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Lactobacillus plantarum 1074: A POTENTIAL PROBIOTIC STRAIN ISOLATED FROM *KESONG PUTI* (FILIPINO SOFT WHITE CHEESE)

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ABSTRACT

***Kesong puti* is a Filipino soft white cheese made usually from carabao or cow's milk using rennet or acid. It is home to different species of beneficial microbes that may potentially display probiotic traits. This study aimed to evaluate the acid and bile resistance, NaCl tolerance, cell surface hydrophobicity (CSH), auto-aggregation, hemolytic, antibacterial, and antibiotic susceptibility characteristics of *Lactobacillus plantarum* 1074, a rod-shaped, Gram-positive, lactic acid bacterium isolated from *kesong puti*. The isolate exhibited resistance to pH 3 and 0.5% bile salts. Moreover, it was able to tolerate MRS broth with 8% NaCl. In terms of cell surface properties, the isolate displayed strong auto-aggregation activity. It also showed a higher affinity to xylene indicating cell surface hydrophobicity. When grown to blood agar, the isolate demonstrated gamma (γ)-hemolytic activity suggesting its non-hemolytic nature. In terms of inhibitory activity, it exhibited strong antibacterial activity against *Escherichia coli* (laboratory isolate). The antibiotic susceptibility pattern of *L. plantarum* 1074 displayed resistance to cefaclor and kanamycin. However, it expressed sensitivity to amoxicillin, ampicillin, augmentin, penicillin, streptomycin, chloramphenicol, clindamycin, erythromycin, and tetracycline. The findings of this study revealed the promising potential of *Lactobacillus plantarum* 1074 as a probiotic strain as it exhibited desirable attributes *in vitro*.**

Keywords: beneficial microorganism, *kesong puti*, lactic acid bacteria, *Lactobacillus plantarum*, probiotics

INTRODUCTION

FAO/WHO (2001) defined probiotics as cultures of viable microbes, usually bacteria, molds, and/or yeast, consumed for their beneficial health effects beyond basic nutrition. Recent studies have reported the health benefits of probiotic consumption on individuals suffering from eczema, acute respiratory tract infections, acute infectious diarrhea, infant colic, antibiotic-associated diarrhea, *Clostridium difficile* infection, inflammatory bowel diseases, and necrotizing enterocolitis (Liu *et al*., 2018; Buccigrossi *et al*., 2019; Kalakuntla *et al*., 2019; Kopacz and Phadtare, 2022). Numerous potential health benefits are still being

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investigated including the effect of probiotics on neurodevelopmental and neurological disorders such as autism spectrum disorder (ASD), Alzheimer's (AD), and Parkinson's disease (Cheng *et al*., 2019). The exact mechanisms by which these microorganisms exert these health benefits have not yet been fully explained. However, a number of mechanisms have been postulated such as immunomodulation, cell adhesion to intestinal receptors, competitive inhibition of pathogenic microorganisms, modification of intestinal microflora, and production of mucus, bacteriocin (defensins), and volatile fatty acids (Plaza-Diaz *et al*., 2019).

Most probiotic strains belong to lactic acid bacteria. They are ubiquitous groups of catalase-negative, non-sporulating, Gram-positive rods or cocci-shaped microorganisms that convert lactose to lactic acid (Tamang, 2014). Aside from being resident flora of the human gastrointestinal tract, LAB is commonly found in food-related niches such as fermented food products (Rezac *et al*., 2018). Among the LAB strains, *Lactobacillus plantarum* is one of the most commonly used probiotics in the food industry today. It is normally found in cultured dairy products, cheese, and fermented foods made from meat, fish, vegetables, and cereals (Behera *et al*., 2018). In the Philippines, there are many indigenous fermented food products that may potentially harbor probiotics including local cheeses. *Kesong puti* is an unmatured soft white cheese usually made from carabao or cow's milk using rennet (animal or microbial), vinegar, or food-grade acetic acid. It is also known as Sta Cruz cheese in Laguna and *Kasilyo* in Cavite (Barraquio, 2006). During fermentation, it is home to various groups of microorganisms including many beneficial bacteria.

Many previous studies have concentrated on the isolation, identification, and characterization of the microbial population in *kesong puti*. However, there is very little research on the determination of the probiotic properties of indigenous LAB derived from this local cheese. Therefore, the objective of this study was to evaluate the acid and bile salt resistance, NaCl tolerance, auto-aggregation, cell surface hydrophobicity, hemolytic, antibacterial, and antibiotic susceptibility characteristics of *Lactobacillus plantarum* 1074, a rod-shaped, Gram-positive, lactic acid bacterium isolated from *kesong puti*.

MATERIALS AND METHODS

Lactobacillus plantarum 1074 was acquired from the Dairy Training and Research Institute (DTRI), College of Agriculture and Food Science (CAFS), University of the Philippines Los Baños (UPLB), College, Laguna, Philippines. The isolate was stored at 4°C in de Man, Rogosa, Sharpe (MRS) agar. For probiotic experiments, *L. plantarum* 1074 was reactivated by incubation in MRS broth at 37°C for 18 to 24 hours. The cells were then standardized to a 0.5 McFarland turbidity standard which corresponds to an inoculum of approximately 10^7 to 10^8 CFU/ml. On the other hand, *Escherichia coli* (laboratory isolate) was maintained in Brain Heart Infusion (BHI) agar slants at 4°C and reactivated (1% v/v) in BHI broth for 18 to 24 hours at 37°C for routine analysis.

For the acid resistance assay, *L. plantarum* 1074 was inoculated at 10% (v/v) into modified MRS broth media with different pH levels (1, 2, 3, 4, 5, and 7). MRS broth adjusted to pH 7 served as the control. For the bile resistance assay, the isolate was transferred at 2% (v/v) in MRS broth media (control) and MRS broth with varying concentrations (0.1, 0.3, 0.5, 0.7, and 0.9%) of ox gall bile. All tubes were placed in an incubator for 18 to 24 hours at 37°C. After incubation, the optical density was measured at 560 nm (OD_{560nm}) using a Ultraviolet-Visible Spectrophotometer. The resistance (%) was expressed as the percentage

of bacterial growth in MRS broth media with different pH levels or with various concentrations of ox gall bile as compared to that of their respective controls (Kumar and Kumar, 2015).

The antimicrobial activity of *L. plantarum* 1074 against *Escherichia coli* was assessed by the agar well diffusion assay using Mueller Hinton agar (MHA) plates. Briefly, MHA plates were prepared by streaking the active *E. coli* culture on the solid surface of the media. Using an aseptic technique, a circular well with a 6 mm diameter was prepared using a sterile pipette tip. Cell-free supernatant or CFS (100 µl) of *L. plantarum* 1074 was then loaded in each agar well. CFS was obtained by centrifugation (4,000 g, 15 minutes, 4°C) following the protocol of Saadatzadeh *et al*. (2013). The supernatant was subsequently filter-sterilized by passing through a 0.22 µm syringe filter. Half of the CFS was used for bacteriocin assay by adjusting the pH to 6.5 while the remainder was left with its original pH. All plates were placed at room temperature for 1 hour for diffusion with subsequent incubation for 18 to 24 hours at 37°C. Using the vernier caliper, the diameter of the zone of inhibition (ZOI) was measured in each well and the results were interpreted according to Akabanda *et al*. (2014).

The cell surface hydrophobicity (CSH) of *L. plantarum* 1074 was determined by microbial adhesion to hydrocarbons (MATH) assay using xylene, chloroform, and ethyl acetate as solvents (Ji *et al*., 2015). Briefly, active cells of *L. plantarum* 1074 were harvested by centrifugation (5,000 g, 10 minutes, 4°C) and rinsed twice in sterile phosphate-buffered saline (PBS) with subsequent resuspension in the same buffer solution. The initial absorbance at 580 nm (OD_{580nm}) served as reading 1. Equal amounts of solvents and cell suspension were added into the tubes with successive mixing for 2 minutes using a vortex mixer. The tubes were then placed in an incubator at 37°C for 30 minutes to allow the phase separation. A portion of the aqueous phase (water-soluble layer) was carefully collected, and the optical density was consequently recorded at 580 nm (reading 2). The cell surface hydrophobicity was calculated using the formula below:

$$Hydrophobicity\ (\%) = \frac{(OD_{580nm}\ reading\ 1 - OD_{580nm}\ reading\ 2)}{OD_{580nm}\ reading\ 1}\ x\ 100$$

The auto-aggregation activity of *L. plantarum* 1074 was evaluated according to the protocol by Melgar-Lalanne *et al*. (2015) with slight modifications. Briefly, active cells of *L. plantarum* 1074 were harvested by centrifugation (5,000 g, 10 minutes, 4°C) and rinsed twice in sterile phosphate-buffered saline (PBS) with subsequent resuspension in the same buffer solution. The suspension was then standardized to 0.5±0.01 at 600 nm (OD_{600nm}). Four millimeters of the suspension were dispensed into the test tube and then mixed for 2 minutes using a vortex mixer. Auto-aggregation activity was measured after the 3rd and 5th hour of incubation at room temperature. After incubation, a portion (0.1 ml) of the upper suspension was collected and transferred onto a 3.9 ml sterile PBS solution. The absorbance of the resulting mixture was determined at 600 nm (OD_{600nm}). The auto-aggregation activity of the *L. plantarum* 1074 was computed using the formula below:

$$\text{Auto} - \text{aggregation}\ (\%) = 1 - \frac{\text{Absorbance at time (t)} = \text{3 or 5 hours}}{\text{Absorbance at t} = \text{0 hour}} \times 100$$

The halotolerance of *L. plantarum* 1074 was evaluated using the turbidimetric method (Hoque *et al*., 2010). Briefly, 1% (v/v) of the active culture of the isolate was inoculated into modified MRS broth (10 ml) supplemented with various concentrations of NaCl (1 to 10%, w/v). MRS broth without NaCl served as the control. The inoculated tubes were then incubated at 37°C for 18 to 24 hours. The turbidity of the tubes was checked and interpreted as follows: (-) = no growth; (+) = normal growth; and (++) = maximum growth.

The hemolytic activity of *L. plantarum* 1074 was evaluated using BAP or blood agar plates (Halder *et al*., 2017). Briefly, BAP was prepared from blood agar base (infusion agar) supplemented with 5% sterile and defibrinated sheep blood. One loopful of the isolate was streaked on the solid surface of sterile BAPs. The plates were then placed in an incubator for 72 hours at 37°C. After incubation, the plates were inspected for the signs of hemolytic activity: α-hemolysis (green-hued zones around colonies), β-hemolysis (transparent zones around colonies), and γ-hemolysis (no notable zones around colonies).

Antibiotic susceptibility of *L. plantarum* 1074 was determined against 10 antibiotics using a standard disc diffusion assay. The antibiotics tested were amoxicillin, ampicillin, augmentin (amoxicillin/clavulanate), penicillin G, cefaclor, kanamycin, streptomycin, chloramphenicol, clindamycin, erythromycin, and tetracycline. MRS agar plates were prepared by streaking the active *L. plantarum* 1074 culture on the solid surface of the media. Using an aseptic technique, the antibiotic discs were placed onto MRS agar plates. The plates were then allowed to stand for 1 hour at 40°C for diffusion with subsequent incubation for 24 hours at 37°C. Using the vernier caliper, the diameter of the zone of inhibition (ZOI) was measured and the results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) interpretative standards described previously by Charteris *et al*. (1998).

All determinations were performed in triplicate. Simple descriptive analyses were carried out on all data collected from antimicrobial activity (agar well diffusion), MATH, auto-aggregation activity, hemolytic activity, NaCl tolerance, and antibiotic susceptibility assays. A one-way analysis of variance (ANOVA) in a completely randomized design (CRD) was performed on acid and bile tolerance data with pH levels and bile salt concentrations served as treatments, respectively. A pairwise comparison of treatment means was analyzed using Scheffe's *post hoc* test at 5% significance level. All statistical analyses were done using SAS® OnDemand for Academics software (Statistical Analysis System Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Microorganisms intended for probiotic applications must remain viable after exposure to harsh stomach acids. It is recommended that the ideal strain must persist in an environment with a pH of at least 3.0 with a 50% resistance rate (Kumar and Kumar, 2015). In this study, *L. plantarum* 1074 demonstrated relatively the highest rate of resistance at pH 5.0 (Table 1). It differed significantly ($P<0.05$) from the resistance rate observed in MRS broth adjusted to pH 1, 2, 3, and 4. However, it is worth noting that the isolate was able to resist pH 3.0 with a resistance rate of 54.93%. The result indicates that *L. plantarum* 1074 has met the standard set for acid resistance of probiotic strains. Several acid-resistant strains have also been identified in previous studies including *L. plantarum* 15HN, *L. plantarum* FH185, *L. plantarum* KR6-DSM 28780, and *L. plantarum* M5 (Park and Lim, 2015; Šeme *et al*., 2015;

Table 1. Optical density and resistance rate of *L. plantarum* 1074 in MRS broth adjusted to different pH levels.

pH Level	Optical Density (absorbance at 560 nm)[1]	Resistance Rate (%)[1,2]
1	0.7805±0.0139	26.74±0.48[e]
2	0.8743±0.0107	29.95±0.37[d]
3	1.6035±0.0009	54.93±0.29[c]
4	2.1908±0.0119	75.57±0.41[b]
5	2.7895±0.0171	96.22±0.59[a]
	P-value:	<0.0001

[1]Data are presented as mean ± standard deviation.
[2]Means within column with different superscript are significantly different using Scheffe's *post hoc* test ($P \leq 0.05$).

Haghshenas *et al*., 2016). The acid resistance of microorganisms could be attributed to various mechanisms including intracellular proton consumption (glutamate decarboxylation system), activation of H^+-ATPases, cell envelope remodeling, alkali production (urease or arginine deiminase activities), or macromolecules protection and repair (Liu *et al*., 2015). These mechanisms would permit probiotics to persist and remain viable during their transit in the acidic gastric environment.

Probiotics must also persist in the intestinal tract where bile is secreted directly into the first segment and upper part of the small intestine called the duodenum. Bile acid is highly toxic for bacterial cells since it can disrupt the lipid packaging in the cell membrane, can alter cellular components such as proteins and DNA, and can chelate iron and calcium (Begley *et al*., 2005). It is recommended that the ideal strain must survive an environment with a bile salt concentration of at least 0.3% with a 50% resistance rate (Kumar and Kumar, 2015). In this study, *L. plantarum* 1074 displayed the highest resistance rate at MRS agar with 0.1% (w/v) bile salt (Table 2). It differed significantly ($P<0.05$) from the resistance rate obtained in 0.5%, 0.7%, and 0.9% (w/v) bile-supplemented MRS broth. However, it was not significantly ($P>0.05$) different from the resistance rate observed in 0.3% (w/v) bile-supplemented MRS broth. It is noteworthy that *L. plantarum* 1074 was still able to survive 0.5% bile salt concentration with an 80.63% resistance rate. This indicates that the isolate has surpassed the standard set for bile resistance of probiotic strains. Several bile-resistant strains have also been documented in previous studies including *L. plantarum* E680, *L. plantarum* FH185, *L. plantarum* K10, *L. plantarum* K21, and *L. plantarum* ZS07 (Belicová *et al*., 2013; Park and Lim, 2015; Zheng *et al*., 2020). The bile resistance of microorganisms could be ascribed to various mechanisms including bile salt hydrolysis (BSH), the presence of efflux pumps or transporters, alteration of the cell membrane architecture and composition, and regulation of intracellular homeostasis (Bustos *et al*., 2018). These mechanisms would allow probiotics to survive and exert their biological activities within the small intestine.

The gastrointestinal (GI) tract is a complex ecosystem containing trillions of microorganisms. Some of these microorganisms can be beneficial or harmful to the host. A good probiotic must competitively inhibit the growth and proliferation of harmful pathogens in the GI tract. Table 3 presents the antibacterial activity of *L. plantarum* 1074 against *Escherichia coli* using an agar well diffusion assay. The isolate demonstrated strong antagonistic activity

Table 2. Optical density and resistance rate of *L. plantarum* 1074 in MRS broth containing different concentrations of bile salts.

Bile Salt Concentration (%)	Optical Density (absorbance at 560 nm)[1]	Resistance Rate (%)[1,2]
0.1	2.3997±0.0114	89.58±0.42[a]
0.3	2.3247±0.0074	86.78±0.28[a]
0.5	2.1600±0.0459	80.63±1.71[b]
0.7	0.8737±0.0053	32.61±0.20[c]
0.9	0.3653±0.0271	13.64±1.01[d]
	P-value:	<0.0001

[1]Data are presented as mean ± standard deviation.
[2]Means within column with different superscript are significantly different using Scheffe's *post hoc* test ($P \leq 0.05$).

Table 3. Antibacterial activity of *L. plantarum* 1074 against *E. coli* (laboratory isolate).

Supernatant	pH	Inhibition Diameter (mm)[1]	Interpretation[2]
Cell-free Supernatant	4.61	16.67±1.25	Strong inhibition
Neutralized Cell-free Supernatant	6.50	0.00±0.00	No inhibition

[1]Inhibition zone diameters are means from triplicate determination. Diameters of the wells (6 mm) are inclusive.
[2]The diameter of the zone was interpreted according to Akabanda *et al*. (2014): <1 mm = no inhibition (-); 1 to 4 mm = weak (+); 4 to 8 mm = moderate inhibition (++); and 8 to 12 mm = strong inhibition (+++).

towards *E. coli* when its CFS was used. However, this activity was not detected when the pH of CFS was adjusted to pH 6.5 for the bacteriocin (antimicrobial peptides) assay. This suggests that the observed inhibition could be attributed to the synthesis of a variety of metabolites including acetic acid and lactic acid that lower the pH of the medium, thereby creating unfavorable conditions for many organisms, especially those that are not adapted to acidic environments. This condition could lead to various structural and functional alterations in the proteins and DNA that disrupt nutrient transport and energy generation, impairing microbial growth (Cotter and Hill, 2003; Ray, 2004). The antibacterial activity of *Lactobacillus plantarum* strains toward *Escherichia coli* has been previously described in several studies (Yadav *et al*., 2016; Mao *et al*., 2020; Zheng *et al*., 2020). However, the mechanism and degree of inhibition vary from strain to strain, indicating that antibacterial activity is a strain-specific property.

Probiotics must also compete with pathogens for space. They should be able to block pathogens from colonizing the intestinal epithelial cells (Bermudez-Brito *et al*., 2012). Adherence of probiotics to the intestinal mucosal membrane has been reported to be associated with cell surface properties (Sengupta *et al*., 2013). This study evaluated the cell surface properties of *L. plantarum* 1074 such as cell surface hydrophobicity (CSH) and auto-aggregation activity. The CSH was assessed using a variety of solvents such as xylene, chloroform, and ethyl acetate. The adhesion of the isolate to xylene (non-polar solvent) indicates the hydrophobicity of the cell surface. In contrast, affinity to electron acceptor (acidic)

solvent (e.g., chloroform) and electron donor (basic) solvent (e.g., ethyl acetate) correspondingly reflect the electron donor (basic) and electron acceptor (acidic) properties of bacterial cell surfaces (Sharma *et al*., 2017). In this study, the affinities of *L. plantarum* 1074 to xylene and chloroform were relatively high compared to ethyl acetate (Table 4). It is suggested that probiotic strains must adhere to non-polar solvents with at least 40% affinity for them to be considered hydrophobic (Sharma and Sharma, 2017). Therefore, the MATH assay reveals that the cell surface of *L. plantarum* 1074 has hydrophobic and electron donor (basic) properties. Previous studies have also described several highly hydrophobic strains including *L. plantarum* Dad-13, *L. plantarum* KCC-32, *L. plantarum* Mut-7, and *L. plantarum* RYPR1 (Yadav *et al*., 2016; Srigopalram *et al*., 2017; Darmastuti *et al*., 2021).

Some probiotics can also inhibit pathogenic adherence on the intestinal epithelial cells by barrier formation through auto-aggregation. The auto-aggregation activity of microorganisms allows members of the same strain to clump together in high quantities (Janković *et al*., 2012). This enables them to promote gut colonization of the host, provide protection against pathogen infections and modulate the intestinal barrier function (Isenring *et al*., 2021). The auto-aggregation activity of *L. plantarum* 1074 was measured photometrically after a 5-hour incubation (Table 5). Microorganisms with activity higher than 40% are considered strongly auto-aggregating (Wang *et al*., 2010). In this study, the highest activity was measured after 5 hours of incubation. It registered a high percentage of 89.77% proving that the isolate exhibits strong auto-aggregation activity. Similar activity has been reported in previous studies describing the strong auto-aggregation phenotype of *Lactobacillus plantarum* (Malik *et al*., 2013; Handa and Sharma, 2016; Zheng *et al*., 2020). However, the observed activity differs between strains.

The salt tolerance of *L. plantarum* 1074 was evaluated on MRS agar with different concentrations of NaCl (Table 6). The isolate was found to grow well at 8% NaCl-supplemented MRS broth. However, no cell growth was observed at 9% NaCl indicating that the survival of the isolate was greatly reduced at NaCl concentrations higher than 8%. Similar tolerance has been observed in *L. plantarum* UCC 43364 when grown at MRS broth with 8% NaCl (Yao *et al*., 2020).

L. plantarum 1074 exhibited gamma (γ)-hemolytic activity when cultivated in blood

Table 4. Cell surface hydrophobicity of *L. plantarum* 1074.

Solvents	Property of the Solvent	Hydrophobicity (%)[1]
Xylene	Non-polar solvent	84.90±0.67
Chloroform	Acidic solvent and electron acceptor	24.35±0.39
Ethyl acetate	Basic solvent and electron donor	2.44±0.27

[1]Values are means from triplicate determination. Data are presented as mean ± standard deviation.

Table 5. Auto-aggregation activity of *L. plantarum* 1074.

Incubation Time	Auto-Aggregation Activity (%)[1]
3 hours	88.13±0.21
5 hours	89.77±0.75

[1]Values are means from triplicate determination.

Table 6. Salt tolerance of *L. plantarum* 1074.

NaCl Concentration	Results	Interpretation[1]
1%	++	Maximum growth
2%	++	Maximum growth
3%	++	Maximum growth
4%	++	Maximum growth
5%	++	Maximum growth
6%	+	Normal growth
7%	+	Normal growth
8%	+	Normal growth
9%	-	No growth
10%	-	No growth

[1]Maximum growth was indicated as double positive sign (++), normal growth as single positive sign (+) and no growth as negative sign (-) for NaCl.

agar with 5% sheep blood as indicated by the lack of notable zones around the colonies. This suggests the non-hemolytic nature of the tested strain. The finding of this study corroborates with those of previous reports which described the gamma (γ)-hemolytic nature of *Lactobacillus plantarum* (Yadav *et al*., 2016; Jung *et al*., 2019; Wang *et al*., 2020).

Table 7 shows the antibiotic susceptibility of *L. plantarum* 1074 against 11 antibiotics. The isolate expressed susceptibility to the class of antibiotics that inhibit the synthesis of the cell wall (i.e., amoxicillin, ampicillin, augmentin, and penicillin G) and proteins (i.e., streptomycin, chloramphenicol, clindamycin, erythromycin, and tetracycline). However, it was resistant to cephalosporins (cefaclor) and kanamycin. The phenotypic resistance towards cefaclor could be attributed to the ability of some microorganisms to produce cephalosporinase, an enzyme capable of inactivating cephalosporins (Brook, 2016). A similar observation has been reported by Charteris *et al*. (1998) where *L. plantarum* UCC 43364 displayed resistance to cefaclor. In contrast, the resistance of *L. plantarum* 1074 towards kanamycin could be caused by the lack of cytochrome-mediated electron transport through the electron transfer chain (ETC), a system that is normally involved in the uptake of antibiotics in microorganisms (Narayanan and Narayanan, 2019). This intrinsic resistance to kanamycin has been observed in several strains of *L. plantarum* including *L. plantarum* Dad-13, *L. plantarum* Mut-7, and *L. plantarum* T-3 (Andriani *et al*., 2021).

The findings of this research revealed the promising potential of *Lactobacillus plantarum* 1074 derived from *kesong puti* (Filipino soft white cheese) as a probiotic strain as it showed desirable *in vitro* performance with regard to acid and bile resistance, antagonistic activity towards *Escherichia coli*, cell surface hydrophobicity, auto-aggregation activity, NaCl tolerance, γ-hemolytic activity, and antibiotic susceptibility to various cell wall and protein synthesis inhibitors. This study provides baseline information regarding the probiotic potential of *Lactobacillus plantarum* 1074. Further works, however, should be conducted to validate the results *in vivo*. Furthermore, the behavior of *Lactobacillus plantarum* 1074 in various food systems should be investigated to determine its potential application for the development of novel functional foods.

Table 7. Antibiotic susceptibility pattern of *L. plantarum* 1074 using disc diffusion assay on MRS agar.

Antibiotics	Concentration (µg)	Zone of Inhibition (mm)[1]	Interpretation[2]
Cell wall synthesis inhibitors			
Amoxicillin	10	27	Susceptible
Ampicillin	10	30	Susceptible
Augmentin	30	28	Susceptible
Penicillin G	10	38	Susceptible
Cefaclor	30	14	Resistant
Protein synthesis inhibitors			
Kanamycin	30	8	Resistant
Streptomycin	10	15	Susceptible
Chloramphenicol	30	22	Susceptible
Clindamycin	2	29	Susceptible
Erythromycin	15	20	Susceptible
Tetracycline	30	29	Susceptible

[1]Inhibition zone diameters are means from triplicate determination. Diameters of the discs (6 mm) are inclusive.
[2]Susceptibility was expressed as susceptible, moderately susceptible, and resistant (Charteris *et al*., 1998).

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