

***Lactobacillus plantarum* as Biopreservative Agent in Paneer for Dietary Food of Diabetic and Coronary Heart Disease Patients**

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Abstract

Paneer is a food product that is obtained by heating milk followed by acid coagulation. It is known as a diet food that is recommended for diabetic and coronary heart disease patients. A relatively shorter shelf life of paneer is considered to be a major hurdle in its production. The present preliminary study was conducted to determine antibacterial activity of *Lactobacillus plantarum* against *Escherichia coli* and *Bacillus cereus*, to develop it as biopreservative agent in paneer before using it for dietary food of diabetic and coronary heart disease patients in next clinical study. The antibacterial activities were tested through Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) using microdilution well method. Biopreservative activity in paneer was tested using total plate count method based on time and temperature variations. Result showed that *Lactobacillus plantarum* inhibited *Bacillus cereus* and *Escherichia coli* with MIC of 3125 and 1562.5 µg/mL whereas MBC was in a value of >6250 and >3125 µg/mL, respectively. As a biopreservative agent, addition of *Lactobacillus plantarum* to paneer showed no bacterial growth until 7 days in room temperature and 9th day in cold temperature. It is concluded that *Lactobacillus plantarum* could be used as a natural biopreservative agent for extending the shelf life of paneer. The paneer with addition of *Lactobacillus plantarum* as biopreservative will then be consumed by diabetic patient and coronary heart patients in next clinical study.

Keywords: Biopreservative, *Lactobacillus plantarum*, MBC, MIC, paneer

***Lactobacillus plantarum* sebagai Biopreservatif pada Paneer untuk Makanan Diet Pasien Diabetes dan Jantung Koroner**

Abstrak

Paneer adalah produk makanan yang diperoleh dengan pemanasan susu diikuti dengan koagulasi asam. *Paneer* dikenal sebagai makanan diet yang direkomendasikan untuk pasien dengan penyakit diabetes dan penyakit jantung koroner. *Paneer* memiliki ketahanan yang relatif lebih pendek sehingga dianggap menjadi masalah utama dalam produksi. Penelitian pendahuluan ini dilakukan untuk mengetahui aktivitas antibakteri *Lactobacillus plantarum* terhadap *Escherichia coli* dan *Bacillus cereus*, untuk mengembangkannya sebagai biopreservatif sebelum digunakan sebagai makanan diet pasien dengan penyakit diabetes dan penyakit jantung koroner pada uji klinik. Pengujian antibakteri diuji melalui *Minimum Inhibitory Concentration* (MIC) dan *Minimum Bactericidal Concentration* (MBC) menggunakan mikrodilusi metode susia. Selanjutnya, pengembangan sebagai biopreservatif pada paneer diuji menggunakan metode angka lempeng total berdasarkan waktu dan variasi suhu. Hasil penelitian menunjukkan bahwa *Lactobacillus plantarum* menghambat *Bacillus cereus* dan *Escherichia coli* dengan MIC 3125 dan 1562,5 µg/mL sedangkan nilai MBC masing-masing adalah 6250 dan 3125 µg/mL. Sebagai biopreservatif, penambahan *Lactobacillus plantarum* untuk *paneer* tidak menunjukkan pertumbuhan bakteri sampai 7 hari pada suhu kamar dan hari ke-9 di dalam lemari pendingin. Kesimpulannya, *Lactobacillus plantarum* dapat digunakan sebagai agen biopreservatif alami untuk memperpanjang masa simpan *paneer* yang akan dikonsumsi untuk pasien dengan penyakit diabetes dan penyakit jantung koroner pada tahap uji klinik.

Kata kunci: Biopreservatif, *Lactobacillus plantarum*, MBC, MIC, *paneer*

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Introduction

Milk produced in India is being utilized for preparation of various indigenous dairy products like paneer, chhana and chhana-based sweets. Paneer is an important indigenous product which is obtained by heat treating the milk followed by acid coagulation using suitable acid such as citric acid. Paneer is characterized by a mild acidic flavor with slightly sweet taste, soft, cohesive and compact texture. Paneer is a great value in diet because it contains a fairly high level of fat and proteins as well as some minerals, especially calcium and phosphorus. Paneer is recommended by the clinicians for diabetic and coronary heart disease patients.^{1,2}

One of the major disadvantages of these traditional milk products, especially with paneer, is the low keeping quality and shelf life. The use of chemical preservatives to inhibit growth of spoilage bacteria in these products has been recognized as a deterrent to health because of their potential toxicity.³ Paneer is a highly perishable product. At room temperature, paneer does not keep good for more than one day. Hence, there is a considerable interest in the possible use of natural biopreservatives in these traditional milk products. Biopreservation is the use of natural/controlled microbes or antimicrobials as a way of preserving food and extending its shelf life. Lactic acid bacteria have antagonistic properties which make them particularly useful as biopreservatives.^{4,5,6} Studies suggest *Lactobacillus plantarum* has a great potential to be used as biopreservative in extending the shelf life of dairy products such as cheese or also in preservation of meat products.^{7,8}

Paneer is a food product that is obtained by heating milk followed by acid coagulation. General preparation procedure across other countries is same procedure, which is by acid coagulation by heat treating milk. However, cow milk as ingredient can be replaced

by sheep milk, goat milk, low fat milk and buffalo milk. Various types of milk have been used for the manufacture of paneer. The quality of paneer is determined by the quality of milk from which it is produced.⁹

Paneer manufacture product involves the coagulation of milk proteins to form curd. The coagulation of milk occurs when pH of milk reaches 4.6 which is the isoelectric point of its major protein, casein. Some coagulants have been used such as lemon juice, yoghurt and lactic cultures. Calcium lactate has also been used as coagulant.^{10,11,12}

Paneer contains low carbohydrates and high protein. It makes a potential candidate for nutrition of any diet for diabetics. American Diabetes Association lists paneer as a healthy food choice to ensure energy releasing into the body slowly, without causing any of the spikes in blood sugar levels. American Journal of Clinical Nutrition published that those who ate at least 55 g of cottage cheese a day—around two slices—were 12 percent less likely to develop type 2 diabetes. In addition, bacteria in cheese and yoghurt shows to decrease cholesterol level and produce certain vitamins which prevent diabetes.^{13,14}

Prior conducting a clinical study of benefits of paneer as a dietary food for diabetic patient and coronary heart patients, this preliminary study investigates the use of *Lactobacillus plantarum* as natural biopreservative agent for extending the shelf life of paneer.

Methods

Paneer production

Paneer was prepared from milk and lemon juice.^{1,15} Briefly, 1 L of milk was allowed to boil and 2 teaspoons of lemon juice was added to milk. The milk was stirred until it curdled and formed whey. The whey was filtered using muslin cloth then tied and kept until all water was strained. Paneer weight average is

205±1 g with thickness of 0.55±0.03 cm.

Antibacterial screening

Antibacterial screening was generally performed by disc diffusion method. Briefly 20 mL quantities of nutrient agar were plated in petri dish with 1 mL of bacterial suspension. The sterilized filter paper discs (6 mm in diameter) placed on solidified agar plates, which filled with 10 µL of *Lactobacillus plantarum* suspension and potassium sorbate solution. Water was used to dissolve the potassium sorbate. Blank filter paper disc with 10 µL of solvent water was used as negative control. The activity was determined after 24-hour of incubation at 37 °C. The zone of inhibition was measured using Vernier’s caliper. Each microbe was prepared for triplo set and average inhibition zone is taken.¹⁶

Determination of MIC and MBC

Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC) of *Lactobacillus plantarum* suspension dilution were determined by using micro dilution method. Potassium sorbate was used as positive control.¹⁶

Determination of effects of *Lactobacillus plantarum* in extending shelf life of paneer

Determination of effects of *Lactobacillus plantarum* in extending shelf life of Paneer based on time and temperature variation was using plate count method. One mL contained 108 CFU of *Lactobacillus plantarum*

suspension added to 10 mL of paneer sample. The samples, positive and negative control were subjected to storage studies both at room temperature (25±1 °C) and cold temperature (4±1 °C), respectively. The analysis of the stored samples was carried out for 7 days in room temperature and 9 days in cold temperature. The samples were also tested for time variation in which the colonies formed was calculated in different time intervals which is 0 hour, 3 hours, 6 hours, 24 hours, 3 days, 6 days, 9 days and 12 days in both room temperature (25±1 °C) and cold temperature (4±1 °C).

Organoleptic properties

Organoleptic properties of paneer were tested before and after addition of *Lactobacillus plantarum* suspension. The parameters evaluated in organoleptic properties were appearance, flavor/odor, texture/color and taste.

Data collection

The data were obtained from three dependent experiments and representative as mean±SD. Data was statistically significant using t-test (p<0.05).

Results

The antibacterial screening was performed by disc diffusion method to determine the zone of inhibition of *Lactobacillus plantarum*, potassium sorbate and solvent water against two bacteria *Escherichia coli* and *Bacillus*

Table 1 Result of Antibacterial Screening of *Lactobacillus plantarum* Suspension and Potassium Sorbate

Strains	Diameter of Zone of Inhibition (mm)	
	<i>Lactobacillus plantarum</i> suspension	Potassium Sorbate 50 (mg/mL)
<i>Bacillus cereus</i>	20.3±0.2	19.7±0.1
<i>Escherichia coli</i>	18.5±0.3	19.5±0.4

The control disc used for solvent (water) had no zone of inhibition, so the data was omitted from the above data.

Table 2 Results for MIC of *Lactobacillus plantarum* and Potassium Sorbate Against *Escherichia coli* and *Bacillus cereus*

Strains	<i>Lactobacillus plantarum</i> 104 (%)		Potassium Sorbate ($\mu\text{g/mL}$)	
	MIC	MBC	MIC	MBC
<i>Bacillus cereus</i> (13.6×10^4 CFU/100 μL)	3.13 7.72x103 CFU/mL	>6.25 15.4x103 CFU/mL	3125	>6250
<i>Escherichia coli</i> (10.9×10^4 CFU/100 μL)	3.13 7.72x103 CFU/mL	>6.25 15.4x103 CFU/mL	1562.5	>3125

cereus, and values to evaluate the bacterial response to each compound (Table 1).

MIC and MBC results showed that *Lactobacillus plantarum* inhibited *Bacillus cereus* and *Escherichia coli* with MIC of 3125 and 1562.5 $\mu\text{g/mL}$ whereas MBC was in value of >6250 and >3125 $\mu\text{g/mL}$, respectively. From the result of MIC, *Lactobacillus plantarum* with dilution 104 showed inhibitory effect against both Gram-positive bacteria and Gram-negative bacteria.

The results of the effect of *Lactobacillus plantarum* and potassium sorbate on the microbial growth is shown in Table 3. Control sample, which is paneer, only showed total bacterial count of 58 ± 3 to 256 ± 3 respectively from 1st to 7th day in room temperature (25 ± 1 °C) and 40 ± 2 to 167 ± 3 respectively from 6th to 9th day in cold temperature (4 ± 1 °C). Addition of *Lactobacillus plantarum* to paneer showed no bacterial growth until 7 days in room temperature and 9th day in the cold temperature.

Discussion

This preliminary study demonstrated that *Lactobacillus plantarum* has a potential as antibacterial and biopreservative agent. Addition of *Lactobacillus plantarum* to paneer showed no bacterial growth until 7 days in room temperature and 9th day in the cold temperature. The total bacterial was increased proportionally every day. This clearly shows that *Lactobacillus plantarum* in paneer can

inhibit the microbial growth. Normal paneer without any preservatives can be kept for only 1 day at ambient temperature and 6 days at refrigerated temperature. However, addition of *Lactobacillus plantarum* extends its shelf life up to 7 days in room temperature and 9 days in cold temperature.

Antibacterial screening was conducted to confirm that *Lactobacillus plantarum* can inhibit both *Escherichia coli* and *Bacillus cereus*. The screening was conducted through disc diffusion method which is used to test whether the bacteria is affected by antimicrobial agent. The highest activity of *Lactobacillus plantarum* was 20.3 ± 0.2 mm diameter of zone inhibition found against *Bacillus cereus* followed by 18.5 ± 0.3 mm diameter of zone inhibition against *Escherichia coli* whereas the control disc used for solvent (water) had no zone of inhibition. In the comparison to positive control, potassium sorbate 50 mg/mL, the diameter of zone inhibition against *Bacillus cereus* was 19.7 ± 0.1 mm and 19.5 ± 0.4 mm against *Escherichia coli*. The diameter zone of inhibition of *Lactobacillus plantarum* suspension and potassium sorbate against both bacteria was more than 16 mm which is in category as susceptible according to antibiotic sensitivity testing methods.¹⁶

Lactobacillus plantarum inhibited *Bacillus cereus* and *Escherichia coli* with MIC of 3125 and 1562.5 $\mu\text{g/mL}$ whereas MBC was in a value of >6250 and >3125 $\mu\text{g/mL}$, respectively. The results indicated that

Table 3 Results of Total Plate Count for Temperature Variation at Room and Cold Temperature

Sample/ CFU-Day	CFU																	
	Day 1 (°C)	Day 2 (°C)	Day 3 (°C)	Day 4 (°C)	Day 5 (°C)	Day 6 (°C)	Day 7 (°C)	Day 8 (°C)	Day 9 (°C)									
Temperature	25±1	4±1	25±1	4±1	25±1	4±1	25±1	4±1	25±1	4±1								
Paneer only	58±3	-	85±2	-	107±4	-	128±2	-	198±3	-	215±2	40±2	256±3	67±3	NA	96±1	NA	67±3
Paneer + <i>Lactobacillus plantarum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Paneer + Potassium sorbate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 4 Results of Organoleptic Properties of Paneer Before and After Addition of *Lactobacillus plantarum*

	Appearance	Flavor/Odor	Texture/Color	Taste
Paneer (Before addition of <i>Lactobacillus plantarum</i>)	Clear from dirt	Mild acidic flavor/ Pleasant odor	Smooth homogenous texture/ Milky white color	Pleasant taste
Paneer (After addition of <i>Lactobacillus plantarum</i>)	Clear from dirt	Acidic flavor/ Pleasant odor	Smooth homogenous texture/ Milky white color	Slight sour taste

both bacteria *Escherichia coli* and *Bacillus cereus* were sensitive towards *Lactobacillus plantarum* with the smallest MIC population which was 3.13% containing 7.72×10^3 CFU/mL. Meanwhile, for potassium sorbate the results indicated that the most sensitive bacteria were *Escherichia coli* with the smallest MIC which was 1562.5 $\mu\text{g/mL}$, followed by *Bacillus cereus* with MIC of 3125 $\mu\text{g/mL}$. From these results, it is shown that *Lactobacillus plantarum* can inhibit both Gram-positive and Gram-negative bacteria, while potassium sorbate has high ability in inhibiting Gram-negative bacteria than Gram-positive bacteria.

Based on the organoleptic properties of paneer there was no significant change between the control paneer and paneer treated with *Lactobacillus plantarum*. However, there was slightly sour taste due to production of acetic acid. The overall acceptability of paneer samples treated with *Lactobacillus plantarum* did not vary significantly and were similar to the control. Hence it could be inferred that *Lactobacillus plantarum* could be incorporated in paneer samples without significant changes in the sensory attributes of paneer.

Based on other preservation technique than adding *Lactobacillus plantarum* suspension, there are many ways to preserve paneer, for example using freezing, heat sterilization and addition of brine solution. Blast freezing has recently been used to enhance the shelf life of paneer. Although the refrigerated shelf life improves markedly by the various treatments given to raw paneer, the shelf life at room temperature does not improve noticeably. Heat sterilization of paneer is an effective treatment for improving its shelf life at room temperature. Paneer packed in tins along with water/brine and sterilized in an autoclave at 15 psi for 15 minutes lasts for 4 months. The perception of an oxidized flavor renders the product unacceptable afterwards. Paneer

dipped in 5% brine solution lasts for nearly 20 days as against control that is spoiled after 6 days of storage at 8–10 °C. The sensory attributes are rated higher for salted samples. Other techniques are seen to be effective in increasing the shelf life of paneer but there is change in organoleptic properties of the paneer.¹⁷ However, the preservation technique using *Lactobacillus plantarum* suspension improve the shelf life and also the taste and smell of paneer hence it is suggested to be the best technique to preserve paneer.

Paneer has a great value in diet. It has a fairly high level of fat and proteins as well as some minerals, especially calcium and phosphorus and also a good source of fat soluble vitamins A and D. It is considered that the nutritive value is fairly high. Superior nutritive value of paneer is attributed to the presence of whey proteins that are rich source of essential amino acids. Due to its high nutritive value, paneer is an ideal food for the expectant mothers, infants, growing children, adolescents and adults. Paneer is also recommended by the clinicians for diabetic and coronary heart disease patients.^{2,13,14} However, the evidence for those purposes is still not sufficient. To investigate that paneer has a valuable diet for diabetic and coronary heart disease patients, we will use paneer that has been enriched with *Lactobacillus plantarum* as natural biopreservative agent for diabetic and coronary heart disease patients in near future clinical study.

Conclusions

This preliminary study concluded that *Lactobacillus plantarum* has a potential as antibacterial and biopreservative so that it could be used as a natural biopreservative agent for extending the shelf life of paneer. This paneer with *Lactobacillus plantarum* will be investigated for dietary food of diabetic and coronary heart disease patients

in the next clinical study.

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Conflict of Interests

The authors have no competing interests to declare.

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