

DETERMINATION OF ANTIMICROBIAL ACTIVITY ON BACTERIAL ISOLATES FROM SELECTED YOGURT SAMPLES

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Abstract

Yogurt is among the most popular fermented foods in the world. In the present study, ten samples of yogurt from South Okkalapa Township and North Dagon Township were collected. The study period was lasted from January 2022 to August 2022. This study was conducted at the Microbiology Laboratory, Department of Zoology, Dagon University. In the present study, the objectives were to enumerate and identify bacterial isolates and access their antimicrobial activities. The enumeration of total bacteria count in ten yogurt samples was duplicated carried out. The maximum bacteria count was found in sample code YgI of South Okkalapa Township at 9.62×10^8 cfu/ml and the minimum count in sample code YgA of South Okkalapa Township was 5.32×10^6 cfu/ml. This research was used streak plate method for isolation on MRS agar for their colony morphology and biochemical characters. According to the colony morphology, gram-staining and biochemical tests, six bacteria genera were identified as *Leuconstoc* (14.81%), *Lactococcus* (18.52%), *Pediococcus* (3.71%), *Clostridium* (18.52%), *Lactobacillus* (33.33%) and *Streptococcus* (11.11%). All of the isolated bacteria did not show antimicrobial activity against tested targets *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* bacteria. Yogurt is good for health because it contains beneficial live bacteria so it should be consumed every day.

Keywords: Yogurt, colony forming unit, bacteria

Introduction

Yogurt is a dairy product produced by lactic fermentation of milk (Hui, 1992). Any sort of milk may be used to make yogurt, but modern production is dominated by cow milk. It is the fermentation of the milk sugar (lactose) into lactic acid that gives yogurt its gel-like texture and characteristics tang (Davis, 1974). It is a widely consumed as functional food due to its good taste and nutritional properties (rich in potassium, calcium, protein and vitamin B) and excellent vehicle to deliver probiotics to consumers (Reid *et al.*, 2003). Regular consumption of yogurt is thought to be beneficial in the strengthening of the immune system, improvement in lactose digestion, blood glucose management (Yadav *et al.*, 2007). The reduction of constipation, diarrhea, colon cancer, inflammatory bowel disease and allergies (Adolfsson *et al.*, 2004).

Yogurt is a fermented dairy product and have highly digestible proteins. Fermented dairy products, also categorized in functional foods group, are considered to have functional properties because of its enhanced nutritional values and the presence of probiotics (friendly bacteria). Among fermented dairy product, the most important fermented food is yogurt. Therefore, yogurt bacteria are very important to human nutrition. In addition, having antimicrobial activity increases the importance of yogurt bacteria (Suskovic *et al.*, 2010).

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Lactic acid produced on fermentation of lactose contributes to the sour taste of yogurt by decreasing its pH and enables the formation of the characteristic texture by acting on milk proteins (Guarner *et al.*, 2005).

One of the most dairy products for the delivery of viable *Lactobacillus* sp. cells is yogurt (Analie and Bennie, 2001). Viable bacteria in yogurt are believed to actively enhance health by improving the balance of microflora in the gut (Fuller, 1989 and Fuller, 1992). Due to this yogurt by itself has been recognized as a healthy food by virtue of the beneficial action of its viable bacteria that compete with pathogenic bacteria for nutrients and space

In Myanmar, yogurt is the most popular dairy products and sold in many areas. People widely consumed it as soft drink. Therefore, the present study was undertaken with the following objectives :

- to enumerate of bacterial isolates in yogurts
- to identify the isolates their specific genus levels
- to access the antimicrobial activities of isolates

Materials and Methods

Study sites

A total of ten yogurt samples were purchased from South Okkalapa and North Dagon Townships in Yangon Region. The samples were analyzed carried to the Microbiology Laboratory, Department of Zoology, Dagon University.

Study period

The study period was from January, 2022 to August, 2022.

Materials and Methods

The materials used for research were yogurt samples. The apparatus and equipment used for the laboratory work were autoclave, water distiller, hot air oven, incubator, analytical balance, stirrer hotplate, refrigerator, compound microscope, vortex mixer, biosafety cabinet, colony counter, various kinds of glassware, pipettes, inoculation nichrome wire loop, aluminium foil, sterilized screw-cap bottle, microscope glass slides, disposable gloves, masks, sterilized cotton and cotton buds.

The culture media and biochemical test media used in bacteriological study consisted of Lactobacillus Man Rogosa Sharpe (MRS) Agar (M641), Triple Sugar Iron (TSI) Agar, Simon's Citrate Agar, Methyl-Red Voges-Prokauer (MR-VP) Broth, Urea agar, Sulphide Indole Motility (SIM) Medium, Gelatinase, Methyl Red solution, α -naphthol solution, 40% KOH solution containing 0.3% creatine, 3% H₂O₂ and Kovac's reagent. The test bacteria, Gram-negative bacteria *Escherichia coli*, Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*.

A total of ten yogurt samples were purchased from South Okkalapa Township and North Dagon Township and brought to the laboratory for isolation and identification of bacteria.

Preparation of samples

1mL of each yogurt sample was serially diluted with 9ml of distilled water by using a vortex mixer.

Enumeration of total bacteria

Serial dilution and pour plate method were used to enumerate the total bacteria from various collected materials (Dubey and Maheshwari, 2002). The suspension was diluted into 1:10 serial dilutions to the seventh dilution level. 1 ml of suspension from each dilution was inoculated onto MRS agar by using pour plate method. The petri dish cultures were inoculated at 35°C for 24-48 hrs in an anaerobic jar. Subsequently, the number of growing colonies on each plate was counted for estimation of the number of total viable bacteria. The colony numbers only between 30 and 300 in each plate were used to calculate the colony forming unit (cfu/ml). Then the number of colonies was multiplied with the dilution factor and the bacteria counts in cfu/ml were calculated (Dubey and Maheshawri 2002).

Isolation and identification of bacteria

Streak plate methods were used to obtain a pure culture of bacteria. According to streak plate method, one loopful of bacteria from a colony on the plate from pure plate method was streaked onto the surface of MRS agar. Then, these plates were incubated at 35°C for 24-48 hrs and the colonies growing on the surface of the culture plates were examined for its purity by detailed characterization. Only the pure colonies growing the streak line without coalescing with another colony was picked out to examine or store as a stock culture for further studies. The isolated bacteria were characterized by observing colonial morphology, Gram staining, catalase test, motility test and some biochemical characteristics (Bisen and Verma, 1998),

All isolates were subjected to the following standard biochemical tests described using dehydrated media. These tests included (i) triple sugar iron (TSI) test, (ii) citrate utilization test, (iii) methyl-red (MR) test, (iv) Voges-Proskauer (VP) test, (v) gelatinase test, (vi) indole formation test, (vii) H₂S production, and (viii) catalase test. The characteristic features of isolated bacteria like colony and cell morphology, gram staining nature and biochemical properties obtained in the present work were compared to the standards described in Bergey's Manual for Determinative Bacteriology (Breed *et al.*, 1994), Cowan and Steel's Manual for Identification of Medical Bacteria (Cowan, 1975).

Antimicrobial activity

A perpendicular streak method was used for determining antimicrobial activities of each isolate on MRS agar (Egorov, 1987). The target bacteria were cross streaked as single lines on solidified MRS media in a petridish and were incubated at 35°C for 24-48hrs. The isolated bacteria were then cross streaked perpendicular to the original streaks of bacteria isolates (Dubey and Maheshwari, 2002).

The different test as target microorganisms used in this study were Gram-negative bacteria *Escherichia coli*, Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*.

Results

Bacteria counts of the yogurt samples

The value of the standard viable total plate count of bacteria in the sample code YgA from South Okkalapa Township was 10.65×10^6 cfu/ml, YgB from North Dagon Township was 7.36×10^7 cfu/ml, YgC from South Okkalapa Township was 14.48×10^8 cfu/ml, YgD from North Dagon Township was 19.76×10^7 cfu/ml, YgE from South Okkalapa Township was 10.30×10^7 cfu/ml, YgF from North Dagon Township was 10.84×10^8 cfu/ml, YgG from South Okkalapa Township was 15.75×10^8 cfu/ml, YgH from North Dagon Township was 8.80×10^8 cfu/ml, YgI

from South Okkalapa Township was 19.23×10^8 cfu/ml and YgJ from North Dagon Township was 2.11×10^8 cfu/ml were found in present study (Table 1).

Cell morphology, Gram staining reaction and Biochemical reactions of the isolates bacteria

A total of 27 isolates were isolated from ten yogurt samples. Among them, 15 isolates Gram positive bacilli (rod) shaped bacteria (55.56%) were most abundant, 8 isolates cocci (spherical) shaped bacteria (29.63%) were second abundant and 4 isolates coccobacilli (oval) shaped bacteria (14.81%) were abundant and when grow onto De Man, Rogosa and Sharpe (MRS) agar (Table 2).

The biochemical properties of the isolates were designated six genera: *Leuconostoc* sp., *Lactococcus* sp., *Pediococcus* sp., *Clostridium* sp., *Lactobacillus* sp. and *Streptococcus* sp. (Table 3).

Identification of isolates

According to the biochemical tests, six genus bacteria namely *Leuconstoc* sp., *Lactococcus* sp., *Pediococcus* sp., *Clostridium* sp., *Lactobacillus* sp. and *Streptococcus* sp. were identified. Among them, *Leuconstoc* sp. was isolated in the samples codes YgA, YgC and YgE. *Lactococcus* sp. also isolated in the sample codes YgC, YgD, YgG and YgI. *Pediococcus* sp., was only found in the sample code YgE. *Clostridium* sp. was found in the sample codes YgB, YgE, YgF and YgH. *Lactobacillus* sp. was found in the sample codes YgB, YgC, YgE, YgF, YgG, YgH and YgI. *Streptococcus* sp. was isolated from the sample codes YgG, YgH and YgJ (Table 4 and Fig 1). Morphological features and some biochemical characteristics of isolated bacteria from the yogurt samples are shown in Fig.2.

Composition of identified bacteria isolated from yogurt samples

Among ten samples of yogurt, *Leuconstoc* spp., *Lactococcus* spp., *Pediococcus* spp., *Clostridium* spp., *Lactobacillus* spp. and *Streptococcus* spp. were selected 14.81%, 18.52%, 3.71%, 18.52%, 33.33% and 11.11% respectively (Table 4).

Antimicrobial activity

All of the isolates did not show antimicrobial activity against tested targets *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* bacteria .

Table 1. Total bacteria count of yogurt samples from different markets

Sr No	Sample code	Single bacteria count	Duplicate bacteria count	Total bacteria count	Average bacteria count(cfu/ml)
1	YgA	2.07×10^6	8.58×10^6	10.65×10^6	$5.32 \times 10^{6*}$
2	YgB	3.96×10^7	3.40×10^7	7.36×10^7	3.68×10^7
3	YgC	9.30×10^8	5.18×10^8	14.48×10^8	7.24×10^8
4	YgD	8.20×10^7	11.60×10^7	19.76×10^7	9.90×10^7
5	YgE	6.50×10^7	3.80×10^7	10.30×10^7	5.15×10^7
6	YgF	6.45×10^8	4.39×10^8	10.84×10^8	5.42×10^8
7	YgG	10.75×10^8	5.00×10^8	15.75×10^8	7.87×10^8
8	YgH	4.50×10^8	4.30×10^8	8.80×10^8	4.40×10^8
9	YgI	9.58×10^8	9.66×10^8	19.23×10^8	$9.62 \times 10^{8**}$
10	YgJ	1.22×10^8	0.89×10^8	2.11×10^8	1.06×10^8

Note: counts on MRS agar

** = maximum

* = minimum

Table 2. Number and composition of types of isolates from the yogurt samples

Isolate code	Number of Gram positive, rod shaped isolates	Number of Gram positive, cocci shaped isolates	Number of Gram positive, coccobacilli shaped isolates	Total
YgA	-	-	2	2
YgB	3	-	-	3
YgC	1	1	1	3
YgD	1	1	-	2
YgE	2	1	1	4
YgF	2	-	-	2
YgG	2	2	-	4
YgH	2	1	-	3
YgI	2	1	-	3
YgJ	-	1	-	1
Total	15	8	4	27
%	55.56	29.63	14.81	100

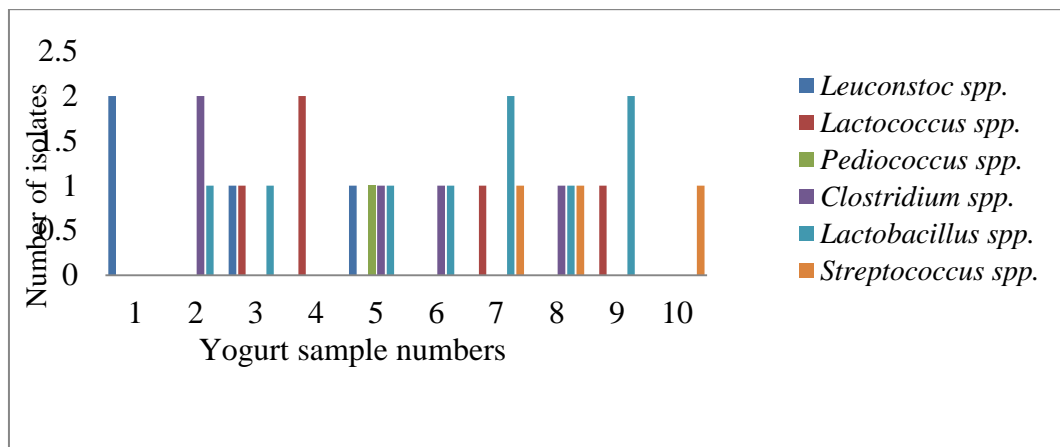
**Figure.1** Number of isolates from the yogurt samples

Table 3. Biochemical reactions of the isolates from the yogurt samples

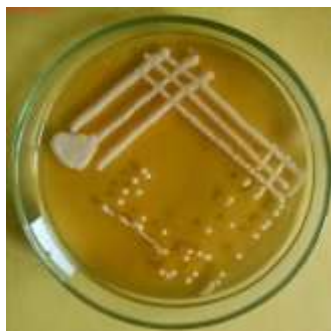
Sr No.	Isolate code	TSI			Cit	MR	VP	Gel	Urea	SIM			Ca	Tentative genera
		B	S	H ₂ S						Indo	Motile	H ₂ S		
1.	YgA-2	A	K	-	-	+	-	-	+	-	-	-	-	<i>Leuconstoc</i> sp.
2.	YgC-3	A	A	-	-	+	-	-	+	-	-	-	-	<i>Lactococcus</i> sp.
3.	YgE-1	K	A	-	-	+	-	-	+	-	-	-	-	<i>Pediococcus</i> sp.
4.	YgF-2	K	K	-	-	-	-	-	+	-	-	-	-	<i>Clostridium</i> sp.
5.	YgI-1	A	A	-	-	-	-	-	-	-	-	-	-	<i>Lactobacillus</i> sp.
6.	YgJ-1	K	A	-	-	-	-	-	+	-	-	-	-	<i>Streptococcus</i> sp.

(+) = positive reaction, (-) = negative reaction

S = Slant, B = Butt, G = Gas, Cit = Citrate utilization, MR = Methyl Red, VP = Voges Proskauer, Gel = Gelatinase, Urea = Urease test, SIM=Sulphide Indole Motility, Indo = Indole, M = Motility, Ca = Catalase, A = Acid, K = Alkaline, TSI = (Triple Sugar Iron)

Table 4. Number of isolates identified from the yogurt sample

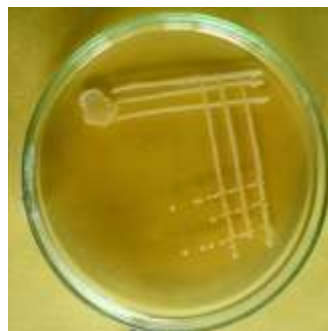
Sample code	Identified bacteria						Total
	<i>Leuconstoc</i> spp.	<i>Lactococcus</i> spp.	<i>Pediococcus</i> spp.	<i>Clostridium</i> spp.	<i>Lactobacillus</i> spp.	<i>Streptococcus</i> spp.	
YgA	2	-	-	-	-	-	2
YgB	-	-	-	2	1	-	3
YgC	1	1	-	-	1	-	3
YgD	-	2	-	-	-	-	2
YgE	1	-	1	1	1	-	4
YgF	-	-	-	1	1	-	2
YgG	-	1	-	-	2	1	4
YgH	-	-	-	1	1	1	3
YgI	-	1	-	-	2	-	3
YgJ	-	-	-	-	-	1	1
Total	4	5	1	5	9	3	27
%	14.81	18.52	3.71	18.52	33.33	11.11	100



A. *Leuconstoc* sp. colonies on MRS agar



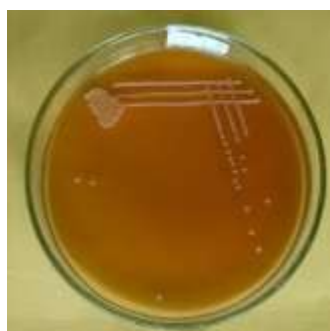
B. *Lactococcus* sp. colonies on MRS agar



C. *Pediococcus* sp. colonies on MRS agar



D. *Clostridium* sp. colonies on MRS agar



E. *Lactobacillus* sp. colonies on MRS agar



F. *Streptococcus* sp. colonies on MRS agar



G. Biochemical reactions of *Leuconstoc* sp.



H. Biochemical reactions of *Lactococcus* sp.



I. Biochemical reactions of *Pediococcus* sp.



J. Biochemical reactions of *Clostridium* sp.



K. Biochemical reactions of *Lactobacillus* sp.



L. Biochemical reactions of *Streptococcus* sp.

Figure.2 Morphological features and some biochemical characteristics of isolated bacteria from the yogurt samples

Discussion

In the present study, the maximum bacteria count was found in sample code YgI of South Okkalapa Township at 9.62×10^8 cfu/ml and the minimum count in sample code YgA of South Okkalapa Township was 5.32×10^6 cfu/ml. Abrar *et al.*, 2009 reported that the total bacterial counts of the registered and non-registered samples were in the range of 3.0×10^3 - 9.0×10^4 cfu/ml and 8.2×10^4 - 28.4×10^5 cfu/ml, respectively. Compare with these statements, according to the result of present study, colony forming unit was a more higher and this might be due to different selective agar used in this research and also to temperature and environment conditions of yogurt production sites and markets.

Anwarul Hasan *et al.*, 2016 stated that the yogurt samples collected from different district of Bangladesh showed a large number of total bacterial counts. Among the ten samples total viable bacterial count (TVBC) ranged from 1.72×10^2 to 5.04×10^8 cfu/ml. Therefore, the present study is similar to the total bacterial count of his finding.

In the present study, a total of twenty seven isolates were obtained from the ten yogurt samples. All isolates were characterized as being identified at the six genus level but not at the species level because their biochemical reactions and morphological changes were complicated. The isolates were grouped into six genera of bacteria based on the results of morphological, physiological and biochemical characterization. The isolates were identified as *Leuconstoc* spp., *Lactococcus* spp., *Pediococcus* spp., *Clostridium* spp., *Lactobacillus* spp. and *Streptococcus* spp.

Isolation of bacteria is also possible from other substrates like traditional fermented foods, beverages and sourdough. Generally, bacteria genera identified in the present study were comparable to that of other studies. Sawsan *et al.*, 2010 reported that the genera *Lactobacillus*, *lactococcus* and *Pediococcus* had been isolated from raw cow milk, white cheese and rob in Sundan. Vuysta and Vancanneyt, 2007 also reported that the genera *Leuconstoc*, *Streptococcus*, *Pediococcus* and *Lactobacillus* were isolated from borde and shamita. Mayeux *et al.*, 1962 stated that *Lactobacillus plantarum*, *Lactococcus lactis* ssp. *lactis*, *Lactobacillus delbrueckii* subsp. *lactis*, *Leuconstoc lactis* and *Leuconstoc citreum* were identified in South African traditional fermented milks. Furthermore, Ei Thandar Khaing (2021) described the occurrence of *Lactobacillus xylosus*, *Leuconstoc citreum*, *Streptococcus acidominimus*, *Lactobacillus plantarum*, *Lactococcus lactis* and *Pediococcus petosaceus* in yogurt of Yangon area.

Calculations for percentage of occurrence regarding bacterial isolates consisted of bacilli, cocci and coccobacilli groups present in all samples: high occurrence at 55.56% in rod-shaped groups, 29.63% in cocci groups and coccobacilli groups were low in occurrence at 14.81% in yogurt samples. The result indicated that bacilli groups were dominant.

In this study, *Lactobacillus* spp. (33.33%) was the most abundant and *Lactococcus* spp. (18.52%) and *Clostridium* spp. (18.52%) were the second most abundant in equal numbers. *Leuconstoc* spp. (14.81%), *Streptococcus* spp. (11.11%) and *Pediococcus* spp. (3.71%) were found. The present findings indicated that the geneus *Lactobacillus* was the most abundant among all isolates.

Lianou *et al.*, 2016 state that lactic acid bacteria are the major microbes found in yogurt and dairy fermentation although a diverse range of other organisms used in other fermentation processes. Among the lactic acid bacteria, *Lactobacillus*, *Streptococcus*, *Lactococcus* and *Leuconostoc* are most frequently found in fermented dairy foods, either as starter cultures or as naturally occurring members of the raw material. However, some fermented foods, especially

yogurt and other fermented milk products, may also contain added probiotic species of *Bifidobacterium* and *Lactobacillus*.

Antimicrobial activity is one of the most important selection criteria for probiotics. Bacteriocins can be broken down by some proteolytic enzymes leading to a loss in their antimicrobial activity. In the present study, twenty seven isolates were tested for their antimicrobial effects on one gram-negative and two gram-positive bacteria. The experimental results of the current research showed that all bacteria isolates were unable to inhibit the target test organisms of *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*. This might be due to different concentration of isolates and test organisms. Another reason may be due to temperature and time.

Sanders *et al.*, 2014 reported that five European Union member states currently have national guidelines or recommendations that include yogurt with live bacteria. Nonetheless, Ebner *et al.*, 2014, Rezac *et al.*, 2018, Hill *et al.*, 2017, Sanders *et al.*, 2014 and Bell *et al.*, 2018 also stated that there appears to be emerging interest in including fermented foods as part of dietary guidelines. While the US dietary guidelines, as well as national recommendations from other countries, recommend the consumption of yogurt for its nutrient content. Therefore, yogurt should be consumed for health.

Conclusion

In vitro results showed that maximum bacteria count 9.62×10^8 cfu/ml was found in the yogurts samples. A total of six bacteria genus *Leuconstoc*, *Lactococcus*, *Pediococcus*, *Clostridium*, *Lactobacillus* and *Streptococcus* were isolated and identified from the yogurt samples. Among fermented dairy product, one of the most important fermented food is yogurt. Therefore, yogurt bacteria are very important to human nutrition. In addition, having antimicrobial activity increases the importance of yogurt bacteria. Lactic acid produced on fermentation of lactose contributes to the sour taste of yogurt by decreasing its pH and enables the formation of the characteristic texture by action on milk protein. This findings indicate that the isolates are able to survive in the stomach and intestine of human. Based on the overall results, the bacteria isolates found in the yogurt sample should be suitable to supplement in normal diet in humans.

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