

Isolation and Identification of Potent Probiotics with High Lead Removal Capability

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PROBIOTICS play an effective and significant role in human health. The aim of this study was to isolate potential probiotics from Egyptian sources. Among the 14 different bacterial isolates recovered from 8 different sources on Man-Rogosa-Sharp (MRS) agar medium, 5 isolates exhibited tolerance to pH 3 and survived at bile concentration of 0.3% for 3h. The selected isolates were resistant to amikacin, vancomycin, ciprofloxacin and bacitracin. In addition, they showed high antibacterial activity against 7 pathogens. Hydrophobicity using xylene and toluene showed high hydrophobic property for M isolate with toluene and xylene ($80.43 \pm 0.95\%$ and $78.2 \pm 0.73\%$, respectively). The potent isolate was identified by 16S rRNA gene as *Lactobacillus plantarum* strain M (KY508300). This strain was able to remove $71.28 \pm 0.4\%$ of lead (1mg/ml) after 5min. The probiotic strain M showed count stability in yoghurt up to 21 days. The characteristic features of *L. plantarum* strain M as potent probiotics entitled it to be used in industrial and environmental applications.

Keywords: Probiotics, pH, Antimicrobial, Bile, Hydrophobicity, Lead.

Introduction

Probiotics are defined by FAO/WHO (2001) as live microorganisms that confer health benefits to the host when administered in adequate amounts (Rafael et al., 2015). There are many beneficial effects attributed to probiotics, including improvement of intestinal health such as prevention of antibiotic-associated diarrhea (AAD), preventing infectious diarrhea, treatment of lactose intolerance and eradication of *Helicobacter pylori* (Schmid et al., 2006). Other applications of probiotics include enhancement of the immune response, reduction of serum cholesterol, cancer prevention as well as radioprotectors (Khademi & Abdollahi, 2014).

Probiotics may exert their beneficial effects on the host health through several mechanisms: Adhesion to epithelial cells, colonization, production of biosurfactants, co-aggregation of pathogens, production of antagonistic metabolites (organic acids, hydrogen peroxide, bacteriocins and other inhibitory compounds), competition for nutrients and supporting the immune system (Pellegrino et al., 2018).

Lactic acid bacteria (LAB) are Gram-positive, catalase-negative, oxidase negative, non-sporulating microaerophilic bacteria whose main fermentation product from carbohydrates is lactate and they are important organisms recognized for their fermentative ability as well as their health and nutritional benefits (Gilliland, 1990 and Sablon et al., 2000). Many lactic acid bacteria including; *Lactobacillus* sp., *Bifidobacterium* sp., *Enterococcus* sp., *Streptococcus* sp., *Lactococcus* sp., *Propionibacterium* sp., *Pediococcus* sp., *Leuconostoc* sp., *Saccharomyces cerevisiae* are applied in probiotic products (Hatice, 2007). They produce various antimicrobial compounds such as organic acids, di-acetyl, hydrogen peroxide and bacteriocin or bactericidal proteins during lactic fermentations (Lindgren & Dobrogosz, 1990 and Sablon et al., 2000).

Due to the antimicrobial activities and human health benefits, many lactobacilli were referred as probiotics (Nishida et al., 2016). Lactobacilli exert strong antagonistic activities against many microorganisms, including food spoilage organisms and pathogens (Ogunbanwo et al., 2003 and Malheiros et al., 2015).

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Heavy metals are a unique group of naturally occurring compounds released into the environment by various processes including mining, smelting, Industry, agriculture and many activities generating heavy metals (Thevenon et al., 2011). Lead is one of the abundant heavy metal in the earth. It has a long history of human use in buildings, pipes, sinks, construction projects, batteries, radiation shielding, gasoline and paints (Gorospe & Gerstenberger, 2008). Lead toxicity cause neurobehavioral problems, mild fatigue, headaches, nausea, vomiting, impaired hemoglobin synthesis, impaired renal function and blindness (Gracia & Snodgrass, 2007). *Lactobacillus* sp. was reported to bind and sequester heavy metals via different mechanisms such as ion exchange with peptidoglycan and teichoic acid, precipitation through nucleation reactions and complexation with nitrogen and oxygen ligands (Monachese et al., 2012).

Yogurt is classified as dairy product that is produced by fermentation of lactic acid bacteria. Its production and consumption is growing continuously due to its therapeutic properties beside its high nutritive value. However, there are some studies recorded the poor survival of the probiotics in dairy products during storage and distribution, due to the overly produced lactic acid (Sun & Griffiths, 2000 and Shima et al., 2012).

This investigation was carried out to isolate probiotic strains with competitive features for industrial applications.

Materials and Methods

Sampling and isolation

Natural and commercial yogurt, baby stool, sausage, pickle, cow, sour milk and breast milk samples were collected in sterile screw-capped glass containers and transferred to the laboratory in insulated cooling bag for the microbiological analysis (Sharma et al., 2013; Tulumoglu et al., 2013 and Serrano-Niño et al., 2016). Ten folded dilutions in phosphate buffer saline solution (PBS) were carried out for all samples and then inoculated onto MRS agar plates. The plates were incubated at 37°C for 24h (Jafari et al., 2011).

Screening isolates for probiotic properties

Probiotic colonies were counted and purified by re-subculturing on MRS medium. (Ashraf et al., 2009 and Hoque et al., 2010). The pure

isolates were tested for the major probiotics criteria including; tolerance to low pH and bile, antibiotic resistance, the antimicrobial activity and hydrophobicity property.

pH tolerance

Isolates were evaluated for survival at low pH, as described by Shaikh & Shah (2013). Overnight active cultures in MRS broth (1.3×10^9 CFU/ml) were centrifuged for 10min at 5000rpm. Pellets were re-suspended in PBS solution adjusted at pH 3 and pH 6 (control) then incubated at 37°C for 3h. After incubation, 1% of each culture was inoculated into fresh MRS broth (pH 6) then incubated at 37°C for 24h. The growth was measured at OD₆₀₀.

Isolates that failed to tolerate pH 3 for 3h, were gradually adapted by successive overnight growing on MRS adjusted at different pH values; 6, 5, 4 and 3 (Sa´nchez et al., 2007).

Bile tolerance

Overnight cultures (10ml) were centrifugation at 5000rpm for 10min. The pellets were suspended in MRS broth containing 0.3% bile and incubated for 3h at 37°C. An inoculum was transferred (1%) to fresh MRS broth (without bile) then incubated at 37°C for 24h. The growth was measured at OD₆₀₀ (Shaikh & Shah, 2013).

Isolates that showed high tolerance for pH 3 and bile for 3h were selected for further experiments.

Antibiotic resistance assay

The selected isolates were tested for their resistance against 14 antibiotics using kirby bauer disc diffusion assay. The studied antibiotics were ampicillin (AM 10µg), amikacin (AK 30µg), cefotaxime (CTX 30µg), rifampin (RA 5µg), vancomycin (V 30µg), trimethoprim/sulphamethoxazole (SXT 25µg), streptomycin (S 10µg), erythromycin (E 15µg), gentamycin (GM 10 µg), ciprofloxacin (CIP 5µg), imipenem (IPM 10µg), chloramphenicol (C 30µg), amoxicillin/clavulanic acid (AMC 30µg) and bacitracin (B 10µg) (Chang et al., 2009). Plates containing MRS agar medium were inoculated with 1ml of overnight cultures (1.3×10^9 CFU/ml). After solidification, antibiotic discs were loaded onto the surface of the media. The plates were left in refrigerator for 2h then incubated for 24h at 37°C. The inhibition zones were recorded and tabulated (Amin et al., 2014).

Antibacterial activity

Antibacterial activity against different pathogenic bacteria was examined by the agar-well diffusion assay (Cheesbrough, 2000). The tested pathogenic bacteria (*Staphylococcus aureus*, *Streptococcus* sp., *Pseudomonas* sp., *Salmonella* sp., *Proteus* sp., *E. coli* and *Klebsiella pneumoniae*) were kindly supplied from Microbiology Department, Faculty of Science, Ain Shams University. Cell-free supernatants (CFS) of the selected bacterial isolates grown on MRS broth for 24h at 37°C were centrifuged at 5000rpm for 15min. A volume of 200µl of CFS was placed in 12mm diameter wells cut into nutrient agar medium. The plates were kept at 4°C for 2h to allow diffusion then incubated at 37°C for 24h. The inhibition zone was measured after incubation time (Akabanda et al., 2014).

Cell surface hydrophobicity

Cell surface hydrophobicity of isolates was determined by microbial adhesion to hydrocarbons (MATH) method using xylene and toluene as solvents (Amin et al., 2014). Isolates were grown in MRS broth (1%v/v of 1.5x10⁹ CFU/ml) for 24h at 37°C. Cells were collected by centrifugation at 5000rpm for 15min, then washed twice by phosphate urea magnesium sulfate (PUM) buffer (Duary et al., 2011) and finally resuspended in the same buffer to achieve initial absorbance (A_0) 0.7±0.02 at 600nm. Cell suspension (5ml) was mixed with 1ml of hydrocarbon (xylene or toluene) in dry rounded bottom test tubes. The contents were vortexed for 2min and left undisturbed for 1h at 37°C to allow phase separation. The lower aqueous phase was carefully separated with a sterile Pasteur pipette and the absorbance (A_1) was measured at 600nm. Cell surface hydrophobicity in terms of percent (H %) was calculated using the following equation:

$$H \% = (1 - A_1/A_0) \times 100$$

Identification of potential probiotic isolate by 16S-rDNA sequencing

Amplification of 16S rRNA gene was performed by a modified method of Massol-Deya et al. (1995). A loopful of overnight grown cells was transferred to 50µl TE buffer and boiled for 5min then, 1µl of cell suspension was used as template for PCR reaction. PCR was performed using Premix *Taq* (Ex *Taq* Version, Takara, Japan) according to instruction manual. A pair of flanking sequences was used for primer

binding sites to partially amplify target 16S rRNA gene from the bacterial isolates 16S 1F (5'-AGAGTTTGATCCTGGCTCAG-3') and 16S 517R (5'-ATTACCGCGGCTGCTGG-3'). Amplification reactions were performed in total volume of 25µl containing 1µl of each primer (10pmol), 12.5µl of Premix *Taq* and 1µl of DNA. PCR was performed in genius model FGENO2TD thermal cycler (Techne, England). The PCR conditions were adjusted to 5min for initial denaturation at 94°C then 35 cycles of 1min at 94°C, 1min at 54°C and 1min at 72°C, then finally 10min at 72°C. The amplified genes were run on 1% agarose gel and visualized by GelDoc. (InGenius 3).

Direct Sequencing of PCR product for 16S rRNA gene was carried out in GATC Company, Sigma. Nucleotide sequence were analyzed using Blastn (Altschul et al., 1997) (<http://www.ncbi.nlm.nih.gov/BLAST/>) to compare the nucleotide sequence with their similar sequences on the data base. Multiple sequence alignment was performed using ClustalW. Phylogenetic tree was constructed using neighbor-joining method of Saitou & Nei (1987) with MEGA 6 software (Tamura et al., 2013). Nucleotide sequence was submitted to *GenBank* to obtain the accession numbers.

Measurement of lead removal by the selected strain

Overnight culture of the selected strain grown on MRS broth was centrifuged at 5000rpm for 5min then washed twice with ultra-pure water. Bacterial pellet was resuspended in 2ml ultra-pure water containing 1mg/ml of Pb (lead acetate). The suspension was mixed thoroughly, and left for 5min at room temperature. After that, the tubes were centrifugation for 5min at 5000rpm. The supernatants were analyzed by Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (Teledyne Leeman, USA) (Halttunen et al., 2007). Operation condition of ICP-OES was RF power 1.2 KW, coolant gas flow 20L/min, auxiliary gas flow 0.3L/min, nebulizer gas flow 36psi, solution uptake rate 1ml/min, Mg II/MgI ration (robustness) 6, replicates 3, integration 10sec.

Yoghurt production and shelf time of the selected strain

Overnight culture of the selected strain was inoculated into sterile cups containing sterile

Juhayna milk and incubated at 37°C for 6h. The fermented milk (the produced yoghurt) was refrigerated and stored at 4°C, then the count of probiotic stain was recorded separately at 0, 7, 14 and 21 days. Ten folded serial dilutions of the manufactured yoghurt were prepared. One ml of each dilution was cultivated on MRS agar plate. All plates were incubated at 37°C for 24h the count was recorded.

Results

Isolation and screening of isolates for the probiotic properties

From the 8 sources, 14 bacterial isolates were recovered according to their morphological

characteristics on MRS medium. All isolates were screened for the following probiotic properties.

pH tolerance

The 14 selected bacterial isolates were tested for their tolerance to pH 3. Heavy growth was detected with 5 bacterial isolates M, St3, Sa2, Sa3 and Sa4 (Fig. 1).

Bile tolerance

The isolates that showed pH 3 tolerance were tested for their tolerance to bile (0.3%). All isolates showed bile tolerance, the results were illustrated in Fig. 2. Growth absorbance of the tolerated isolates ranged between 0.760 ± 0.010 and 1.093 ± 0.028 .

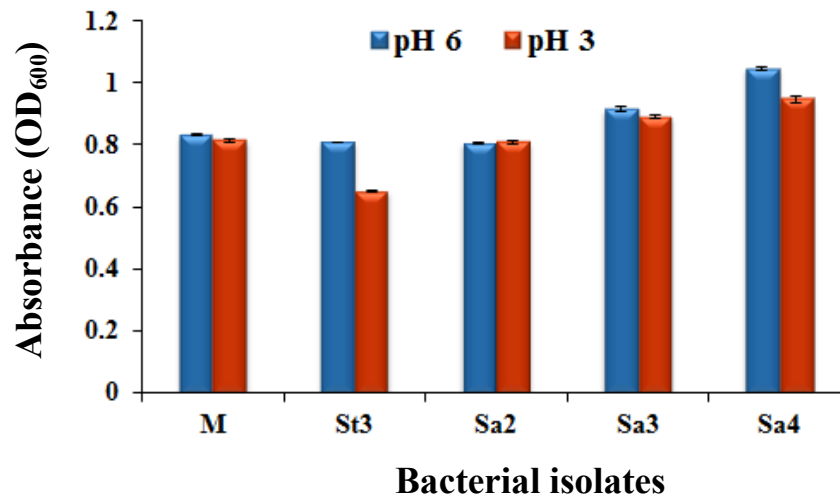


Fig. 1. pH tolerance of the selected bacterial isolates.

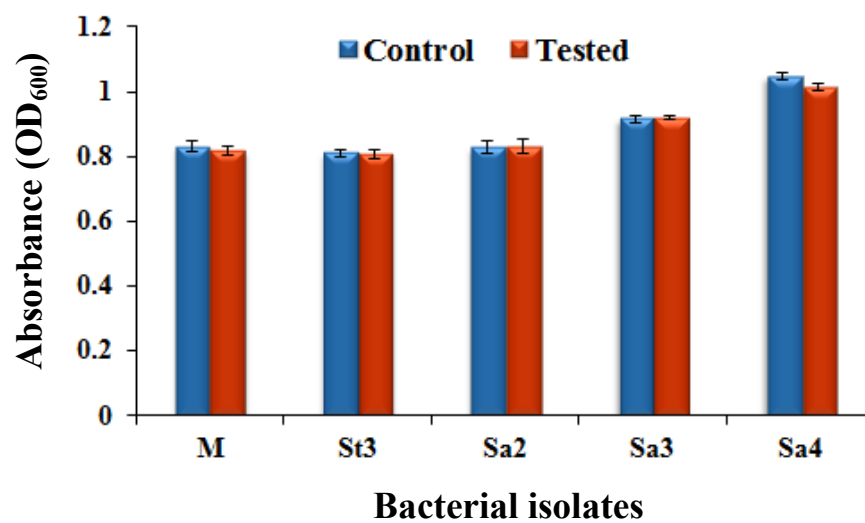


Fig. 2. Bile tolerance of the selected bacterial isolate.

Antibiotic resistance of the selected isolates

Isolates that showed tolerance towards acidity and bile were tested for their antibiotic resistance against 14 antibiotics. All bacterial isolates were resistant to amikacin, vancomycin, ciprofloxacin and bacitracin while sensitive to rifampin, trimethoprim/sulphamethoxazole, erythromycin, imipenem, chloramphenicol and amoxicillin/clavulanic acid. Also, isolates St3, Sa2 and Sa4 share a common resistance towards both streptomycin and gentamycin (Table 1).

Antibacterial activity

The antibacterial activities of the isolates were examined against 7 pathogenic bacteria (Fig. 3). All supernatants from the bacterial isolates showed inhibitory activity against the tested pathogens. *Staphylococcus aureus* was the most sensitive pathogen toward the supernatants of all bacterial isolates. Isolate M was the most potent isolate.

Cell surface hydrophobicity

Cell surface hydrophobicity of the selected isolates was determined by microbial adhesion to hydrocarbons using xylene and toluene as solvents. The highest hydrophobicity was recorded for M isolate with both solvents toluene and xylene (80.43±0.95% and 78.2±0.73%, respectively). While moderate hydrophobicity was detected with xylene for other isolates (Fig. 4).

Identification of the selected probiotic bacterial isolates by 16S rRNA gene

According to the results obtained from previous experiments, isolate M was selected as the most potent probiotics and identified by 16S rRNA gene as *Lactobacillus plantarum* (*L. plantarum*) with 99% similarity. The nucleotide sequence was submitted to *GenBank* under accession number KY508300.

Neighbor joining phylogenetic tree of 16S rRNA gene was constructed using related members of genus *Lactobacillus* and *Enterococcus durans* R03-16 as an out group. The phylogenetic tree revealed that isolate M was grouped with *Lactobacillus plantarum* strains with high bootstrap value (91). While other *Lactobacillus* spp. (*Lactobacillus casei* 0108, *Lactobacillus acidophilus* VDLB02 and *Lactobacillus pentosus* strain TEP12) were separated in another clade with bootstrap value 91 (Fig. 5).

TABLE 1. Antibiotic resistance of the selected isolates .

Bacterial isolate	Inhibition zone (mm)													
	Am	AK	CTX	RA	VA	SXT	S	E	GM	CIP	IPM	C	AMC	B
M	0	0	0	13±1.4	0	12±0.7	13±0.7	13±0.7	9±0.7	0	35±0.7	25±1.4	19±0.7	0
St3	0	0	13±1.4	11±0.7	0	13±1.4	0	11±0.7	0	0	34±1.4	18±1.1	23±1.4	0
Sa2	15±1.4	0	11±0.7	11±0.7	0	12±0.7	0	12±0	0	0	31±0.7	16±0.7	20±3.5	0
Sa3	16±0	0	26±0.7	14±0.7	0	16±0.7	8 ± 0.7	14±2.8	8 ±0.7	0	36±0.7	20±0	26±0.7	0
Sa4	11±0.7	0	14±0.7	12±0.7	0	14±0.7	0	15±0	0	0	31±0.7	18±0.7	22±0.7	0

Am: Ampicillin 10µg, AK: Amikacin 30µg, CTX: Cefotaxime 30µg, RA: Rifampin 5µg, VA: Vancomycin 30µg, SXT: Trimethoprim/sulphamethoxazole 25µg, S: Streptomycin 10µg, E: Erythromycin 15µg, GM: Gentamycin 10µg, CIP: Ciprofloxacin 5µg, IPM: Imipenem 10µg, C: Chloramphenicol 30µg, AMC: Amoxicillin/clavulanic acid 30µg, B: Bacitracin 10µg.

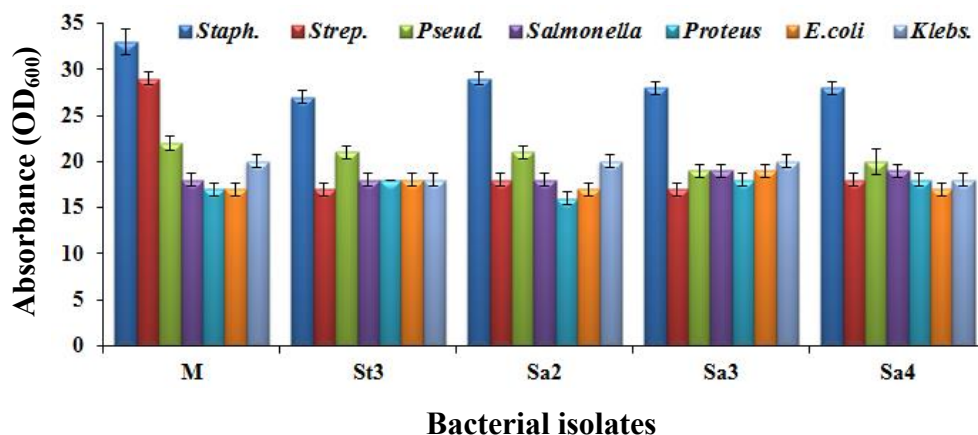


Fig. 3. Antimicrobial activities of the bacterial isolates against some pathogens.

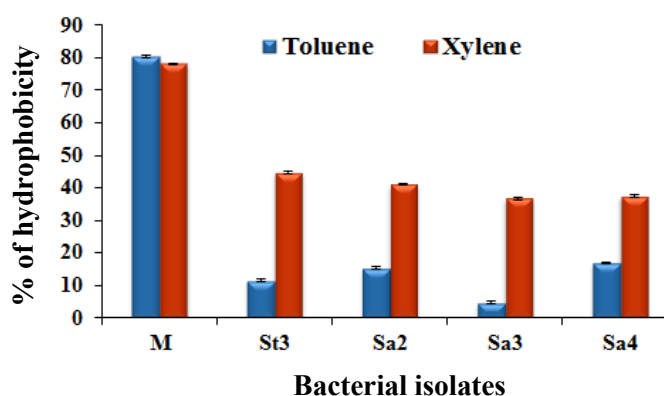


Fig. 4. Hydrophobicity percentages of the selected probiotic isolates using toluene and xylene.

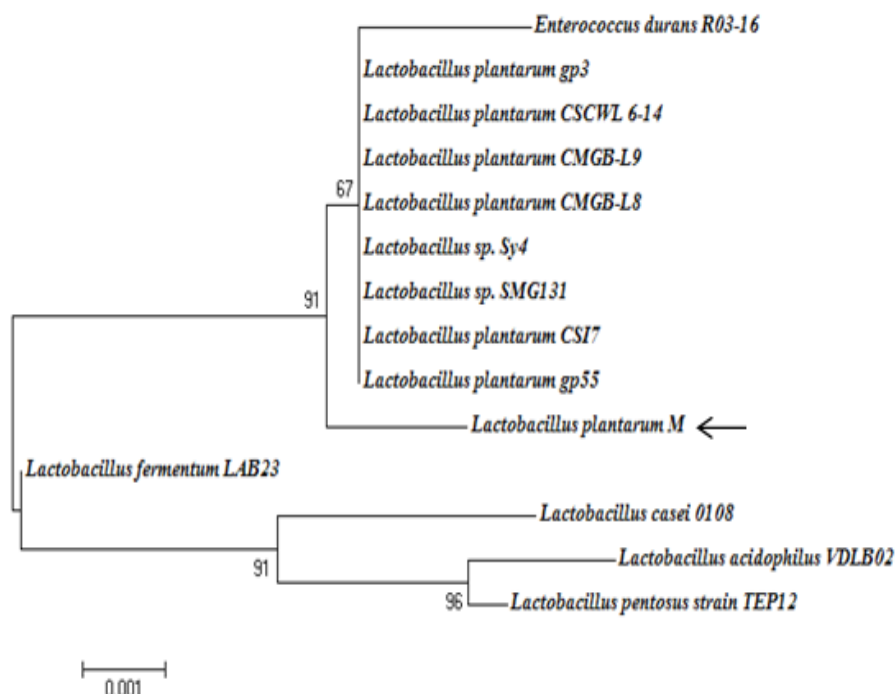


Fig. 5. Neighbor joining phylogenetic tree of 16S rRNA genes. The numbers at the nodes are bootstrap values recovered from 100 trees, the bar indicates 0.1% nucleotide substitution. Arrow indicate the identified strain in this study.

Lead removal by *L. plantarum* strain M

The ability of strain M to remove lead was calculated and the percentage of lead removal after 5min was 71.28 ± 0.4 .

Yoghurt production and shelf time of *L. plantarum* strain M

The ability of *L. plantarum* strain M to survive in a product was evaluated after milk fermentation and yoghurt production. The count of strain M was enumerated after 0, 7, 14 and 21 days in the formed yoghurt sample. The probiotic strain showed count stability in formed yoghurt after refrigeration for 7 and 14 while slight decrease started after 21 days (Fig. 6).

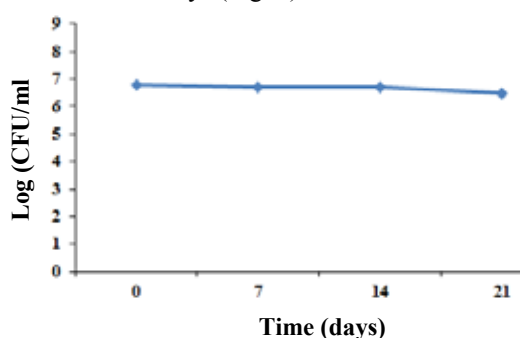


Fig. 6. Shelf time of *L. plantarum* strain M in the manufactured yoghurt sample.

Discussion

Probiotics were used in many different products worldwide. In addition to food products, probiotics were also used in pharmaceuticals and animal feed. The criteria used for *in vitro* selection of probiotics, include pH and bile tolerance that enable them to survive, grow in the gastrointestinal tract (GIT) (Amin et al., 2014).

In this study, 14 bacterial isolates from 8 different sources were grown on MRS agar medium. Only 5 bacterial isolates, M, St3, Sa2, Sa3 and Sa4 exhibited high tolerance to pH 3. The survival of isolates in low pH is very important for bearing initial stress in the stomach (pH 2-3) (Jafari et al., 2011). Meanwhile, all isolates grew and survived at bile concentration of 0.3% for 3h. This was consistent with Shaikh & Shah (2013) who demonstrated the tolerance of probiotic isolates to pH 3 and bile (0.3%).

The antimicrobial susceptibility as well as antibiotics resistance may serve as suitable criteria for probiotic culture selection (Tulumoglu et al.,

2013 and Curto et al., 2011). The susceptibility of all studied isolates towards different antibiotics was examined by agar disc diffusion method. The selected isolates showed resistance against amikacin, vancomycin, ciprofloxacin and bacitracin while, showed sensitivity against rifampin, trimethoprim/sulphamethoxazole, erythromycin, imipenem, chloramphenicol and amoxicillin/clavulanic acid. This was in accordance with Botes et al. (2008) who reported that *L. casei* Shirota was inhibited by several commercial antibiotics such as ciprofloxacin, amoxicillin, cefadroxil, roxithromycin, doxycycline and norfloxacin. Lactobacilli are usually sensitive to the cell wall-targeting penicillin and β -lactamase but resistant to cephalosporins (Gueimonde et al., 2013). Many *Lactobacillus* species showed a high resistance to vancomycin. Most inhibitors of nucleic acid synthesis seem to have a low inhibitory effect among most *Lactobacillus* species. On the other hand, lactobacilli were generally susceptible to low concentrations of many protein synthesis inhibitors, such as chloramphenicol and tetracycline due to the presence of their resistant genes that sometimes found in combination with each other (Ammor et al., 2008).

Since probiotic bacteria played a significant role in human health through control of pathogenic strains in the digestive system and improving the immune system (Noori et al., 2016), the antibacterial activity is one of the main features of probiotics. Accordingly, the selected bacterial isolates were tested for their antibacterial activity against seven pathogens; *Staphylococcus aureus*, *Streptococcus* sp., *Pseudomonas* sp., *Salmonella* sp., *Proteus* sp., *Escherichia coli* and *Klebsiella pneumonia*. The isolates showed high antibacterial activity against all pathogens. This was consistent with Tulumoglu et al. (2013) who observed the antibacterial activity of probiotic isolates against *E. coli*, *P. aeruginosa* and *S. aureus*. The antibacterial activity of probiotic strains may be due to pH levels, competition for substrates, the production of substances with a bactericidal or bacteriostatic action, including bacteriocins and bacteriocin-like substances (Pan et al., 2009). The antimicrobial metabolites as a primary metabolic end-product such as lactic acid and acetic acid, diacetyl, hydrogen peroxide, carbon dioxide, aldehydes and other metabolites also act as inhibitory compounds for pathogenic bacteria (Yuksekdag et al., 2004).

Hydrophobicity is considered a potential probiotic marker along with other criteria for screening of novel probiotic isolates that can adhere to human intestinal cells (Duary et al., 2011). Cell surface hydrophobicity of the selected isolates was performed by MATH method to determine the cell surface characteristics. M isolate showed high hydrophobicity percentage to toluene (80.43±0.95%) and xylene (78.2±0.73%). This hydrophobicity percentage was higher than previously reported by Duary et al. (2011) and Amin et al. (2014) who demonstrated lower hydrophobicity percentages for some *Lactobacillus* species as 34% for xylene and 34 to 42% for toluene.

The most potent probiotic isolates M was identified by 16S rRNA gene as *Lactobacillus plantarum* strain M (KY508300). Previous reports demonstrated that *L. plantarum* was isolated from fully ripened, white brined Bulgarian homemade cheeses (Georgieva et al., 2008).

Probiotics have the capacity to bind many toxic heavy metals therefore, was used in bioremediation of environmentally contaminated sites (Srivastava et al., 2012). The concentration used in the present study (1mg/ml) exceeds the normal food residue level (0.01 to 0.1 µg/ml) by 10 folds. The results demonstrated that high capacity of strain M to remove lead from aqueous solution in short time. The strain M elucidated high removal percentage (71.28±0.4) of lead in 5min. Previous report by Yin et al. (2016) demonstrated lower removal percentage (36.66±1.01%) of lead by *Lactobacillus plantarum* CCFM8661.

The ability of the probiotics to survive in food products is one of the important factors to be considered after addition of the probiotics to food till delivery to consumer gut. Effective probiotics depend on their stability during production, formulation and storage conditions (Noori et al., 2016). Yogurt has been introduced as a safe and healthy food. The shelf life of yogurt was strongly affected by the cell number of lactic acid bacteria (Kim et al., 2009). During storage and distribution, the cell number significantly decreases due to the overproduced lactic acid (Sun & Griffiths, 2000). In this study, strain M showed stability of the cell number after yoghurt formation and during refrigeration up to 21 days. After that a slight decrease in count was observed which may be due to the lowering in pH as a result

of organic acids production as well as cold storage of yogurt (Kim et al., 2009). The potent Egyptian strain is promising probiotics for industrial and bioremediation applications.

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عزل وتعريف عزلات بروبيوتيك قوية ذات قدرة عالية فى التخلص من الرصاص

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تلعب البروبيوتيك دورا هاما وفعالا فى صحة الإنسان. تهدف هذه الدراسة إلى عزل بروبيوتيك قوية من مصادر مصرية. من بين 14 عزله بكتيرية مختلفه تم عزلها من 8 مصادر مختلفة على وسط الأجار (Man Rogosa Sharp)، أظهرت 5 عزلات مقاومة لدرجة الحموضة 3 كما تحملت تركيز 0.3% من العصاره الصفراوية لمدة 3 ساعات. قاومت العزلات المختاره المضادات الحيوية مثل الاميكاسين، فانكوميسين، سيبروفلوكساسين و باسيتراسين. بالإضافة إلى ذلك أظهرت العزلات نشاط ضد بكتيرى قوى ضد 7 من مسببات الأمراض. بتقييم خاصيه الكراهيه للماء باستخدام الزيلين والتولوين، اوضحت العزله M كراهيه عاليه للماء مع كلا من الزيلين والتولوين (80.43±0.95% و 78.2±0.73% على التوالي). تم تعريف العزله القوية باستخدام 16S rRNA على انها *Lactobacillus plantarum* strain M KY508300. استطاعت هذه العزله التخلص من 71.28±0.4% من الرصاص (1ملجرام/مل) بعد 5 دقائق. كما اوضحت عزل البروبيوتيك M ثبات عددي فى الزبدي لمدة 21 يوم. هذه الصفات المميزة للعزله M تؤهلها للاستخدام كبروبيوتيك قوى فى التطبيقات الصناعية والبيئية.