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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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The Influence of Royal Jelly Addition on the Growth and Production of Short Chain Fatty Acids of Two Different Bacterial Species Isolated from Infants in Jordan

Zaid M.O.Y. Nabas[1], Malik S.Y. Haddadin[1] and Ibrahim K. Nazer[2]
[1]Department of Nutrition and Food Technology, Faculty of Agriculture, University of Jordan, Amman, Jordan
[2]Department of Plant Protection, Faculty of Agriculture, University of Jordan, Amman, Jordan

Abstract: This research was conducted to evaluate the effect of Royal Jelly (RJ) on the growth and metabolism of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* in skim milk. The highest count of *B. bifidum* was 9.89 $\log_{10}$ CFU/ml when 7.5% RJ was added to skimmed milk, while the highest count of *L. acidophilus* was 8.93 $\log_{10}$ CFU/ml at 2.5% level of RJ. The productivity of Short Chain Fatty Acids (SCFAs) and antimicrobial activity against *Salmonella typhimurium* (ATCC 14028), *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) for both bacterial species were investigated. Results showed significant amounts of SCFAs. The final values for acetic acid (1027.6 ppm) butyric acid (1050.1 ppm) and propionic acid (222.8 ppm) produced by *L. acidophilus* were much higher than control, whereas, *B. bifidum* produced detectable amounts of acetic acid (131.7 ppm) butyric acid (177.2 ppm) and propionic acid (146.1 ppm). *L. acidophilus* mixed with 2.5% RJ exhibited antimicrobial activity against all the three pathogenic bacteria, while *B. bifidum* mixed with 7.5% RJ exhibited antimicrobial activity only against *E. coli*. Fermented milk samples were refrigerated at 5°C for 2 weeks intervals. Counts of both probiotic bacteria remained above 5-6 $\log_{10}$ CFU/ml. Based on the obtained results, RJ found to promote the growth and SCFAs production of both probiotic bacteria, thus satisfying the major requirement to produce functional foods.

Key words: *Lactobacillus acidophilus, Bifidobacterium bifidum,* Royal Jelly, short chain fatty acids

INTRODUCTION

Bee keeping is considered as a specialized type of farm enterprise since ancient times. Moreover, it plays an important role in crop pollination, thus, increases the productivity of agricultural crops and improves their quality. On the other hand, it is considered as a suitable practical work which generates extra income to beekeepers. Moreover, honey bee products such as honey, propolis and RJ have been used worldwide for many years as medicinal products, health foods and cosmetics (Tamura *et al*., 1987; Krell, 1996; Matsui *et al*., 2002; Martos *et al*., 2008). Honey bee hive products such as honey, propolis, pollen, Royal Jelly (RJ), venom and bees wax gained more importance. RJ is one of the most important products of honeybees. It is a natural substance secreted from the hypopharingeal glands of worker bees (nurse bees from 8-12 days) which serves as food for the queen bee and to the young larvae (queens, workers and drones) and the adult queen (Grout, 1992). RJ can be included directly in our food and dietary supplements (Garcia and Almaida, 2007). Generally, fresh RJ is composed of (60-70%) water (9-18%) proteins (7-18%) sugars (3-8%) lipids, vitamins, free amino acids and oligosaccharides (Sabatini *et al*., 2009). Moreover, RJ is traditionally known to have some pharmacological and nutritional functions such as antibacterial activity (Eshraghi and Seifollahi, 2003); immunoregulatory effect (Okamoto *et al*., 2003); antioxidant activity against lipid peroxidation (Guo *et al*., 2005); antitumor activity (Nakaya *et al*., 2007); and it can improve metabolism in human (Guo *et al*., 2007).

RJ is relatively acidic (pH 3.1-3.9) with a high buffering capacity ranges between 4 and 7 (Sauerwald *et al*., 1998). It is composed of proteins, lipids, sugars, vitamins and amino acids (Howe *et al*., 1985). Moreover, it contains different minerals (P, S, Ca, Mg, K, Na, Zn, Fe, Cu, Mn) and trace elements with biological functions such as (Al, Ba, Sr, Bi, Cd, Hg, Pb, Sn, Te, Tl, W, Sb, Cr, Ni, Ti, V, Co, Mo) (Stocker *et al*., 2005). In addition, it contains B complex vitamins such as B1, B2, B6 (Moreschi and Almeida-Muradian, 2009) and Biotin (Nandhasri *et al*., 1990). The main fatty acid present in RJ is 10-hydroxy-2-decenoic acid (10-HDA), it plays an important role in boosting immune system, anticancer activity and antibacterial activity (Eshraghi and Seifollahi, 2003; Popescu and Marghitas, 2007).

RJ contains 5 major proteins MRJPs. It was found that MRJP3 modulates immune response both *in vitro* and *in vivo* (Okamoto *et al*., 2003). Nakaya *et al*. (2007) have demonstrated that RJ had inhibitory effect on human breast cancer MCF-7 cell proliferation activity. Also, RJ is effective in reducing blood pressure (Matsui *et al*., 2002).

Corresponding Author: Malik S.Y. Haddadin, Department of Nutrition and Food Technology, Faculty of Agriculture, University of Jordan, Amman, Jordan

Honey bee hive products can be used also as prebiotics such as: Honey, propolis and Royal Jelly (RJ) and can be incorporated with probiotics that affect the host in beneficial manner (Haddadin *et al*., 2007, 2008; Jan *et al*., 2010; Haddadin *et al*., 2012).

It is believed that polyphenolic compounds and flavonoids from RJ might have desirable effect in stimulating both bacterial species growth and metabolism. Therefore, the aim of this study was to evaluate the effect of five different concentrations of RJ on the behavior of two different probiotic bacteria *Lactobacillus acidophilus* and *Bifidobacterium bifidum* isolated locally from infants.

MATERIALS AND METHODS

Source of royal jelly: Langstroth hives with colonies of the most common honeybee species *Apis mellifera* headed by Italian Queen bee were used as source of RJ during the spring and summer of the year 2011. Royal Jelly (RJ) samples were collected from hives located at the University of Jordan Apiary-Faculty of Agriculture using the artificial cups method according to Grout (1992). Strong hives were chosen and their queens were isolated, thus encouraging the workers to produce new queen bee cells to rear queens. For grafting, young larvae (1-2 days old) and small readily available quantities of RJ were taken diligently from their hexagonal cells by a proper needle. The larvae were transferred to an artificial wood frame contains two bars. Each bar contains 11 wax queen cells cups (large cell) for queen rearing. These queen cell cups were slightly glued by melted wax and fitted into each wooden bar of 2.2 cm width x 6.35 mm thickness. These queen cell cups were filled with one drop of diluted RJ with distilled water (1:1) to prepare grafting. After grafting, extra sugar syrup was fed to the colony in order to encourage nurse bees (8-12 days old) to produce maximum amount of RJ. Three days after grafting, queen cell cups were removed for RJ harvesting and was stored at -20°C immediately after collecting for few hours until analysis (Grout, 1992).

Probiotic bacteria: *L. acidophilus* and *B. bifidum* isolates used in this research were previously isolated from new born breast fed infants stool (Haddadin, 2004), at Queen Alia hospital. One gram of each freeze-dried powders of these isolates were transferred aseptically into 50 ml sterile MRS broth supplemented with 0.05% L (+) cystiene -HCL (99.6% purity, Sigma, USA), then incubated at 37°C for 20 hrs in an anaerobic jar (Oxoid, UK). Repeated streaking onto MRS agar plates were used for purification for both isolates, the isolates of *L. acidophilus* and *B. bifidum* were identified physiologically and biochemically according to Bergey's Manual (Kandler *et al*., 1986) and Prokaryotes (Hammes *et al*., 1992). The isolates were activated making subculturing twice in MRS broth containing 0.5% L (+) cystiene -HCL, using 1% inoculum and 18-20 hrs of incubation at 37°C. Each isolates was subcultured twice to three times prior to every test (Walker and Gilliland, 1993).

Preparation of milk containing royal jelly: Samples of RJ were diluted with distilled water and filtered under a vacuum, using, Grade No. 1 filter paper and Grade No. 40 filter paper (Whatman membrane, England) and 0.45 µm nylon membrane. Cold sterilization was performed via micro-filtration unit using 0.20 µm sterile cellulose-ester membranes (Advantec MFS, Japan) (Haddadin *et al*., 2007). A 9% skimmed milk was reconstituted in distilled water and sterilized at 115°C for 10 min. After cooling to 37°C, five different concentrations of sterilized RJ (0, 2.5, 5, 7.5, 10% m/v) were added to reconstituted milk in 100 ml Duran bottles. A control sample was used without the addition of RJ (Haddadin *et al*., 2007).

pH of royal jelly: The pH of RJ was measured using digital pH meter (Hanna instrument model HI 8519, Italy). The pH was directly measured after adding 2g of RJ to 4 ml of distilled water (pH 7.00).

Determination of total flavonoids of royal jelly: The total flavonoid content of RJ was determined according to Zhishen *et al.* (1999). Five grams of RJ were added to 50 ml of distilled water and filtered using Grade No. 1 Filter Paper (Whatman membranes, England). 0.5 ml of the solution was mixed with 0.3 ml of sodium nitrite solution (5g/L). After 5 min, 0.3 ml of aluminum chloride (1g/L) was added. After another 5 min, 2 ml of 1M of sodium hydroxide was added to the mixture. Total volume was made up to 10 ml with distilled water and sonicated immediately after preparation. The absorbance was measured at 510 nm against water blank using UV/Visible Spectrophotometer (Jasco-V-530, Japan). Calibration curve was prepared by preparing rutin solution (0-200 µg/ml). Concentrations are expressed as (µg rutin equivalent/100g RJ).

Determination of total phenolic content of Royal Jelly: Total phenolic content of RJ was determined by Folin-Ciocalteu method according to Singleton *et al.* (1999). 0.5 ml from the previous solution of RJ used in flavonoid assay was mixed with 2.5 ml of 0.2 N Folin-Ciocalteu reagents (Sigma-Aldrich, USA) for 5 min and then 2 ml of 7.5% sodium carbonate were added. The absorbance was measured at 760 nm after 2 hrs of incubation at room temperature against methanol blank using UV/Visible spectrophotometer (Jasco-V-530, Japan). The concentration between 0.01-0.05 mg/ml of gallic acid was used as standard for the calibration curve. The total phenolic content was expressed as µg gallic acid equivalent/100g RJ.

Estimation of probiotic growth: One percent % (v/v) of *L. acidophilus* and *B. bifidum* was added to the prepared milk containing the different concentrations of RJ. Inoculated RJ-milk samples were incubated at 37°C in anaerobic jars for 24 hrs followed by a serial dilution (10^{-1}-10^{-7}) of fermented milk realized using 0.1% sterilized peptone broth and plated on MRS agar supplemented with 0.5% L (+) cystiene -HCL and incubated at 37°C in anaerobic jar for 48 hrs to determine the Colony Forming Unit (CFU) (Harrigan *et al*., 1996).

pH and titratble acidity: Ten mL of fermented milk were used to measure the pH at 23°C using digital pH meter (Hanna instrument model HI 8519, Italy). Titratable Acidity (TA) was determined after adding three drops of phenolphthalein as an indicator to the previous samples used in the pH measurement and titrated with 0.1 N NaOH. After titration, titratable acidity was calculated as a lactic acid percentage (%) (Dave and Shah, 1997).

Determination of Short Chain Fatty Acids (SCFAs) in fermented milk: The organic acids available in the fermented were measured using the method proposed by Qureshi *et al*. (2011). High Pressure Liquid Chromatography (HPLC) was used. The chromatographic system was equipped with a manual 20 µL loop injector, a variable wavelength ultraviolet/visible detector (Jasco model 875, Japan) using an integrator recorder (Shimadzu C-R2AX, Japan) and an insulated column oven (Jasco model 865, Japan). Column effluents were monitored at a wavelength of 222 nm. The HPLC column used in the analysis was C18, 25 cm x 4.6 mm with a mobile phase consisting of 20 mM potassium phosphate buffer (pH 2.5) at a flow rate of 1ml/min and a temperature of 30°C. Acetic butyric and propionic acid (Sigma, USA) were used as a standard at concentrations of 400, 500, 600, 800 and 1000 ppm. Each concentration was injected into the HPLC to obtain the Retention Time (RT) and Area Under Peak (AUC). The coefficient of correlation (r), regression equation and standard curves for each acid were calculated using Microsoft Office Excel 2007. Fermented milk samples were micro-filtered using 0.45 µm nylon membrane. The experiment was carried out in triplicates (Qureshi *et al*., 2011).

Antimicrobial activity: The antimicrobial activity of fermented milk with *L. acidophilus* and *B. bifidum* and the most effective concentrations of RJ were determined by an agar spot test method (Jacobsen *et al*., 1999). Three strains of pathogenic bacteria were used, *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* (ATCC 14028) and *Staphylococcus aureus* (ATCC 25923). Concentrations of pathogenic bacteria were adjusted using a 0.5 McFarland standard to an initial concentration of 1.5 x 10^8 CFU/ml. A 3 µl of each probiotic cultures were spotted on the surface of MRS agar and incubated for 24 hrs at 37°C. A 0.1 ml of overnight culture of the pathogenic bacteria was mixed with 7 ml of (0.7%) soft agar of brain heart infusion agar and poured over the MRS agar plates. Plates were incubated anaerobically at 37°C for 48 hrs and the inhibition zones were observed. Clear zones of more than 1mm around a spot were scored as positive.

Statistical analysis: The General Linear Model (GLM), produced by the Statistical Analysis System (SAS) version 7 (SAS® system for Microsoft® Windows®. 2001), was used to analyze the data. All the data of this research were designed to apply two replicates of each level for every experiment. Different among the means of treatments were tested using the Least Significant Difference (LSD) test. Levels of significance were at ($p<0.05$).

RESULTS AND DISCUSSION

Results of the physiological and biochemical tests are presented in Table (1). The results obtained were in accordance with the main features described in Bergey's Manual of Systematic Bacteriology (Kandler and Weiss, 1986) and Prokaryotes (Hammes *et al*., 1992). Based on these results, the isolates were confirmed as *L. acidophilus* and *B. bifidum*.

The tests that were done in this research on the harvested RJ samples are: pH, Total phenolic and flavonoids content. The pH value of RJ was 3.42 which agreed with the pH value reported by Krell (1996) as RJ contained special organic acids which were known to have antibacterial potential (Eshraghi and Seifollahi, 2003).

The highest total phenolic content was 215 µg/g were found in RJ obtained from Taiwan collected 24 hrs after grafting, while the total phenolic contents of RJ collected after 48 and 27 hrs was 194.4 µg/g and 131.7 µg/g, respectively (Liu *et al*., 2008). On the other hand, relatively similar results obtained from Chinese bees with 21.2 µg/mg (Nagai and Inoue, 2003). This variation of the total phenolic contents is attributed to several factors such as climate and floral source as the phenolic compounds of RJ could be originated from plants where they widely distributed in nature (Bravo, 1998; Wongchai and Ratanavalachai, 2002; Liu *et al*., 2008).

Five different concentrations of RJ were used to estimate the highest count of *L. acidophilus* and *B. bifidum*. Most of the results revealed that RJ has significant effect on the growth of *L. acidophilus* and *B. bifidum*. The level of 7.5% RJ had the most significant effect on *B. bifidum*, with a total viable count of 9.89 Log_{10} CFU/ml and 8.77 Log_{10} CFU/ml, for control, respectively. The level 2.5% of RJ gave significantly the highest count of *L. acidophilus* 8.93 compared with control 8.30 (Table 3). These results

Table 1: The physiological and biochemical reactions of *B. bifidum* and *L. acidophilus*

	Results			
Test	*Bifidobacterium bifidum*	Prokaryotes Book	*Lactobacillus acidophilus*	Bergey's manual
1. Gram Stain	+	+	+	+
2. Catalase test	-	-	-	-
3. Cell morphology	Branched Y shape	Branched Y shape	Straight rod	Straight rod
4. NH_3 production from arginine	-	-	-	-
5. Growth at 15°C			-	-
6. Growth at 45°C			+	+
7. Growth at aerobic conditions	-	-		
8. Glucose (gas production)	-	-	-	-
9. Acid production from Amigdaline		+	+	
Arabinose	-	-	-	-
Cellobioses	-	-	+	+
Fructose	+	+	+	+
Galactose	+	+	+	+
Glucose	+	+	+	+
Lactose	+	+	+	+
Maltose	-	D	+	+
Mannitol	-	-	-	-
Mannose	-	-	+	+
Melibiose	+	D	+	D
Rafinose	-	-	+	D
Rhamnose			-	-
Ribose	+	+	-	-
Starch	-	-	-	-
Sorbitol	-	-	-	-
Sucrose	+	D	+	D
Xylose	-	D	-	-
10. Hydrolysis of Esculin			+	+
11.Fructose-6-Phosphate Phosphoketolase		+	+	

+: Positive, -: Negative, D: Positive or negative

Table 2: The pH, phenolic content and flavonoid content of Royal Jelly from honeybee

Analysis	Collected RJ sample
pH	3.42±0.02
Total phenolic (µg galic acid/mg)	23.3±0.92
Total flavonoids (µg rutin/mg)	1.28±0.09

Table 3: Effect of different concentrations of royal jelly (g/100 ml skimmed milk) on the growth of *B. bifidum* and *L. acidophilus* incubated anaerobically at 37°C for 24 hr; expressed as Log_{10} CFU/ml and means of duplicate samples from two bottles of milk

Concentration of Royal Jelly	*B. bifidum*		*L. acidophilus*	
(g/100 ml)	0 hr	24 hr	0 hr	24 hr
0.0	7.00^{c}	8.77^{d}	6.26^{d}	8.30^{c}
2.5	7.96^{b}	9.15^{c}	7.30^{a}	8.93^{a}
5.0	8.05^{b}	9.54^{b}	6.78^{b}	8.79^{b}
7.5	8.30^{a}	9.89^{a}	6.44^{c}	8.78^{b}
10.0	7.95^{b}	8.82^{d}	6.27^{d}	8.03^{d}

Means within a column with a different superscript letter are significantly different at ($p<0.05$)

are in general in agreement with those reported by Haddadin *et al.* (2007). The level of 7.5% for three kinds of honey collected from Al Salt, Al Azraq and from Hives in the University of Jordan, had the most significant effect on the counts of *B. Infantis*, while lower concentrations 1 and 2.5% had the best effect on the growth *L. acidophilus*. On the other hand, another study by Haddadin *et al.* (2008), found that the level 16% of propolis extract had significant effect on the growth of *L. acidophilus* with a count of 7.99 in log_{10} CFU/ml than in the control with a count of 6.84 log_{10} CFU/ml but with inhibitory effect on *B. Infantis*. Unfortunately, there are no enough studies available for using RJ as prebiotic or if the effect of RJ concentrations on *Lactobacilli* and *Bifidobacteria* are dependent on the strain (Within the same species) Haddadin *et al.* (2012). The obtained results are generally in agreement and go with those obtained by Haddadin *et al.* (2012). It was reported that the effect of 1 and 5% of RJ enhanced the growth and viability of *L. acidophilus* and *B. bifidum*, respectively. However, this can be explained that the different behavior between the two different probiotic bacteria, where *B. bifidum* showed better growth to high concentration of RJ. Shin *et al.* (2005) reported that three kinds of oligosaccharides and inulin (as polysaccharide) enhanced the growth and viability of *B. bifidum*. A decline of growth was noticed at high concentration of RJ 10%, This could be due to the presence of antimicrobial compounds in RJ such as l0-hydroxy-decenoic acid (Eshraghi and Seifollahi, 2003). The obtained results

Table 4: The pH value and Titratable acidity expressed as lactic acid percentage % of skimmed milk with royal jelly (7.5 g/100 ml) inoculated with *B. bifidum* and (2.5 g/100 ml) inoculated with *L. acidophilus* incubated anaerobically at 37°C for 24 hr, means of duplicate samples from two bottles of milk

Probiotic	pH value	Titratable acidity
B. bifidum	4.06±0.02[a]	0.92±0.01[a]
Control	4.52±0.01[b]	0.63±0.02[b]
L. acidophilus	3.64±0.02[a]	1.09±0.01[a]
Control	4.30±0.02[b]	0.68±0.01[b]

Means within a column with a different superscript letter are significantly different at ($p<0.05$)

Table 5: Effect of royal jelly (7.5 g/100 ml of skimmed milk for *B. bifidum* and 2.5 g/100 ml for *L. acidophilus*) on the production of organic acids (expressed as ppm) incubated anaerobically at 37°C for 24 hr, means of duplicate samples from two bottles of milk

Probiotic	Acetic acid	Butyric acid	Propionic acid
B. bifidum	131.7±45.6[a]	177.2±11.80[a]	146.1±24.20[a]
Control	42.2±8.1[b]	0±0.00[b]	12±1.00[b]
L. acidophilus	1027.6±56.8[a]	1050.1±54.20[a]	222.8±42.00[a]
Control	0±0.00[b]	0±0.00[b]	0±0.00[b]

Means within a column with a different superscript letter are significantly different at ($p<0.05$)

showed that *L. acidophilus* is more susceptible to high concentrations of RJ in contrast with *B. bifidum* (Table 3).

The pH value and titratable acidity are closely related. The lower pH value indicates higher lactic acid amounts. (Ustunol, 2000). The pH results of treated milk with most effective concentration of RJ are listed in Table (4). With the experiment done using *L. acidophilus* and 2.5% RJ, pH values had significantly low pH value (3.46) than control (4.30). The decrease in the pH of fermented milk could be attributed to the metabolic activities of the lactic acid bacteria such as the production of lactic acid and other organic acids. Fermented milk containing *B. bifidum* with 7.5% RJ had significantly lower pH value (4.06) than the control (4.52). For lactic acid percentage, fermented milk with *L. acidophilus* and 2.5% RJ had significantly higher percentage (1.09) in comparison with control (0.68). In the case of *B. bifidum* with 7.5% RJ, the Lactic acid percentage (0.92) was significantly higher than control (0.63). The obtained results are in agreement with Haddadin *et al.* (2012).

RJ beneficially influenced the metabolism of the probiotic bacteria at certain levels. RJ was responsible for the production of Short Chain Fatty Acids (SCFA) with an appreciable amount (Table 5). Acetic acid and butyric acid were the predominant SCFA produced by *L. acidophilus* with 1050.1 ppm/ml, when 2.5% RJ was used. Results showed that fermented milk incubated for 24 hrs containing *B. bifidum* and 7.5% RJ produced low amounts of SCFA, with 177.2 ppm of propionic acid, followed by 146.1 ppm butyric acid and 131.7 ppm acetic acid. Variations between *L. acidophilus* and *B. bifidum* in the productivity of SCFA could be attributed to the differences in the biochemical and physiological

Table 6: Effect of royal jelly on the viability of *B. bifidum* and *L. acidophilus* in skimmed milk at different time intervals at 5°C

Incubation duration	*B. bifidum*	Control	*L. acidophilus*	Control
After 24 hrs at 37°C	9.89[a]	8.72[a]	8.93[a]	8.30[a]
After 1 week at 5°C	7.52[b]	7.87[b]	8.51[b]	8.17[b]
After 2 weeks at 5°C	7.21[c]	7.42[c]	8.22[c]	8.06[c]

Means within a column with a different superscript letter are significantly different at ($p<0.05$)

Table 7: Antimicrobial activity of Royal jelly (7.5 g/100 ml of skimmed milk for *B. bifidum* and 2.5 g/100 ml for *L. acidophilus*)

Probiotic	*Salmonella typhimurium*	*Staphylococccus aureus*	*Esherichia coli*
B. bifidum	-	-	+
Control	-	-	-
L. acidophilus	+	+	+
Control	-	-	-

(+) inhibition zone more than 1mm (-) no inhibition or inhibition zone less than 1mm

properties of both *L. acidophilus* and *B. bifidum*. It is worth noting that the rate of SCFA production could be affected by fermentation period, with an appreciable amount of lactic acid had been produced.

The initial counts of *L. acidophilus* and *B. bifidum* after 24 hrs of anaerobic incubation were 8.93 and 9.89 Log_{10} CFU/ml, respectively which were significantly ($p<0.05$) higher than the controls. After two weeks of refrigeration at 5°C, counts of *L. acidophilus* and *B. bifidum* decreased significantly ($p<0.05$) to 8.22 and 7.42 Log_{10} CFU/ml, respectively but did not reach to a low probiotic potential of 5-6 log_{10} (CFU/ml) according to Dave and shah (Table 6). The reduction in the count of *L. acidophilus* and *B. bifidum* during the cold storage at 5°C could be attributed to the production of antimicrobial and inhibitory substances produced such as organic acid sensitivity to oxygen, metabolites such as H_2O_2 and bacteriocins (Shimamura *et al.*, 1992; Bozanic *et al.*, 2001; Wang *et al.*, 2009).

Investigation of the antimicrobial activity of fermented milk with the most effective concentrations of RJ showed an inhibitory effect on growing of pathogenic Gram positive bacterial test cultures *S. aureus*. Also, it showed inhibitory effect on negative bacterial test culture *Salmonella typhimurium* and *E. coli* (Table 7). Fermented milk with *L. acidophilus* and 2.5% RJ showed inhibitory effect for all the pathogenic tested cultures. These results go with what was reported on the antimicrobial activity of RJ against Gram negative bacteria *E. coli* and *Pseudomonas aeruginosa* and Gram positive such as *S. aureus* and *Streptomyces griseus*. The antimicrobial activity of RJ is attributed to that low pH and the presence of l0-hydroxy-decenoic acid (Mercan *et al.*, 2002). Eshraghi and Seifollahi (2003) examined the antibacterial effect of RJ on *Escherichia coli* (ATCC 29532), *Staphylococcus aureus* (ATCC 14776), Streptomyces griseus (ATCC 11746) and three different

Streptomyces sp. (S.46) (S.F8) and (S.66). They found that the application of ether soluble fraction of RJ was more effective than pure RJ. On the other hand, it was found that the inhibitory effect of 30 mg/ml ether soluble fraction of RJ was stronger than the same concentration of ether non soluble fraction which has no effect even at 300 mg/ml of RJ.

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