cell pellet was then washed and reconstituted in 10 mL of saline solution. At a wavelength of 600 nm (A0), the optical density of the cell suspension in saline was adjusted to 0.6. One ml of the isolate's cell suspension was stirred with 1 ml of xylene to obtain a volume-equal mixture, then the mixture was homogenized for two minutes. The aqueous phase was allowed to remain separate from the other phase for 30 minutes at room temperature, and the optical density (A1) at 600 nm has been measured after carefully removing the aqueous phase. The following formula was used for estimating the hydrophobicity percentage of solvent-adherent (xylene) isolate:

Hydrophobicity% = (A0-A1)/A0 ×100.

Identification of the most potent LAB isolate

Based on the screening of anti-bacterial activity of probiotic, a 24-hour-old culture of the promising isolate was characterized using the vitek2 automated technique (bioMérieux), and its identity was validated via 16S rRNA gene sequence analysis. According to (Mahmoud et al., 2022), the bacterial isolate's genomic DNA was isolated from 50ml of liquid bacterial culture using the Gene Jet genomic DNA extraction kit (Thermo K0721) per the manufacturer's instructions. Polymerase chain reaction (PCR) was used to amplify the 16S ribosomal DNA using the primers Bact 27f (50-GTTTGATCCTGGCTCAG-30) and 1492r (50-CGGCTA CCTTGT TACGAC-30). The PCR product was then sequenced bidirectionally with an ABI 3730× DNA sequencer (GATC Biotech, Konstanz, Germany) using forward, reverse, and internal primers (27 f and 1492 r). The obtained sequence was blasted on GenBank to find and compare similarities between the isolates and the available sequences on the GenBank database. The phylogenetic tree was constructed in order to determine the phylogenetic position of the selected isolate.

RESULTS

Obtained LAB isolates

From the collected samples, ten white, creamy, and convex-shaped colonies were isolated, picked up and purified as promising probiotics (Fig.1). Further probiotics characterizations were observed; they are clearly Gram +ve, rod in shape, and catalase -ve (Fig. 2).

Characteristics of LAB

Viability at Different pH

Although the growth at pH 2 was retarded for all isolates, they could survive at different pH (2-8). However, except for isolate 5, the optimum growth was exhibited at pH 6 and 8 (Fig.3).

Bile salt tolerance

The selected isolates could grow and survive in 0.3% bile salt. Isolates M1b and M3c showed more susceptibility against bile salts while others were resistant even at 0.5% of bile salts concentration (Fig.4).

NaCl Tolerance

The LAB isolates from food samples tolerated 2% NaCl except isolate Y3d that showed slow growth at different NaCl%. Isolates M3c, CH2a and CH3c showed no growth at 8% and the growth of all isolates was affected by increasing NaCl concentrations except isolate P2f (Fig.5.). However, isolates that are able to survive at different concentrations of NaCl are more probable to be probiotic.

The anti-bacterial activity of the LAB

Eighty percent (80%) of the isolates displayed antimicrobial activity against one or more of the test bacteria (Fig.6). However, the degree of antagonistic effects varied among the isolates. All the Isolates exhibited inhibition zone with diameter between (11–

32 mm), the most potent isolate was Y2c with inhibition zone (24 – 32 mm) and were selected as most potent isolate (Fig. 7).

The MIC of isolate Y2c

The Y2c filtrate exhibited different MICs according to test organisms under investigation. The MIC was 3 mg/ml against both *E. coli* and MRSA, while was 1.5 mg/ml against other test strains (Fig.8).

Characterization of antimicrobial substances

According to the results, supernatants of Y2c culture treated with 1 mg/ml trypsin did not impact their inhibitory activities against test strains. This suggests that the inhibitory potential of the isolate was not related to bacteriocin formation. Also, addition of catalase to Y2c supernatant did not affect the inhibitory behavior of the isolate against same strains. This confirmed that inhibition was not due to releasing of hydrogen peroxide. Conversely, neutralized supernatant of Y2c did not exhibit any inhibitory effect on the test strains. Accordingly, organic acid production is responsible of the inhibitory effect of Y2c isolate. (Fig.9).

Quantification of organic acid and determination of pH value

Isolate Y2c coagulated the skim milk and organic acids were produced gradually and increased during the first 48 h. After that, the quantity of organic acids showed no noticeable increasing with 0.76 % (Table 1).

Autoaggregation and coaggregation percentages

The autoaggregation behavior of isolate Y2c were estimated based on its deposition ability. The results showed that the isolate showed the highest autoaggregation percentage after 18 h of incubation (72%) (Table 2.).

The results of coaggregation of the Y2c isolate investigated with different test pathogens (*E. coli, B. cereus,* MRSA and *K. pneumonia*) at different time intervals (4,12and 18 h) are shown in Table 3. The isolate showed effective coaggregation with all pathogens at all different time intervals with high Coaggregation percentage reached 62 % with *E. coli* and more than 50 % with other pathogens after 18 h.

Hydrocarbon adherence efficiency of Y2c

Isolate Y2c have hydrophobic nature towards xylene with 38% (Table 4). This suggests that Y2c has the ability to adhere to intestinal mucous of human supporting its capacity to be used as probiotic supplements with high viability in GIT.

Vitek2 and molecular Identification of Y2c isolate

The probiotic bacterial isolate Y2c was identified using the vitek2 automated system as *lactobacillus fermentum*, with an excellent ID message confidence level and 97% probable similarity. In addition, Analysis of 16S rDNA partial sequences (840 bp) of Y2c revealed its homology (97%) with *Limosilactobacillus fermentum* strain Cip102980 and *Limosilactobacillus fermentum* strain NBRC 15885 which found in GenBank databases forming separate clade with the later (Fig.10). Therefore, Y2c isolate was designated as *Limosilactobacillus fermentum* strain HwOs.

DISCUSSION

The provided study was proposed to isolate and identify potential probiotic bacterial strains from different fermented food samples collected from commercial market in Giza Governorate, Egypt. The methodology of the study includes various techniques to isolate and screen for probiotic strains, involving cultural characteristics, Gram staining, physiological and biochemical characterization. The outcome of the isolation process is ten white, creamy, and convex-shaped colonies, they were picked up and purified as promising probiotics. Further characterization of the probiotics showed that they are clearly Gram-positive, rod-shaped, and catalase negative. This result is consistent with other research studies that have characterized probiotic bacteria using morphological and biochemical tests(Sørensen et al., 2022), (Amelia, Philip, Pratama, Purwati, & Technology, 2020)and (Salam et al., 2021).

The use of Gram staining and colony morphology to identify probiotic bacteria is a common practice in microbiology. The identification of probiotic bacteria is important for the development of functional foods and the selection of potential probiotic strains for use in different applications. Additionally, the characteristics of the probiotic bacteria were evaluated in terms of their viability at different pH, bile salt concentrations, and tolerance to NaCl. The results showed that all isolates could survive at different pH levels (2-8), with optimum growth exhibited at pH 6 and 8 that is a criterium for probiotics. The selected isolates could grow and survive in 0.5% bile salt which is strongly suggested that the isolates have such unique characteristic of

probiotics. The LAB isolates from food samples tolerated 2% NaCl except isolate Y3d that showed slow growth at different NaCl%. Although all isolates showed NaCl tolerance, the increase in salt concentration highly affected the growth of LAB isolates. The suggested data from other research studies have characterized probiotic bacteria in terms of their tolerance to different environmental conditions which are relevant with our findings (Karnwal & Malik, 2023), (Menconi et al., 2014)and(Ayyash et al., 2021)

The study also evaluated the antibacterial activity of the probiotic isolates, the result of the antibacterial activity of the LAB isolates against test strains showed that 80% of the isolates displayed antimicrobial activity against one or more of the test bacteria. The degree of antagonistic effects varied among the isolates, with all isolates exhibiting inhibition zones with a diameter between 11-32 mm. The most potent isolate was Y2c with an inhibition zone of 24-32 mm and was selected as the most potent isolate. In contrast, the results revealed by the study of (Yang, Fan, Jiang, Doucette, & Fillmore, 2012) are shown lower activities among 28 LAB isolates against test strains with maximum inhibition zone with dimeter of 11.24 mm. However, other research study reported similar results between LAB isolates from human milk that exhibited maximum inhibition zone with 25 mm but still lower than our findings (Sharma et al., 2017) . Further research is needed to determine the mechanism of action of the LAB isolates and to investigate their potential as antimicrobial agents which is fulfilled in this study.

The study also examined the (MIC), the result of the minimum inhibitory concentration (MIC) of isolate Y2c showed that its filtrate exhibited different MICs according to the test organisms under investigation. The MIC ranged from 1.5 - 3 mg/ml that is considered low concentrations according to results reported by the other studies. For instance, in a study of antimicrobial activity of probiotics against food borne pathogens, the MICs of 6 different microorganisms against 8 pathogens were more than 16 mg/ml with little exceptions of 3 mg/ml against only 2 strains (Acharjee et al., 2022b). MIC is a vital feature in the assessment of the antimicrobial activity of LAB isolates and their potential as probiotics and antimicrobial agents. The characterization of antimicrobial substances is important in the selection of potential probiotic strains for use in different applications.

The results of this study suggest that the Y2c isolate has the potential to be used as an antimicrobial agent due to its organic acid production. This result is also reported by other studies that have investigated the antimicrobial substances produced by LAB isolates(Lin, Pan, & Infection, 2019)and(Yang et al., 2012). Further examination evaluated to quantify the organic acid and determine the pH value, the result of the quantification of organic acid and determination of pH value of isolate Y2c showed that the isolate coagulated the skim milk and produced organic acids that gradually increased during the first 48 hours. After that, the quantity of organic acids showed no noticeable increase, with 0.76%. The pH value decreased from 5.99 to 4.93 during the incubation period. According to previous studies, the organic acid production was extremely decreased after 48 h, this may be due to that LAB utilize organic acid as carbon source after consuming the main constituent of the culture media (Branson, Broadbent, & Carpenter, 2022)and(Heyen, Scholz-Böttcher, Rabus, Wilkes, & chemistry, 2020).

The production of organic acids is an important factor in the selection of potential probiotic strains for use in different applications, including the development of functional foods. Further research is needed to determine the specific organic acids produced by the Y2c isolate and their mechanism of action against the test strains.

The study also examined the autoaggregation and coaggregation abilities of the selected isolate. The result of the autoaggregation and coaggregation percentages of isolate Y2c showed that the isolate had a high percentage of autoaggregation after 18 hours of incubation (72%). The coaggregation results of the Y2c isolate tested with different test pathogens (*E. coli*, *B. cereus*, MRSA, and *K. pneumonia*) at different time intervals (4, 12, and 18 hours) showed that the isolate have an effective coaggregation with all pathogens at all different time intervals, with a high coaggregation percentage of 62% with *E. coli* and about 50% with other pathogens after 18 hours. The autoaggregation of strains reported by (Khemaleelakul, Baumgartner, & Pruksakom, 2006) was 56.45% indicating that our findings exhibited higher autoaggregation percentage. In contrast, in another report, coaggregation between *E. duran* recovered from wild Boar and *S. aureus* was more than 80% that showed stronger coaggregation

percentage than our results (Li et al., 2020). The autoaggregation and coaggregation behavior of LAB isolates are important factors in their selection for use as probiotics and in the development of functional foods. The results of this study suggest that the Y2c isolate has the potential to be used as a probiotic due to its high autoaggregation and coaggregation percentages.

Additionally, the study evaluated the cell surface hydrophobicity of the Y2c isolate and found that it has a highest hydrophobic nature. The attachment efficacy of Y2c to hydrophobic surfaces is high compared to enteropathogenic bacteria with 38% cell-surface hydrophobicity. This finding is supported by data derived from research of (MladenoviĆ, GrujoviĆ, D NIKODIJEVIĆ, ČomiĆ, & Health, 2020) who reported that *K. oxytoca* KGPMF 1, *K. pneumoniae* KGPMF 13 and *E. coli* KGPMF 17 exhibited cell-surface hydrophobicity percentage with 9.01 ± 0.50, 13.10 ± 0.59 and 6.97 ± 0.33, respectively.

The result of characterization of the selected isolate Y2c using vitek2 (*Lactobacillus* ID card) indicated it as *lactobacillus fermentum* while the 16S rDNA analysis named it as *Limosilactobacillus fermentum.* latterly, whole genome studies have been performed, and the taxonomy of Lactobacillaceae has been newly reevaluated. Therefore, the former namenclature, *Lact. fermentum*, has been reconstructed to *Limosilactobacillus fermentum* (Zheng et al., 2020). *Limosilactobacillus fermentum* is a probiotic bacterium that had been isolated from milk products. The identified strain recorded in GenBank as *Limosilactobacillus fermentum* HwOs showed effective probiotic characteristics as the lactobacilli strains are the major group of bacteria among probiotics (El Hage et al., 2022)

Overall, the study provides valuable information about the potential probiotic properties of bacterial strains isolated from fermented foods.

CONCLUSION

The selected strain *Limosilactobacillus fermentum* HwOs has exhibited promising features promoting it to be used as potential probiotic to enhance the defense against human pathogens. It also showed excellent probiotic properties, such as pH, NaCl and bile tolerance, auto- and co- aggregations, control of pathogen growth under *in vitro* conditions. More studies are required to discover new potential probiotic features as the identified strain showed more efficient potentiality as probiotic than earlier data reported. Finally, these findings support the importance of discovering novel probiotics as a biotherapeutic cure to safely combat bacterial threats.

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Table. 1: Quantification of organic acid and determination of pH value of Y2c

Sources of bacteria	Incubation time (h)	Incubation temp. (°C)	Organic acid (%)	pH
Yoghurt	12	37	0.18	5.99
	24	37	0.49	5.33
	48	37	0.73	4.98
	72	37	0.76	4.93

Table 2: The percentage of autoaggregation of isolate Y2c

Isolate	Time (h)	OD (600nm) after A time (h)	Autoaggregation %
Y2c	0	1.851	0
	4	1.55	16.26
	12	0.531	71.31
	18	0.5	72.98

Table 3: Percentage of isolate Y2c coaggregation with test pathogens

Time (h)	*E. coli*	*B. cereus*	*MRSA*	*K. pneumonia*
4	12.9068	10.9393	14.166	14.17
12	52.0994	49.581	42.498	42.5
18	62.96	59.025	50.368	50.37

Table 4: Percentage of cell-surface hydrophobicity of Y2c

Selected isolate	Cell-surface hydrophobicity (%)
LAB Y2c isolate	38.0 ± 0.11

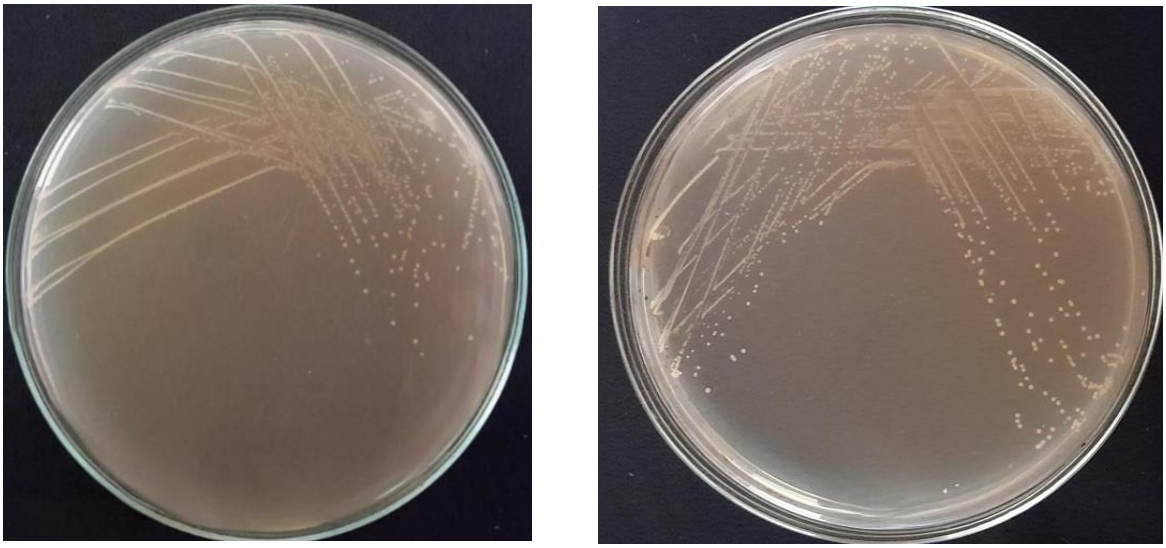

Figure 1: Purified promising LAB cultures on MRS after 24 h of incubation at 37°C

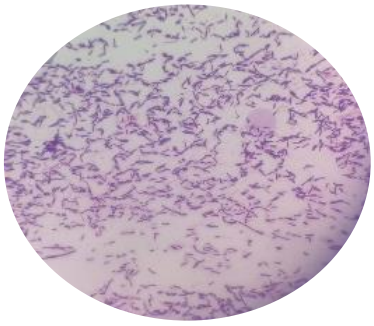

Figure 2: Shows Gram-positive rod-shaped bacteria under oil lens (1000x)

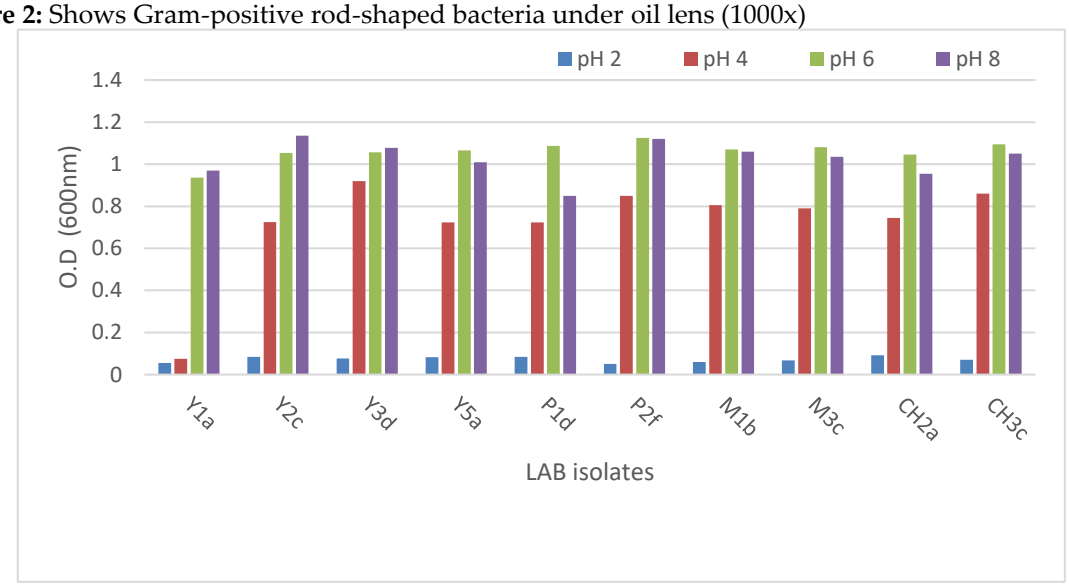

Figure 3: Effect of pH on the growth of LAB isolates

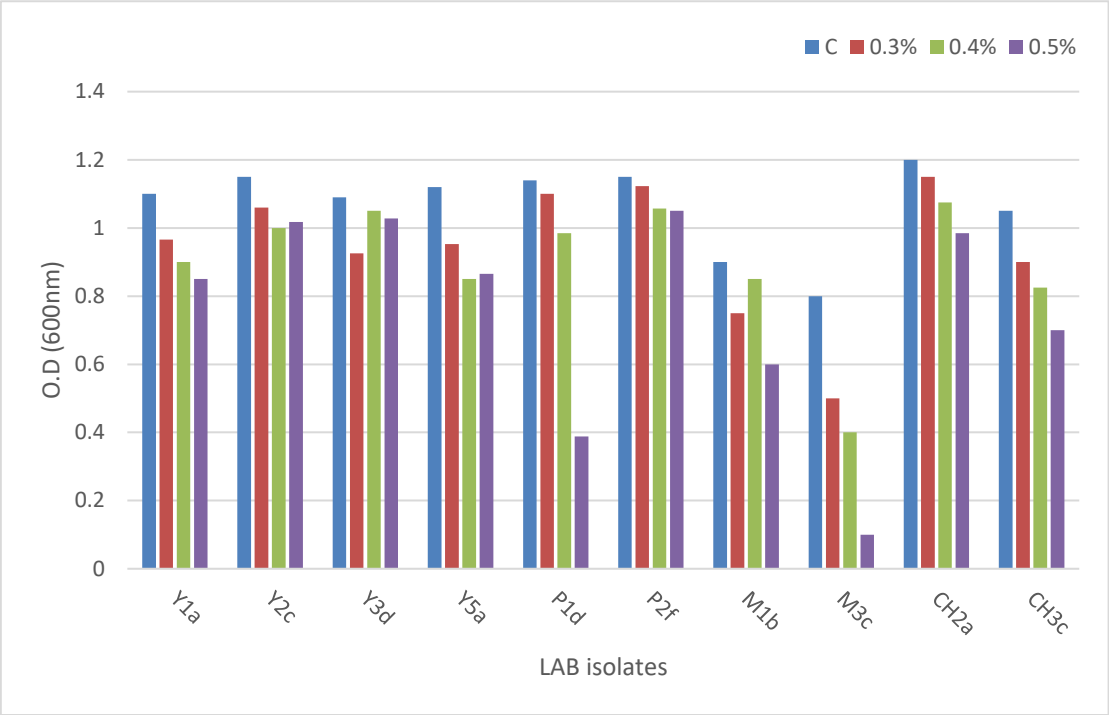

Figure 4: Influence of bile salts on the growth of LAB isolates

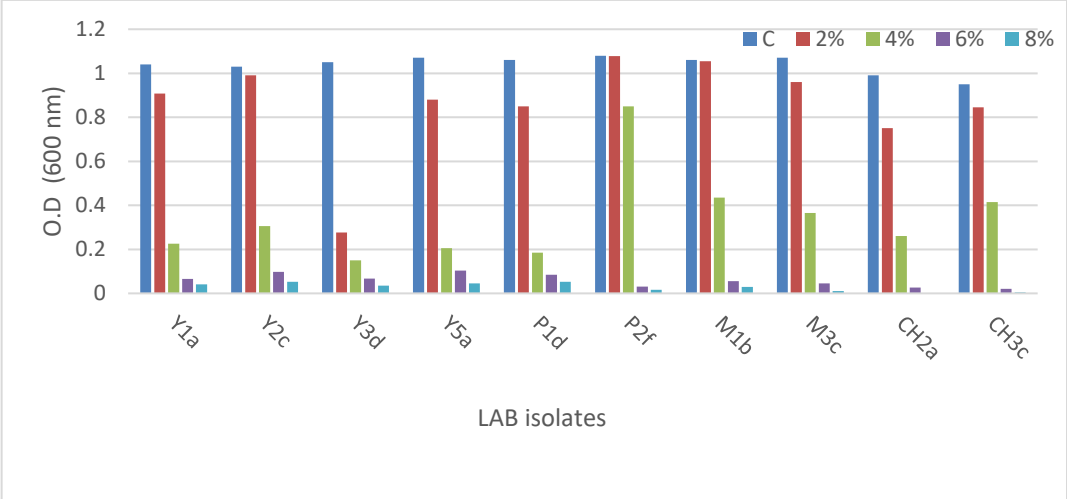

Figure 5: Effect of NaCl % on the growth of LAB isolates

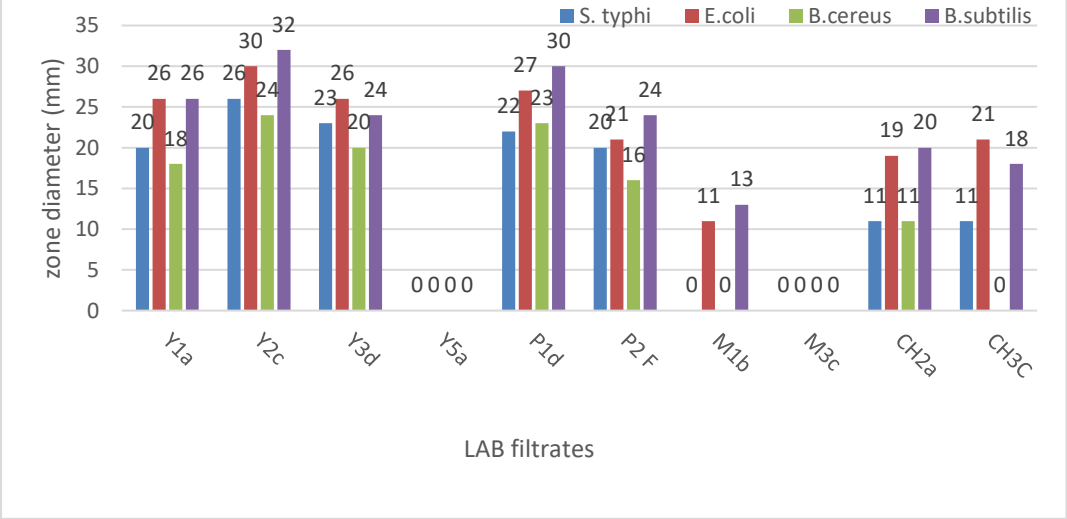

Figure 6: The antibacterial activity of the LAB isolates against test strains

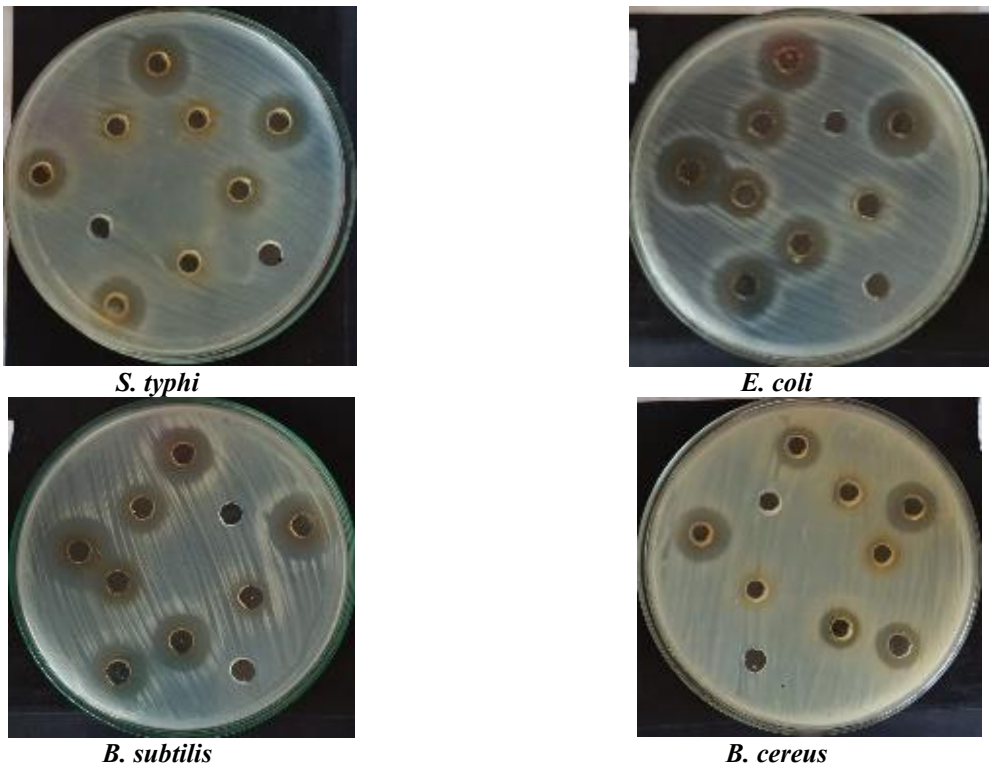

Figure 7: shows test organisms growth clear zones around LAB isolates filtrates

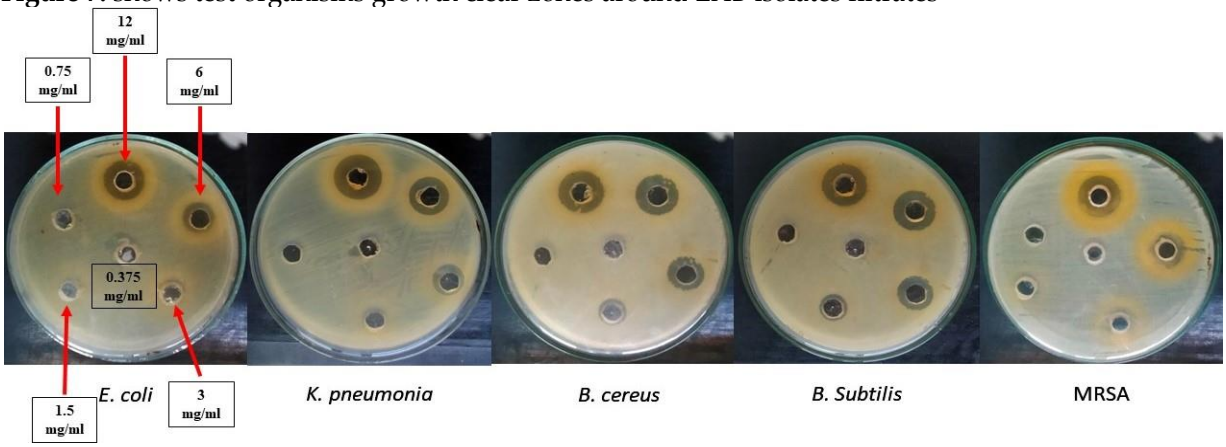

Figure 8: The minimum inhibitory concentration of Y2c filtrate against both Gram +/- bacteria

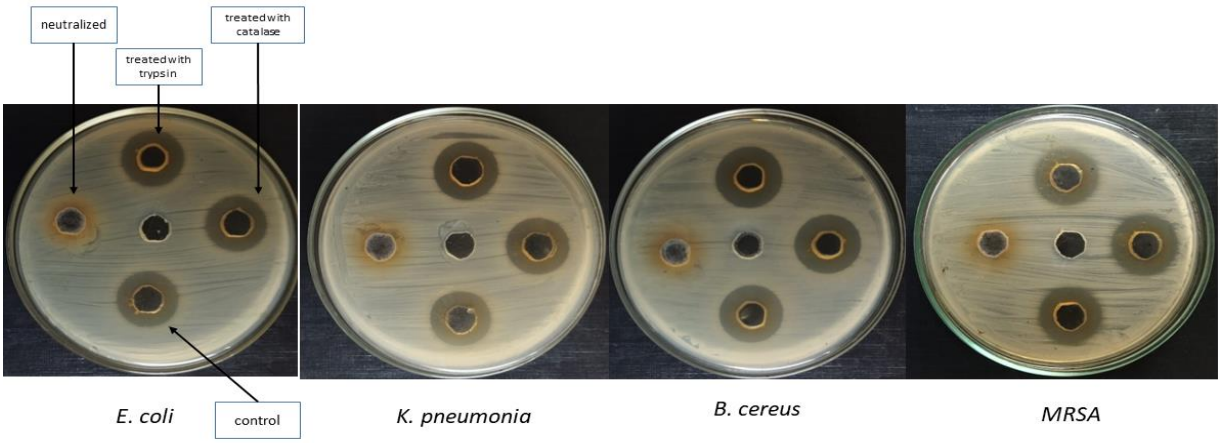

Figure 9: Characterization of the antimicrobial substances of Y2c isolate (well in the middle is for -ve control)

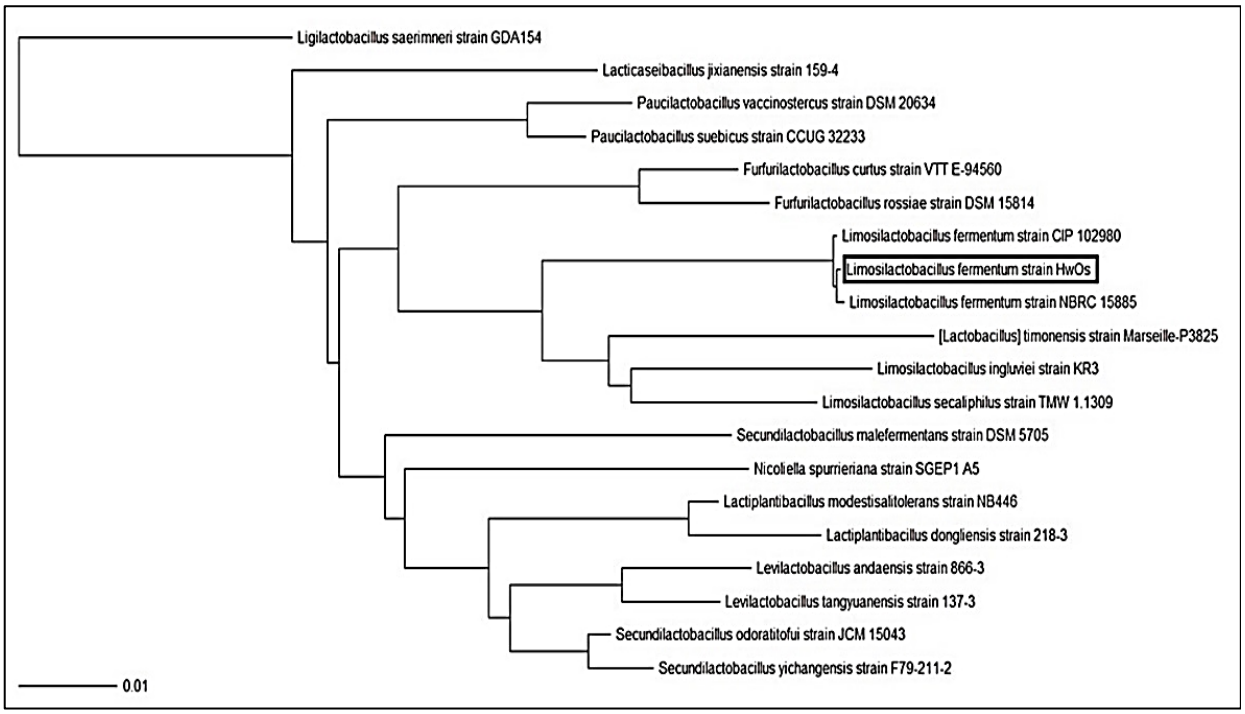

Figure 10: Neighbour joining Phylogenetic tree of *Limosilactobacillus fermentum* HwOs

بكتيريا حمض اللاكتيك بروبيوتيك: نهج بديل واعد للسيطرة على التهديدات البشرية

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الملخص

تم استخدام منتجات الألبان والمنتجات المخمرة من قبل البشر منذ العصور المبكرة وكانت مصدرًا مألوفًا لبكتيريا حمض اللاكتيك .(LAB) اكتسبت التأثيرات القيمة للبكتيريا بروبيوتيك القابلة للحياة كمكملات غذائية مذكرة بحثية ضخمة، .Lactobacillus sppتستخدم على نطاق واسع ذات خصائص البروبيوتيك لتحضير منتجات الألبان مثل الزبادي والجبن وما إلى ذلك. الهدف من هذه الدراسة هو تقييم أنواع LAB ذات الأنشطة المستقبلية، تم جمع 22 عينة من عينات الأغذية المتخمرة من الأسواق المحلية بالجيزة لعزل ميكروبات البروبيوتيك. من العينات، تم عزل 10 سلالاتLAB ، وتم اختيار العزلات الواعدة عن طريق الفحص الأولي باعتبارها بروبيوتيك محتمل مع نشاط مضاد للميكروبات ضد مسببات الأمراض البكتيرية. تم تشخيص عزلات LABمختارة في المختبر لنمطها البروبيوتيك وأنشطتها المضادة للميكروبات ضد مسببات الأمراض. وجد أن العزلات مقاومة لـ (6%) NaCl% والملح الصفراوي (0.5%) وأظهرت نمواً جيداً في الحالة الحمضية، في حين لوحظ أعلى نمو عند الرقم الهيدروجيني حوالي 6.0. أظهرت العزلة LAB الأكثر فعالية نشاطاً مضاداً للميكروبات ضد أربعة ممرضات مختلفة ذات قطر منطقة تثبيط (24-32) ملم. بالإضافة إلى ذلك، كان الحد الأدنى التداخلي لنفس العزلة (1.5-3 ملغم/مل) وهي نتيجة كبيرة بالمقارنة مع الدراسات التي أجريت في نفس المجال. وجد أن إنتاج الأحماض العضوية بكمية 0.76% هو المسؤول عن النشاط المضاد للميكروبات. كما أظهرت العزلات خصائص تجمعية وكارهة للماء ملحوظة. وفقا للنتائج، يعتقد أن العزلات LAB المختارة (Y2c) هي بكتيريا بروبيوتيك محتملة. بعد ذلك، تم تحديد عزلة Y2c بشكل تقليدي ووراثي وكشف أنها Limosilactobacillus vermentum وتم تسجيلها في GenBankتحت سلالة.Limosilactobacillus Fermentum HwOs

الكلمات الاسترشادية: البروبيوتيك، العصيات اللبنية، منتجات الألبان والمنتجات المخمرة، نشاط مضاد للميكروبات.