JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

IN VITRO EVALUATION OF PROBIOTIC POTENTIAL OF *Lactiplantibacillus plantarum* TRG1 ISOLATED FROM AN ALGERIAN'S GOAT RUMEN

*Tarek Khennouf**[1,2], *Amina Bouchefra*[2], *Tayeb Idoui*[2]

Address(es):
[1] Department of Microbiology, Faculty of Nature and Life Sciences, University of Bejaia, 06000 Bejaia, Algeria.
[2] Laboratory of Biotechnology, Environment and Health, University Mohamed Seddik Benyahia of Jijel, 18000 Jijel, Algeria.

*Corresponding author: khennouf.t@gmail.com

https://doi.org/10.55251/jmbfs.4278

ARTICLE INFO

Received 29. 1. 2021
Revised 4. 7. 2023
Accepted 4. 7. 2023
Published 1. 10. 2023

Regular article

OPEN ACCESS

ABSTRACT

This present paper is intended to demonstrate the desirable probiotic properties of *Lactiplantibacillus plantarum* TRG1 isolated from the Algerian goat rumen. The study focused on assessing the strain's survival rate in stress conditions, namely in phenol 0.4%, bile salts 0.3%, in saliva and the Gastrointestinal tract (GIT) simulated conditions. The adhesion ability of the strain with GIT cells, cell surface properties, the production of EPS and β-galactosidase activity were also analyzed. Besides, biogenic amines production, sensitivity to antibiotics and hemolytic activity were evaluated as safety properties. The results showed that the strain can tolerate phenol 0.4% (13.88 ± 2.54%), bile salts 0.3% (10.91± 0.71%), and displayed good viability in saliva and different parts of the GIT. Viability rates were 99.26 % in saliva, 69.68 % in gastric juice, 37.4 % in the ileum. In the duodenum, 93.09 % of viability was attained after 90 min and 53.73% after 180 min of incubation. Furthermore, the strain displayed good adhesion and acceptable surface properties: hydrophobicity (76.94 ±3.36%), auto-aggregation (29.52% ± 6.95), and co-aggregation with *E. coli* (15.38 ± 05.43), *P. aeruginosa* (16.05 ± 2.14) and *S. aureus* (17.33 ± 2.31). A considerable amount of EPS (942.50 ± 32.78 mg/l) was noticed too. Results also showed that the strain did not present hemolytic activity while it can produce the β-galactosidase enzyme. The strain displaced sensitivity to nitroxolin and tetracycline and is resistant to other antibiotics.

Keywords: Adhesion, exopolysaccharide, goat rumen, *Lactiplantibacillus plantarum*, probiotic

INTRODUCTION

The probiotic concept was defined for the first time by Lilly and Stillwell in 1965 as a substance produced by a microorganism stimulating the growth of another microorganism **(Lilly and Stillwell, 1965)**. After several definitions have been proposed, it is only in 2002 that the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) gave an official definition of probiotics, which defines them as: live microorganisms which when administered in adequate amounts confer a health benefit on the host **(FAO/WHO, 2002; Vasiljevic and Shah, 2008)**. To be considered as probiotics, lactic acid bacteria (LAB) should meet some functional, technological, physiological and safety criteria, namely: the ability to survive in gut conditions, like gastric acid juice and bile, good adhesion to the mucosal surface, validated and documented health effects, immunomodulation ability, the strain must be genetically stable, desired viability during processing and storage, phages resistant and antagonistic activity towards gastrointestinal pathogens. Moreover, LAB should be Generally Recognized As Safe (GRAS), should be free from virulence, hemolytic activity, enterotoxins and free from antibiotic resistance genes, as well the strains should be free from biogenic amines production activity **(Ammor and Mayo, 2007; de Melo Pereira *et al.*, 2018; Vinderola *et al.*, 2017)**. The consumption of LAB as probiotics has proven a beneficial effect on human health, they can be an anti-cholesterol activity, alleviate the risk of some cancers **(de Melo Pereira *et al.*, 2018)**, moreover, reduce lactose intolerance, prevent and treat diarrhea symptoms, relieve and prevent allergy, inhibit *Helicobacter pylori* and intestinal pathogens, prevent inflammatory bowel disease and modulate the immune system **(Vasiljevic *et al.*, 2008)**. Moreover, LAB are used also in several food fermentations both to provide good organoleptic characteristics and to preserve different food preparations **(Melgar-Lalanne *et al.*, 2013)**.
The objective of the current paper was to assess some *in vitro* probiotic capabilities of the strain *Lactiplantibacillus plantarum* TRG1, previously isolated from goat's rumen including growth in 0.3% bile salts and 0.4% of phenol, the growth in gastrointestinal tract (GIT) conditions, surface properties and adhesion, production of exopolysaccharides (EPS) and biogenic amines, then finally the study of β-galactosidase, antibiotic resistance and hemolytic activity.

MATERIAL AND METHODS

Strain isolation and Identification

Several strains were isolated from different ecosystems, the *Lactiplantibacillus* strain used in this study was isolated in May 2013 from the rumen of the local goat (Jijel, Algeria) and was grown on MRS broth (Biokar Diagnostics, Allonne, France). To isolate strains, from the decimal dilutions prepared from the ruminal product, 0.1 ml was plated on MRS agar and then incubation at 37°C from 24 to 48 h in anaerobic conditions. After the isolation and purification of the strains we looked for Gram-positive and negative catalase bacilli, characteristics of lactic acid bacteria bacilli. The identification of this strain was carried out by sequencing the 16S rRNA gene. The DNA was extracted from an overnight culture using the Instagene matrix (Bio-Rad Laboratories, Italy) using the instructions of the manufacturer. The 16S rRNA gene sequence was amplified using the primers LpigF/LpigR (5'-TACGGGAGGCAGCAGTAG-3' and 5'-CATGGTGTGACGGGCGGT-3', Eurofins MWG Operon, Germany). The PCR amplification was carried out using the Taq DNA polymerase kit (Fisher Scientific, Italy). The constitution of the mixed reaction was, Buffer 5X, 2 mM $MgCl_2$, 50 mM, each of the four deoxynucleotide triphosphates (dNTP), 1.25U of Taq polymerase, 0.5 mM of each primer, and 0.5 µl of bacterial DNA. BiometraT3000 thermocycler (Germany) was used for DNA amplification. The PCR products were sequenced using the same primers **(Montanari *et al.*, 2015)**.

Artificial saliva simulation

Artificial saliva (PBS in the presence of 0.1 mg/l of lysozyme and 1.0 mg/l of α-amylase) sterilized using a Millipore filter (0.2 µm) was inoculated with 10% of the cell suspension of TRG1 and incubated at 37°C and 50 rpm in a shaker incubator for 15 min. Microbial count (at 0 and 15 min) was performed after incubation on MRS agar for 48 hours at 37°C. Results were expressed as log CFU/ml and survival rate as percent survival (%), where, the survival rate (%) = $\log N / \log N_0 \times 100$, ($N_0$ was the cell number at time 0 min and N, was the cell number after 15 min of incubation) **(Melgar-Lalanne *et al.*, 2013)**.

Gastrointestinal tract simulation

The viability of TRG1 in artificial gastric juice was carried out using **de Valdez and Taranto (2001)** method with minor modifications. The artificial gastric juice: 2.0 g/l NaCl, 3.2 g/l pepsin, adjusted to a final pH 2.0-2.2 and sterilized by filtration through a 0.2 µm membrane (Millipore), was inoculated with 10% of microbial cell suspension and incubated for 3 hours in a shaker incubator at 37°C and 150 rpm. Microbial count at 0 and 90min was performed after the incubation on MRS agar for 48 hours at 37°C. Results were expressed as CFU/ml and survival rate as described above. Duodenum and ileum simulations were carried out according to the method described by **Melgar-Lalanne *et al.* (2013)**. A pancreatic–bile suspension (1.9 mg/ml pancreatin, 5 mg/ml bile salts, 9 g/L NaCl), adjusted to pH 5.0 (duodenum simulation) or 6.5 (ileum simulation) with HCl 1N and sterilized by filtration using a 0.2 µm membrane, was inoculated with 10% of bacterial cell suspension and incubated at 37°C and 45 rpm in a shaker incubator for 30 min for ileum simulation and 180 min for duodenum simulation. The microbial count was carried out on MRS agar and incubated for 48 hours at 37°C. Results were expressed as CFU/ml and survival rate (%).

Growth in 0.3% bile salts and 0.4% of phenol

Bile salt tolerance was measured according to the method described by **(Lin *et al.*, 2007)**. MRS broth with 0.3% of bile salts (Institut Pasteur, Algeria) was inoculated with 1% of an overnight culture of TRG1, incubated for four hours at 37°C. MRS without bile salts was used as a control. OD at 600 nm of each MRS broth was measured after incubation time. Survival rate (%) was expressed as the percentage of the growth of the strain in the presence of bile salts compared to the control. Resistance to phenol was performed according to the method of **(Ji *et al.*, 2013)**. MRS broth with 0.4% of phenol was inoculated with 1% of an overnight culture then incubated for four hours at 37°C. MRS broth without phenol was used as control. The bacterial growth was measured at 600 nm and the results were expressed as the percentage of growth in the presence of 0.4% phenol compared to the control.

Cell surface hydrophobicity

An overnight culture of TRG1 was harvested by centrifugation at 10000×g for 5 min, washed twice with PBS buffer (pH 6.5) then resuspended in the same buffer. The initial absorbance was adjusted to 1.0 at 450 nm ($OD_{initial}$), then 0.6 ml of a hydrocarbon was added to 3 ml of the cell suspension, the mixture was incubated for 10 min at 37°C followed by vortexing for 2 min and allowed to stand for 15 min. The aqueous phase was taken, and the final absorbance was measured (OD_{final}) using a spectrophotometer (SPECORD® 50 plus, Analytik Jena, Germany). The used hydrocarbons were ethyl acetate, chloroform (BIOCHEM Chemopharma, France), and xylene (Sigma-Aldrich, Germany). The percentage of cell surface hydrophobicity was calculated using the following equation:

$$\text{Cell surface hydrophobicity (\%)} = \frac{OD_{initial} - OD_{final}}{OD_{initial}} \times 100.$$

Where $OD_{initial}$ and OD_{final} were the absorbances before and after using hydrocarbons **(Iyer *et al.*, 2010)**.

Auto-aggregation assay

Auto-aggregation was measured using the method described by **Tomás *et al.* (2005)**. An overnight culture of TRG1 was centrifuged at 6000×g for 15 min, washed twice with PBS and then resuspended in the same buffer. The initial absorbance was adjusted to 0.6 ± 0.05 at 600 nm (OD_0). The absorbance of the suspension was measured after four hours of incubation at room temperature without agitation (OD_{final}) and the auto-aggregation of the cell strain was calculated using this equation:

$$\text{Auto} - \text{aggregation (\%)} = \frac{OD_0 - OD_{final}}{OD_0} \times 100.$$

Where OD_0 was the OD at time t = 0 hour and OD_{final} was the OD after four hours of incubation.

Co-aggregation assay

Co-aggregation of TRG1 with three pathogenic bacteria, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Laboratory of Biotechnology, Environment, and Health, University of Jijel, Algeria), was carried out using the method of **Collado *et al.* (2008)**. TRG1 and pathogenic bacterial cell suspension were harvested separately by centrifugation at 6000×g for 15 min, washed twice with PBS buffer, and resuspended in the same buffer, and then the absorbance was adjusted to 0.25 ± 0.05 at 600 nm for each strain. An equal volume of TRG1 and each pathogenic bacterium were mixed and incubated at 37°C. After four hours of incubation, co-aggregation was calculated using this equation:

Co-aggregation (%) = $[[(OD_{TRG1} + OD_{path})/2 - OD_{mix}] / [(OD_{TRG1} + OD_{path})/2]] \times 100$. Where OD_{TRG1} is the OD of *Lactiplantibacillus plantarum* TRG1, OD_{path} is the OD of pathogenic bacteria, OD_{mix} is the OD of the mixture of TRG1 and the pathogenic bacteria.

Exopolysaccharide (EPS) production

Isolation of EPS produced by TRG1 was carried out according to the method described by **Lai *et al.* (2014)**. MRS broth was inoculated with TRG1 and incubated at 37°C for 24 hours. Trichloroacetic acid (TCA) 50% was added to the culture to give a final concentration of 4% and allowed to stand for 30 min at room temperature. The culture was harvested by centrifugation at 10000×g for 20 min at 4°C to precipitate cells and proteins. To one volume of the supernatant, two volumes of cold ethanol were added and stored 24 hours at 4°C to precipitate EPS. EPS was collected by centrifugation at 10000×g for 20 min. The recovered pellet was dissolved in water. Quantification of EPS was carried out by the phenol-sulfuric acid method as described by **Dubois *et al.* (1956)**. MRS broth supplemented with 2% of different carbon sources (glucose, sucrose, fructose, maltose, and galactose) was used to study the production of EPS.

β-galactosidase activity

The β-galactosidase activity was carried out using the method of **(Jeronymo Ceneviva *et al.*, 2014)**. Briefly, an overnight culture was harvested by centrifugation (6000×g), the pellet was washed twice with PBS buffer then resuspended in the same buffer. The disk of *O*-nitrophenyl-β-D-galactopyranoside (ONPG) was added to the cell suspension. The yellow color indicates the release of *O*-nitrophenyl due to β-galactosidase activity.

Adherence to epithelial cells

Adherence to epithelial cells was performed according to the method of **(Lin *et al.*, 2007)**. Briefly, to prepare epithelial tissue, an ileum segment of chicken was opened and washed with PBS (pH 7.2), allowed to stand in the same buffer for 30 min at 4°C to eliminate the mucus, then washed up to five times with PBS. Epithelial cells were scraped into PBS, the suspension should be examined for gut contaminating bacteria. To prepare bacterial cells, an overnight culture of TRG1 was centrifuged at 6000×g for 10 min, washed twice with PBS, and resuspended in the same buffer. The cell number was adjusted approximately to 1×10^8 CFU/ml. One milliliter of epithelial cells was mixed with one milliliter of bacterial cell suspension in a glass tube. The mixture was incubated at 37°C for 30 min in a shaker incubator. The adhesion ability of bacterial cells to epithelial tissue was observed using a light microscope (magnification ×100, Olympus, Philippines) after staining with crystal violet (5%). An epithelial cell smear was done as a control to confirm the absence of the native gut bacteria.

Biogenic amines production

Biogenic amines production was carried out in two steps, a screening on agar medium and confirmation of production using HPLC. The screening of biogenic amines production from four amino-acids, histidine, lysine, tyrosine, and ornithine was performed using the agar medium described by **Maijala (1993)**, where the composition was: 5.0 g tryptone, 8.0 g meat extract, 4.0 g yeast extract, 0.5 g Tween 80, 0.2 g $MgSO_4$, 0.05 g $MnSO_4$, 0.04 g $FeSO_4$, 0.1 g $CaCO_3$, 0.06 g bromocresol purple as pH indicator, 20.0 g agar and 20 g of amino acid, the pH was adjusted to 5.3 and the ingredients were mixed in 1 liter of distilled water then autoclaved at 120°C for 10 min. The medium was inoculated by the strain, the purple color of bromocresol displayed after 24 hours of incubation at 37ºC, indicates the activity of decarboxylase, hence the production of biogenic amines. For biogenic amines production, the broth medium described above supplemented with 2% of each amino acid was inoculated with an overnight culture of TRG1 and incubated at 37°C for 24 hours. For HPLC analysis, the extraction and derivatization of biogenic amines were carried out using the method described by **Martuscelli *et al.* (2000)**. Bacterial biogenic amines production was analyzed using a PU-2089 Intelligent HPLC quaternary pump, Intelligent UV-VIS multiwavelength detector UV 2070 Plus (Jasco Corporation, Tokyo, Japan), and a manual Rheodyne injector equipped with a 20 µl loop. An Analytical Cartridge Waters Spherisorb ODS-2, 150 x 4.6 mm, 3 µm column (Waters Corporation, Milford, MA, USA), coupled with Guard Cartridge Waters Spherisorb S5 ODS2, column, 4.6 x 10 mm (Waters Corporation), was used for chromatographic separation. The calibration curve was prepared using histamine standards.

Hemolytic activity

Hemolytic activity was assayed on a Columbia agar plate (Institut Pasteur, Algeria) containing 5% of fresh blood from laboratory rats and spotted with an overnight culture. The plate was incubated at 37°C for 48 hours. Hemolytic activity was recorded by the observation of a clear zone of hemolysis around the colony (β hemolysis), green zone (α hemolysis), and no zone around the colony (γ hemolysis) **(Arena *et al.*, 2014)**.

Antibiotic Resistance

For antibiotic resistance, the disc diffusion method was used. An overnight culture of *Lactiplantibacillus plantarum* TRG1 was standardized at 0.5 McFarland at 600 nm, and then plated evenly over the entire surface of the MRS agar using a sterile cotton wool swab. Antibiotic discs of kanamycin (30µg), ciprofloxacin (5µg), tetracycline (30 µg), gentamicin (10 µg), streptomycin (10 µg), and nitroxoline (30 µg) were placed on MRS agar and incubated for 24 hours at 37°C. The diameter (mm) of the zone of inhibition around the discs was measured. The sensitivity/resistance to antibiotics was carried out according to the Antibiogram Committee of the French Microbiology Society (Comité de l'Antibiogramme de la Société Française de Microbiologie in French) **(SFM, 2020)**.

RESULTS AND DISCUSSION

Strain Identification

The obtained strain's sequence was analyzed by the BLAST program and was then deposited in GenBank (accession number MG770887). The strain was identified as *Lactiplantibacillus plantarum* TRG1. The strain stored at -20°C in MRS broth supplemented with 20% of glycerol.

Artificial saliva and GIT simulation

In oral cavity simulated conditions, we noticed a very slight decrease in the survival rate of the strain as illustrated in Table 1, while in stomach simulated conditions, the strain displayed a very good resistance to the gastric juice (pH 2) after 90 min of incubation. Also, in the ileum simulation, TRG1 displayed a good tolerance after 30 min of incubation and in the small intestine simulated conditions, the strain showed high viability after 90 min and even after 180 min of incubation (Table 1). The obtained results revealed that TRG1 tolerated stress conditions, as stomach acidic pH, bile salts, and enzymes (α-amylase, lysozyme, pepsin and pancreatin) from the oral cavity and along with the GIT. This resistance is required for the LAB to survive in GIT, and it is one of the very important selection criteria of probiotic microorganisms **(de Melo Pereira *et al.*, 2018)**. Tolerance to GIT stress involves several mechanisms as cell membrane functionality maintain, intracellular pH, and the stability of nucleic acids and proteins as reported by **(Sirichokchatchawan *et al.*, 2018)**. Besides, the strain's production of EPS is also one of the mechanisms used by LAB bacteria to tolerate GIT conditions as demonstrated by **(Bengoa *et al.*, 2018)**, which reported also that this resistance is strain-dependent. Therefore, the strain meets the viability criterion in GIT.

Table 1 Viability of *Lactiplantibacillus plantarum* TRG1 in gastrointestinal conditions simulation

	Time (minutes)	Viable counts[a] (log CFU/ml)	Survival rate (%)
Oral cavity	0	07.42 ± 0.60	
	15	07.38 ± 0.12	99.26 %
Gastric juice	0	12.47	
	90	08.69	69.68 %
Duodenum	0	06.30 ± 0.42	
	90	05.86 ± 0.12	93.09 %
	180	03.38 ± 0.12	53.73 %
Ileum	0	05.65 ± 0.021	
	30	02.11 ± 0.021	37.4 %

[a]Log mean counts of two trials (Mean ± SD)

Growth in 0.3% bile salts and 0.4% phenol

The strain survival rate in the presence of bile salts was 10.91 ± 0.71% after four hours as seen in Figure 1 indicates that the strain could tolerate bile salts encountered while crossing the GIT. In a similar study, several strains of *Lb. plantarum* isolated from Nigerian fermented cereal-based foods displayed high viability in the presence of bile salts **(Adesulu Dahunsi *et al.*, 2018)**. Phenolic compounds can affect the viability of LAB **(Devi and Anu Appaiah, 2018)**, therefore tolerance to some antibacterial compounds like phenol, that can be found during passage through the GIT, formed by LAB deamination of some aromatic amino acids from dietary and endogenous proteins should be investigated **(Aswathy *et al.*, 2008; Ji *et al.*, 2013)**. As illustrated in Figure 1, the survival rate of TRG1 in presence of 0.4% phenol was 13.88 ± 2.54%, which indicates that the strain can grow and resist during four hours of incubation at 37°C. Several studies revealed a good tolerance to phenol, as reported by **Sirichokchatchawan *et al.* (2018)**, where they demonstrated the tolerance to 0.4% phenol by several *Lb. plantarum* strains isolated from pig feces. For the resistance to phenolic compounds, **Chan *et al.* (2018)** suggested that LAB can tolerate this environment by detoxification of phenolic acids, or these bacteria can get acclimated to the phenolic acids as reported by **Fritsch *et al.* (2016)**.

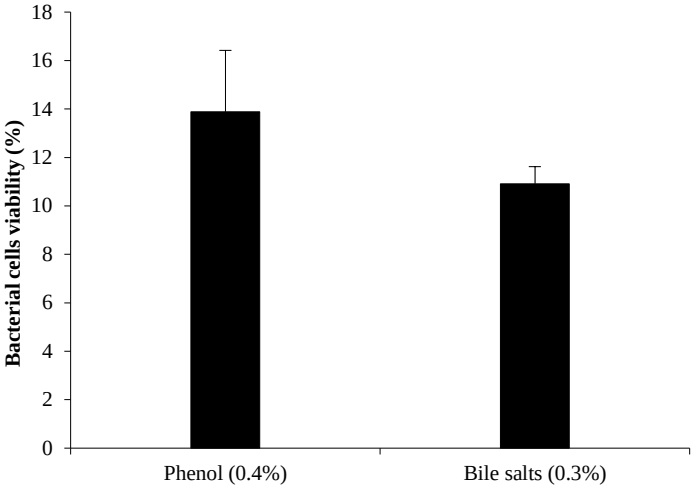

Figure 1 Growth of *Lactiplantibacillus plantarum* TRG1 in the presence of 0.4% of phenol and 0.3% of bile salts. Results are expressed as the mean ± SD of three independent experiments.

Cell surface hydrophobicity

Cell surface hydrophobicity was performed using three solvents and the obtained results were 76.94 ± 3.36%, 73.03 ± 0.52%, and 80.85 ± 1.59% with xylene, chloroform and ethyl acetate, respectively (Figure 2). According to **Burgain *et al.* (2014)**, the bacterial surface is hydrophobic when the percentage of adherence to hexadecane (nonpolar solvent) is greater than 50%, hydrophilic when this percentage is less than 20% and moderately hydrophobic when the percentage is between 20 and 50%. The TRG1 surface is hydrophobic since the percentage of adherence to xylene (nonpolar solvent) was higher than 50%. Chloroform (acidic solvent) was used to determine if the strain is electron-donating while ethyl acetate (basic solvent) was used to know if strains are electron-accepting **(Khanji *et al.*, 2018)**. The results demonstrated that the TRG1 surface was strongly electron-accepting since the adhesion to ethyl acetate was higher than the adhesion to chloroform. Hydrophobicity is the ability of bacteria to adhere to hydrocarbons, this feature is used to study the strain's adhesion ability, which is an especially important factor for microbial cells to persist in GIT, furthermore, the cell surface properties are involved in biofilms formation, colonization and adhesion to intestinal mucosal cells. This parameter can also affect the auto-aggregation of microorganisms, so to have good adhesion abilities, strains should, therefore, have a hydrophobic surface **(Abid *et al.*, 2018; Kang *et al.*, 2018; Sirichokchatchawan *et al.*, 2018)**. The strain tested could be considered hydrophobic and could have a good adhesive capability.

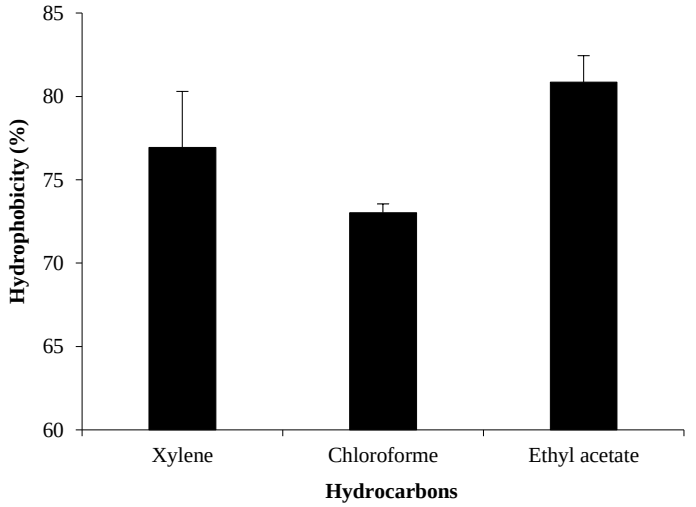

Figure 2 Hydrophobicity of *Lactiplantibacillus plantarum* TRG1. Results are expressed as the mean ± SD of three independent experiments.

Auto and co-aggregation assay

Auto-aggregation's result of TRG1 is presented in Figure 3. According to **Rodríguez-Sánchez *et al.* (2021)**, if the auto-aggregation percentage lower or equal to 10%, the strain is considered as unable to auto-aggregate and strongly auto-aggregate if the percentage is equal to or greater than 80%. The result showed good auto-aggregation ability (29.52% ± 6.95) after four hours of incubation at room temperature. Values of co-aggregation with the tested pathogenic bacteria were, 15.38% ± 05.43 with *E. coli*, 16.05% ± 2.14 with *P. aeruginosa* and 17.33%

± 2.31 with *S. aureus* as shown in Figure 3. **Rodríguez-Sánchez *et al.* (2021)** reported also that percentages below 20% indicate a weak co-aggregation, so the strain TRG1 displayed a weak co-aggregation ability with the three pathogenic bacteria. The Auto-aggregation ability of LAB should be assessed because of its relationship with cell adherence, good auto-aggregation ability indicates a good adherence ability, it is also involved in biofilm formation and colonization mechanisms **(Sirichokchatchawan *et al.*, 2018; Zhang *et al.*, 2018)**. Furthermore, the co-aggregation ability of LAB may play an important role in attaching pathogenic bacteria and eliminate them outside the GIT, consequently decreasing the pathogenic microorganism's adherence opportunities **(Tuo *et al.*, 2013)**.

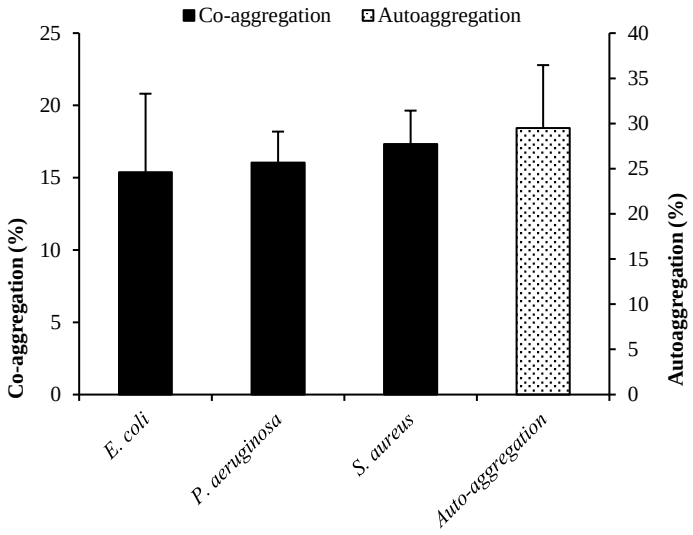

Figure 3 Auto-aggregation and co-aggregation of *Lactiplantibacillus plantarum* TRG1. Results are expressed as the mean ± SD of three independent experiments.

Exopolysaccharide production

The EPS production level of *Lactiplantibacillus plantarum* TRG1 is illustrated in Figure 4. It is noticed that the strain displayed the highest amount of EPS (942.50 ± 32.78 mg/l) using glucose as a carbon source compared to EPS amounts produced from sucrose (930.83 ± 23.76 mg/l), fructose (903.33 ± 12.58 mg/l), maltose (935.83 ± 14.64 mg/l) and galactose (937.50 ± 9.01 mg/l). These findings are agree with those obtained by **Imran *et al.* (2016)**, where, they found that the use of glucose as a carbon source by two strains of *Lb. plantarum* provided the highest amount of EPS (320 ± 20 mg/l) and (310 ± 10 mg/l) respectively, compared to lactose, sucrose, and galactose. Unlikely, **Cirrincione *et al.* (2018)** found that the highest amount of EPS was produced from lactose by three species of *Lactobacillus* (*Lb. rhamnosus* and *Lb. helveticus*). It seems that LAB exopolysaccharides biosynthesis might be dependent on both the carbon source and the bacterial species.

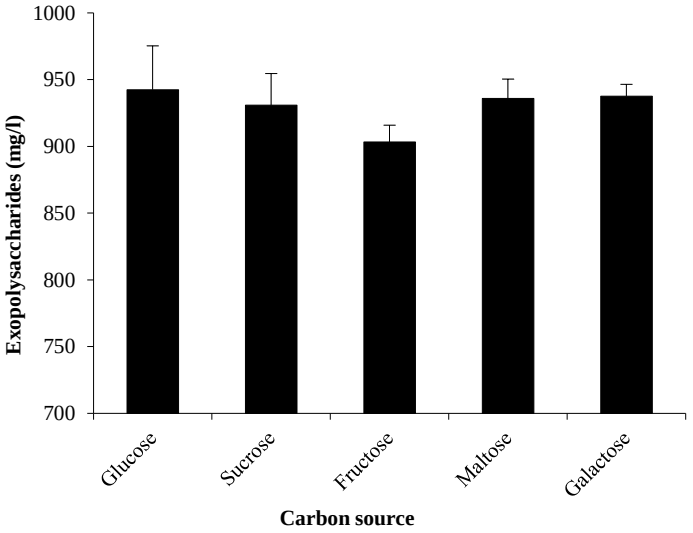

Figure 4 Exopolysaccharides production by *Lactiplantibacillus plantarum* TRG1. Results are expressed as the mean ± SD of three independent experiments.

β-galactosidase activity

TRG1 strain showed a positive response for β-galactosidase activity, which can justify its ability to improve lactose tolerance in people with deficit production of this enzyme. The β-galactosidase activity is very important for probiotic strains making it able to break down lactose into galactose and glucose, which makes it easier to be absorbed **(Jeronymo Ceneviva *et al.*, 2014)**.

Adherence to epithelial cells

The strain used in this study displayed good adhesion to mucosal tissue of chicken GIT as shown in Figure 5. One of the most important criteria for probiotic selection is the adherence ability to the mucosal surface of GIT, this is why it is so strongly recommended to assess the strain's adhesion behavior **(de Melo Pereira *et al.*, 2018)**. Strain adhesion is influenced by the composition and the structure of the microbial cell wall that can influence the surface properties of the strain as auto-aggregation, co-aggregation, and cell surface hydrophobicity **(Melgar-Lalanne *et al.*, 2013)**. Results of surface properties already described previously support the good adhesion of the LAB strain. Furthermore, the strain produces a high amount of EPS, which can be involved in the adhesion mechanisms as reported by some authors **(Bengoa *et al.*, 2018; Karasu and Ermis, 2019)**. It can be said that the strain can adhere to the GIT tissue, so it meets the criterion of adhesion.

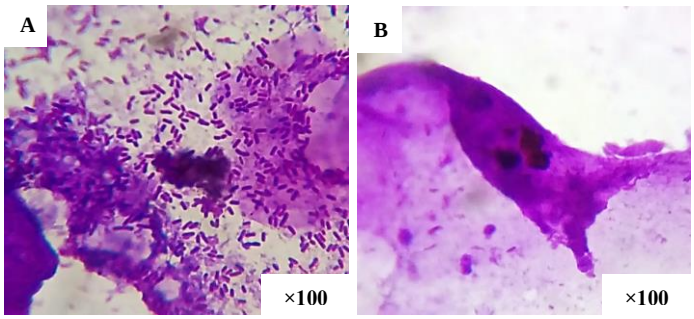

Figure 5 Adherence of *Lactiplantibacillus plantarum* TRG1 to chicken's ileum epithelial tissue (**A**), control (**B**).

Biogenic amines production

Results showed that TRG1 was not a producer of biogenic amines from histidine, lysine, tyrosine, and ornithine used in this study, the strain is therefore considered as safe. Biogenic amines are low-molecular-weight nitrogenous organic bases such as histamine, tyramine, putrescine, cadaverine, and phenylethylamine, which can be produced by the decarboxylation of histidine, tyrosine, ornithine, lysine, and phenylalanine, respectively. The presence of biogenic amines in food is closely related to microbial activities among LAB through the decarboxylation of amino acids found there and can cause several forms of toxicity such as headaches, hypotension, digestive problems, and even formation of carcinogenic nitrosamines **(Ordóñez *et al.*, 2016)**. Then it should be recommended to search for their presence.

Hemolytic and antibiotic resistance

The assessment of hemolytic activity is recommended for strains intended for usage in food. The strain TRG1 showed neither α nor β blood hemolysis, so the strain is considered as safe. The strain TRG1 was found to be sensitive to two antibiotics, nitroxoline (30 µg) and tetracycline (30 µg). On the other hand, the strain displaced a resistance towards streptomycin (10 µg), ciprofloxacin (5µg), kanamycin (30µg) and gentamicin (10 µg), (Table 2). The presence of antibiotic resistance genes in LAB was reported by several authors **(Ji *et al.*, 2013)**. These findings are agree with those reported by **Das *et al.* (2020); Shao *et al.* (2015)** and **Wang *et al.* (2019)**, where, they found that *Lactobacillus* species are resistant to kanamycin, gentamicin, and streptomycin, on the other hand, **Abriouel *et al.* (2015)** and **Shao *et al.* (2015)** reported a variable sensitivity of *Lactobacillus* species toward ciprofloxacin and tetracycline.

Table 2 Antibiotics susceptibility testing results of *Lactiplantibacillus plantarum* TRG1

Antibiotics	Zone of inhibition (mm)	Results
Kanamycin (30µg)	0	R
Ciprofloxacin (5µg)	0	R
Tetracycline (30 µg)	18	S
Gentamicin (10 µg)	13	R
Streptomycin (10 µg)	0	R
Nitroxolin (30 µg)	30	S

Legend: S –susceptible, R – resistant

The risk of transmitting antibiotic resistance from probiotic strains to pathogenic strains and vice versa always exists. For this, we must use probiotic strains devoid of resistance genes transmitted via mobile elements (plasmids, transposons, and integrons) **(Imperial and Ibana, 2016)**.

CONCLUSION

The current study demonstrated that the TRG1 strain possessed desirable probiotic properties *in vitro*. It could survive in stressful conditions such as gastric acidity, phenol and bile salts. The strain had good adherence to epithelial cells, good surface properties and may produce the β-galactosidase enzyme. Moreover, it does not produce biogenic amines and has no hemolysis activity. In terms of antibiotic resistance, the strain displaced sensitivity to nitroxoline and tetracycline, and resistant to other antibiotics. The primary objective of this work is to produce a probiotic product, which is safe for human and animal use. According to the aforementioned results, the strain can be said to satisfy the selection criteria for probiotic microorganisms. However, further studies should be performed, together with an *in vivo* study and an assessment of the stability and viability of the strain under manufacturing conditions and in the different food environments.

Acknowledgment: This work was supported by the Ministry of Higher Education and Scientific Research of Algeria under Grant [Project PRFU: D01N01UN180120200001].

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