Characterization of Lactic Acid Bacteria Isolates from Pickled Tea Leaves (La-phet)

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Abstract

In the present study, lactic acid bacteria (LAB) were isolated from commercial pickled tea leaves (La-phet). Isolation of lactic acid bacteria was carried out by selective MRS agar incorporated with 1%$CaCO_3$. The morphology, gram staining nature, biochemical reactions, carbohydrate and sugar fermentation patterns and physiological characteristics (NaCl, phenol, pH, temperature tolerances) were employed to identify the lactic acid bacteria isolates. Nine isolates were identified as LAB: two isolates (22.22%) having rod shape consisted of *Lactobacillus plantarum* and *Lactobacillus acidophilus*; and seven isolates (77.78%) with cocci shape cells consisted of *Leuconostoc mesenteroides*, *Pediococcus* sp. and *Streptococcus* sp. Hence, some beneficial LAB isolates were recorded in La-phet.

Keywords: pickled tea leaves (La-phet), lactic acid bacteria (LAB), identification

Introduction

La-phet, Burmese pickled tea, is a Myanmar national food processed and produced by anaerobic fermentation. Nowadays, it is well known because eating La-phet may have many beneficial effects for human health. There are many species under the La-phet genus *Camellia*, but *Camellia sinensis* is the popular species used to produce tea products (Hnin Htet Htet Shein, 2016).

Tea plant products are mainly consumed as drinks and tea leaves are not consumed in general. However, there is a traditional kind of pickled tea processed by anaerobic fermentation; this kind of tea is produced and drunk or eaten in many Asian countries and is given different names such as Yancha (or Suancha) in China, La-phet (or leppet-so) in Myanmar, Miang tea in Thailand and Laos and Goishi cha and Awaban cha in Japan (Huang *et al*., 2016).

Myanmar, a country in South East Asia, has the tradition of consuming pickled tea leaves by preparing as salad. In the daily life of Myanmar people, La-phet is consumed as snacks, side dish, salads and as a treat for guests. The cuisine of Myanmar has a popular expression: "Of all the fruits, mango is the best; and of all the meat, pork is the best; and of all the leaves, La-phet is the best". La-phet products can be easily found everywhere in Myanmar and Myanmar markets around the world (Han and Aye, 2015).

The most popular salad or dessert La-phet is the one produced by bacterial fermentation process under anaerobic conditions. The traditional La-phet fermentation process has three steps, including pre-fermentation, fermentation and modification of the fermented tea leaves. The anaerobic fermentation is driven by naturally forming lactic acid bacteria. This naturally anaerobic fermentation promotes the spontaneous growth of naturally existing microorganisms in which the LAB are dominant. LAB have been isolated from specific habitats like dairy products, plants, meat products, sewage and manure of humans and animals (Noonpakdee *et al*., 2009; Pelinescu *et al*., 2009) and also from tea leaves (*Camellia sinensis*) (Fathabad and Elamifar, 2011). LAB are non-pathogenic, generally recognized as safe (GRAS) and probiotic microorganisms beneficial for human health. Moreover, the LAB have been used in the processing of fermented food for centuries (Fathabad and Elamifar, 2011).

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In recent years, microbial fermented tea has been paid more and more attention in the world because of many unique functions such as losing weight and lowering blood pressure. There are some studies on pickled tea (La-phet) of Myanmar but much research still needed to be done. And, there is a need to make La-phet products by artificial inoculation of indigenous LAB that ensure health safety leading to mass commercial production. Hence, the current research was carried out to generate some data to contribute to innovative processing technology ensuring high quality production of safe fermented La-phet.

The present study aims to isolate and identify the indigenous lactic acid bacteria, naturally occurring in pickled tea leaves (La-phet), for future use as starter cultures to ferment tea products in the future.

Materials and Methods

Study area and Study sites

The La-phet samples were randomly purchased from retail markets and supermarkets in North Dagon and Kamayut Townships of Yangon Region. These samples were only pure La-phet, not being mixed with other ingredients. These collected samples were carried in sterile plastic bags to the Microbiology Laboratory, University of Yangon for the microbial analysis.

Study period

The study was made from December, 2019 to December, 2020.

Sample collection

Myanmar traditional pure pickled tea leaves (La-phet) samples of 10 branded products, without any added ingredients samples were transported aseptically in sterile plastic containers at room temperature and then were immediately analyzed for lactic acid bacteria in the Microbiology laboratory of Zoology Department, University of Yangon.

Sample preparation and Serial dilution

After weighing 50g of La-phet sample, it was transferred into the glass container already filled with 450 mL of sterile normal saline. And then, this container was thoroughly shaked to get the homogenous mixture. A 10 fold serial dilutions were made to the ninth level by using prepared La-phet samples.

Isolation and Purification of lactic acid bacteria (LAB) from the collected La-phet samples

The diluted mixture (0.1mL) was taken from each dilution with a micropipette under aseptic conditions in a laminar flow biosafety cabinet and inoculated on MRS agar medium by the spread plate method (Dubey and Mashwari, 2002). After incubation, LAB isolates were subcultured by observing their colony morphology. Then, each single isolated colony was further transferred aseptically to the MRS agar containing 1%$CaCO_3$ by streak plate method to obtain the pure culture of each LAB isolate (Dubey and Mashwari, 2002). These plates were incubated at 37^{o}C for 48 hours (Bisen and Verma, 1998). Colonies that formed clear zone was further subcultured using the same methods and media.

Characterization of lactic acid bacteria isolated from La-phet samples

The identification and further study on the characters of LAB isolates grown on MRS agar were performed by determination of their morphological, physiological, biochemical properties as well as by carbohydrate fermentation tests as described by Buchanan *et al*., (1974).

Maintenance of stock culture

These pure LAB cultures were kept in MRS agar slants (containing 20% glycerol) in sterile stock bottles with caps and stored at 4ºC for long term use.

Results

A total of 67 isolates of LAB are obtained from ten different La-phet samples. The colonies are initially characterized by their physiological appearance followed by Gram staining for cell morphology together with the microscopic examination. Gram staining showed that all nine isolates were Gram positive. Under microscopic examination, some isolated LAB cells were rods (single, in pairs or chains) but most were spherical or cocci (single, tetrads, in pairs or chains) (Table 1).

Among 67 isolates, nine LAB isolates showed clear zones with gram-positive, spherical and rod-shaped characters in addition to colony morphology were selected for further study on physiological, biochemical characters and the ability to ferment carbohydrates and sugars.

Biochemical characters were recorded from the responses of the selected LAB isolates whereby the *In vitro* experimentation (Table 2). Both 'positive' and 'negative' results had been observed in the Methyl Red test from each different isolate. All nine isolates showed their negative results for the catalase, oxidase, citrate utilization, gelatinase, Indole, H_2S production, gas production, Voges Proskauer and motility tests. All isolates could ferment the sugars such as glucose, sucrose, lactose in the Triple Sugar Iron (TSI) test. In addition, the isolates had negative responses in catalase, oxidase, H_2S production, indole, citrate utilization and motility tests.

A total of 24 kinds of carbohydrate and 11 sugars provided by the KB009 HiCarbo^{TM}Kit were used to determine the fermentation capability of the selected LAB isolates (Table 3 and Plate 1). The discoloration of basal medium showed that there was the ability to ferment carbohydrate and sugar. In the present study, all LAB isolates fermented and produced acid from lactose, xylose, maltose, fructose, dextrose, galactose, raffinose, trehalose, melibiose, sucrose, L-arabinose, mannose, inulin, sodium gluconate, glycerol, salicin, dulcitol, inositol, sorbitol, mannitol, adonitol, arabitol, erythritol, alpha-methyl-D-glucoside, rhamnose, cellobiose, melezitose, alpha-methyl-D-mannoside, xylitol, D-arabinose and sorbose. But, no change was observed in ONPG, citrate and malonate for all isolates whereas LI9, LJ4 and LJ7 isolates gave positive reaction by changing color (cream to black) in esculin, other six isolates (LA2, LI4, LI7, LI8(s2), 32 LJ1, LJ1(s2)) were not able to hydrolyze it.

The growth condition of the selected LAB isolates was studied by performing the test on physiological characters that involved examining the effect of NaCl, phenol, pH and temperature (Table 4). The selected isolates were tested against 1% to 8% NaCl concentrations to know their NaCl tolerance. Five isolates (LI4, LI7, LI8 (s2), LJ4, LJ7) were unable to tolerate all NaCl concentrations. Isolates (LA2, LJ1, LJ1(s2)) showed changes in indicator colour (purple to yellow) at 1%, 2% and 3% NaCl concentrations indicating growth and thus, can survive in these ranges. Moreover, the growth of (LI9) isolate was not inhibited by 1-8% NaCl except 6%. Hence, it was observed that the LAB isolates from La-phet had different NaCl tolerance. In the phenol tolerance test, 0.1-0.4% phenol concentrations were used.(LA2 and LJ1(s2)) isolates that were rod-shaped LAB, could grow in 1% and 2% phenol but others indicated no change in colour and hence were unable to grow in all of the tested percentages of phenol. Isolates were tested at different pH to determine whether the isolates could survive in acidic and alkaline conditions. All isolates could grow in all acidic levels, as indicated by purple to yellow color change, ranging from pH 2.0-6.0. All isolates showed no growth at 10ºC

and 15ºC. But (LJ1 and LJ1(s2)) isolates grow at 45ºC as indicated by purple to yellow colour 33 change while the rest of the isolates were not able to survive at 10ºC, 15ºC and 45ºC.

Overall data identified that these nine isolates were found to belong to four genera of LAB: *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Leuconostoc mesenteroides*, *Pediococcus* sp. and *Streptococcus* spp.. The remaining 58 isolates could not be unidentified. Among the selected nine LAB isolates, *streptococcus* spp. were dominant (56%), but the other four LAB species (*L.plantarum*, *L.acidophilus*, *L.mesenteroides*, *Pediococcus* sp.) occurred at equal percentage of 11% respectively (Table 5 and 6).

Table 1. Morphological characterization of lactic acid bacteria colonies isolated from pickled tea leaves (La-phet)

Isolate Code	**Colony morphology**	**Gram stain**	**Cell shape**	**Clear zone on MRS agar with 1% $CaCO_3$**
LA2, LJ1(s2)	Circular, entire, raised, small, smooth, glistening, white, opaque	+	Rod	+
LI4, LI7, LJ1, LJ4	Circular, entire, raised, small, smooth, waxy, cream, opaque	+	Cocci	+
LI9, LJ7	Circular, entire, raised, small, smooth, waxy, white, opaque	+	Cocci	+
LI8(s2)	Circular, entire, flat, small, smooth, waxy, yellowish-cream, opaque	+	Cocci	+

+ = positive, small = 1-2mm, $CaCO_3$ = calcium carbonate

Table 2. Biochemical characterization of lactic acid bacteria isolated from pickled tea leaves (La-phet)

Characteristics	Isolate code								
	LA2	LI4	LI7	LI8(s2)	LI9	LJ1	LJ1(s2)	LJ4	LJ7
Oxidase	-	-	-	-	-	-	-	-	-
Catalase	-	-	-	-	-	-	-	-	-
Gelatinase	-	-	-	-	-	-	-	-	-
Methyl Red	+	-	-	-	+	+	+	-	-
Voges-Proskauer	-	-	-	-	-	-	-	-	-
Citrate utilization	-	-	-	-	-	-	-	-	-
Triple Sugar Iron	A/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A
Indole production	-	-	-	-	-	-	-	-	-
Motility	-	-	-	-	-	-	-	-	-
Gas production	-	-	-	-	-	-	-	-	-
H_2S production	-	-	-	-	-	-	-	-	-

\+ = positive
\- = negative
A/A = acid/acid (slant/butt)
H_2S = hydrogen sulphide gas

Table 3. Carbohydrate and sugar fermentation patterns of the isolated LAB from La-phet

Sr No.	Medium	Isolate code								
		LA2	LI4	LI7	LI8(s2)	LI9	LJ1	LJ1(s2)	LJ4	LJ7
1.	Lactose	+	+	+	+	+	+	+	+	+
2.	Xylose	+	+	+	+	+	+	+	+	+
3.	Maltose	+	+	+	+	+	+	+	+	+
4.	Fructose	+	+	+	+	+	+	+	+	+
5.	Dextrose	+	+	+	+	+	+	+	+	+
6.	Galactose	+	+	+	+	+	+	+	+	+
7.	Raffinose	+	+	+	+	+	+	+	+	+
8.	Trehalose	+	+	+	+	+	+	+	+	+
9.	Melibiose	+	+	+	+	+	+	+	+	+
10.	Sucrose	+	+	+	+	+	+	+	+	+
11.	L-Arabinose	+	+	+	+	+	+	+	+	+
12.	Mannose	+	+	+	+	+	+	+	+	+
13.	Inulin	+	+	+	+	+	+	+	+	+
14.	Sodium gluconate	+	+	+	+	+	+	+	+	+
15.	Glycerol	+	+	+	+	+	+	+	+	+
16.	Salicin	+	+	+	+	+	+	+	+	+
17.	Dulcitol	+	+	+	+	+	+	+	+	+
18.	Inositol	+	+	+	+	+	+	+	+	+
19.	Sorbitol	+	+	+	+	+	+	+	+	+
20.	Mannitol	+	+	+	+	+	+	+	+	+
21.	Adonitol	+	+	+	+	+	+	+	+	+
22.	Arabitol	+	+	+	+	+	+	+	+	+
23.	Erythritol	+	+	+	+	+	+	+	+	+
24.	alpha-Methyl-D-glucoside	+	+	+	+	+	+	+	+	+
25.	Rhamnose	+	+	+	+	+	+	+	+	+
26.	Cellobiose	+	+	+	+	+	+	+	+	+
27.	Melezitose	+	+	+	+	+	+	+	+	+
28.	alpha-Methyl-D-Mannoside	+	+	+	+	+	+	+	+	+
29.	Xylitol	+	+	+	+	+	+	+	+	+
30.	ONPG	-	-	-	-	-	-	-	-	-
31.	Esculin	-	-	-	-	+	-	-	+	+
32.	D-Arabinose	+	+	+	+	+	+	+	+	+
33.	Citrate	-	-	-	-	-	-	-	-	-
34.	Malonate	-	-	-	-	-	-	-	-	-
35.	Sorbose	+	+	+	+	+	+	+	+	+

+ = positive, - = negative

Table 4. Physiological characterization of lactic acid bacteria isolated from pickled tea leaves (La-phet)

Tolerance level		Isolate Code								
		LA2	LI4	LI7	LI8(s2)	LI9	LJ1	LJ1(s2)	LJ4	LJ7
NaCl (%)	1.0	+	-	-	-	+	+	+	-	-
	2.0	+	-	-	-	+	+	+	-	-
	3.0	+	-	-	-	+	+	+	-	-
	4.0	-	-	-	-	+	-	-	-	-
	5.0	-	-	-	-	+	-	-	-	-
	6.0	-	-	-	-	-	-	-	-	-
	7.0	-	-	-	-	+	-	-	-	-
	8.0	-	-	-	-	+	-	-	-	-
Phenol (%)	0.1	+	-	-	-	-	-	+	-	-
	0.2	+	-	-	-	-	-	+	-	-
	0.3	-	-	-	-	-	-	-	-	-
	0.4	-	-	-	-	-	-	-	-	-
pH	2.0	+	+	+	+	+	+	+	+	+
	3.0	+	+	+	+	+	+	+	+	+
	3.5	+	+	+	+	+	+	+	+	+
	4.0	+	+	+	+	+	+	+	+	+
	4.5	+	+	+	+	+	+	+	+	+
	5.0	+	+	+	+	+	+	+	+	+
	6.0	+	+	+	+	+	+	+	+	+
	7.0	-	-	-	-	-	-	-	-	-
	8.0	-	-	-	-	-	-	-	-	-
	9.0	-	-	-	-	-	-	-	-	-
	9.6	-	-	-	-	-	-	-	-	-
Growth at	10^{o}C	-	-	-	-	-	-	-	-	-
	15^{o}C	-	-	-	-	-	-	-	-	-
	45^{o}C	-	-	-	-	-	+	+	-	-

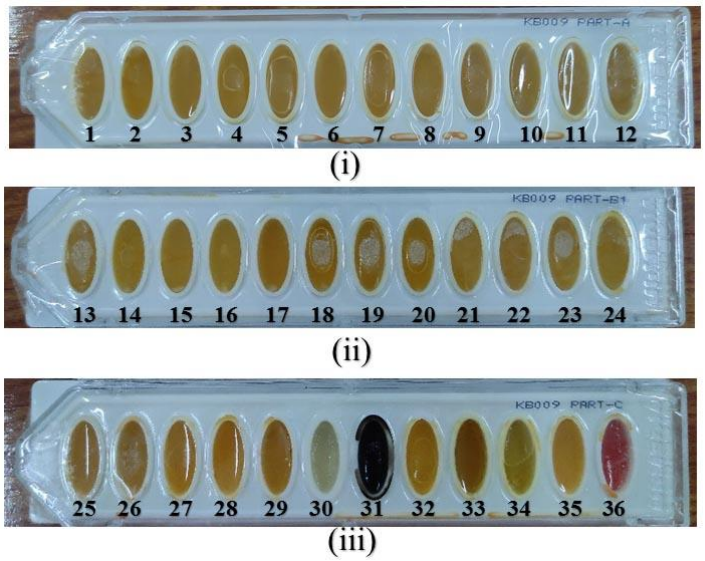
+ = positive (growth)
- = negative (no growth)

Table 5. Classification of the identified lactic acid bacteria isolated from La-phet

Phylum	Class	Order	Family	Genus	Species
Firmicutes	Bacilli	Lactobacillales	1.Lactob-acillaceae	1.*Lactobacillus*	1.*L.plantarum*
					2.*L.acidophilus*
				2.*Pediococcus*	3.*Pediococcus* sp.
			2.Leuco-nostocaceae	3.*Leuconostoc*	4.*L.mesenteroides*
			3.Streptoco-ccaeae	4.*Streptococcus*	5.*Streptococcus* spp.

Table 6. Number of LAB species isolated from the pickled tea leaves (La-phet)

Sample code	Species						
	Lactobacillus plantarum	*Lactobacillus acidophilus*	*Leuconostoc mesenteroides*	*Pediococcus* sp.	*Streptococcus* spp.	Unidentified spp.	Total
1LP	1	-	-	-	-	6	7
2LP	-	-	-	-	-	6	6
3LP	-	-	-	-	-	9	9
4LP	-	-	-	-	-	8	8
5LP	-	-	-	-	-	4	4
6LP	-	-	-	-	-	4	4
7LP	-	-	-	-	-	6	6
8LP	-	-	-	-	-	6	6
9LP	-	-	1	-	3	5	9
10LP	-	1	-	1	2	4	8
Total	1	1	1	1	5	58	67
%	1.49	1.49	1.49	1.49	7.46	86.57	100.00



(i) KB009A

(1) (+), (2) (+), (3) (+), (4) (+), (5) (+), (6) (+), (7) (+), (8) (+), (9) (+), (10) (+), (11) (+), (12) (+)

(ii) KB009B1

(13) (+), (14) (+), (15) (+), (16) (+), (17) (+), (18) (+), (19) (+), (20) (+), (21) (+), (22) (+), (23) (+), (24) (+)

(iii) KB009C

(25) (+), (26) (+), (27) (+), (28) (+), (29) (+), (30) (-), (31) (+), (32) (+), (33) (-), (34) (-), (35) (+), (36) control

Plate 1 Carbohydrate and sugar fermentation patterns of *Leuconostoc mesenteroides* from the pickled tea leaves (La-phet).

KB009A, KB009B1, KB009C=KB009 HiCarbo^{TM} Kit tests

(1) Lactose, (2) Xylose, (3) Maltose, (4) Fructose, (5) Dextrose, (6) Galactose, (7) Raffinose, (8) Trehalose, (9) Melibiose, (10) Sucrose, (11) L-Arabinose, (12) Mannose, (13) Inulin, (14) Sodium gluconate, (15) Glycerol, (16) Salicin, (17) Dulcitol, (18) Inositol, (19) Sorbitol, (20) Mannitol, (21) Adonitol, (22) Arabitol, (23) Erythritol, (24) alpha-Methyl-D-glucoside, (25) Rhamnose, (26) Cellobiose, (27) Melezitose, (28) alpha-Methyl-D-Mannoside, (29) Xylitol, (30) ONPG, (31) Esculin, (32) D-Arabinose, (33) Citrate, (34) Malonate, (35) Sorbose, (36) control

Discussion

The present study is carried out to observe indigenous LAB from Myanmar traditional pickled tea leaves (La-phet). The nine isolates were selected depending on morphology, cell shape and biochemical results among the total of 67 isolates obtained. The clear zones were recognized around the isolates because of the reaction between the acid produced by the lactic acid bacteria isolates with $CaCO_3$ in the MRS medium; resulting in soluble calcium-lactate in the medium (Hartayanie *et al*., 2016). The selected LAB isolated from the La-phet samples were identified as *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Leuconostoc mesenteroides*, *Pediococcus* sp. and *Streptococcus* spp. by determination on the physiological characters and the capability to ferment carbohydrates and sugars. The selected LAB isolates were categorized depending on the characters mentioned in Buchanan *et al*., (1974); Carr *et al*., (2002); Whitman *et al*., (2009), Holzapfel and Wood, (2014).; Kanauchi, (2019).

Among nine isolates, two (*Lactobacillus plantarum* and *Lactobacillus acidophilus*) were gram-positive rod-shaped LAB. *L.plantarum* is not only found in milk but also in meats, fermented sausage, pickles and pickled cabbage (Holzapfel and Wood, 2014). In other work, *Lactobacillus* species were isolated from fermented tea leaves (Miang) (Tanasupawat *et al*., 2007). *L.plantarum* is reported to be dominant in pickled tea processed by submerged fermentation of leaves (Huang *et al*., 2016) and it was also isolated from fresh tea leaves (Hnin Htet Htet Shein, 2016).

Another *Lactobacillus* sp. found in the present La-phet samples was *L.acidophilus* although literatures describe that it is isolated from the intestinal tract of humans and animals,

the human mouth, vagina, sourdough and wine (Whitman *et al*., 2009; Holzapfel and Wood, 2014).

In addition, the finding of *Leuconostoc mesenteroides* from the pickled tea leaves (La-phet) was not reported by the previous researches. But, in the present study, the characters of the (LI9) isolate confirmed it to be *L.mesenteroides* according to the references (Buchanan *et al*., (1974); Carr *et al*., (2002); Whitmen *et al*., (2009); Kanauchi, (2019)).

The six LAB isolates (LI4, LI7, LI8(s2), LJ1, LJ4 and IJ7) from La-phet samples could only be identified to the genus level. The (IJ7) isolate was identified as *Pediococcus* sp. Pickled tea leaves (La-phet) can be the habitat for pediococci as they are found commonly associated with various plants and their products such as cabbage and saukeraut, cucumbers and pickles, grapes and wine and wort and grain mashes (*Carr et al*., 2002). And also, *Pediococcus* species were also isolated from fermented tea leaves (Miang) produced in the northern part of Thailand (Tanasupawat *et al*., 2007).

The isolates (LI4, LI7, LI8(s2), LJ1 and LJ4) were found to be *Streptococcus* spp. However, members of the genus *Streptococcus* are especially difficult to distinguish from those of the genera *Enterococcus* and *Lactococcus* only by using phenotypic tests alone; thus, molecular identification methods should be used for genus and species identification to be precise (Holzapfel and Wood, 2014).

Apart from *Lactobacillus* spp. and *Pediococcus* sp., other LAB of *Leuconostoc* and *Streptococcus* species were isolated from the La-phet in the present study. The diversity of LAB in pickled tea can depend on the processing method, the chemical composition, handling technique and quality of tea leaves. *Lactobacillus acidophilus, Leuconostoc mesenteroides* and *Streptococcus* spp. from pickled tea (La-phet) in the present study may be new findings in terms of LAB isolation from La-phet as they were not previously reported. Among them, *Streptococcus* spp. isolates could contain pathogenic strains because streptococci can cause localized and systemic infections under appropriate conditions although they usually live as commensals on warm-blooded animals. In contrast, *Leuconostoc mesenteroides* are non-pathogenic to humans, animals and plants, and they play an important role in the fermentation of vegetables and are also associated with a wide variety of meat and d airy products (Whitman *et al*., 2009). The genus *Lactobacillus* is generally recognized as safe and the most important and beneficial group of living organisms ingested in human food. Thus, *Lactobacillus acidophilus* is also free from pathogenicity. In this study, although two other beneficial LAB (*Leuconostoc mesenteroides* and *Lactobacillus acidophilus*) were recorded from La-phet in the present study, there is still no information on their mass production and usage of in food industry. Nevertheless, from the present results, it was clearly shown that there are some beneficial LAB in La-phet.

Conclusion

Five groups of LAB (*Lactobacillus plantarum, Lactobacillus acidophilus, Leuconostoc mesenteroides, Pedicoccus* sp. and *Streptococcus* spp.) were isolated and identified from the La-phet. Hence, the consumers could, on the other hand, receive the beneficial bacteria from eating the La-phet. Among them, *Lactobacillus plantarum, Lactobacillus acidophilus* and *Leuconostoc mesenteroides* can be considered as the suitable starter cultures especially in food production focusing on the fermentation technology. But, precise molecular identification and genetic structures of these LAB should be done. For the sake of health safety, they must be tested with antibiotics whether they occupy the ability of antibiotic resistance even though they are recognized as safe bacteria because genetic modification and mutation rapidly occur in different bacterial species in nowadays. From the findings, the future study can be continued to

genetic engineering on these LAB isolates with the intention of developing the innovative and effective alternative technology for food industry.

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References

Bisen, P.S. and Verma, K. 1998. *Handbook of Microbiology*. CBS Publishers and Distributors, New Delhi, India.

Buchanan, R.E. and Gibbons, N.E., eds. 1974. *Bergey's Manual of Determinative Bacteriology*, Eight edition. Williams and Wilkins Company, United States of America.

Carr, F.J., Chill, D. and Maida, N. 2002. The Lactic Acid Bacteria: A Literature Survey. *Critical Reviews in Microbiology* 28, (4): 281–370.

Dubey, R.C. and Maheshwari, D.K. 2002. *Practical Microbiology*. S.Chand and Company Ltd., Ram Nagar, New Delhi.

Fathabad, E.G. and Eslamifar, M. 2011. Isolation and applications of one strain of *Lactobacillus paraplantarum* from tea leaves (*Camellis sinensis*). *American Journal of Food Technology* 6, (5): 429-434.

Han, T. and Aye, K.N. 2015. The legend of laphet: A Myanmar fermented tea leaf. *Journal of Ethnic Foods* (2): 173-178.

Hartayanie, L., Lindayani and Murniati, M.P. 2016. Antimicrobial activity of lactic acid bacteria from bamboo shoot pickles fermented at 15ºC. *Microbiology Indonesia* 10, (2): 71-77.

Hnin Htet Htet Shein, 2016. Study on microbial fermentation of tea leaves, *Camellia sinensis*, in Myanmar traditional salad (Laphet). *MFT.6*, Yangon Technological University, Yangon.

Holzapfel, W. H and Wood, B. J. B., 2014. *Lactic Acid Bacteria: Biodiversity and Taxonomy*. John Wiley and Sons, Ltd, UK.

Huang, Y., Liu, C. and Xiao, X. 2016, Quality characteristics of a pickled tea processed by submerged fermentation. *International Journal of Food Properties* 19, (6): 1194-1206.

Kanauchi, M., 2019. *Lactic Acid Bacteria: Methods and Protocols*. Department of Food Management, Miyagi University, Sendai, Miyagi, Japan.

Noonpakdee, W., Jumriangrit, P., Wittayakoml, K., Zendo, J. and Nakayama, J. 2009. Two-peptide bacteriocin from *Lactobacillus plantarum* PMU 33 strain isolated from som-fak, a Thai low salt fermented fish product. *Asia Pac.J.Mol.Biol.Biotechnol*. 17: 19-25.

Pelinescu, D.R., Sasarman, E., Chifiriuc, M.C, Stoica, I., Nohit, A.M., Avram, I., Serbancea, F. and Dimov, T.V. 2009. Isolation and identification of some *Lactobacillus* and *Enterococcus* strains by a polyphasic taxonomical approach. *Romanian.Biotechnol. Lett*. 14: 4225-4233.

People Daw Amar, 1992. The inequivalent development and other articles. *Da Na* 2, (8): 25-61.

Tanasupawat, S., Pakdeeto, A., Thawai, C., Yukphan, P. and Okada, S. 2007. Identification of lactic acid bacteria from fermented tea leave (miang) in Thailand and proposals of *Lactobacillus thailandensis* sp.nov,. *Lactobacillus camelliae* sp.nov., and *Pediococcus siamensis* sp.nov. *J.Gen.Appl.Microbiol.* (53): 7-15.

Whitman, W.B., Vos, P.D., Garrity, G.M., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A. and Schleifer, K.H. 2009. *Bergey's Manual of Systematic Bacteriology: Second Edition, Volume Three, The Firmicutes*. Springer Science+Business Media, New York, USA.