IDENTIFICATION OF A BACTERIAL STRAIN ISOLATED FROM PICKLED MELON SOLUTION AND DETERMINATION OF ITS ABILITY TO PRODUCE LACTIC ACID

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Abstract. Bacterial strain LB7 isolated from pickled melon solution was identified as Lactobacillus plantarum using molecular marker *16S* rRNA *gene* (rDNA) sequences. The content of lactic acid in the isolate culture was relatively high (33.7 g/l) when quantified using HPLC system.
Keywords. Lactobacillus, *16S* rRNA *gene* (rDNA) sequences, lactic acid.

1. INTRODUCTION

Lactic acid producing bacteria are called *Lactobacillus.* They are characterized by the ability to produce high amount of lactic acid from various types of sugars, especially lactose. Most of them belong to family Lactobacillaceae containing four genera, including *Streptococcus*, *Pediococcus*, *Lactobacillus* and *Leuconostoc*. They are rod or spherical shaped, gram-positive, non-spore-forming and non-motile bacteria [5].

Lactobacillus belongs to the group of lactic acid producing bacteria, which plays a very important role in food technology as well as biological products [4]. This group contains about 150 species [1, 14] which are now widely studied all over the world. Most studies focused on their potential in production of probiotic [2, 4, 6], while some other studies focused on the isolation and selection of those beneficial strains [5].

In Vietnam, *Lactobacillus* are mostly found in traditionally pickled foods such as pickled cucumbers, yogurt, pickled pork roll and pickled rice, etc. Therefore, in order to better understand them as well as to increase their applicability in the food industry, in this study, bacteriual strain LB7 isolated from pickled melon solution was identified using biomolecular technique. In addition, the results of lactic acid quantification by HPLC (High performance liquid chromatography) proved its ability to produce lactic acid.

2. MATERIALS AND METHODS

2.1. Materials

Isolate LB7 was isolated from pickled melon solution purchased in Big C supermarket, Go Vap, Ho Chi Minh City. In addition, this study also used some 16S rRNA gene (rDNA) sequences of some bacterial strains from GenBank to identify the isolate (Table 1).

Table 1: Sequences from GenBank database used in this study

Taxa	Accession number	Taxa	Accession number
Lactobacillus insectis	AY667699	*Bacillus subtilis*	MF351830
Lactobacillus kunkeei	JQ009345	*Lactobacillus reuteri*	JN813102
Lactobacillus casei	KC456363	*Lactobacillus sakei*	EU081017
Lactobacillus curvatus	EU081014	*Lactobacillus salivarius*	KT371516
Lactobacillus fermentum	AB932537	*Lactobacillus plantarum*	KJ187148
Lactobacillus paracasei	KM096826		

2.2. Methods

2.2.1. Isolation and enrichment

1 ml of pickled melon solution was added into a test tube containing 9 ml of sterile distilled water which was then gently shaken. 100 µl of the dilute solution from the test tube was spread on petri dish containing MRS medium and incubated at room temperature for 48 hours. The appearance of colonies was observed. The colonies surrounded with transparent circles ($CaCO_3$ decomposed by acid formed transparent cirle) were isolated and purified. The isolates were preserved in MRS agar slants [6].

The enrichment of *Lactobacillus* isolate was conducted in liquid MRS medium at pH 6.2 at room temperature. After 24 hours, the culture was transferred to a liquid MRS medium supplemented with glucose 0.02 g / l, pH = 6.2 and incubated at room temperature. After 72 hours, the culture was centrifuged at 13000 rpm to collect the supernatant which was then diluted 10 times, thorougly shaken and filtered through filter paper to collect the filtrate for quantification of lactic acid [6]. The lactic acid quantification was conducted using HPLC system at the Center for Chemical Analysis of the University of Natural Sciences, Ho Chi Minh City. The analyses was performed using high performance liquid chromatography coupled with ultraviolet detection (HPLC-UV). The HPLC equipment consisted of an integrated system of Agilent 1100. Chromatographic separation was achieved using C18 reverse phase HPLC column (4.6×150 mm, 5 mm) operating at 30 °C. The mobile phase was phosphoric buffer (pH = 2.25) pumped through the coulumn at a flow rate of 1 mL/min. 20 µL of sample was injected into the system to pass through the column. The detection was conducted at wavelength 220 nm.

2.2.2. Analysis of 16S *rRNA* gene *(rDNA) sequence*

DNA extraction:

Total genomic DNA was extracted using the method described by Tran (2003) [11]. The eppendorf containing 500 µl of the enrichment culture was centrifuged at 13000 rpm to decant the supernatant. The tube was added with 500 µl TE solution, vortexed, incubated at 95^0C for 10 min and then centrifuged at 15000 rpm for 5 min to collect the supernatant containing DNA.

Polymerase chain raction (PCR):

Lac1 and Lac2 primers: Lac1 forward, 5'-AGCAGTAGGGAATCTTCCA-3', and Lac2 reverse, 5'-ATTCCACCGCTACACATG-3' were used in the PCR process to amlify the 16S rRNA gene (rDNA) sequence [3]. PCR was conducted in an eppendorf containing 12,5 µl Go-Taq green master mix (Promega, USA), 1,25µl of each forward and reverse primers (10 µM), 9.5µl nuclease-free water and 0.5µl DNA template (25 µg/ml). The target DNA sequence was amplified using the following program: initial denaturation for 3 min at 96°C; 35 cycles of denaturation (1 min at 94°C), annealing (1 min at 40^oC) and extension (90 sec at 72°C); and a final extension at 72°C for 10 min. The PCR products were visualized on 1% agarose gel and sent for purification and sequencing by Nam Khoa Biotek Ltd. Company (Vietnam) using ABI 3130 XL Sequencer.

The homology between sequences was recognised using the ClustalW software to have a multiple sequence alignment [10]. PAUP* ver. 4.0 Beta [9] and MrBayes [8] using parsimonious and Bayesian methods, respectively, were employed to construct a phylogenetic tree rooted with *Bacillus subtilis* [1]. The cluster supports in the phylogenetic tree were considered to be significant only when the bootstrap values was 50% or higher. MEGA6 was used to calculate the pairwise genetic distances [10].

3. RESULT AND DISCUSSION

3.1 Isolation

Figure 1A showed that strain LB7 formed small spherial and milky white colonies standing alone, pairing or gathering. Furthermore, gram staining result from Figure 1B showed that strain LB7 was gram positive (+) when all of the cells were stained purple. The cells were described to be short or long rods, single or double or string forms. This results were consistent with characteristics of *Lactobacillus* [1-2, 6-7].

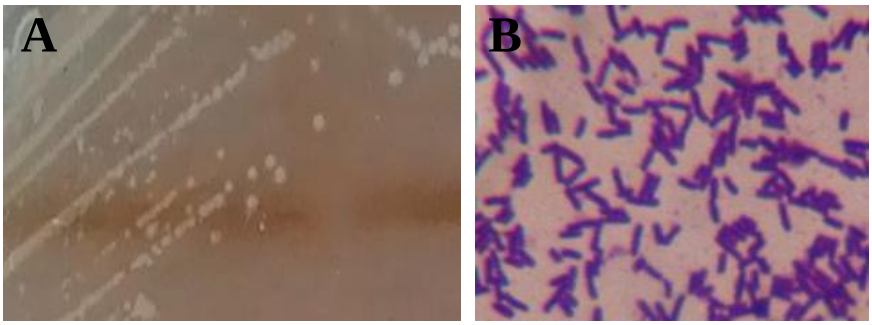

Figure 1: Colonies of LB7 on solid MRS (A) and LB7 cells stained purple under light microscope (B).

3.2. PCR reaction

PCR products were visualized on agarose gel as band sized between 300 and 400 bp. The result was in accordance with the theoretical size of PCR product amplified by Lac1 and Lac2 (340 bp) [3]. There was no band visualized in negative control which indicated that there was no infection during conduction of the PCR process.

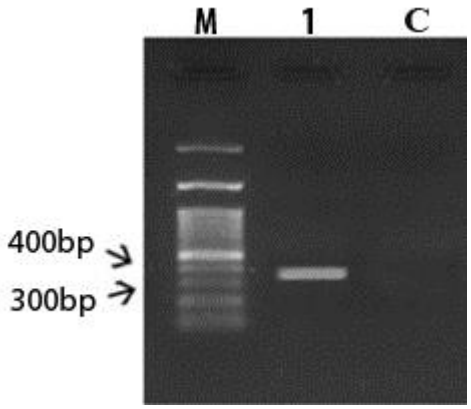

Figure 2: PCR products visualized on agarose gel. M: DNA ladder; 1: LB7; C: Negative control

3.3. Phylogenetic analysis of *16S* rRNA *gene* (rDNA) sequences

Phylogenetic tree in Figure 3 showed that strain LB7 was grouped with *Lactobacillus plantarum* (KJ187148) with bootstrap value of 100% in both Bayesian and Parsimony method (Figure 3A and B). In addition, the pairwise genetic distance value calculated based on the 16S rRNA gene (rDNA) sequence (Table 2) was zero which indicated that there was no difference between LB7 and *Lactobacillus plantarum* (KJ187148).

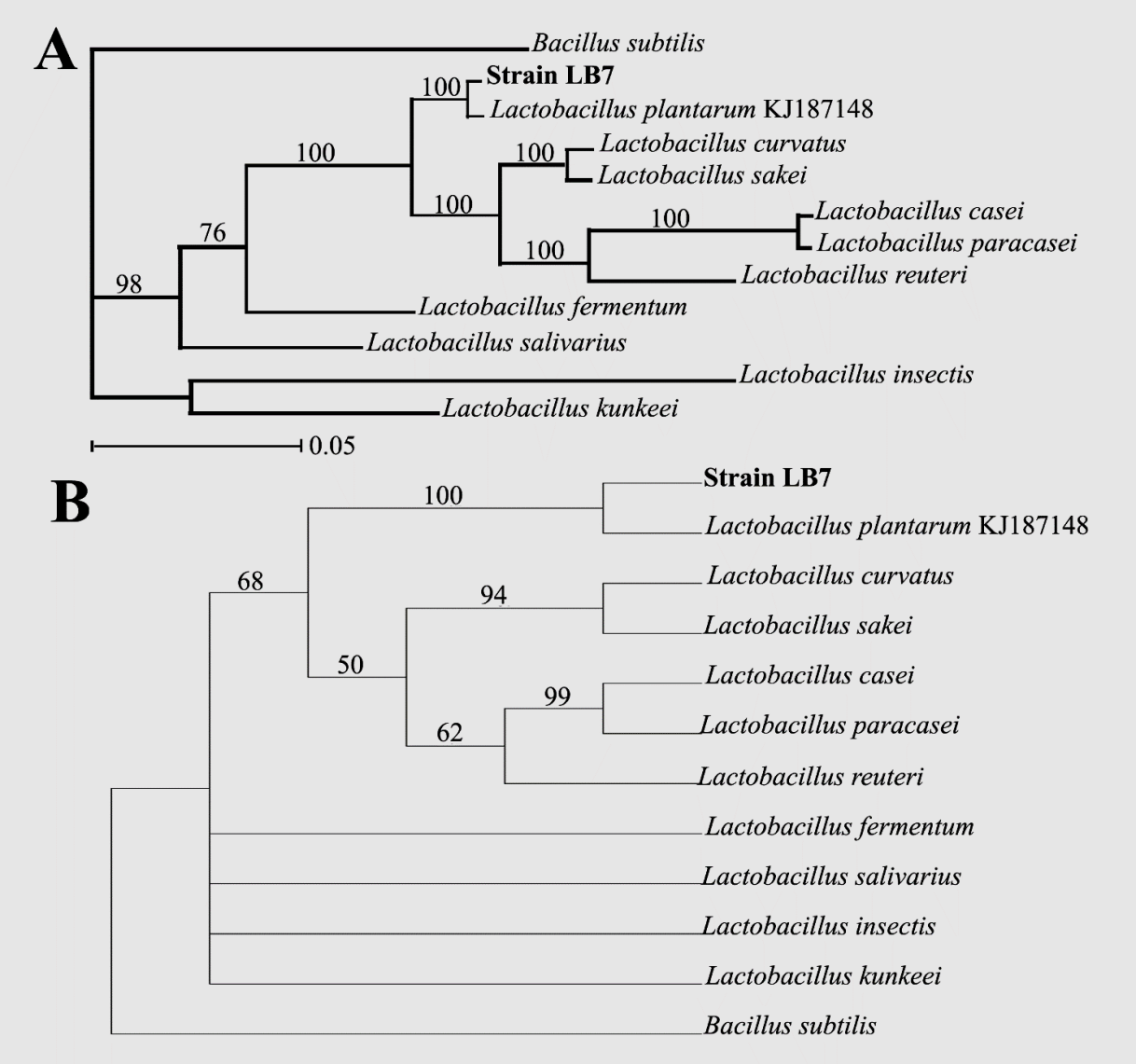

Figure 3: Rooted Bayesian (A) and rooted most parsimonious (B) tree constructed based on 16S rRNA gene (rDNA) sequences showed the phylogenetic relationship between strain LB7 and 11 taxa belonging to Lactobacillus. Bootstrap values of >50% were mentioned above the branches and beside the nodes

Table 2: Pairwise genetic distances between 16S rRNA gene (rDNA) sequence of strain LB7 and those of taxa belonging to Lactobacillus.

	Strain LB7	*L. plantarum*	*L. sakei*	*L. casei*	*L. reuteri*	*L. fermentum*	*L. insectis*	*L. kunkeei*
Strain LB7								
L. plantarum	0.000							
L. sakei	0.043	0.043						
L. casei	0.093	0.093	0.077					
L. reuteri	0.080	0.080	0.073	0.085				
L. fermentum	0.073	0.073	0.120	0.133	0.091			
L. insectis	0.198	0.198	0.175	0.187	0.207	0.203		
L. kunkeei	0.113	0.113	0.146	0.149	0.172	0.138	0.192	

Therefore, the phylogentic analysis result of 16S rRNA gene (rDNA) sequence amplified by primer pairs Lac1 and Lac2 proved that strain LB7 isolated was completely matched with *Lactobacillus plantarum* which is one of the most versatile species extensively used in the food industry. This is the first research which uses phylogentic analysis (16S rRNA gene) in order to identify the scientific name of *Lactobacillus* strain in Vietnam.

3.4. Lactic acid content quantified using HPLC system

Chromatogram of LB7 sample in figure 4B and chromatogram of standard in figure 4A were compared. The fact that peak in chromatogram of LB7 with retention of 4,752 and peak in chromatogram of standard with retention of 4,750 were nice sharp symmetrical shapes and almost had the same retention time indicated that the pickled product from *Lactobacillus plantarum* isolated in this study was lactic acid. The concentration of lactic acid produced in culture was 33,7g/l. The result was consistent with the results from previous studies on the ability of production of lactic acid in some *Lactobacillus* strain, for instance, Cock and Rodríguez (2006) [2, 14] reported that *Lactobacillus subslactis* strain isolated in the study had the ability to produce 13,7g/l acid lactic in optimal condition. Strain HN11 in a study of Nguyen and Tran (2008) [7] could produce 10.94 g/l within 48–60 h of cultivation in MRS medium supplemented with 15g/l glucose, pH 6 at 30^0 C. Recently, Nguyen and Do (2012) [6] reported that lactic acid content in culture of *Lactobacillus fermentum* isolated from pickled melon solution collected in Hue City was 20,93 g/l.

By using HPLC system, this study showed the high ability to produce lactic acid of *Lactobacillus plantarum* (LB7 strain) which we isolated from pickled melon solution, a popular food of Vietnamese. Thus, this result showed that LB7 strain will be able to applied to food industry in future.

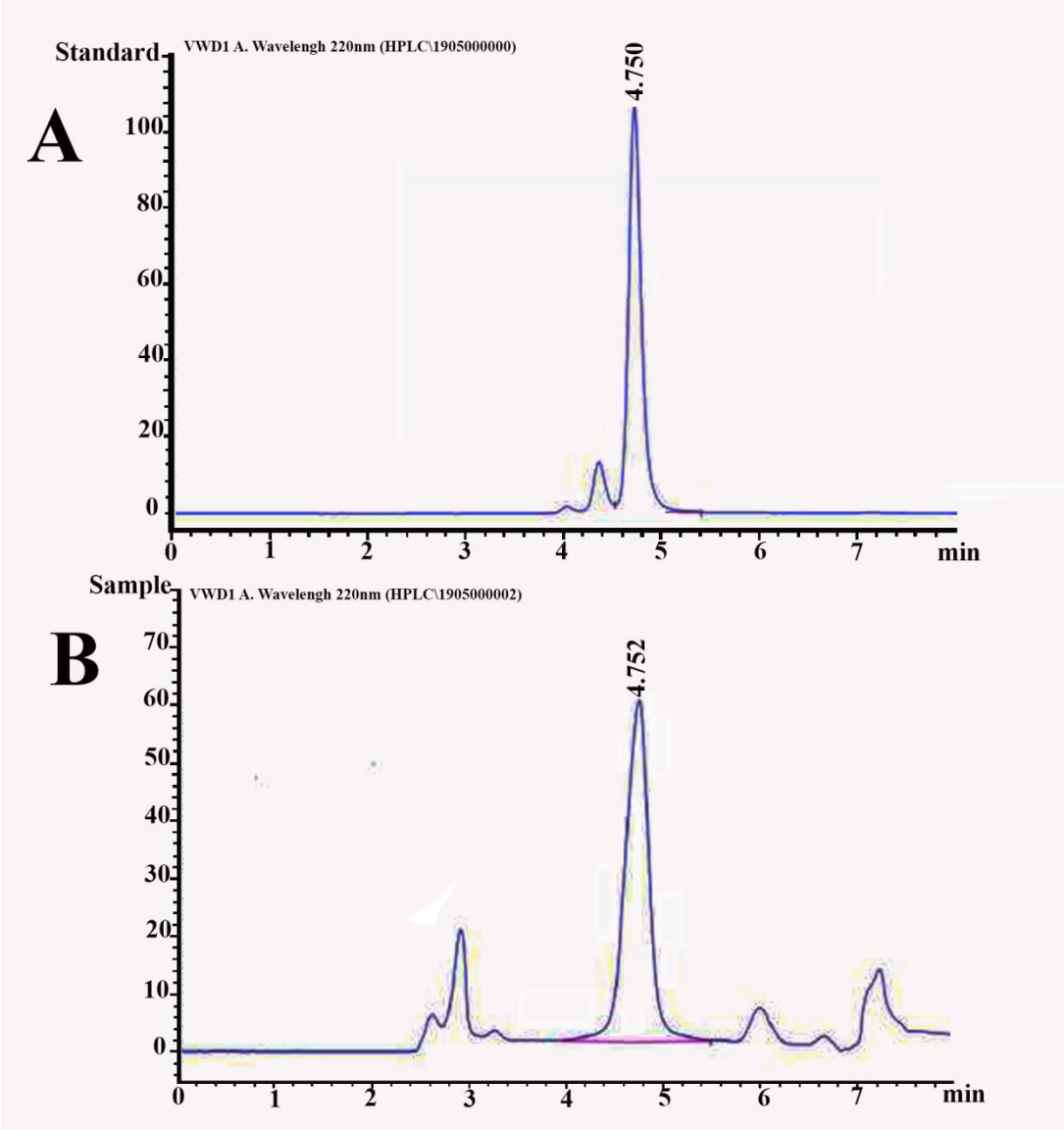

Figure 4: Chromatogram of lactic acid. A: Standard; B: LB7 sample

4. CONCLUSION

By using molecular technique, strain LB7 isolated from pickled melon solution was identifed as *Lactobacillus plantarum.* Besides, this study also showed the high ability to produce lactic acid of the isolate LB7 using HPLC system.

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ĐỊNH DANH VÀ XÁC ĐỊNH KHẢ NĂNG SINH ACID LACTIC CỦA VI KHUẨN PHÂN LẬP TỪ NƯỚC DƯA CẢI CHUA

Tóm tắt. Bằng kỹ thuật phân tích vùng trình tự DNA 16S, chủng vi khuẩn LB7 được phân lập từ nước dưa cải chua được xác định là loài Lactobacillus plantarum. Phân tích hàm lượng acid lactic trong dịch nuôi cấy tế bào vi khuẩn Lactobacillus plantarum phân lập được cho thấy, hàm lượng acid lactic đạt mức khá cao là 33,7g/l.

Từ khóa. Lactobacillus, *16S* rRNA *gene* (rDNA), acid lactic.

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