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DUAL OPTIMIZATION OF MEDIUM COMPONENTS USING WEIGHTED STANDARDIZATION FOR ANTIBACTERIAL SPECTRUM AND CELL YIELD OF *LACTOBACILLUS PLANTARUM* SK1305 ISOLATED FROM PICKLED PEPPER

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ABSTRACT

The present study aimed to optimize medium component which maximize antibacterial spectrum and cell yield of *Lactobacillus plantarum* SK1305 at the same time. *L. plantarum* isolated from Korean traditional pickled pepper was used and its antibacterial activity against *Salmonella typhimurium*, *Listeria monocytogenes*, *Burkholderia* sp., *Enterococcus faecalis*, *Salmonella gallinarum*, and *Staphylococcus aureus* were investigated. Alteration of medium components was performed using a fractional factorial design and weighted standardization method was applied in optimization of medium components. Inversed probability of medium component effect was employed in weighting. Total of 15 medium ingredients: sucrose, maltose, molasses, yeast extract, corn steep liquor, whey, K_2HPO_4, $MnSO_4$, $MgSO_4$, tween 80, NaCl, CH_3COONa, $C_6H_{11}NO_7$, Na_2SO_4, and FeSO4, were used as variables. The significant positive effects on cell yield were found for maltose and $FeSO_4$. The effects of ingredients on the antibacterial spectrum were summarized using weighted standardization, and sucrose and CH_3COONa were determined as the most important ingredients. These results provide useful information about medium ingredients for the improvement of the antibacterial spectrum of *L. plantarum* SK1305.

Key words: *Lactobacillus plantarum*; medium ingredients; antibacterial activity; cell yield; fractional factorial design.

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INTRODUCTION

Lactic acid bacteria (LAB) have been widely used in various industrial fields such as fermented dairy food, direct fed microorganism for livestock and food preservatives. These broad applications are possible since LAB are regarded as an organism involved in Generally Regarded As Safe (GRAS) , which can suppress the growth of pathogenic bacteria (Castellano *et al*., 2017). Antibiotics have been used to control pathogenic infection and to improve animal performance (Forte *et al*., 2016). However, many concerns induced by its abuse such as cross-resistance of antibiotics and occurrence of multidrug resistant pathogenic bacteria have been reported. And the use of antibiotics in animal husbandry is now strictly controlled in many countries. Probiotics have been developed to alternate the use of antibiotics as growth promoters, and LAB is the most-well known microorganism for probiotics. The beneficial roles of LAB are immune enhancement, competitive exclusion, production of organic acids and direct antibacterial activities (Castellano *et al*., 2017; Vieco-Saiz *et al*., 2019; Wang *et al*., 2015). The antibacterial activity or those spectrums against various pathogenic bacteria are reported to be varied depending on the genus and/or species of LAB (Ren *et al*., 2018). Other factors that can influence the antibacterial activity have been known as culture conditions consisting of temperature, pH, growth stage and medium compositions (Yang *et al*., 2018). Therefore, selection of LAB (Castellano *et al*., 2017) and optimizing culture condition are crucial for getting an effective antibacterial activity from culture. Investigation of the optimum medium, which is specialized to a certain LAB is a primary step in development of fermentation process (Srivastava *et al*., 2015; Yoo *et al*., 2018). An one factor at a time (OFAT) method considering the effect of one factor while the others are kept at a fixed point has been traditionally applied because it did not require any complicated statistical experimental design. It is obviously an excellent procedure unless there are many factors that should be considered at the same time (Coman and Bahrim, 2011; Zhang *et al*., 2012). Fractional factorial design (FFD) is a statistical tool applicable to experimental design and analysis of system's responses with only few trials compared to OFAT (Cho *et al*., 2010; Soni *et al*., 2007). Plackett-Burman design (PBD) is a frequently used FFD when only the main effects of variables are matter of interest (Cho *et al*., 2010).

In the present study, LAB showing antibacterial

activity isolated from Korean traditional pickled pepper and investigated the effect of various medium ingredients on the antibacterial activity for animal pathogens using statistical methods.

MATERIALS AND METHODS

Chemical and media: The chemical and medium ingredients were purchased from Sigma Chemical Co. (St. Louis, Mo, USA) and Becton, Dickinson and Company (BD, Le Pont-de-Claix, France), respectively unless otherwise stated.

Isolation of LAB: Korean traditional pickled pepper was used as a source for bacterial isolation. It was serially diluted with sterilized 0.8% NaCl solution and spread on MRS agar plates. The medium was incubated at 30 °C until a colony was detected, and the colony was transferred into MRS broth and then incubated for overnight (30 °C, 150 rpm). Culture supernatant was prepared after incubation by centrifugation (4 °C, 1,028 x 10g for 10 min) and then used for antibacterial activity assay. Finally, the strain showing antibacterial activity was selected.

Determination of antibacterial activity: Antibacterial activity was estimated using agar well diffusion assay according to Basualdo *et al*. (2007). Briefly, 100 µL of pathogenic bacterial culture (optical density was about 1.0 at 600 nm) was added to 10 mL of 0.8% water agar solution, which was kept at 60 °C to avoid solidification. And then the mixture was immediately poured on LB plate medium and let it be solidified at room temperature. A 6 mm hole in diameter was drilled and 100 µL of culture supernatant was infused into the hole. The assay plates were then incubated at 37 °C for 24 h and the diameter of the clear zone was measured. For pathogenic bacteria in assay, *Salmonella typhimurium* KCTC 2514 (KCTC: Korean Collection for Type Cultures), *Listeria monocytogenes* KACC10550 (KACC: Korean Agricultural Culture Collection), *Enterococcus faecalis* KCTC 2011, *Sal. gallinarum* ATCC 9184, *Staphylococcus aureus* SK1213 (wild type) and *Burkholderia* sp. SK875 (wild type) were used. All strains were maintained using LB medium at 37 °C

Taxonomical identification: Identification of the isolated strain was performed based on 16S ribosomal RNA gene sequence. The sequence was analyzed by polymerase chain reaction (PCR) with two universal primers: 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'-GGT TAC CTT GTT ACG ACT T- 3') (Weisburg *et al*., 1991). The PCR products were sequenced using ABI PRISM 3730XL DNA analyzer (Applied Biosystem, Franklin Lakes, NJ, USA). The identity of an isolated sequence was searched in GenBank. A multiple alignment was performed using CLUSTRAL_W program (Thompson *et al*., 1994) with 16S rRNA gene sequences of type strains involved in same genus with the bacteria. Phylogenetic tree of isolated strain was built based on the evolutional distance analysis using Maximum Composite Likelihood method (Lindsay, 1988). All identification procedures were performed using MEGA (Molecular Evolutionary Genetics Analysis) 4 program (Tamura *et al*., 2004; Tamura *et al*., 2007). Biochemical test was carried out using API 50 CHL kit (bioMérieux, Inc, NC, France), and the analysis was followed manufacturer's guide (Baradaran *et al*., 2012).

Cell yield: Cell yield was determined via viable cell counts on MRS agar plates. The number of colonies was enumerated and cell yield was expressed after logarithmic transformation of CFU (colony forming unit) based on 10.

Screening of media ingredients for antibacterial spectrum: The effects of medium ingredients on the antibacterial activity of the isolated strain were screened using PBD. For variables, sucrose, maltose, molasses yeast extract, corn steep liquor, whey, K_2HPO_4, $MnSO_4$, $MgSO_4$, NaCl, $FeSO_4$, CH_3COONa, $C_6H_{11}NO_7$, Na_2SO_4, and tween 80 were applied. All variables were assigned to two concentration levels (high and low) as summarized in Table 1. Total of 20 experimental run were constructed based on PBD (for X1, +, -, +, +, -, -, -, -, +, -, +, -, +, +, +, +, -, -, +, -). Four dummy variables were set after 15 variables for the calculation of standard error. High and low levels for variables were denoted as +1 and -1, respectively.

The effects of variables and their significance were calculated according to Chauhan *et al*. (2007). The main effect of variables were calculated by the difference between the sum of responses assigned to high levels of variables and the sum of responses from low levels of variables.

$$E(X_i) = \frac{2(\sum A_i^+ - \sum A_i^-)}{N}$$

where, $E(X_i)$ is main effect of variable X_i. A_i^+ and A_i^- are the responses from the trial where high and low levels of X_i variable, respectively. The responses are antibacterial activities against various pathogenic bacteria. N is the total number of trials. Experimental error was estimated by the variance that was calculated from dummy variables.

$$V = \sum (E_d)^2 / n$$

where, E_d is main effect from dummy variable, and n are number of dummy variables. The standard error of concentration effects is the square root of variance. The significance of each concentration effects of variables is calculated using student's *t*-test.

Standardized effect on various pathogenic bacteria: To summarize the effects of variables on antibacterial activity against various pathogenic bacteria simultaneously, standardized effects were calculated by transformation of effects using standard normal distribution and inversed probability.

$$Z_i^n = \frac{(E_i^n - \mu_i)}{\sigma_i} \times \frac{1}{p_i^n}$$

where, Z_i^n and E_i^n is the standardized effect and the effect of nth variable against i^{th} pathogenic bacteria. The μ_i and σ_i are mean and standard deviation from all effects of variables against i^{th} pathogenic bacteria. The p_i^n is the probability of the effect of nth variables against i^{th} pathogenic bacteria. Finally, overall effects of variables were calculated by summing all standardized effects.

RESULTS AND DISCUSSION

Isolation and identification: In this study, SK1305 strain showing antibacterial activity was isolated and it was determined to be closely related to *Lactobacillus plantarum* ATCC14431^T (AF429479) with 99% identity based on 16S rRNA gene sequence analysis (Fig. 1). The isolated strain was then designated as *L. plantarum* SK1305 (GenBank accession number: JX501236). Carbohydrate utilization patterns of *L. plantarum* SK1305 are summarized in Table 2. *L. plantarum* SK1305 is known to not be able to utilize glycerol, and the utilization of glycerol can be used for the distinguishable feature for *L. plantarum* from its intra-species, *L. pentosus* that can utilize glycerol (Tajabadi *et al*., 2013).

Effects of medium ingredients on cell yield of *L. plantarum* SK1305: In the cell yield, positive effects were found at sucrose, maltose, molasses, whey, $MnSO_4$, tween 80, sodium sulfate and $FeSO_4$ (Table 3). Molasses (p=0.007) and $FeSO_4$ (p=0.025) had significant positive effects on the cell yield, respectively. Molasses is a frequently used industrial medium ingredient for the fermentation of various bacteria including Lactobacilli because of its cheap price and suitable for energy supply (Prado *et al*., 2016). It was reported that molasses could increase the growth efficiency in *L. rhamnosus* and *L. delbrueckii* (Senedese *et al*., 2015; Srivastava *et al*., 2015).

Concentration effects of medium ingredients on antibacterial activity: Broad antibacterial spectrums were found in runs of 3, 4, 11, 12, 14, 15 and 19 (Table 4). With these results, it could be supposed that the used medium ingredients and their concentrations could considerably influence the antibacterial activity in culture of *L. plantarum* SK1305. Sucrose showed a positive effect on antibacterial activity against all pathogens and it showed a particularly great effect against *List. monocytogenes*. All these effects were significant except for *Sal. gallinarum* (p=0.082). In *L. plantarum,* sucrose is known as a primary carbon source for improving cell growth, bacteriocin, and exopolysaccharides which are high molecular weight carbohydrate polymers synthesized by microorganisms including *Lactobacillus* (Cheng *et al*., 2019). However, there was a study reporting no effect of sucrose (15 or 30 g/L) on the production of bacteriocin (Sabo *et al*., 2019). In the present study, sucrose demonstrated as an important medium ingredient for the antibacterial activity of *L. plantarum* SK1305. Maltose is an important carbon source for LAB that can hydrolyze starch for the productions of lactic acid and bacteriocin (Reddy *et al*., 2008). It can be utilized for the bacteriocin production in *L. plantarum* that does not have amylolytic activity (Todorov and Dicks 2006). However, it was reported that bacteriocin production was not stimulated by 20 g/L maltose (Todorov *et al*., 2013). In this study, maltose showed positive antibacterial effects against *List. monocytogenes* (E=3.3, p=0.044) and *Ent. faecalis* (E=0.2, p=0.047). Molasses was shown as an important medium component in terms of cell yield and reported to be an important ingredient for the growth and bacteriocin production in *L. delbrueckii* (Srivastava *et al*., 2015). Sucrose is known as a major constituent in molasses (Prado *et al*., 2016). In the present study, high concentration of sucrose had positive effects on antibacterial activity against all pathogens in all runs. Therefore, molasses is expected to be a positive variable. However, the average effect of molasses was 0.7, which was lower than the maltose (E=1.45). It had a negative antibacterial effect against *Sal. typhimurium*, whereas it had a significant positive effect against *Sal. gallinarum* (p=0.045). Yeast extract had a negative effect on cell yield, while having positive effects against all tested pathogenic bacteria. In particular, yeast extract showed significant antibacterial effect against *Sal. typhmurium* (p=0.026). Yoo *et al.* (2018) reported that yeast extract was positively influencing ingredient on cell yield. On the other hand, it was not effective on bacteriocin production in LAB according to Todorov and Dicks (2006).

Corn steep liquor (CSL) is rich in nutrient and cheap. The CSL has been reported as an essential medium component for the production of lactic acid from *Lactobacillus* sp., *L. casei*, and *L. rhamnosus* (Hwang *et al*., 2012; Li *et al*., 2016; Wee and Ryu 2009). The CSL showed a positive effect on antibacterial activity of *L. plantarum* SK1305 culture against all pathogenic bacteria except for *Sal. typhimurium* in this study. Whey showed positive antibacterial effects against *List. monocytogenes*, *Burkholderia* sp., *Ent. Faecalis,* and *Sal. gallinarum*, whereas its effects against *Sal. typhimurium* and *Staph. aureus* was negative. In addition, whey is known as a cheap and effective medium ingredient for the culture of

LAB as like molasses and CSL. Whey is a by-product from milk processing and contains a lot of nutrients such as lactose, protein, fat, and minerals. Hence, whey can be utilized as both nitrogen and carbon source for the growth of LAB. Particularly, it is regarded as important for the productions of lactic acid and cell yield of LAB (Kim *et al.,* 2006; Sweta and Samir 2016). Potassium phosphate is a phosphate source, essential for bacterial metabolism and it can improve the various fermentation efficiencies, such as cell yield and production of metabolites, via its buffering activity (Penna *et al*., 2005). However, potassium phosphate did not show positive effects on antibacterial activity against all tested pathogenic bacteria in this study (p>0.05). Manganese and magnesium ions are important cofactors in the carbohydrate metabolism of LAB (Lew *et al*., 2012). Brillet-Viel *et al*. (2016) reported that these ions were also important for the production of bacteriocin. In this study, the effect of manganese sulfate was positive on cell yield, whereas it was negative on antibacterial activity. Contrary to the manganese sulfate, magnesium sulfate showed a negative effect on cell yield and a positive effect on the antibacterial activity. Tween 80 showed a positive effect against all pathogenic bacteria except *Burkholderia* sp. However, no significance was found (p>0.05). NaCl showed antimicrobial effects against *Sal. typhimurium* and *Sal. gallinarum* but no significant effect (p>0.05). Sodium acetate had positive antibacterial effects against all pathogenic bacteria and antibacterial effects against *Sal. typhimurium*, *Burkholderia* sp. and *Sal. gallinarum* were significant (p<0.05). Ammonium citrate had positive antibacterial effect against all pathogenic bacteria but was not significant (p>0.05). Sodium sulfate showed positive effects against *Ent. faecalis* and *Staph. aureus* but their significances were not found (p>0.05). Ferrous sulfate showed positive effects on the antibacterial activity of *L. plantarum* SK1305 against *Sal. typhimurium*, *List. monocytogenes,* and *Ent. faecalis,* but those effects were not significant (p>0.05).

Standardized effects of media components on antibacterial spectrum: Summarized effect of ingredients against different pathogens are shown in Table 5. As the results, two ingredients, sucrose and sodium acetate,were detected as essential for the improvement and acquisition of antibacterial activity in the culture of *L. plantarum* SK1305. It was also found that sucrose and sodium acetate could get *L. plantarum* SK1305 specific antibacterial activity against *Listeria monocytogenes* and *Burkholderia* sp., respectively.

Table 1. Variables showing medium components used in Plackett-Burman design.

Variables	Medium components	+ Values (g/L)	- Values(g/L)
X1	Sucrose	10	1
X2	Maltose	10	1
X3	Molasses	10	1
X4	Yeast extract	10	1
X5	Corn steep liquor	10	1
X6	Whey	10	1
X7	K_2HPO_4	2	0.2
X8	$MnSO_4$	0.05	0.025
X9	$MgSO_4$	0.05	0.025
X10	Tween 80	1	0.1
X11	NaCl	5	0.5
X12	CH_3COONa	5	0.5
X13	$C_6H_{11}NO_7$	2	0.2
X14	Na_2SO_4	2	0.2
X15	$FeSO_4$	0.05	0.0025

Table 2. Carbohydrate utilization profiles of *L. plantarums* SK1305.

Substrate	Reactivity	Substrate	Reactivity
Control	-[1]	Galactose	+
Glycerol	-	Glucose	+
Erythritol	-	Fructose	+
D-Arabinose	-	Mannose	+
L-Arabinose	+	SorbosE	-
Ribose	+	Rhamnose	-
D-Xylose	-	Dulcitol	-
L-Xylose	-	Inositol	-

Adonitol	-	Mannitol	+
Methyl-D-xyloside	-	Sorbitol	+
Methyl-D-mannoside	-	Melibiose	+
Methyl-D-glucoside	-	Sucrose	+
N-acetyl-glucosaine	+	Trehalose	+
Amygdalin	+	Inulin	-
Arbutin	+	Melezitose	+
Esculin	-	Raffinose	+
Salicin	+	Starch	-
Cellobiose	+	Glycogen	-
Maltose	+	Xylitol	-
Lactose	+	Gentiobiose	+
D-Turanose	+	D-Arabitol	-
D-Lyxose	-	L-Arabitol	-
D-Tagatose	-	Gluconate	+
D-Fucose	-	2-keto-Gluconate	-
L-Fucose	-	5-keto-Gluconate	-

[1]+ and – mean utilization and non-utilization of related carbohydrates, respectively

Table 3. Viable cell yield and antibacterial activity results obtained from Plackett-Burman design configuration.

Trials	Cell yield Log_{10}(CFU/ml)	Clear zone diameter (mm) against pathogenic bacteria[1]					
		A	**B**	**C**	**D**	**E**	**F**
1	9.43	A	B	C	D	E	F
2	9.83	ND[2]	16	15	15	12	16
3	9.70	ND	16	10	13	12	13
4	9.65	12	20	14	15	13	17
5	9.36	13	20	10	15	12	16
6	9.68	ND	17	12	15	12	15
7	7.60	12	12	12	15	12	ND
8	9.23	ND	ND	10	ND	ND	13
9	9.51	ND	13	12	16	12	15
10	9.28	ND	16	ND	14	ND	14
11	9.45	ND	10	ND	13	ND	11
12	8.11	10	14	14	13	13	14
13	9.54	12	17	14	15	13	16
14	9.92	ND	14	10	15	12	15
15	9.92	13	20	13	16	12	16
16	9.96	10	18	13	16	12	15
17	9.91	ND	18	12	16	10	14
18	9.64	ND	15	10	14	10	14
19	8.04	ND	15	10	13	10	13
20	8.65	12	16	14	13	10	14
		ND	ND	ND	ND	ND	ND

[1] A, *Salmonella typhimurium*; B, *Listeria monocytogenes*; C, *Burkholderia* sp., D, *Enterococcus faecalis*; E, *Salmonella gallinarum*, F, *Staphylococcus aureus*.
[2] ND: not detected.

Table 4. Calculated effect and probability of media components for cell yield and antibacterial activity of *L. plantarum* SK1305.

Media components	Cell yield		Antibacterial activity											
			Salmonella typhimurium		*Listeria monoytogenes*		*Burkhorderia* sp		*Enterococcus faecalis*		*Salmonella gallinarum*		*Staphylococcus aureus*	
	Effect	***P* value**	**Effect**	***P* value**	**Effect**	***P* value**	**Effect**	***P* value**	**Effect**	***P* value**	**Effect**	***P* value**	**Effect**	***P* value**
Sucrose	0.90	0.087	1.80	0.011	4.30	0.001	1.80	0.049	2.00	0.029	1.80	0.082	2.70	0.012
Maltose	1.00	0.110	0.20	0.442	3.30	0.044	0.60	0.172	2.00	0.047	0.90	0.079	1.70	0.056
Molasses	1.76	0.007	-0.60	0.288	1.70	0.119	0.80	0.256	1.40	0.100	1.70	0.045	-0.80	0.333
Yeast extract	-0.44	0.231	2.60	0.026	1.90	0.148	1.20	0.179	0.20	0.440	0.30	0.397	0.50	0.358
Corn steep liquor	-0.40	0.280	-1.00	0.172	0.10	0.476	0.60	0.327	0.20	0.432	0.30	0.332	1.70	0.086
Whey	0.64	0.141	-0.60	0.343	1.70	0.156	0.80	0.265	1.60	0.076	0.30	0.391	-0.10	0.461
K_2HPO_4	-0.14	0.332	-0.20	0.442	-0.90	0.329	-0.20	0.440	-0.60	0.528	-1.10	0.149	-0.70	0.293
$MnSO_4$	0.25	0.385	-1.00	0.204	-0.30	0.433	-0.50	0.391	-0.40	0.370	0.10	0.464	0.90	0.202
$MgSO_4$	-0.20	0.391	1.00	0.197	2.70	0.094	0.80	0.270	1.00	0.199	1.30	0.098	2.10	0.068
Tween 80	0.32	0.354	0.40	0.373	1.30	0.280	-0.40	0.382	1.40	0.117	0.50	0.315	0.50	0.357
NaCl	-0.39	0.109	1.20	0.159	-0.90	0.320	-0.20	0.445	-0.60	0.315	0.30	0.397	-0.70	0.293
CH_3COONa	-0.38	0.331	1.60	0.024	0.70	0.331	3.20	0.002	1.00	0.235	2.10	0.028	1.30	0.115
$C_6H_{11}NO_7$	-0.09	0.459	0.00	0.500	0.10	0.473	-0.60	0.314	0.80	0.270	0.30	0.351	0.10	0.469
Na_2SO_4	0.36	0.334	-1.60	0.133	-0.10	0.480	-1.20	0.163	0.60	0.320	-1.10	0.161	1.30	0.166
$FeSO_4$	1.36	0.025	0.80	0.278	0.50	0.372	-1.00	0.174	1.40	0.066	-0.10	0.441	-0.50	0.309
Standard error	1.55		7.81		5.45		5.26		5.80		4.25		9.53	

Table 5. Standardized effect[1] of media components on antibacterial activity and their sum.

Media Components	Pathogenic bacteria						Sum of effect
	Salmonella typhimurium	*Listeria monocytogenes*	*Burkholderia* sp.	*Enterococcus faecalis*	*Salmonella gallinarum*	*Staphylococcus aureus*	
Sucrose	118.31	2177.09	25.97	48.77	17.40	157.36	2544.89
Maltose	-0.21	34.14	1.15	30.09	5.49	17.14	87.80
Molasses	-2.74	3.55	1.47	7.07	29.25	-4.09	34.51
Yeast extract	76.87	3.77	4.10	-1.61	-0.57	-0.43	82.13
Corn steep liquor	-6.62	-1.38	0.60	-1.64	-0.69	11.16	1.44
Whey	-2.30	2.71	1.42	12.41	-0.58	-1.54	12.10
K_2HPO_4	-1.00	-4.05	-1.18	-3.12	-11.89	-4.33	-25.58
$MnSO_4$	-5.58	-2.14	-2.02	-3.82	-0.97	1.07	-13.45
$MgSO_4$	3.07	11.68	1.39	1.18	8.93	19.57	45.83
Tween 80	0.22	0.55	-1.83	6.04	-0.02	-0.43	4.52
NaCl	4.90	-4.16	-1.17	-5.24	-0.57	-4.33	-10.58
CH_3COONa	46.96	-0.76	1263.37	1.00	62.77	5.11	1378.45
$C_6H_{11}NO_7$	-0.53	-1.39	-2.80	0.00	-0.65	-1.12	-6.49
Na_2SO_4	-12.49	-1.65	-8.69	-0.74	-11.01	3.54	-31.03
$FeSO_4$	1.55	-1.04	-7.11	10.71	-1.52	-3.51	-0.91

[1]Individual effects were standardized using average effects and their standard deviation and the application of weighting to each standardized effect was performed by dividing their probability.

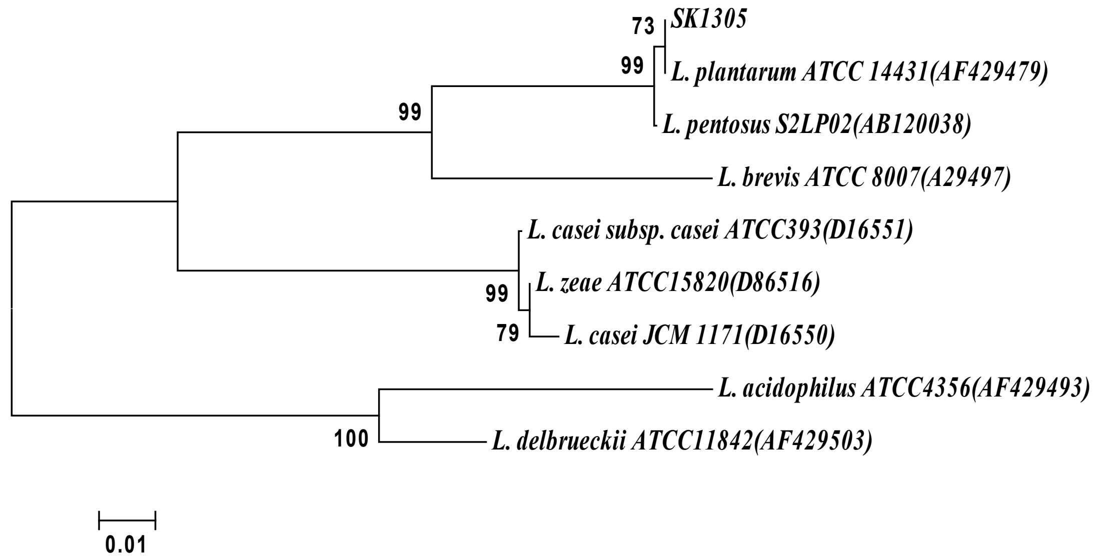

Fig. 1. Phylogenetic tree of *L. plantarum* SK1305 with 8 type strains of *Lactobacillus* genus. Tree was built based on 16S rRNA gene sequences.

Conclusion: The present study isolated a bacteria, which was shown antibacterial activity, and it was identified as *L. plantarum* SK1305 based on 16S rRNA gene sequence analysis. Total of 15 medium ingredients were investigated for their effects on cell yield and antibacterial activity. Total of 20 experimental run based on PBD were employed. Various medium ingredients showed different effects on cell yield and antibacterial activities of *L. plantarum* SK1305. Through standardization of individual effects of ingredients, sucrose and sodium acetate were found as essential medium elements for the culture of isolated *L. plantarum* SK1305.

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