SHORT COMMUNICATION

Isolation of *Lactobacillus HBUAS56719* from Black Raspberry Leaves and its *in-vitro* antidiabetic activity

Amira Khaled Kasser[1,2], A. B. Abeer Mohammed[1], *Mohammed A. Hussein[3*]

[1]Microbial Biotechnology Department, Genetic Engineering and Biotechnology Research Institute, University of Sadat City, Egypt
[2]Medical Lab. Department, Faculty of Applied Medical Sciences, October 6 University, Egypt
[3]Biotechnology Department, Faculty of Applied Health Sciences Technology, October 6 University, Egypt

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*** Corresponding author: Mohammed A. Hussein**
Email: prof.husseinma@o6u.edu.eg

ABSTRACT

Probiotics are living bacteria that, when given in sufficient quantities, help the host's health. They are currently recognized as appropriate substitutes for antibiotics in medical practice of animal diseases and the improvement in animal productivity. It has several different pharmacological properties, including as anti-diabetic, anti-inflammatory, and antioxidant actions. The aim of this article was to characterize of *L. plantarum HBUAS56719* isolated from fermented black raspberry and estimate its antidiabetic activity. In the current investigation, *L. plantarum HBUAS56719* was isolated from black raspberry leaves aqueous extract (BRLAE) and characterized using bile salt tolerance assay, cell-surface adhesion to solvents affinity, hemolytic test, 16S rDNA sequencing, and PCR-amplified product electrophoresis on agarose gel. On the other hand, survival rates, adhesion assay, of *L. plantarum HBUAS56719* in bile salt, hemolytic test *L. plantarum HBUAS56719* were estimated. However, α-glucosidase, pancreatic lipase, lipoprotein lipase inhibitory activity of *L. plantarum HBUAS56719* were evaluated to access its anti-obesity activity. The results of our study showed that the agarose gel electrophoresis of *L. plantarum HBUAS56719* using PCR results, a fragment (1500 bp) of 16S rRNA. Also, survival rates of *Lactiplantibacillus sp.* in bile salt (0.1 – 0.8%) showed that 0.8% oxgall produces the highest ratio of bile acid tolerance 95%. Also, adhesion assay of *L. plantarum sp.* to solvents (n-hexadecane chloroform and ethyl acetate) revealed that low acidic characteristics (less than 40% affinity to n-hexadecane and ethyl acetate) and highest basic characteristics (more than 60 % affinity to chloroform). In addition, *L. plantarum HBUAS56719* was γ-hemolytic and did not exhibit any hemolysis on the agar blood plates. Our results showed that α-glucosidase, pancreatic lipase and lipoprotein lipase inhibitory activities of *L plantarum HBUAS56719* was in the order of 91, 29 and 48%, respectively. The findings imply that *L. plantarum HBUAS56719* could be explored to produce α-glucosidase, pancreatic lipase and lipoprotein lipase inhibitory peptides from fermented black raspberry. Fermented black raspberry produced by *L. plantarum HBUAS56719* could be a novel source of antidiabetic peptides.

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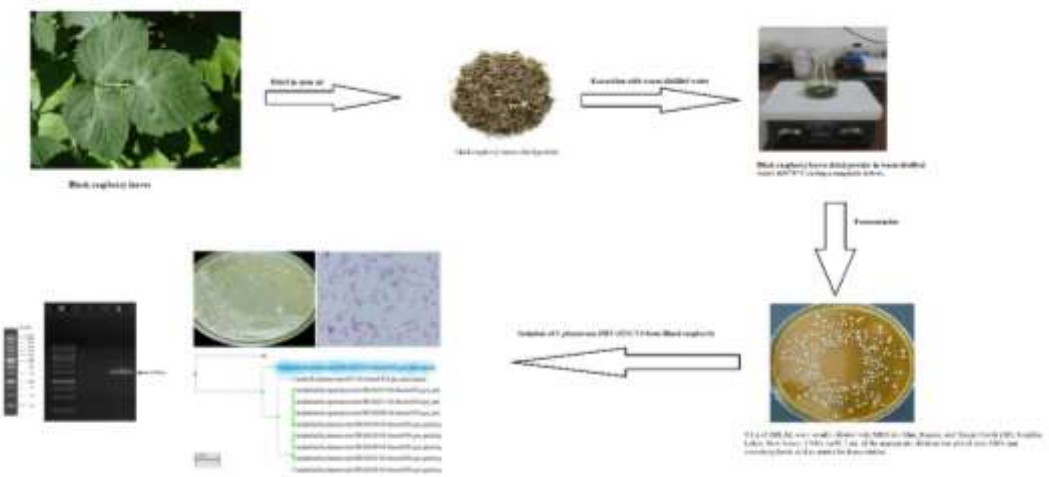

Keywords: *L. plantarum HBUAS56719*, 16S rDNA, Black raspberry, Agarose gel electrophoresis, α-glucosidase, Pancreatic lipase and lipoprotein lipase.

1. INTRODUCTION

Hyperglycemia, or elevated blood sugar levels, is the primary clinical sign of diabetes, a metabolic illness (Wild et al., 2004). About 90–95 percent of all cases of diabetes are type 2 diabetes mellitus (T2DM) (Einarson et al., 2018). However, a variety of diabetic medications, including sulfonylureas, thiazolidinediones, guanidines, -glucosidase inhibitors, and others, have been created to treat T2DM. However, these therapies are unable to provide ideal blood glucose control for a considerable amount of time due to the significant adverse effects of these medications, such as weight gain and hypoglycemia (Patel et al., 2014). On the lumen surface of intestinal epithelial cells, dipeptidyl peptidase-4 (DPP-4), a serine protease, is abundantly expressed. Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) can be inactivated by DPP-4 at the postprandial stage, which results in the loss of their insulin-promoting function (Lacroix and Li-Chan, 2016). However, synthetic DPP-4 inhibitors have some adverse effects, such as mild infection and headache (Kushner and Gorrell, 2010). Therefore, screening for DPP-4 inhibitors from natural sources has attracted considerable attention in recent years (Feng et al., 2018).

The role of gut symbiotic bacteria has been the subject of several research on obesity and diabetes (Soliman *et al*., 2022 and Mohamad *et al.*, 2022). The gut microbiota contains 2,000 different kinds of bacteria, and about 10^{13} - 10^{14} microbial cells in the human gastrointestinal system (Delzenne et al., 2011 and Gerritsen et al., 2011). The human host's microbial flora gradually develops mutualism, stabilizing its colonization of the body (Kang et al., 2013), and carefully controlling the physiological condition of the host by fending off invasive pathogens (Lee et al., 2013) and assisting with intestinal digestion and nutritional absorption (De Souza et al., 2017). Several studies reported that the host's energy balance and metabolic processes may be impacted by the gut microbiota, which suggests that intestinal bacteria may contribute to obesity by

affecting human metabolism (Zhang et al., 2014 and Swanson et al., 2020).

There are several commensal Lactobacillus species in the gut microbiota, which help restore intestinal homeostasis and hence prevent ulcerative colitis (Ghorab et al., 2010 and Hudson et al., 2017). Probiotics can control the makeup of the gut microbiota and treat aberrant responses of the mucosal immune system to chronic gut inflammation since they are living bacteria (Hussein et al., 2017). By modulating cytokine production, encouraging the release of regulatory T cells, and promoting the survival of intestinal cells, probiotics can help improve the function of the gut barrier (Chen et al., 2015).

Lactobacillus plantarum is the most prevalent probiotic that may be discovered in the mouth and intestines (Ghouri et al., 2014). It's also found in fermented foods that may aid in food digestion, nutritional absorption, and the defense against pathogenic organisms. Probiotic supplements also contain specific strains of *L. plantarum*, which are frequently added to fermented foods like yoghurt (Qian et al., 2021). Also, *L. plantarum* was used as a treatment against eczema, high cholesterol, to treat ulcerative colitis and avoid getting the flu or other respiratory diseases (Qian et al., 2020). Studies have demonstrated the detrimental effects of black raspberry on human health, but nothing is known about the isolated Lactobacillus strains from black raspberry (Kaewnopparat 2013). Black raspberries have anti-inflammatory, anti-oxidative, and anti-atherosclerotic properties (Vicariotto et al., 2014). Black raspberry-derived Lactobacillus plantarum may possibly reduce oxidative stress, adhesion molecules, and pro-inflammatory cytokines while boosting endothelial nitric oxide synthase (Falagas et al., 2007, Afifirad et al., 2022 and Jia et al., 2021). The present study sought to evaluate the antidiabetic peptides of L. *plantarum HBUAS56719* isolated from black raspberry leaves.

2. MATERIALS AND METHODS

Black raspberry leaves were collected from local farms Sharqia Governorate, Egypt.

2.1.Preparation of aqueous extract:

Using a magnetic stirrer, 50 gm of dried black raspberry leaves powder was dissolved in 250 mL of warm distilled water (60–70 ^{o}C) to create the aqueous extract of black raspberry leaves (Ryu et al., 2015). It was subsequently filtered and dried under low pressure.

2.2.Growth conditions of lactic acid bacteria

Lactic acid bacteria are MRS (Difco, Detroit, MI, USA) liquid media. After subculture more than twice in the culture medium, the precultured strain is inoculated at a concentration of 1% for 6 hours at 37°C.

2.3.Isolation of *L. plantarum HBUAS56719* from Black raspberry leaves aqueous extract (BRLAE)

Black raspberry extract (0.1 gm in 0.9 mL saline) was sterilized at 80°C for 3 minutes to remove contaminated bacteria if present, the added to 1 mL culture medium contained lactic acid bacteria used as fermentation starters, mixed, diluted by a 10-fold dilution. 100 µL smeared onto MRS flat medium at 30°C for 48 hours of incubation at pH 8.3 (Ryu et al., 2015). After being incubated for 24 hours at 30 °C, the plates were checked for bacterial growth. Furthermore, the separated and purified morphologically distinct colonies were kept at 80 °C in MRS broth supplemented with 20 percent (v/v) glycerol.

2.4.Molecular identification of isolated *L. plantarum HBUAS56719*

According to Cheng and Jiang (2006), 16S rDNA sequencing was used to identify the isolated strain of *L plantarum HBUAS56719*. The 16S rRNA gene was

amplified and sequenced using universal primers 27f and 1492r, which were used to amplify the partial 16S rRNA. Briefly, the genomic DNA of the isolated L plantarum was extracted using a DNA extraction kit from Qiagen, United States. The amplicons were sequenced automatically using a DNA analyzer (SeqStudio Flex DNA analyzer, Life Technologies Ltd 3 Fountain Drive Inchinnan Business Park Paisley PA4 9RF, UK), and the Basic Local Alignment Search Tool of the National Center for Biotechnology Information was used to analyses the sequences (NCBI) (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Utilizing the whole 16S rDNA data and the neighbor-joining technique, the phylogenetic tree was produced using the BLAST software programme.

2.5.Estimation of bile salt tolerance

With some modifications, Bile Salt Tolerance estimation of *L. plantarum HBUAS56719* was determined according to the method of Leite et al, (2015). Briefly, overnight culture of *L Lactiplantibacillus sp.* was harvested by centrifugation at 10,000 rpm for 5 min and cells were resuspended in PBS (pH 6.5) (absorbance = 0.5 at 600 nm). Cell suspensions were adjusted to 0.1%, 0.2%, 0.4, 0.6, 0.8% (w/v) oxgall (Difco, Detroit, MI, USA), and incubated at 37 ˚C for 3 h. The bile tolerance was estimated by enumerating the viable cells on MRS agar plate and comparing viable cell counts in MRS with and without bile (oxgall). The percentage of *L. plantarum HBUAS56719* survival rate was calculated using Equation (1).

Survival rate (%) = [log A1/ log A0] x 100

Where: A1 = Final (Log CFU/mL)
A0 = Initial (Log CFU/mL).

2.6.L. plantarum HBUAS56719 cell surface adhesion to solvents assay

The cell surface hydrophobicity assay of *L. plantarum HBUAS56719* was determined according to the method of Abbasiliasi et al. (2017). Briefly, 10^8 CFU ml/L of *L. plantarum HBUAS56719* was created in PBS (pH 7.2) and 1 mL organic solvents (n-hexadecane chloroform and ethyl acetate). Exactly 5 mL of *L. plantarum HBUAS56719* suspension was vortexed for 1 min then standing for 5–10 min, the aqueous phase was isolated, and absorbance measured at zero and after 10 min of incubation at 37 °C using Shimadzu UV-Visible spectrophotometer (600 nm). Cell surface adhesion to solvents (affinity %) was expressed using the equation below:

Affinity (%) = (1- [A2/A0]) x 100
Where: A0 = absorbance of the *L plantarum HBUAS56719* suspension at zero time
A1 = absorbance of the *L plantarum HBUAS56719* suspension after 10 min.

2.7.Hemolytic assay

Hemolytic test of *L. plantarum HBUAS56719* was estimated as reported by Leite et al, (2015). 50 µL of 10^8 CFU/mL of *L. plantarum HBUAS56719* was incubated for 48 h at 37 ˚C in blood agar plates, containing sheep blood (5% *w/v*) (Fisher Scientific, Fairlawn, NJ, USA). Blood agar plates were examined for signs of β-hemolysis (clear zones around colonies), α-hemolysis (green-hued zones around colonies), or γ-hemolysis (no zones around colonies).

2.8.Preparation *L. plantarum HBUAS56719* supernatant

10 mL MRS broth was autoclaved and then allowed to cool down at 37 °C. *Lactiplantibacillus sp.* cells were inoculated at 37 °C, centrifuged for 15 minutes at 4000

xg and resuspended in 50 µL of PBS and adjusted to get 10^8 CFU/mL. After mixing, culture was incubated at 37 °C for 24 h. then centrifugated at 10,000 rpm for 15 min at 4 °C, and supernatant were collected. Each activated culture's supernatant was stored further used.

2.9.α–Glucosidase inhibition of *L. plantarum HBUAS56719*

The ability of the *L. plantarum HBUAS56719* to α–Glucosidase was assayed using the method Zheng et al. (2010) with some modification. 50 µL of culture supernatant or acarbose (0.4 mg/mL) were pre-incubated with 50 µl α-glucosidase for 1 h, the enzymatic reaction was allowed to proceed at 37 °C for 30 min, then 50 µL Na_2CO_3, 0.1 M was added to stop the reaction. The absorbance was measured using microplate reader at 405 nm using a UV-Visible spectrophotometer (BioTek Synergy HT, Winooski, VT, USA). The activity of the negative control was also examined with and without an inhibitor.

the inhibition of α-glucosidase activity of *L. plantarum HBUAS56719* according to the following formula:

Inhibition (%) = (1- [A2/A1]) x 100
Where: A1 = absorbance of the reactants with the sample
A2 = absorbance of blank (reactants without the sample).

2.10.Pancreatic lipase inhibition assay of *L. plantarum HBUAS56719*

Porcine pancreatic lipase (PPL, type II) activity was measured using p-nitrophenyl butyrate (p-NPB) as a substrate. The method used for measuring the pancreatic lipase activity was modified from that previously described by Kim, et al. (2010). PPL stock solutions (1 mg/mL) were prepared in a 0.1 mM potassium phosphate buffer (pH 6.0) and the solutions were stored at −20 °C. 50 µL of *L. plantarum HBUAS56719* supernatant or orlistat (50 µg/mL) as a standard drug were pre-incubated with PPL (1000 ppm) for 1 h in a potassium phosphate buffer (0.1 mM, pH 7.2, 0.1% Tween 80) at 30 °C before assaying the PPL activity. The reaction was then started by adding 0.1 µL NPB as a substrate, all in a final volume of 100 µL. After incubation at 30 °C for 5 min, the amount of p-nitrophenol released in the reaction was measured at 405 nm using a UV-Visible spectrophotometer (BioTek Synergy HT, Winooski, VT, USA). The activity of the negative control was also examined with and without an inhibitor. The inhibitory activity (I) was calculated according to the following formula:

Inhibitory activity (I%) = 100 − ((B − b)/ (A − a) × 100)

where A is the activity without inhibitor; a is the negative control without inhibitor; B is the activity with inhibitor; and b is the negative control with inhibitor. DMSO was used as negative control and its activity was also examined.

2.11. Lipoprotein lipase (LPL) inhibition assay of *L. plantarum HBUAS56719*

The ability of the *L. plantarum HBUAS56719* to inhibit LPL was measured using the modified method previously reported by Schotz et al. (1970). An activator consisted of Apo C-II from human plasma and diluted to 1 µg/mL with 0.002 M Tris HCl (pH 8.0) was prepared. In the preparation of substrate, 0.6 mL triolein, 24 mL apo C-II, 3.6 mL of 1% BSA solution, 3.6 mL of 1% triton X-100, and 28.8 mL of 0.2 Tris HCl buffer (pH 8.0) were mixed. The mixture was then sonicated in ice for 3 minutes. Enzyme LPL from bovine milk was prepared by diluting with 0.02 M Tris HCl (pH 8.0) to a concentration of 25 units/mL. The LPL activity was then determined using a method reported by Chung and Scanu.[35]. Briefly, 50 µL of *L. plantarum HBUAS56719* supernatant or epicatechin (25.0 µg/mL) as a standard drug in test tubes followed by pre-incubation at 4°C for 30 minutes. Then, 1 mL of substrate

emulsion was added to the mixture of enzyme and samples, followed by incubation at 37°C for 1 h, to initiate the reaction. The reaction was stopped with the addition of 1 mL of 1 M NaCl. Control samples were consisted of mixture of enzyme and substrate only. The liberated free fatty acids (FFA) were titrated with 0.01 M NaOH until pH 9.4. The amount of liberated free fatty acid (FFA) was reflected by the amount of base required by the incubation mixture which is equivalent to LPL activity. Control sample was equivalent to 100% enzyme activity. The experiment was repeated three times for each sample, and percent inhibition was calculated.

3. RESULTS AND DISCUSSION

Isolates were phenotypically recognized as *L. plantarum HBUAS56719* which were distinguished by their round, white, smooth, and moist surface characteristics. After gram staining, the morphology of cells was examined under a microscope and revealed that they were rods arranged in chains, doubles, and singles (Fig.1). In addition, several biochemical assays were run to determine which isolates of *L. plantarum HBUAS56719* came from the identified LAB.

L. plantarum HBUAS56719 is one of the most widely used and promising LAB species in food production as a probiotic and starter culture (Zheng et al., 2020). It is safe for human consumption, most of the LAB genera, notably the L. plantarum genus, are covered by the qualified presumption of safety (QPS) recommendation of the European Food Safety Authority (Tosukhowong et al., 2011). In the current investigation, *Lactiplantibacillus sp.*, was isolated from black raspberry, as the method reported by Tosukhowong et al. (2011), who found that L. plantarum GBL17 had the maximum proliferation after 24 hours of incubation and the medium's pH dropped to pH 3.8. Other research has demonstrated that L. plantarum AF1 has the greatest growth proliferation under identical conditions within 20 hours (Yilmaz et al., 2022). These findings suggest that *L. plantarum HBUAS56719* might be cultured at 30 ^{o}C for 24 hours with a 5 percent inoculation ratio to MRS broth to get the best growth possible. These settings were used to develop the cells for the investigations that followed.

3.1. 16s rRNA identification of Lactiplantibacillus sp.

By sequencing the 16s rRNA, phylogenetic analysis was used to identify the isolated bacteria. It was performed with BLAST. A fragment of the 16S rRNA gene (1500 bp) used for genotypic identification by gene sequencing had a maximum sequence similarity of 99.35 percent to the GenBank reference strain *Lactiplantibacillus sp.* with accession number (OM301856) (fig. 2a and b) and had been deposited in the Gen-Bank database (https://www.ncbi.nlm.nih.gov/nuccore/OP 103741). Additionally, following agarose gel electrophoresis of amplified PCR products, the L plantarum HBUAS56719 investigated were positive with 1500 bp size (Fig. 3). A partial 16S rRNA sequence analysis of L plantarum HBUAS56719 demonstrated 99 percent similarity with L plantarum R503 and the predicted 0.9 K amplicons were generated by PCR. Phylogenetic research confirmed the findings after the isolate L. plantarum GBL16 and GBL17 strains were discovered in the leaves of black raspberry (Ryu et al., 2015). The findings are in line with a prior investigation that found L. plantarum in salted fish products (Yang et al., 2008). 16S rRNA sequences of *L. plantarum HBUAS56719 was* uploaded to Genbank (https://www.ncbi.nlm.nih.gov/nuccore/OP 103741, accession no. OP103741). Also, *L. plantarum HBUAS56719* identified by agarose gel electrophoresis of amplified PCR products according to the method described by Zeng et al., (2014) and Jung et

al. (2015). Our findings support prior recommendations that 16S rRNA sequencing be used to identify precisely and accurately.

3.2. Bile Salt Tolerance Test

The isolated *L. plantarum HBUAS56719* was subjected to different oxgall concentrations, 0.1- 0.8%, at an incubation time of 3 h. As shown in Figure 4, the isolated *L. plantarum HBUAS56719* sample conferred the highest survived rate (%) more than 80 % at 0.6 and 0.8% bile concentration after incubation. Given these data, it has been established that *L. plantarum HBUAS56719* has good, survival rate and in confirmed with the results of Salvador et al. (2021), who identified other probiotic genes, such as bile salt hydrolases and beta-galactosidases, which lower cholesterol levels (Valeriano et al. 2019) and ameliorate lactose intolerance in the host (Jia et al. 2020), respectively, noting its probiotic potential.

3.3. Affinity of *L. plantarum HBUAS56719* to solvents (n-hexadecane, chloroform and ethyl acetate)

Figure 5 showed that the affinity of *L. plantarum HBUAS56719* to solvents was increased significant in order of ethyl acetate, n-hexadecane and chloroform by 17, 61 and 79%, respectively. Hydrophobicity and Lewis acid-base characteristics are known to initiate bacterial adhesion to surfaces and, thus, it is important to characterize favorable interactions that significantly affect bacterial adhesion. For the affinity assay, hexadecane, a polar solvent, was used to evaluate the hydrophobicity or hydrophilicity of the cell surface, while ethyl acetate (basic solvent and electron donor) and chloroform (acidic solvent and electron acceptor) (Fan et al. 2022) were used to explore Lewis acid-base characteristics. Lactobacilli strains showed hydrophobic surface character (>50%). Also, our results showed weak acidic characteristics (less than 40% affinity to ethyl acetate) and highly basic characteristics (60–90% affinity to chloroform), like *Lactobacillus acidophilus* (Motey et al. 2021) and *Lact. rhamnosus* (Chen et al. 2020). Most adhesive probiotic lactobacilli strains showed strong basic and electron-donating properties, demonstrating preferential monopolarity towards chloroform (Rai et al. 2022).

The isolated *L. plantarum HBUAS56719* examined for hemolytic activity were β-haemolytic and was screened for probiotic properties. Our results indicated that *L. plantarum HBUAS56719* did not present any hemolysis on the agar blood plates. *L. plantarum HBUAS56719* was γ-hemolytic. However, hemolysis is another well-known virulence factor among pathogenic microorganisms. *L. plantarum HBUAS56719* was γ-hemolytic, which indicated no hemolysis on blood agar plates. This finding was similar to a previous study that revealed *Lactobacillus spp.* possess no hemolytic activity (Leite et al., 2015).

The α-glucosidase inhibitory activity results of *L. plantarum HBUAS56719* is shown in Fig. 6. The present results indicate that *L. plantarum HBUAS56719* showed inhibitory effects (91%) more than acarbose (84%) against α-glucosidase activity. Although α-glucosidase inhibitory activity is strain specific, our results was in confirmed with the results reported by Kim et al., 2018, who, showed that the *Lactobacillus spp.* strains from the intestine showed the highest percentage (92.0) of activity while activity by other strains ranged between 8.3 to 45.0%. This activity could result from *Lactobacillus spp.* ability to produce exopolysaccharides (EPS). These *Lactobacillus spp.* strains could reduce the absorption of intestinal carbohydrates (Park et al., 2018).

Figure 7 shows inhibitory effects of *L. plantarum HBUAS56719* and orlistat on pancreatic lipase. Based on a comparison of

the absorbance at 700 nm, the inhibitory effects of *L. plantarum HBUAS56719* and orlistat are equal 29 and 76%, respectively. On the other hand, figure 8 shows inhibitory effects of *L. plantarum HBUAS56719* and epicatechin on lipoprotein lipase. The inhibitory effects of *L. plantarum HBUAS56719* and epicatechin are equal 48 and 91%, respectively.

Lipase is an enzyme that catalyzes the hydrolysis of fats. Lipases perform essential roles in digestion, transport and processing of dietary lipids in most living organisms. Pancreatic lipase is the main enzyme that breaks down dietary fats in the human digestive system. The major role of lipase inhibitors is to decrease the gastrointestinal absorption of fats. Fats then tend to be excreted in feces rather than being absorbed to be used as a source of caloric energy, and this can result in weight loss in individuals. These inhibitors could be used for the treatment of obesity. This test was performed because obese are prone to having diabetes and heart-related problems (Maqsood et al. 2017). Although pancreatic lipase inhibitory activity in our study was in confirmed with the results reported by Gil-Rodríguez and Beresford et al., (2019), who, reported that the *Lactobacillus spp.* had expressed 23.15% lipase inhibition. Our research may be the first of its kind since, to the best of our knowledge, the evaluation of antidiabetic activity of *L. plantarum HBUAS56719 isolated from* black raspberry leaves have never been reported.

4. CONCLUSION

L. plantarum HBUAS56719 isolated from black raspberry leaves was characterized using 16S rRNA sequencing and agarose gel electrophoresis which may be pave in medical applications. Additionally, it produces a good survival rates of L. plantarum HBUAS56719 in bile salt and adhesive activity to organic solvent as well as inhibits beta-galactosidase, pancreatic and lipoprotein lipases enzymes that may aid in alleviating the effects of lactose intolerance in the host. This strain in combination with other probiotics may prove useful in future applications in treatment of obesity and other carbohydrates disorders.

Declarations

Consent to participate and approval of ethics. Data collection received ethical approval from the research ethics committee of the faculty of applied health sciences technology at October 6 University, Egypt (20210611).

Conflict of interest

The authors declare that they have no financial or other conflicts of interest.

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Table1: Universal primers

Primer Code	**Sequence**	**Product Size**
27F	5′- AGAGTTTGATCCTGGCTAG -3′	1500bp
1492R	5′- GGTTACCTTGTTACGACTT -3′	

Figure 1: Gram stain results of *L plantarum HBUAS56719*

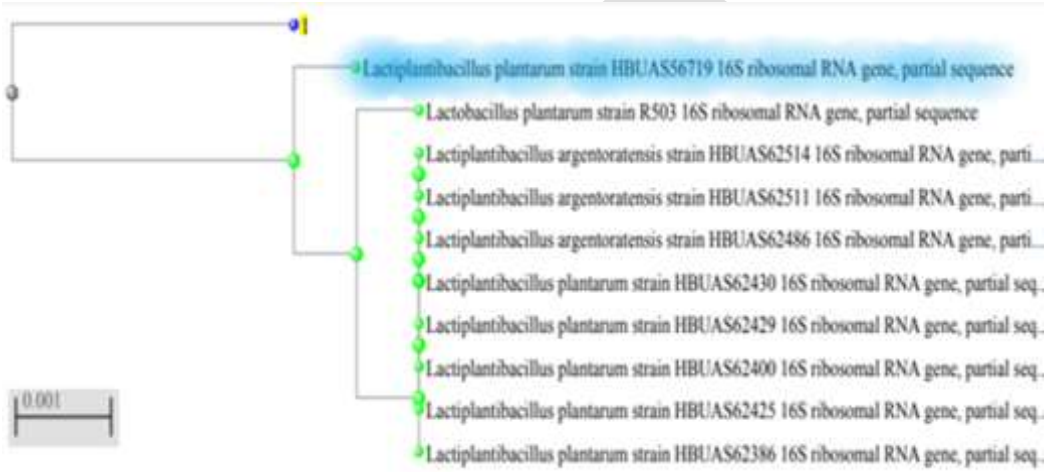

Figure 2a: Phylogenetic tree using BLAST "NCBI, U.S." of *L plantarum* strains using *16S rRNA*, showing names of bacteria species and accession numbers.

```
     rRNA            <1..>760
                     /product="16S ribosomal RNA"
ORIGIN
        1 gtgccaatac tgcaagtcga acgtctctgg tattgaatgg tgctgcatca tgatttacat
       61 ttgagtgagt ggcgaactgg tgagtaacac gtgggaaacc tgcccagaag cgggggataa
      121 cacctggaaa cagatgctaa taccgcataa caacttggac cgcatggtcc gagcttgaaa
      181 gatggcttcg gctatcactt ttggatggtc ccgcggcgta ttagctagat ggtggggtaa
      241 cggctcacca tggcaatgat acgtagccga cctgagaggg taatcggcca cattgggact
      301 gagacacggc ccaaactcct acgggaggca gcagtaggga atcttccaca atggacgaaa
      361 gtctgatgga gcaacgccgc gtgagtgaag aagggtttcg gctcgtaaaa ctctgttgtt
      421 aaagaagaac atatctgaga gtaactgttc aggtattgac ggtatttaac cagaaagcca
      481 cggctaacta cgtgccagca gccgcggtaa tacgtaggtg gcaagcgttg tccggattta
      541 ttgggcgtaa agcgagcgca ggcggttttt taagtctgat gtgaaagcct tcggctcaac
      601 cgaagaagtg catcggaaac tgggaaactt gagtgcagaa gaggacagtg gaactccatg
      661 tgtagcggtg aaatgcgtag atatatggaa gaacaccagt ggcgaaggcg gctgtctggt
      721 ctgtaactga cgctgaggct ccaaagtatc cgtagcaaac
//
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Figure 2b: the sequence alignment using BLAST "NCBI, U.S." of *L. plantarum HBUAS56719* using *16S rRNA*, showing names of bacteria species and accession numbers.

SHORT COMMUNICATION

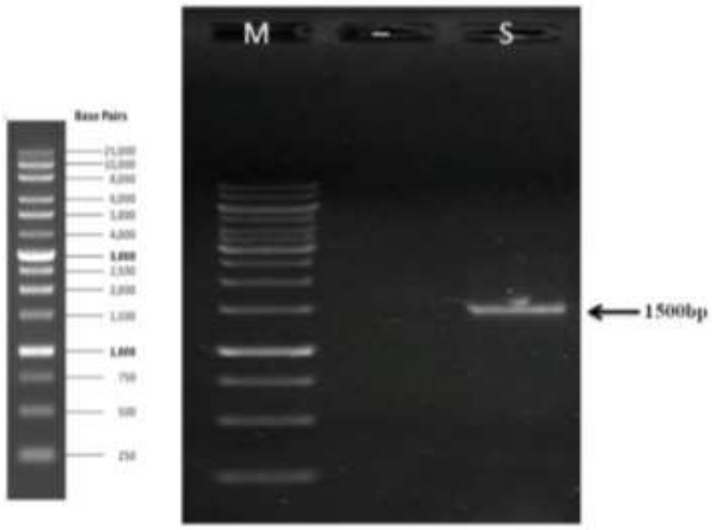

Figure 3.The bands corresponding to the amplified sequences in the PCR reaction (M: DNA ladder: 250 bp, S: band *L. plantarum HBUAS56719,* about 1500 bp., (-) =Lactococcus lactis ssp. cremoris MG 1363 (negative control), agarose gel's percentage 1%.

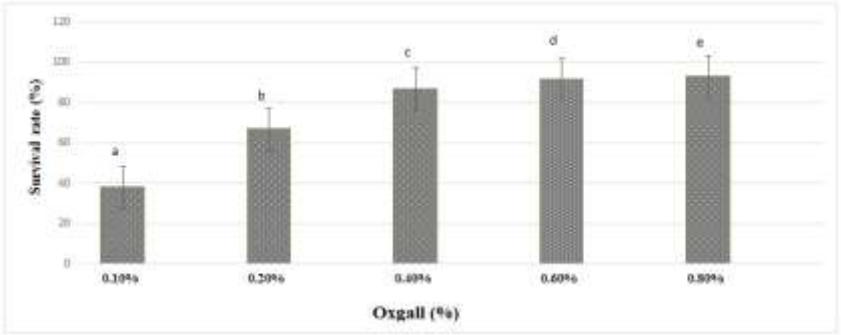

Figure 4. Survival rates of *L. plantarum HBUAS56719* in bile salt (0.1 – 0.8%) conditions for 3 h. Values represent the mean ± SD (n=3). Data followed by the same letter are not significantly different at P ≤ 0.05.

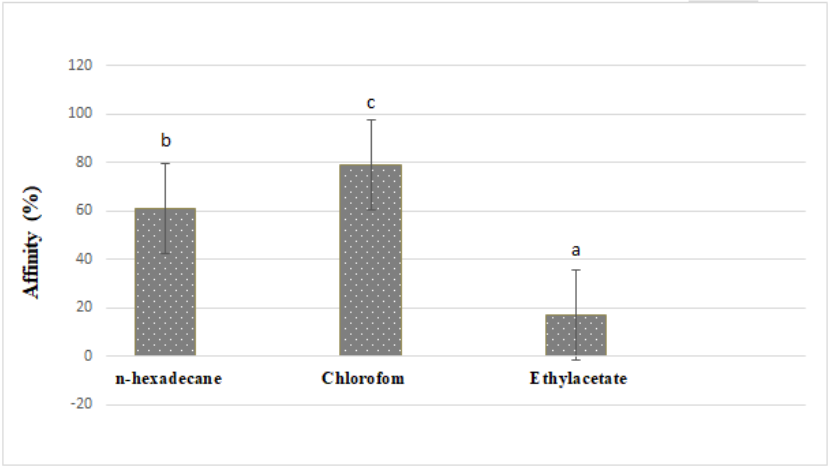

Figure 5: Adhesion assay of *L. plantarum HBUAS56719* to solvents (n-hexadecane chloroform and ethyl acetate). Values represent the mean ± SD (n=3). Data followed by the same letter are not significantly different at P ≤ 0.05.

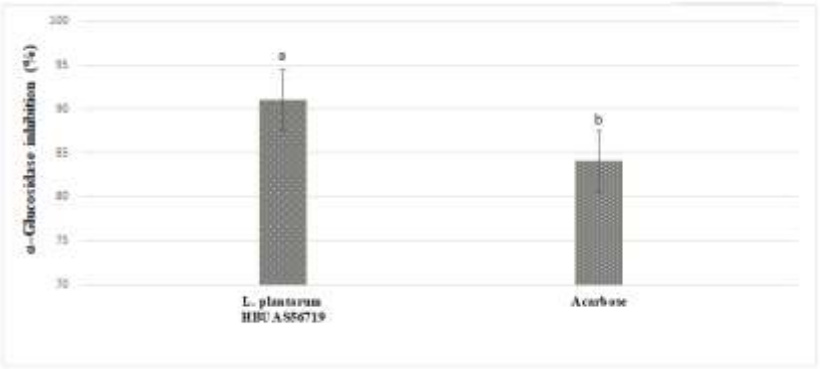

Figure 6: α-glucosidase inhibitory activity of *L. plantarum HBUAS56719* and acarbose (0.4 mg/mL). Values represent the mean ± SE (n=3). Data shown are mean ± standard deviation of number of 3 observations. Data followed by the same letter are not significantly different at P ≤ 0.05.

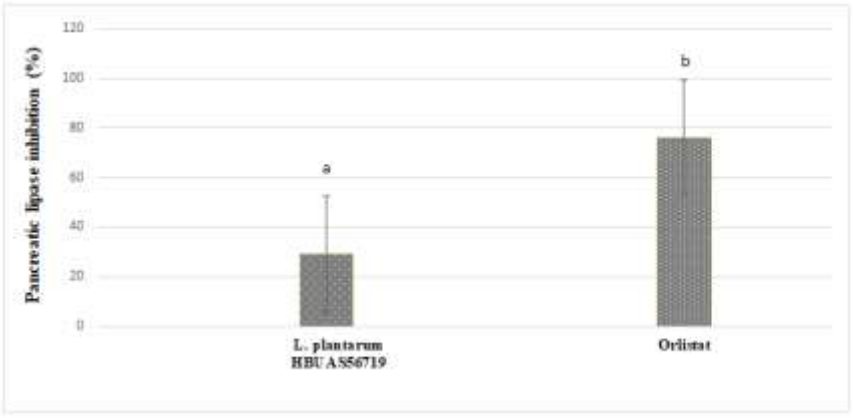

Figure 7: Pancreatic lipase inhibitory activity of and of *L. plantarum HBUAS56719* and orlistat (50.0 µg/mL). Values represent the mean ± SD (n=3) of number of observations within each treatment.

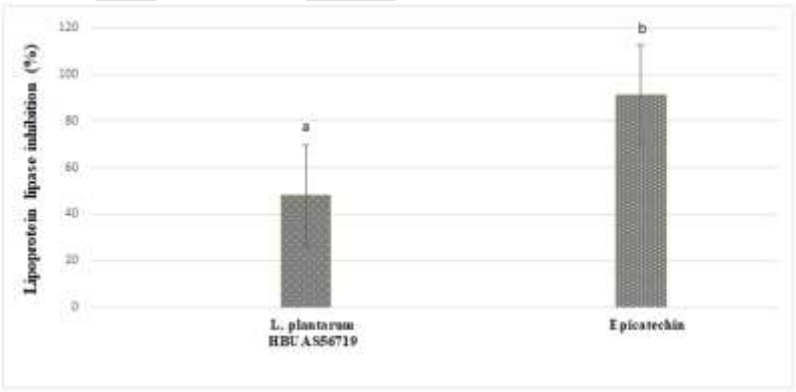

Figure 8: Lipoprotein lipase inhibitory activity of *L. plantarum HBUAS56719* and epicatechin (25.0 µg/mL). Values represent the mean ± SD of number of observations within each treatment. Data followed by the same letter are not significantly different at P ≤ 0.05.