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MYANMAR POPULAR PLANT-BASED FERMENTED FOODS, THEIR PROCESSING, AND THEIR MICROORGANISMS POTENTIAL FOR PROBIOTICS

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ABSTRACT

For traditional processing, six Myanmar popular plant-based fermented foods were investigated. Additionally, chemical and microbiological investigations of fermented foods from local markets were conducted, followed by the isolation of potential probiotic bacteria from these foods. All of the fermented foods investigated were found to be processed by natural spontaneous fermentation accomplished by adding rice water, rice juice, and salt. The total acidity of the fermented foods examined ranged from 0.57 ± 0.04 to 1.42 ± 0.06 g/100g of sample, while the pH ranged from 3.32 ± 0.18 to 4.44 ± 0.11. Aerobic bacteria count was between 7 and 9 log CFU/g, while *lactobacilli* count was between 6.63 ± 0.03 and 8.72 ± 0.10 log CFU/g. After 2 hours of incubation, six isolates demonstrated a survival rate of between 34.06 ± 6.68 percent and 72.38 ± 13.20 percent at pH 2.0, and between 55.73 ± 2.29 percent and 88.35 ± 7.42 percent in 0.3 percent in bile salt solution after 4 hours of incubation. Six isolates were identified based on sequencing results:three *Lactobacillus helveticus*, two *Leuconostoc lactis*, and one *Leuconostoc mesenteroides*. This study could help develop probiotics from Myanmar fermented foods and learn more about how Myanmar fermented foods are processed.

Keywords: Myanmar fermented foods, traditional food processing, lactic acid bacteria, probiotics

INTRODUCTION

Because of consumer familiarity with these beneficial microbes, dairy products are the most prevalent source of probiotics, and European countries are leading in dairy-based probiotics food manufacturing **(Bansal *et al*., 2016).** Although fermented dairy products are generally good carriers and suppliers of probiotic microbes, the increasing number of individuals with lactose intolerance, dyslipidemia, and vegetarianism emphasizes the role of developing non-dairy probiotic products like fruit and vegetables **(Martins *et al*., 2013).**

Furthermore, a surge in vegetarian or vegetable-preferred consumers in both developed and emerging countries is driving up market demand for plant-based products **(Panghal *et al*., 2018)**. Traditional plant-based fermented meals can be created from any plant material, including vegetables, fruits, cereals, and legumes, and they provide dietary fiber, minerals, vitamins, and phytochemicals, along with other nutritional benefits **(Tamang, 2012; Dey, 2018)**. As a result, a large quantity of plant-based fermented foods is available, and it has been consumed all over the world. Plant-based fermented foods also give microbiological advantages and opportunities to human health, because they are large reservoirs of bacteria that can be employed as candidates for isolating new probiotics and can be used as a carrier for the administration of health-promoting probiotics (**Molin, 2001; Kumar *et al*., 2015**).

Myanmar, or Burma, is a country in Southeast Asia that has borders with Bangladesh, China, India, the Lao People's Democratic Republic, and Thailand. Myanmar is 261,227 square miles in size, with a population of 60 million people **(Jafari, 2002).** Myanmar is mostly an agricultural country, with various tropical fruits and vegetables growing throughout the year. Myanmar meals often include fermented foods, particularly fermented vegetables. Fermented foods, such as fermented mustard, are used as a noodle sauce replacement at breakfast. Fermented vegetables are served raw or pickled together with rice, meat, and soup for lunch and dinner. As a result, fermented vegetables might be considered indispensable in Myanmar cuisine. There are primarily two types of fermented foods: year-round fermented vegetables and seasonal fermented vegetables.

Individual small-scale producers make Myanmar's popular plant-based fermented foods, which are primarily sold in the local market. We investigated at how some common fermented vegetables are processed in this article. The chemical and microbiological features of Myanmar plant-based fermented products were then investigated. Lactic acid bacteria were isolated from fermented foods and their probiotic qualities were investigated. This research could look on the development and understanding of Myanmar plant-based fermented foods.

MATERIAL AND METHODS

Study on traditional processing methods

Small manufacturers of fermented foods made from plants were targeted for questioning concerning processing methods. For each type of fermented food, five to eight manufacturers with at least two years of expertise fermenting plant-based material were selected. The questions included a detailed protocol on the raw materials utilized, the involvement of pretreatment procedures, fermentation temperature, fermentation time, specific processes during fermentation, and the consuming style of each fermented foods

General chemical and microbial analysis of fermented foods

Chemical and microbiological studies were performed on six distinct types of fermented foods that are the most popular and widely available. At least five samples from different producers were acquired for each fermented product, and the pH, total titratable acidity, lactic acid bacteria count, and total aerobic count were evaluated.

Fifty grams of each fermented food item was mixed with 200 ml of distilled water. The liquid was filtered, and a 50 ml portion was diluted with 50 ml distilled water before the pH was determined with a digital pH meter. Titration with 0.1 M NaOH was used to determine the total titratable acidity of fermented foods, and the total titratable acidity was expressed as lactic acid equivalent **(Zaika *et al*., 1976).**

Total aerobic bacteria and lactic acid bacteria were counted using nutrient agar and MRS agar, respectively. Ten grams of each sample were diluted 1:10 in saline water (8 g/l NaCl) and thoroughly mixed. Additional tenfold dilutions with the same diluent were prepared, and the following culturing on different agar plates was performed: Total viable counts on nutrient Agar (HiMedia, Mumbai, India) cultured for three days at 37°C; lactic acid bacteria (LAB) on MRS agar (HiMedia, Mumbai, India) incubated for 48 hours at 37°C. Randomly selected microbial colonies on MRS agar were isolated by streaking again onto MRS agar. For further analysis, the isolates were kept at – 20°C in a 50% (v/v) glycerol stock.

Preliminary examination of microbial isolates

The isolates were first determined for the Gram reaction using the Gram staining method and then evaluated under the microscope for morphology. The catalase test was performed on isolated bacteria using the method described elsewhere **(Mulaw *et al*., 2019).** To summarize, the isolated bacteria were cultured on MRS agar for 24 hours at 37°C. A loop of culture aged 24 hours was dripped into two drops of hydrogen peroxide (3%) on a glass slide. The creation of oxygen bubbles is referred to as the positive catalase response, which occurs when isolated bacteria produce catalase. To investigate isolates' motility, the hanging drop method was used **(Jain *et al*., 2020).** Without disrupting the culture drop, a little drop of overnight cultures of isolates in MRS broth was placed on a cover slip and inverted over the glass slide containing the cavity. Under the microscope, the motility of isolates was assessed. The isolates that exhibited a positive catalase reaction and Gram positive were chosen for the following experiments.

Growth at different temperature and NaCl concentration

The isolates were grown on MRS agar at various temperatures (4°C, 37°C, and 45°C) and their growth patterns were observed. Additionally, they were cultivated on MRS agar with varying salt concentrations (sodium chloride: 3, 5, and 7%) to determine their tolerance to osmotic stress.

Tolerance to acid and bile salt

To determine tolerance of isolates for gastric pH and bile salt, *in vitro* acid and bile salt resistance tests were conducted **(Mulaw *et al*., 2019**). Overnight cultures of lactic acid bacteria isolates were inoculated into MRS broth that had been originally adjusted to pH 2.0 with 1N HCl and MRS broth supplemented with 0.3 percent bile salt (HiMedia, India). The control for the acid tolerance test was MRS broth with a pH of 6.5, and the control for the bile tolerance test was MRS broth without bile salt. The samples were incubated at 37°C for 2 hours for acid resistance and 4 hours for bile resistance. After incubation, the samples were rinsed twice with saline solution before to counting. The following equation was used to get the survival rate.

$$Survival\ rate\ (\%) = \frac{Log\ CFU\ N_1}{Log\ CFU\ N_0}\ x\ 100$$

Where N_1 is the viable count of isolates after incubation and N_0 is the initial viable count.

Antibiotic susceptibility test

The antibiotic sensitivity patterns of bacterial isolates were investigated by using antibiotic discs (HiMedia Laboratories Pvt. Ltd, India). The antibiotic discs were inserted on the inoculated plates after the overnight culture of isolates was dispersed on MRS agar. The plates were then incubated for 48 hours at 37°C. According to the CLSI scale, the diameter of the zone of inhibition was measured, and the patterns were classified as R, resistant; S, susceptible; and I, intermediate. The zone standard specified by Performance Standards for Antimicrobial Disc Susceptibility Testing was used to analyze the data **(CLSI, 2021).**

Molecular identification of selected bacterial isolates

The process used to identify selected bacterial isolates included the extraction of genomic DNA from pure bacterial culture, 16S rDNA amplification, sequencing of amplified DNA, and lastly BLAST typing. Chelex-based DNA extraction was used to extract genomic DNA from pure bacterial cultures **(Martín-Platero *et al*., 2010)**. The 16S rRNA gene was then amplified using 27F and 1492R primers **(Lane, 1991)**. DNA fragments were sequenced at SolGent Co., Ltd., Seoul, Korea, using an ABI 3730XL DNA Analyzer. To identify bacterial isolates, DNA sequences were examined using the NCBI's BLAST program **(Altschul *et al*., 1990)**.

Statistical analyses

All values were expressed as means ± standard deviations of triplicate analyses.

RESULTS AND DISCUSSION

***Moanlar Shin* (fermented carrot)**

Moanlar Shin is a famous fermented Myanmar food made with radish, carrots, and other vegetables. It is a fermented sour-acidic fermented vegetable that can be found in most local markets. The cleaned vegetables are cut and placed in a previously used fermentation pot or a new fermentation pot. The required amount of congee is then added (rice water or cooked rice water can also be used). As a minor ingredient, a small amount of table salt is also added. Fermentation takes 1 to 2 days on average. The use of rice water or cooked rice water necessitates a longer fermentation time (Fig.1). *Moanlar Shin* is popular side dish, is eaten together with cooked fish paste (*Nga Pi Ye Cho*). *Moanlar Shin* is comparable to other fermented vegetables like as Kimchi and Sauerkraut, except that *Moanlar Shin* use radish as the primary vegetable, whereas Kimchi and Sauerkraut utilize cabbage. The amount of salt used in the production of *Moanlar Shin* is identical to that used in the production of Kimchi and Sauerkraut. Unlike Kimchi, spices are not utilized in the production of *Moanlar Shin*, however a few people do add a small amount of chile as an ingredient **(Patra *et al*., 2016; Peas *et al*., 2017).** Congee is traditionally used in *Moanlar Shin* processing to provide carbon for microorganisms, whereas rice flour is utilized in Kimchi processing **(Patra *et al*., 2016).**

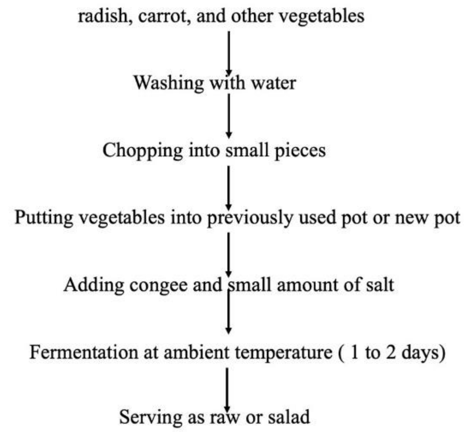

Figure 1 Flow diagram of traditional *Moanlar Shin* processing

Palte Shin (Fermented green gram sprout)

In Myanmar, fermented green gram sprouts are one of the most popular fermented foods. It is accessible throughout the year in the majority of local markets. One important application of fermented green gram sprouts is that they are frequently offered as a side dish at traditional ceremonies. The processing is divided into two stages: germination the green gram and fermenting the sprouted green gram (Fig.2). Green gram seeds were immersed in warm water overnight to initiate germination. After splitting the seed cover, the water is drained and the seeds are transferred to a fresh container. During germination, it is critical to shower the seeds with a suitable amount of water daily, and excessive water should be avoided. Green gram seed germinates in around 5 days, yielding a sprout between 3 and 5 inches long. Concurrently, a liquid suspension of rice water, rice juice, a trace of salt, and turmeric powder allowed to ferment naturally. When the sprouts are ready, they are steeped for one or two days together in fermented liquid suspension. Fermented green bean sprouts can be eaten raw or combined with onion and chile to make a salad. Germination and fermentation enhance the sensory and nutritional qualities of grains by providing nutritional enrichment, removing antinutrients, extending shelf life, increasing safety and stability, and imparting a distinct flavor and aroma **(Wu *et al*., 2019)**. Vitamin C, thiamin, riboflavin, nicotinic acid, biotin, and pyridoxine are all increased in mung bean or green gram sprouts **(Finney, 1983)**. Additionally, germination was observed to boost the mineral content of green gram sprouts **(Embrey, 1921**). Breakfast or snack foods, beverages, spices, weaning foods, and functional foods are all examples of indigenous fermented foods made from sprouted grains **(Wu et al., 2019)**. Thus, fermented green gram sprouts, or *Pal Te Shin*, are one of Myanmar's indigenous fermented foods that can be regarded beneficial to our health.

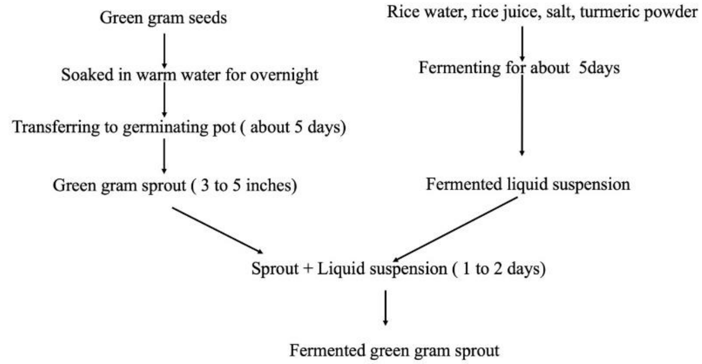

Figure 2 Flow diagram of traditional *Palte Shin* processing

***Nan Phat Shin* (Fermented groundnut or sesame oil cake)**

The oil cake obtained from commercial groundnut or sesame seed oil extraction is inexpensive and nutritious. These oil cakes are only employed as components in a limited number of food processing and animal feed processes. However, in Myanmar, after fermentation, oil cakes are frequently consumed, and the fermented product is referred to as *Nan Phat Shin*. Although the name *Nan Phat Shin* translates as fermented sesame oil cake, it encompasses both fermented sesame and groundnut oil cakes. The majority of fermented groundnut oil cakes are sold in local markets.

It has a sour-acidic flavor and is sold by a tiny family business. The detailed process of making *Nan Phat Shin* is shown in Fig.3. Groundnut or sesame oil cakes are chopped into small pieces and water is poured to them for the fermentation of Nan Phat Shin. The amount of water used is usually 2 to 2.5 times the weight of the oil cake. The oil cake can be easily suspended in water if it is in flake form. If the oil cake is hard and solid, it is steeped in water for 10 to 20 hours to suspend it. After that, a suitable amount of salt is added to the oil cake and water mixture. The mixture is then placed in a plastic container or a clay pot to ferment. To remove extra water from the oil cake to be fermented, some people wrapped it in a clean cloth. Fermentation takes 3 to 5 days on average. By using previously fermented oil cake as a starter, the fermentation time can be cut in half. *Nan Phat Shin* is a popular Myanmar side dish served as a salad with onions, green chilies, cilantro, and lemon leaves. Related to bean fermentation in Asia, most products are fermented soy bean. They are Korean Kanjang, Doenjang, and Chngkukjang, Japanese Shoyu and Miso, and Indonesian Tempe, and they are fermented using molds such as *Aspergillus oryzae* and *Rhizopus* spp. **(Lee et al., 2016; Owens *et al*., 2014)**. In comparison to these fermented soybean products, lactic acid fermentation may be used to make *Nan Phat Shin* due to rapid acidification and the presence of lactic acid bacteria upon isolation on MRS agar. However, validation of *Nan Phat Shin's* major microbial fermentation should be conducted in the future. At the very least, Nan Phat Shin can be described as a distinctive Myanmar fermented cuisine.

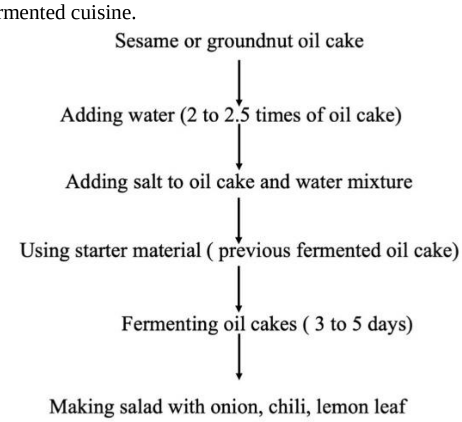

Figure 3 Flow diagram of traditional *Nan Phat Shin* processing

Seasonal plant based fermented foods

Some fermented meals are prepared seasonally, depending on the availability of fruit and vegetables. *Saunggyan Shin* (fermented *Capparis flavicans* Wall fruit), *Khantat Shin* (fermented *Crateva religiosa* leaves), and *Buu Shin* (fermented calabash) are the most regularly encountered seasonal plant-based fermented products in local markets, and they are all manufactured using the same procedure as *Moanlar Shin*. The hard cover of the fruit is removed during *Saunggyan Shin* processing, and the inside part is fermented together with rice water and occasionally cooked rice. Fermentation takes around a week in most cases. *Khantat Shin* is prepared by cleaning and fermenting the leaves with rice water or cooked rice juice and a small amount of salt. Fermentation is accelerated when the previously applied container is utilized (1 to 2 days). Fermentation usually takes no more than one week. Some individuals prepare fermentation liquid by fermenting congee with a modest amount of salt for one to two days. This liquid is used for primary vegetable fermentation. To manufacture *Buu Shin*, calabash fruit is sliced into small and long pieces and fermented in the same way as *Moanlar Shin* . *Buu Shin, Saunggyan Shin,* and *Khantat Shin* are seasonal and only available during Myanmar's hot season (From March to May).

pH, total titratable acidity and microbial counts of fermented foods

In order to investigate the chemical and microbial profiles of six Myanmar fermented foods (Fig. 4), the pH, total titratable acidity, total aerobic bacterial count, and lactobacilli count of six kinds of fermented foods were analyzed (Table 1). In addition, the photograph of these fermented foods is added as Fig. 4. Microbial development during fermentation is linked to the pH and total acidity of fermented foods. The pH of fermented Myanmar foods ranged from 3.32 ± 0.18 (fermented Crateva religiosa leaves) to 4.44 ± 0.11(Fermented groundnut oil cake). The pH of two Myanmar fermented foods was lower than 4, whereas the pH of Myanmar which were comprised of vegetables was higher than 4. The pH of a product manufactured from groundnut oil cake and *Capparis flavicans* Wall fruit was greater than 4. Similarly, Chinese homemade paocai (pH 3.03), Sichuan paocai (pH 3.29) and cabbage kimchi (pH 4.1) all have a wide range of pH values **(Liang *et a*l., 2016; Luo *et al*., 2020; Moon *et al*., 2020).** The maximum overall acidity concentration was found in fermented *Crateva religiosa* leaves, while the lowest was found in fermented *Capparis flavicans* Wall. Myanmar fermented foods had a total acidity range of 0.67 to 1.05. Other optimized fermented foods like Cabbage kimchi (0.7 to 0.9 percent) and Paocai (0.6 percent) exhibited similar acid values, therefore this could be an acceptable range of fermented foods (Moon et al., 2020; Liang et al., 2016). Total aerobic bacterial counts in Myanmar fermented foods ranged from 6.97 ± 0.08 Log CFU/g (fermented radish) to 8.88 ± 0.01log CFU/g (Fermented groundnut oil cake). The fermented radish, on the other hand, had the greatest lactobacilli count. Fermented green gram sprout has the smallest number of lactobacilli. The microbial counts of Myanmar fermented foods were found to be less than 9 log CFU/g. However, the numbers did not fall below 6 log CFU/g. In fact, the microbial count in fermented foods is high in early days of fermentation and gradually decreases with increasing fermentation time. The fermentation times of Myanmar fermented foods collected from the local market were less than 5 days, indicating that the time of fermentation for these items was in the early stages. Tunisian fermented vegetables (6.3 ± 0.02 Log CFU/mL to 8.7 ± 0.03 Log CFU/mL), sauerkrauts (approximately 6 to 8 log CFU/g), and kimchi and teff batter samples (5 to 9 log CFU/g) all had variable range of LAB counts **(Gebru and Sbhatu, 2020; Touret *et al*., 2018; Ziadi *et al*., 2019)**. The large range of counts could be attributed to a variety of factors, including the origin of the veggies, physico-chemical conditions, nutrient composition, and production processes **(Ziadi *et al*., 2019)**. The cell densities in Myanmar fermented foods were similar to those found in most fermented vegetables previously (**Rezac *et al*., 2018).**

Table 1 pH, total titratable acidity and microbial counts of Myanmar fermented foods

No.	Myanmar fermented foods	pH	Total Acidity	Total aerobic bacterial count (log CFU/g)	*Lactobacilli* count (log CFU/g)
1	*Moanlar Shin* (Fermented radish)	3.53 ± 0.30	0.97 ± 0.21	6.97 ± 0.08	8.72 ± 0.10
2	*Palte Shin* (Fermented green gram sprout)	3.47 ± 0.07	1.05 ± 0.09	6.97 ± 0.04	6.63 ± 0.03
3	*Nan Phat Shin* (Fermented groundnut oil cake)	4.44 ± 0.11	0.67 ± 0.06	8.88 ± 0.01	8.71 ± 0.04
4	*Saunggyan Shin* (Fermented *Capparis flavicans* Wall)	4.23 ± 0.12	0.57 ± 0.04	8.46 ± 0.03	7.43 ± 0.02
5	*Khantat Shin* (Fermented *Crateva religiosa* leaves)	3.32 ± 0.18	0.86 ± 0.04	7.55 ± 0.03	7.72 ± 0.01
6	*Buu Shin* (Fermented calabash fruit)	3.72 ± 0.04	0.78 ± 0.04	8.47 ± 0.03	7.12 ± 0.01

Figure 4 A photograph showing Myanmar fermented foods. 1. Fermented radish; 2. Fermented green gram sprout; 3. Fermented groundnut oil cake; 4. Fermented *Capparis flavicans* Wall fruit; 5. Fermented *Crateva religiosa* leaves; 6. Fermented calabash fruit.

Acid and bile resistance of isolated bacteria

The 31 Gram-positive, catalase-negative, and non-motile isolates were chosen for further research as presumptive LAB isolates out of a total of 52 isolates. After that, they're tested for acid and bile resistance. The pH of human stomach juice is low. One of the characteristics of microorganisms recommended for use as probiotics is their resistance to low pH. Furthermore, the ability to tolerate bile salt aids in the evaluation of isolates for metabolic activity and small intestine colonization **(Somashekaraiah *et al*., 2019)**. Six bacterial isolates out of the 31 showed a high level of resistance to low pH and 0.3 percent bile salt. Figure 5 shows the survival of these six isolates in low acidic conditions after 1 and 2 hours of incubation at 37°C. The maximum survival rate of isolates after 1 hr of incubation at pH 2.0 was 82.31 percent (DBR-F4), the lowest rate was 40.60 percent (DBR-F6), and other isolates showed a survival rate of more than 50%. Except for DBR-F6, the survival rate of most isolates declined after 2 hours of incubation, but it did not go below 50%. Figure 6 shows the survival of six bacterial isolates in a 0.3 percent bile salt concentration after 2 and 4 hours of incubation at 37°C. After 2 hours incubation in 0.3 percent bile salt concentration, the greatest survival rate of isolates was 92.94 percent (DBR-F10), while the lowest rate was 69.67 percent (DBR-F4). Except for the bacterial isolate DBR-F4, all of the isolates demonstrated a remarkable survival rate of approximately or more than 70% after 4 hours of incubation (55.73 percent). After *in vitro* testing, it may be considered that the selected bacterial isolates from Myanmar fermented foods exhibited adequate resistance to low pH and bile salt. Selected LAB isolates from Ethiopian fermented food products were also resistant to pH 2.0, with a survival rate of roughly 45 percent after 2 hours of incubation, and 0.3 percent bile salt, with a survival rate of more than 90 percent after 24 hours (**Mulaw *et al*., 2019).** Similarly, after 4 hours of incubation, isolated LAB strains from Neera (fermented coconut palm nectar) had a survival rate of over 50% at pH 2.0 and 0.3 percent bile salt **(Somashekaraiah *et al*., 2019).**

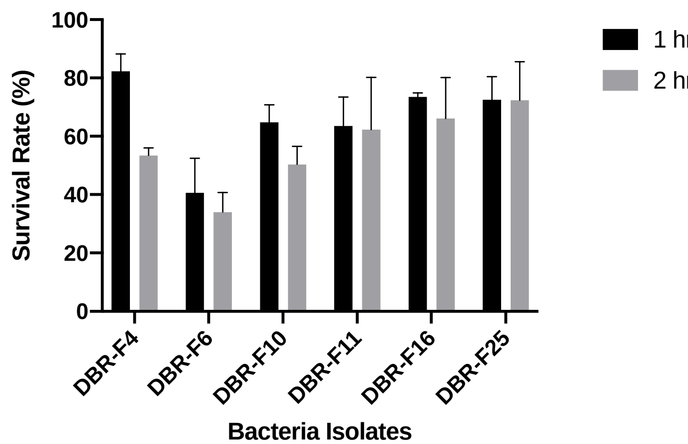

Figure 5 Changes in survival rate of bacterial isolates in MRS broth adjusted to pH 2.0 after 1 and 2 hr incubation

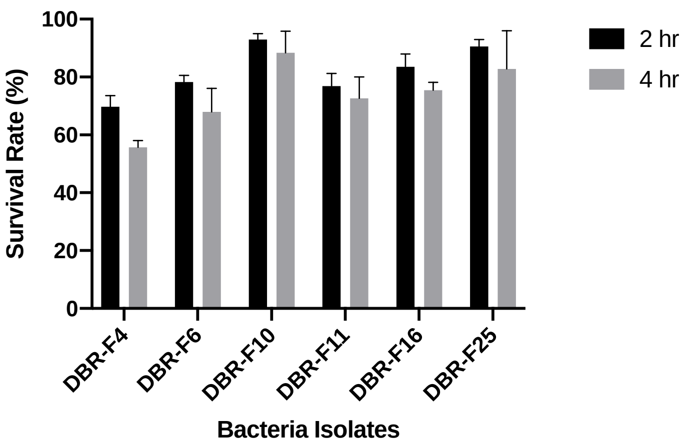

Figure 6 Changes in survival rate of bacterial isolates in MRS broth with 0.3% bile salt after 2 and 4 hr incubation

Physiological, morphological, biochemical and molecular identification of bacterial isolates.

Table 2 shows the growth pattern of some isolates from Myanmar fermented foods which are resistant to low pH and bile salt at various NaCl concentrations and temperatures, motility, catalase reaction, Gram reaction, microscopic appearance, and molecular identification. The isolates were all nonmotile, catalase-negative, and Gram-positive. Only DBR-F4 grew on MRS agar with 7% NaCl, indicating that all of the bacterium isolates are resistant to NaCl. Salt tolerance is important because s alt is used in the production of Myanmar fermented foods in varying amounts. Beneficial bacteria thrive under osmotic stress because they may undergo selective multiplication whereas salt-sensitive microbes, particularly pathogenic bacteria, are repressed **(El-Gendy *et al*., 1983)**. Furthermore, salt tolerance of bacteria utilized as probiotics would be beneficial during the gastrointestinal passage when coupled with injectable foods with high salt concentrations. Three bacterial isolates were not grown at 45°C, while one strain was not grown at 4°C.Similarly, Lactic acid bacteria isolated from fermented coconut palm nectar grew at low and high temperatures and were resistant to a variety of salt concentrations up to 7%. According to microscopic analysis, DBR-F4, DBR-F6, DBR-F16 and DBR-F25 had the shape of rod, whereas DBR-F10 and DBR-F11 had the shape of cocci. The selected six bacterial isolates were three *Lactobacillus helveticus*, two *Leuconostoc lactis*, and one *Leuconostoc mesenteroides*, according to 16S rDNA sequencing and identification. *Lactobacillus helveticus* has a GRAS (Generally Recognized as Safe) designation and is especially well-suited to dairy applications (**Giraffa, 2014).** It's also found in fermented foods like table olives **(Tofalo *et al*., 2014**). *Leuconostoc lactis* is often found in fermented vegetables like kimchi and it provides. a pleasant aroma in fermented foods (**Kim *et al*., 2001;Kim *et al*., 2020)**. Previously, *Leuconostoc lactis* was not thought to be pathogenic to humans, although cases of infection caused by this bacterium have been reported. Therefore, before employing this bacterium in food processing or for human consumption, it should be confirmed that it is safe. *Leuconostoc mesenteroides* can be used as starter microorganism for fermentation of vegetables and it can improve the quality of fermented vegetables **(Jung *et al*., 2012)**. Therefore, selected lactic acid bacteria isolated from Myanmar fermented food could be considered to be applied not only for probiotic uses but also for food fermentation.

Table 2 Physiological, morphological, biochemical, molecular identification and sources of bacterial isolates

Bacterial isolates	Growth on 3% Nacl	Growth on 5% NaCl	Growth on 7% NaCl	Growth at 4°C	Growth at 37°C	Growth at 45°C	Motility	Catalase	Gram reaction	Microscopic morphology	Sequencing and identification	Source
DBR-F4	+	+	–	+	+	–	Non motile	negative	positive	rod	*Lactobacillus helveticus*	Fermented radish
DBR-F6	+	+	+	+	+	+	Non motile	negative	positive	rod	*Lactobacillus helveticus*	Fermented groundnut oil cake
DBR-F10	+	+	+	+	+	+	Non motile	negative	positive	cocci	*Leuconostoc lactis*	Fermented Green Gram Sprout
DBR-F11	+	+	+	+	+	–	Non motile	negative	positive	cocci	*Leuconostoc lactis*	Fermented radish
DBR-F16	+	+	+	+	+	+	Non motile	negative	positive	rod	*Leuconostoc mesenteroides*	Fermented *Crateva religiosa* leaves
DBR-F25	+	+	+	–	+	–	Non motile	negative	positive	rod	*Lactobacillus helveticus*	Fermented *Capparis flavicans* Wall

(+) means growth and (–) means no growth.

Table 3 Antibiotic susceptibility profiles of bacterial isolates.

Antimicrobial agent	Bacterial isolates					
	DBR-F4	DBR-F6	DBR-F10	DBR- F11	DBR- F16	DBR- F25
Penicillin G (10 units/disc)	R	R	R	R	R	S
Ampicillin (10 µg/disc)	R	S	R	S	S	S
Amoxicillin (30 µg/disc)	S	S	R	R	S	S
Vancomycin (30 µg/disc)	R	R	S	S	R	S
Erythromycin (15 µg/disc)	S	I	R	R	R	R
Tetracycline (30 µg/disc)	I	I	S	S	I	S
Ciprofloxacin (5 µg/disc)	R	R	S	S	R	S
Chloramphenicol (30 µg/disc)	S	S	S	S	S	S
Clindamycin (2 µg/disc)	S	I	S	R	I	R
Oxacillin (1 µg/disc)	R	R	R	R	R	R

R, resistant; S, Susceptible; I, Intermediate. The breakpoints for the antibiotic sensitivity/resistant in mm zone of inhibition: Ampcillin and Amoxicillin (≥17/≤16); Vanomycin (≥17/≤14); Erythromycin(≥23/≤13); Tetracycline and Clindamycin (≥19/≤14); Ciprofloxacin (≥21/≤15); Chloramphenicol (≥18/≤12); and Oxacillin (≥18/≤17).

Antibiotic sensitivity of isolated bacteria

The bacterial isolates were tested for antibiotic susceptibility using 10 different antibiotic discs acquired from Hi Media in India (Table 3). All bacterial isolates were susceptible or moderately sensitive to tetracycline and chloramphenicol, according to the results of an antibiotic susceptibility test. Only one bacterial strain was sensitive to penicillin, and all were resistant to oxacillin. Five to seven antibiotics out of ten tested were effective against bacterial isolates in varying degrees. Actually, antibiotic susceptibility of bacteria may decline over time. Antibiotic resistance in microorganisms can lead to a lack of clinical efficacy and/or safety **(CLSI, 2021)**. As a result, the bacteria that will be used as probiotics should be as antibiotic-resistant as possible.

CONCLUSION

Fermentation of plant-derived materials is an important food source for Myanmar people. Plant-based fermented foods, on the other hand, are still produced on a small scale and in a traditional manner. The majority of the productions are carried out using naturally occurring spontaneous fermentations. Furthermore, back slopping fermentation is thought to be used because previously used containers are reused for new fermentation. Furthermore, their manufacturing processes have not been standardized or industrialized. As a result, the disadvantage of fermented foods is inconsistent and low quality, as well as poor hygiene. It is critical to have quality and safety standards in place for all fermented foods. Even between different geographical regions, the most appropriate processing parameters are required for the finest fermentation regimes. Myanmar fermented foods had a wide range of pH (3.32 0.18 to 4.44 0.11), total titratable acidity (0.57 0.04 to 1.42 0.06 g/100g of sample), total aerobic bacterial count (7 to 9 log CFU/g), and lactobacilli count (6.63 0.03 to 8.72 0.10 log CFU/g) according to laboratory tests. Three *Lactobacillus helveticus*, two *Leuconostoc lactis*, and one *Leuconostoc mesenteroides* isolates were chosen from fermented foods and demonstrated survival under low pH and in the presence of bile salt, but only three isolates showed antibacterial activity against pathogenic bacteria. This research could aid in the understanding of Myanmar fermented food processing and the creation of Myanmar fermented food probiotics.

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