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# DIVERSITY IN THE TRADITIONAL FERMENTED FOODS OF MALAYSIA AND THEIR POTENTIAL AS PROBIOTIC SOURCE

Siti Maryam Ahmad Rumaizi[1, a], Shariza Jamek [1, b *], Norazsida Ramli[2, c], Nik Raihan Nik Him [3, d], Mohd Shafiq Mohd Sueb[4, e] and Zatul Iffah Mohd Arshad[4, f]

[1]*Faculty of Industrial Science & Technology, Universiti Malaysia Pahang, Lebuhaya Tun Razak, 26300, Gambang, Kuantan, Pahang*

[2]*Kuliyyah of Allied Health Sciences, International Islamic University Malaysia, 26300, Kuantan, Pahang*

[3]*School of Chemical Engineering, College of Engineering, Universiti Teknologi MARA, Jalan Raja Muda Abdul Aziz, 40450, Shah Alam, Selangor*

[4]*Faculty of Chemical & Process Engineering Technology, Universiti Malaysia Pahang, Lebuhaya Tun Razak, 26300, Gambang, Kuantan, Pahang*

*Corresponding Author: sharizaj@ump.edu.my

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**Abstract**

Fermentation is one of the oldest methods of food preservation. Over the centuries, it has evolved, improved, and diversified. The microbial population, an important component of fermented foods, aids in the preservation of nutrients and extends the shelf life of food. These microbes have attracted further interest due to their genetic similarity to probiotic strains. Probiotics are live microorganisms that, when consumed in sufficient numbers, promote the host's health. Although lactic acid bacteria (LAB) from various genera, such as *Lactobacillus*, *Streptococcus*, and *Leuconostoc*, are the most prevalent in fermented foods, other bacteria, yeast, and fungi also contribute to food fermentations. Local diets have traditionally featured a range of indigenous fermented foods containing probiotic microorganisms. Besides, there is growing interest in commercialising Malaysian-traditional fermented foods as natural prebiotic foods rich in health-promoting bacteria. Probiotic products, such as fermented foods, are becoming more popular due to their health benefits and consumer accessibility. This article aims to provide an overview of recent studies on the potential source of probiotics from Malaysian traditional fermented foods to understand their importance in healthy diets better.

## INTRODUCTION

Fermentation continues to be a viable food processing technique all over the world. This might be attributed to the easiness and simplicity of the process and its numerous other benefits, including providing variety in foods, improving palatability and aesthetic value, detoxification and imparting desirable sensorial properties. Furthermore, it plays a significant role in conferring health promotion and functional benefits to fermented foods [1]. Fermentation is a traditional technique that can be used to preserve food (together with drying and salting), as well as to modify the quality of food and enhance culinary satisfaction (owing to the distinct flavours, aromas and textures of fermented foods). It is the primary strategy for food production in some cultures due to its low cost and low energy consumption [2-3]. Recently, fermentation has been addressed as a viable method for optimising the usage of bio-resources to address the ongoing global food crisis [4].

Moreover, fermentation significantly contributes to the nutritional and functional benefits of fermented foods [1,5]. Fermented foods, whether traditional or innovative, providing not only better nutrition to the general population, but also health functionalities to specific groups of consumers, such as vegans, lactose-intolerant, or cholesterol-restricted diets [3]. The health-promoting benefits of these fermented foods have recently piqued the scientific curiosity of researchers due to consumer awareness of diet-disease links. These fermented foods benefit human health in ways that are not directly related to the starting food materials. [6]. The benefits of fermented foods in terms of food security include a longer shelf life, the removal of harmful or unwanted ingredients from raw materials, and the creation of a safer product [3, 5, 7, 8]. Because of these beneficial effects, fermented foods and drinks have been a staple of the human diet since ancient times, and they continue to be so in many developing nations, where they are an integral part of local cultures and traditions [5]. Moreover, the potential health benefits of fermented foods are significant that several researchers have proposed including them in dietary guidelines [9,41].

## ETHNIC FOOD

Traditional fermented foods that has been existed through generation in every ethnic group is influenced by the cultural tradition and the local food sources in the specific region. Traditional ethnic food is defined as the ethnic group's or a country's cuisine that is originated from a heritage and their culture, which is prepared based on their knowledge on the local ingredients of plants and/or animal sources [10]. People from various countries are eager to introduce their ethnic food to people from around the world. This usually begins with preparing the food and ends with eating it. Malay or *Melayu* is an ethnic that lives in Southeast Asia region. Figure 1 shows the location where Malay or *Melayu* ethnic settle. In the modern days, Malays residents are divided into two countries which are Indonesia and Malaysia. Indonesia and Malaysia share similar ethnic food due to the historic migration of Malays [11].


**Figure 1.** The map shows the location of Malay or *Melayu* ethnic settlement, marked with purple color in Malaysia and Indonesia regions [12]

## MALAYSIAN TRADITIONAL FERMENTED FOODS

Malaysia has a variety of traditional fermented foods and condiments, which have been produced from different raw materials such as meat, fish, fruits, vegetables, and cereals. Fermented foods are either animal-based or plant- based depending on the starting raw materials [13-14]. There are abundant and diverse fermented foods produced from both plant-based raw commodities such as soybeans to produce *tempeh* [15], durian to produce *tempoyak* or *asam durian* [16-26], glutinous rice, cooked rice or cassava to produce *tapai* [15, 18, 27-28], vegetable to produce *jeruk maman* [15], and chili to produce *chili boh*, which is chili puree. Animal-based fermented foods are salted shrimp to produce *belacan* [27], fish to produce *pekasam* [17, 29], fish sauce to produce *budu* [15, 30, 31], and small shrimps to produce *cincalok* [15].

**Malaysian Plant-Based Fermented Foods**

Plant-based fermented foods including fermented cereals and legumes, vegetables and fruits are rapidly growing due to several factors including the low cost of raw materials, diversity of the raw materials, and the claims of health benefits. In addition, the high demands from vegetarians and vegans have led to the increase in the innovation for plant-based fermented foods [32]. Plant-based fermented foods and beverages are regularly consumed in Asia with less frequent in the Western countries where milk-based fermented foods are dominant [33]. However, the increased awareness regarding their health benefits alongside the recent Covid-19 pandemic has led to an increase in the demand for plant-based fermented foods and beverages.

There is abundant plant-based fermented foods in Malaysia. However, fermented durian flesh (*tempoyak*) was the most studied as *tempoyak* is one of the most popular condiments and valuable food heritage products in both Malaysia and Indonesia, which is usually served as a condiment with local herbs [2, 19]. The fermentation process of *tempoyak* is made by the addition of salt into the overripe durian. To produce an acceptable product, durian pulp should be fermented at room temperature. Fermentation at 40 °C was not so favourable for the growth of LAB leaving high residual sucrose after day 4. Also, a long fermentation period at high temperature produced a product that had undesirable aroma and color making it unfit for consumption. Therefore, the best condition for the fermentation of *tempoyak* is at 27 °C, and it is ready to be consumed between days 4 and 6 when the acidity and sugar content had begun to stabilize [2]. Because the fermentation of *tempoyak* is commonly done spontaneously, the microflora that exist in *tempoyak* from some regions in Southeast Asia is diverse as well. However, it relies on natural lactic acid fermentation, by salt addition into the durian pulp, which might inhibit the growth of pathogenic microorganisms and promote the growth of LAB. Previous studies showed that lactic acid bacteria were the predominant microorganisms in *tempoyak*, which would explain its overpowering odour and distinctive flavour upon fermentation where *Lactobacillus plantarum* was known to be the predominant member of the LAB flora [11, 22, 26]. The presence of this LAB in *tempoyak* may exert many beneficial effects including their ability to inhibit spoilage and other pathogenic bacteria due to decrement of pH value and production of metabolites [26].

In Malaysia, where rice is the staple diet but lacking in flavour, fermentation of food is very much involved in improving and adding some varieties to the diet of the population. The diversity of food products produced by fermentation involving microorganisms is considerable. One of the popular traditionally fermented foods in Malaysia is saccharified rice or popularly known as *tapai*, which is consumed without further processing as a dessert or snack. *Tapai* is made from three ingredients, steamed glutinous rice or tapioca, sugar, and a dry starter culture called "*ragi*" (yeast) [33]. This fermented product is a partially liquefied yet cohesive mass, having a sweet acid and mildly alcoholic taste [34]. Besides, fermented soybeans (*tempeh*) is not only popular in Malaysia but also already commercialized in global market.

**Malaysian Animal-Based Fermented Foods**

Fermented fish and fish sauce prepared via spontaneous fermentation process are well known in Asian countries including China, Japan, Malaysia, Indonesia, Thailand, and Philippines. The fermentation is not only to preserve the fish but also to ensure the absence of pathogenic microbes. *Belacan* is a shrimp paste that is spontaneously fermented in the presence of salt and the natural microflora of shrimps. *Budu* is a fermented fish sauce, a traditional seasoning made by mixing anchovies with salt at a ratio of 3:1. It has been consumed as a condiment or flavouring additive in certain food in Southeast and East Asia [30]. *Budu* is known with many names in Asian countries including *nampla* in Thailand, *patis* in Philippines, *terasi* in Indonesia, *teuk trei* in Cambodia, and *nước mˇaˊ ắm* in Vietnam. Aquatic-based fermented foods are prepared based on spontaneous fermentation due to the presence of broad range of microflora on their surface. Besides, Malaysian fermented fish, also known as *pekasam* is also one of the popular Malaysian traditional fermented foods. It is usually made from freshwater fish with ground-roasted uncooked rice. *Pekasam* is widely consumed in Peninsular Malaysia and used as an additive to improve the taste of foods.

## FERMENTED FOODS AS PROBIOTIC SOURCES

Fermentation is a biological technique of food preservation, where the presence of antimicrobial end-products such as organic acids, ethanol, and bacteriocins reduces the chance of contamination by pathogenic microbes [6]. Apart from antimicrobial end-products, fermented foods may contain probiotics, prebiotics, or both [35]. The concept of emerging 'biotics,' such as probiotics, prebiotics, synbiotics, postbiotics, oncobiotics, paraprobiotics, pharmabiotics, and psychobiotics, promoting optimal health and assisting in disease prevention, has garnered considerable attention in recent years. Probiotics are defined as live microorganisms that when consumed in sufficient numbers, promotes the host's health, whereas prebiotics is defined as non-digestible food ingredients that benefit the host by stimulating the growth and/or activity of the host's gut bacteria, thereby improving the host's health [36-37]. Fermented foods and probiotics are closely related and coexist and are gaining significant attention in a variety of fields of microbial biotechnology, including health improvement and therapeutic purposes [37-38]. Moreover, when probiotics are consumed in the form of fermented foods rather than as probiotics alone, their efficacy is increased [39]. Probiotics are usually defined as microbial food supplements with beneficial effects on consumers. Most probiotics fall into the group of organisms known as lactic acid bacteria (LAB) and are normally consumed in the form of yogurt, fermented milk, or other fermented foods [40].

There are a lot of benefits of probiotics as beneficial bacteria that include maintaining intestinal micro-flora balance, promote good digestion, increase resistance to infection, and boost immune functions [40]. Besides, probiotics encourage healthy digestion and enhancing immune function [16, 41]. Additionally, the consumption of fermented foods and probiotics may have a modulatory influence on the brain and central nervous system [7].

**Lactic Acid Bacteria as Probiotic Agents**

Lactic acid bacteria (LAB), the bacterial group to which most probiotic cultures belong, convert fermentable sugars into lactic acid, ethanol, and other metabolites, thus lowering the pH and creating unfavourable conditions for the growth of potentially pathogenic microorganisms [40]. Since LAB exert many potential health and nutritional benefits, they are primarily used by the dairy industries and are essential element in the preservation and production of other fermented foods. Species of LAB and other conserved microorganisms would vary depending on the location of which the fermented product is made [26, 42]. In particular, the *Lactobacillus* species are one of the most widely used groups of bacteria used as probiotics. *Lactobacilli* are found in the gastrointestinal tract (GIT) of humans and animals, fermented animal and plant products, and most of the commercially available fermented foods [23]. Therefore, LAB are thought to be safe bacteria that have been ingested from foods without any problems for many years and are known as GRAS (Generally Recognized As Safe) bacteria that are important for animal health [43].

**Properties and Proposed Functions of Probiotic LAB**

The selection criteria for probiotic LAB include the ability to survive in the GIT environment such as gastric and bile acid conditions, ability to hydrolyse bile salts, resistance to antibiotics, and adhesion to gastrointestinal epithelial lining [43-44]. In addition, probiotics should also have desirable antibiotic susceptibility patterns and antagonistic to inhibit the enteric pathogens enzyme by decreasing the pH value [43, 45-46]. Antagonistic activity and production of antimicrobial compounds are very important probiotic characteristic that are needed to inhibit the growth of spoilage and other pathogenic bacteria as well as increase the shelf life of the fermented foods. The antimicrobial activity of probiotic bacteria is mainly through the production of antimicrobial compounds such as organic acids (i.e., lactic acid, acetic acids, butyric acid, propionic acid, sorbic acid, benzoic-acids, and etc.), hydrogen peroxide ($H_2O_2$), carbon dioxide ($CO_2$), diacetyl, and proteinaceous-compounds [23, 26, 33, 36, 43, 47-49]. However, some strains are able to synthesize antimicrobial substances like bacteriocins. Bacteriocins are one of the important compounds produced by probiotics. Bacteriocins are ribosomally synthesized antimicrobial peptides, which provide a promising technological alternative for food bio preservation, as they can avoid the growth of spoilage and pathogenic microorganisms [50]. The use of bacteriocin-producing bacteria is significantly more effective to improve human gut health as antimicrobial peptides produced by probiotic strains in the intestine can interrelate directly with the sensitive pathogenic organisms in the intestine. Due to their antimicrobial and antioxidant activities, some LAB strains are used in bio-preservation. Furthermore, probiotic strains need to have some functional attributes such as antioxidative effects and be able to decrease toxicity levels through degradation of cyanogens [51]. Other physiological benefits of probiotics include anticarcinogenic from the intestinal tract, immunomodulation of host immune system by reducing allergic reactions and lactose intolerance, as well as enhanced nutrient bioavailability in hosts [43, 46]. Besides bacteriocins, exopolysaccharides (EPS) which are produced by probiotics (LAB) also providing health benefits to the host where it offers protection against the harsh conditions of the gastrointestinal. EPSs may also induce positive physiological responses including cholesterol lowering and reduced formation of pathogenic biofilms modulation of adhesion to epithelial cells [52]. EPS are widely used in the food industry as viscosifying, stabilizing, gelling, or emulsifying agents due to their physical and rheological properties. EPS may also play a role in biofilm formation. The summary of metabolites produced by probiotic strains are tabulated in Table 1. Overall, the proposed mechanisms of the actions of probiotics are summarized in Figure 2.

**Table 1.** Metabolites produced by probiotic strains

| Substances | Related function in health system | Potential industrial application |
|---|---|---|
| Bacteriocins | Improve human gut health | • Natural bio-preservatives<br>• Preventing the growth of spoilage pathogens |
| Exopolysaccharides | Cholesterol lowering, reduced formation of pathogenic biofilms modulation of adhesion to epithelial cells | • Viscosifying, stabilizing, gelling, or emulsifying agents in food industry<br>• Biofilm formation |
| Organic acids (lactic acid, acetic acids, butyric acid, propionic acid, sorbic acid, benzoic acids) | Antimicrobial compounds | Replace functions of chemical preservatives |

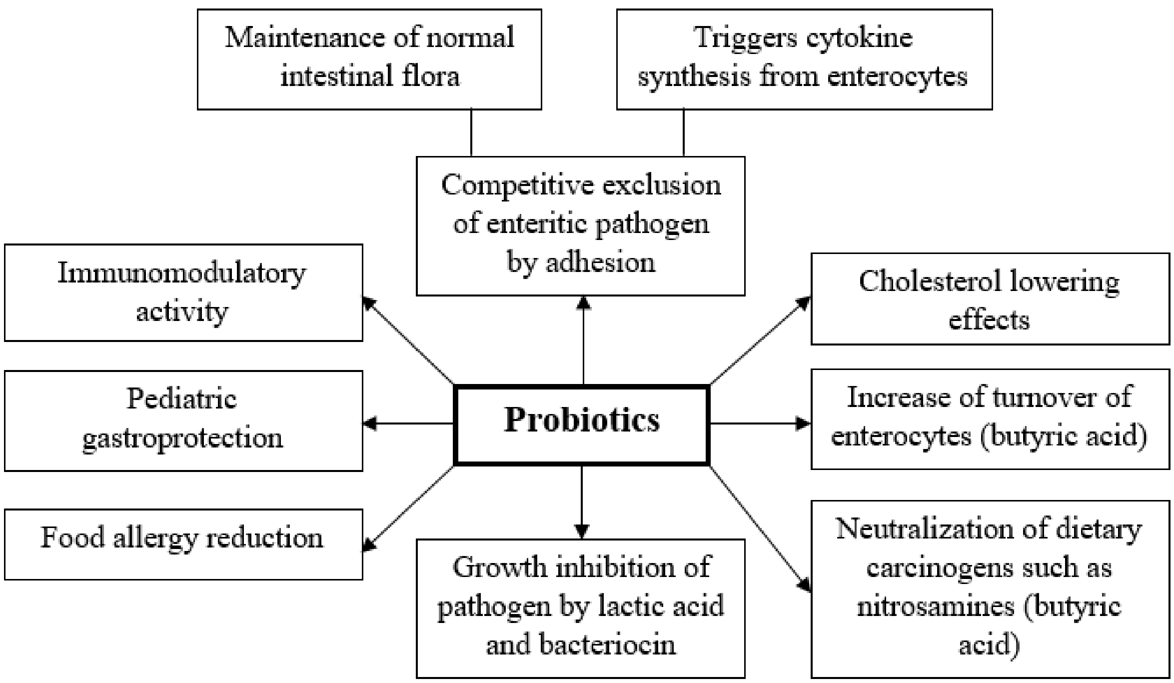


**Figure 2.** Purposed mechanism of action of probiotics [43]

## POTENTIAL PROBIOTIC STRAINS ISOLATED FROM MALAYSIAN TRADITIONAL FERMENTED FOODS

Fermentation improves the taste and aroma of animal-based and plant-based raw materials. In addition, fermented foods have long been thought to provide health benefits to the consumer. Health-promoting components with antimicrobial activity, cholesterol reduction, antioxidant, inhibitory activity, anti-mutagenic, antioxidant, immunostimulatory activity, and antibiotic susceptibility can be derived from both fermented animal-based and plant-based products. Examples of Malaysian animal-based and plant-based fermented foods with the associated probiotic microorganisms, which contribute to the many health benefits are highlighted in Table 2 and Table 3. Among the Malaysia-traditional fermented foods, *tempoyak* is the most studied and various LAB found in *tempoyak* made by spontaneous fermentation from various regions in Southeast Asia such as Indonesia and Malaysia which are described in Table 3.

Research from [26] showed that there were nine acid producing bacteria and were considered as presumptive *Lactobacillus* species isolates based on the main characteristics of LAB, which were gram-positive *bacilli*, non-motile, facultative anaerobes, and catalase negative from *tempoyak*.

**Table 2.** Malaysian animal-based fermented foods, their health benefits, substrates, and the associated probiotic bacteria

| Substrates | Fermented foods | Health benefits | Associated probiotics | References |
|---|---|---|---|---|
| Fish | *Budu* | Antimicrobial activity in vitro | *Bacillus subtilis* | [15] |
| Small Shrimps | *cincalok* | | *Bacillus amyloliquefaciens* | |
| Shrimp | *belacan* | Probiotic ability and antimicrobial activity in vitro | *Weissella confuse* and *Lactobacillus fermentum* | [27] |
| Fish | *Budu* | Probiotic ability and antimicrobial activity in vitro | *Staphylococcus simulans* | [30] |
| Fish | P*ekasam* | Probiotic ability | LAB - unspecified cultures | [17] |
| Fish | *Pekasam* | Antibiotic susceptibility, antimicrobial activity | *Lactobacillus plantarum, Lactobacillus pentosus* | [29] |

**Table 3.** Malaysian plant-based fermented foods, their health benefits, substrates, and the associated probiotic bacteria

| Substrates | Fermented foods | Health benefits | Associated probiotics | References |
|---|---|---|---|---|
| Durian Flesh | *Tempoyak* | Probiotic ability and antimicrobial activity in vitro | *Lactobacillus buchneri, Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus acidophilus* | [22] |
| Durian Flesh | *Tempoyak* | Probiotic ability and antimicrobial activity in vitro | LAB - unspecified cultures | [20] |
| Durian Flesh | *Tempoyak* | Bio preservative or anti-microbial agent into edible film packaging material | LAB - unspecified cultures | [19] |
| Durian Flesh | *Tempoyak* | Probiotic ability | LAB *plantarum, Lactobacillus brevis, Lactobacillus pentosus, Lactobacillus fermentum, Lactobacillus lactis, Lactobacillus raffinolactis, Lactobacillus casei,and Leuconostoc mesenteroides.* | [24] |
| Durian Flesh | *Tempoyak* | Probiotic ability, bile salts tolerance, and antimicrobial activity | LAB - unspecified cultures | [20] |
| Durian Flesh | *Tempoyak* | Antimicrobial Activities and Antibiotic Sensitivity | LAB - unspecified cultures | [26] |
| Durian Flesh | *Tempoyak* | Cholesterol reduction, antioxidant, inhibitory activity, and antimicrobial activity in vitro | *Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus crispatus, Lactobacillus reuteri and Lactobacillus pentosus* | [23] |

| | | | | |
|---|---|---|---|---|
| Durian Flesh | *Tempoyak* | Anti-mutagenic | *Lactobacillus plantarum* | [16] |
| Durian Flesh | *Tempoyak* | Acid and bile salts tolerance, anti- oxidative, antiproliferative effects and antimicrobial activity | *Lactobacillus plantarum* | [16] |
| Durian Flesh | *Tempoyak* | Immunostimulatory activity | LAB - unspecified cultures | [25] |
| Durian Flesh | *Tempoyak* | Acidification capability, antibiotic susceptibility, resistance to phenol, bile salts tolerance,,and antimicrobial activity in vitro | *Pediococcus acidilactici TN1* | [18] |
| Rice | *tapai nasi* | | *Lactobacillus farciminis TY1* | |
| Soybeans | *Tempeh* | Antimicrobial activity | *Bacillus subtilis* | [15] |
| Cassava | *Tapai ubi kayu* | | *Bacillus amyloliquefaciens* | |
| Cassava | *Tapai ubi kayu* | Probiotic ability and antimicrobial activity in vitro | *Pediococcus pentosaceus* | [27] |
| Glutinous Rice | *Tapai pulut* | | *Enterococcus faecium* | |
| Milk | *Kefir* | Probiotic ability | Yeast*: Saccharomyces boulardii, Saccharomyces cerevisiae, Saccharomycetales, Kazachstania unispora* and *Kodamaea ohmeri* | [55] |
| Vegetable | *Jeruk maman* | Probiotic ability | LAB - unspecified cultures | [17] |
| Glutinous Rice | *Tapai pulut* | | | |
| Durian Flesh | *Tempoyak* | | | |

## Antibiotic Susceptibility of Isolated LAB from Malaysian Traditional Fermented Foods

Antibiotics are normally used as drugs to treat bacterial infection prescribed by a medical officer or added as food additives to control microbial spoilage in food product [17]. Any LAB strains need to undergo antibiotic resistance assay before they can be consumed as probiotics. Antibiotic susceptibility was mostly reported from animal-based fermented foods [17, 29]. A study by [29] identified two probionts, the *Lactobacillus plantarum* strain L8 and *Lactobacillus plantarum* strain L20 extracted from *pekasam*, were susceptible to seven antibiotics tested except vancomycin and tetracycline while LAB isolate (S1) was resistant to all antibiotics. The study showed that the probiotics of a strain isolated *pekasam* exhibited the potential probiotic properties to be developed as biotherapeutic agents. Another study by [17] investigated the isolated LAB from *pekasam*, *jeruk maman*, *tapai pulut* and *tempoyak* were antibiotic susceptibility towards penicillin, ampicillin, kanamycin, vancomycin, streptomycin, tetracylin, chloramphenicol, and rifampicin. All twenty LAB strains were resistant to vancomycin and showed different degrees of susceptibility against various antibiotics used. It can be concluded that the results obtained from this study could contribute to the potential use of isolated LAB as a probiotic in the food and pharmaceutical industries. Another study of antibiotic susceptibility in *tempoyak* was also found in research from [26] where it stated that LAB was sensitive to erythromycin and chloramphenicol. All isolates from *tempoyak* were susceptible to β-Iactamase inhibitors, tetracycline, and bacitracin. Besides, LAB strains which are *Pediococcus acidilactici* TN1 (from *tapai nasi*) and *Lactobacillus farciminis* TY1 (from *tempoyak*) showed intrinsic mechanisms of antibiotic resistance towards streptomycin, norfloxacin, erythromycin, amikacin, and nalidixic acid [18].

## Antimicrobial Activity of LAB Isolated from Malaysian Traditional Fermented Foods

LAB is known to produce antimicrobial substances such as metabolites and organic acids [26]. Some organic acids are generally thought to exert their antimicrobial effect by interfering with the maintenance of cell membrane potential, inhibiting active transport, reducing pH and inhibiting a variety of metabolic functions. The developed acidity, low pH and complete sugar removal are the determining factors for the success and safety of the fermentation product [28]. Mostly, supernatants of LAB such as *Lactobacillus casei* and *Lactobacillus rahmnosus* include several antimicrobial components in the forms of organic acids, hydrogen peroxide, aroma components, fatty acids and low molecular-weight compounds that can destroy pathogenic bacteria [16, 23]. Antibacterial compounds produced by lactic acid bacteria are believed to replace functions of chemical preservatives [22].

A few researchers have heavily focused on microbiological aspects in fermented durian pulp. A study from [26] revealed that the *Lactobacillus sp.* isolated from *tempoyak* were able to inhibit most of the pathogenic bacteria and spoilage bacteria with a varied zone of inhibition except for *Staphylococcus aureus* which showed no inhibition zone against all nine isolates. It is noted that *Lactobacillus* strains would produce organic acids such as lactic, acetic, propionic, butyric, caproic, and isobutyric acid to against *Escherichia coli* as the pathogen indicator in *tempoyak*. A recent study by [23] showed the antimicrobial activity of *Lactobacillus plantarum* from *tempoyak* against indicator bacteria such as *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923). In whole culture and cell-free supernatant form, *Lactobacillus plantarum* performed significant inhibition of pathogenic bacteria in antimicrobial assay [16]. Meanwhile, there is no inhibition observed when using *Lactobacillus plantarum* in bacterial cells form. The result indicated that the substances, which are responsible for antimicrobial activities of the strains are bacterial metabolites, which are the organic acids. A similar study about the inhibition capability of unspecified LAB cultures from *tempoyak* against *Escherichia coli* (ATCC 25923) and *Staphylococcus aureus* has also been done [20]. The result indicated that LAB from *tempoyak* has the potential to be widely used in the food industry as bio preservatives due to its antimicrobial activity. Furthermore, research from [24] revealed that ten of the isolated bacteria have been recognized and identified as LAB using biochemical characterization from *tempoyak*. Three of the LAB isolated were confirmed as *Lactobacillus plantarum*, which showed the highest frequency of occurrence in *tempoyak*. Other species that are successfully recognized were *Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus pentosus, Lactobacillus paracasei, Lactobacillus raffinolactis, Lactobacillus fermentum, Lactobacillus lactis* and *Leuconostoc mesenteroides*. During fermentation of the *tempoyak*, lactic acid produced by LAB resulted in lowering of the pH, which made the conditions inhospitable for many pathogenic microbes and inhibit their growth.

Another study compared the inhibitory capability of LAB strain from *tempoyak*, which has the gram-positive and catalase-negative *Bacilli* characteristics against *Listeria monocytogenes*, *Escherichia coli* O157, and *Staphylococcus aureus*. The results showed that LAB from *tempoyak* was more effective to inhibit *Staphylococcus aureus* (19.3 mm of the inhibitory zone), followed by *Listeria monocytogenes* (17.3 mm) and *Escherichia coli* O157 (12.3 mm) [56]. The sequencing results from the isolated *tempoyak* showed that the LAB isolate was *Lactobacillus fermentum* strain CAU6337. The food industry can use these LAB for producing functional food products like fermented milk and others. In addition, more study is being performed into the possible use of *tempoyak* LAB strains as a bio preservative or antimicrobial agent, as well as the incorporation of *tempoyak* LAB into edible film packaging material [19]. The study concluded that the LAB affected the moisture content, pH, time soluble, as well as the total number of LAB from edible film of whey. However, further observation should be done to improve the physical and chemical properties of probiotic's packaging. One of the types of packaging that is environmentally friendly packaging is edible packaging.

LAB can be a potential source for antibacterial agent such as bacteriocins, which has potential to be used as natural bio-preservatives and in preventing the growth of spoilage pathogens in various food products. This can be seen from a study by [57], which showed that 15 LAB strains were isolated from traditional fermented foods namely *tempeh*, *tempoyak*, *tapai ubi* and *tapai nasi*. Out of 15 LAB strains, 2 of them which were identified as *Pediococcus acidilactici* TN1 (from *tapai nasi*) and *Lactobacillus farciminis* TY1 (from *tempoyak*), were able to produce bacteriocin-like inhibitory substances (organic acids, $H_2O_2$, bacteriocins) that were important to inhibit the growth of the food-borne pathogen, *Listeria monocytogenes* ATCC 13932. Even though this study had generated extensive information to validate *Pediococcus acidilactici* TN1 and *Lactobacillus farciminis* TY1 as potential probiotic strains for application in the food industry, the study is no means comprehensive nor complete. More laboratory, particularly in vivo studies are needed before this product could be accepted by the food industry and most importantly to explore its novel health-promoting functions as well as its colonization behaviour in the gut.

Study from [21] showed that there were eight isolates of LAB from *tempoyak* identified with negative catalase and gram-positive bacteria. All LAB isolates from *tempoyak* had inhibitory activity to *Escherichia coli*. The isolate P1 and P3 had the inhibitory zones diameters of 17.35 mm and 16.08 mm, respectively which are interpreted as sensitive, capable to survive in the intestine tract condition at the pH of 2, 4, and 6 and bile salt 0.5%. Besides, the study also concluded that LAB isolates from *tempoyak* have the potential as probiotics for chickens because they inhibit the growth of pathogenic bacteria and can survive in gastrointestinal conditions. The use of antibiotics as a growth promoter for chickens may develop resistance to pathogenic bacteria in the digestive tract of chickens. Other negative impacts on food safety in poultry industry came from antibiotic residues. One alternative that can be used as a substitute for antibiotic is probiotic. Probiotics derived from LAB are safe besides that they are potential to improve chicken growth and health.

LAB derived from fermented foods are also able to inhibit various types of food poisoning bacteria. Food contamination with pathogenic bacteria has become a global concern since it can result in severe hazards to human health. The major causative factor behind food poisoning outbreaks in Malaysia is the hot and humid weather that promotes the growth of pathogenic bacteria. Study from [22] has successfully identified eight isolates of LAB strains, which included *Lactobacillus plantarum*, *Lactobacillus acidophilus* I, *Lactobacillus brevis* and *Lactobacillus buchneri* with similarities of 95–99.9% through phenotypic characterization using API- 50 CHL Kit. Mass spectrum identification using MALDI-TOF/MS biotyper has successfully identified five species of *Lactobacillus buchneri*, including *Lactobacillus buchneri* DSM 20057T, *Lactobacillus paracasei* DSM 2649, *Lactobacillus parabuchneri* DSM 57069, *Lactobacillus paracasei* DSM 20020, and *Lactobacillus farciminis* CIP 103136T. Four LAB strains of *Lactobacillus plantarum*, *Lactobacillus buchneri*, *Lactobacillus brevis* I and *Lactobacillus acidophilus* were able to inhibit various types of *Salmonella* food poisoning and other non-*Salmonella* foodborne pathogens. Crude extracts of LAB produced two major compounds of benzoic and sorbic acids, including potentials to be developed as chemical preservative agents.

The particular interests are due to global outbreaks associated to *Vibrio cholera* and *Escherichia coli* O157, causing tremendously high numbers of fatalities worldwide. Research from [22] revealed that cell-free supernatant extracted from *Lactobacillus plantarum, Lactobacillus buchneri, Lactobacillus brevis I, and Lactobacillus acidophilus I* from *tempoyak* included strong inhibitory effects against *Vibrio cholera* O1 (Inaba type), *Vibrio cholera* O139 (Bengal type), *Vibrio parahaemolyticus* ATCC 17802, *Escherichia coli* ATCC 11795, *Escherichia coli* O157, *Salmonella typhimurium* ATCC 14028, and a total of 23 serotypes of *Salmonella spp.* associated with outbreaks of food poisoning from raw chicken, egg shell, and water samples. However, only *Lactobacillus buchneri* DSM 20057T strain can potentially be used as a food preservative to decrease the growth of foodborne pathogenic bacteria as this strain produce sorbic and benzoic acids.

Moreover, there are also a few studies that heavily focused on microbiological aspects in animal-based fermented food [27, 29-30]. Study by [29] found that three probionts, *Lactobacillus plantarum* strain L8, *Lactobacillus plantarum* strain L20, and *Lactobacillus pentosus* strain S1 extracted from *pekasam* possessed probiotic potential where they showed broad antimicrobial effects towards the pathogenic bacteria: *Esherichia coli, Staphylococcus aureus and Klebsiella sp*. All of the isolates were also γ-hemolytic and tolerant to various pH (pH 3, 5 and 7.5) and 0.3% (w/v) bile salts. While another study by [30] showed that *Staphylococcus simulans* PMRS35 isolated from *budu* have strong anti- microbial activity against foodborne pathogens, particularly *Staphylococcus aureus*. Besides, research from [58] indicated that thirty-three LAB samples were isolated from *tapai ubi*, *tapai pulut* and *belacan*. The phenotypic identifications indicated that 5 LAB isolates (PG, PH, BG, UG and UL) showed typical properties of LAB with the ability to antagonize selected pathogens (*Bacillus subtilis, Escherichia coli, Salmonella typhimurium*, and *Staphylococcus aureus*). Antimicrobial activities studies showed varying degrees of inhibition against pathogenic strains and most of these isolates showed broad-spectrum inhibition against both Gram positive and negative indicator strains. Based on 16S rRNA sequencing analysis, with more than 98% similarity; isolates PG and PH from *tapai pulut* belonged to *Pediococcus pentosaceus*, isolate from *belacan* BG belonged to *Enterococcus faecium*, and those from fermented shrimp, UG and UL belonged to *Weissella confusa* (99%), and *Lactobacillus fermentum* (98%), respectively. Isolate PH are the most potent isolates which is producing antimicrobial agent with potential as food preservatives as PH exhibited the highest antibacterial activity against *Bacillus subtilis*.

Bio-surfactants, or microbial surfactants, can be described as amphipathic molecules that have hydrophilic as well as hydrophobic moieties. Bio-surfactants or microbial surfactants are structurally diverse and contribute to reducing surface and interfacial tension. Apart from being isolated from municipal waste and oil-contaminated soil, bio surfactant-producing *Bacillus spp*. can also be found in fermented foods. *Bacillus subtilis* produce surfactants, have antimicrobial potential against food-borne pathogens and for advancing food safety. This can be proved by a study from [15], where the research indicates that surfactant-producing *Bacillus* was isolated from traditional fermented foods namely *budu*, and *tempeh* are *Bacillus subtilis* while strains from *cincalok* and *tapai ubi kayu* are *Bacillus amyloliquefaciens* with a sequence similarity of 99%. Surfactants produced by the isolated strains exhibited antibacterial activity toward pathogenic bacteria, namely *Bacillus cereus, Listeria monocyto*genes, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Salmonella Typhimurium, Serratia marcescens*, and *Klebsiella pneumoniae.* List of pathogenic organisms that are derived from Malaysian-traditional micros and have demonstrated antimicrobial effects are summarized in Table 4.

**Table 4**. List of pathogenic organisms that Malaysian-traditional fermented foods have demonstrated antimicrobial effects against

| Fermented foods | Microbial species | References |
|---|---|---|
| *Tempoyak* | *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) | [23] |
| *Tempoyak* | *Escherichia coli* (ATCC 25923), *Staphylococcus aureus* | [20] |
| *Tempoyak* | *Listeria monocytogenes*, *Escherichia coli* O157, *Staphylococcus aureus* | [56] |
| *Tempeh*, *tempoyak*, *tapai ubi kayu* and *tapai nasi* | *Listeria monocytogenes* ATCC 13932 | [57] |
| A*sam durian* | *Escherichia coli* | [21] |
| *Tempoyak* | *Vibrio cholera* O1 (Inaba type), *Vibrio cholera* O139 (Bengal type), *Vibrio parahaemolyticus* ATCC 17802, *Escherichia coli* ATCC 11795, *Escherichia coli* O157, *Salmonella typhimurium* ATCC 14028, *Salmonella spp.* | [22] |
| P*ekasam* | *Esherichia coli, Staphylococcus aureus, Klebsiella sp*. | [29] |
| *Budu* | *Staphylococcus aureus* | [30] |
| T*apai ubi*, *tapai pulut* and *belacan* | *Bacillus subtilis, Escherichia coli, Salmonella typhimurium*, *Staphylococcus aureus* | [58] |
| *Budu*, *tempeh*, *cincalok*, *tapai ubi kayu* | *Bacillus cereus, Listeria monocyto*genes, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Salmonella Typhimurium, Serratia marcescens* and *Klebsiella pneumoniae.* | [17] |

## Other Probiotic Properties of LAB Isolated from Malaysian Traditional Fermented Foods

To reach the GIT in a viable form, probiotic strains have to overcome several biological barriers including the presence of lysozyme in the saliva, low pH in gastric juice, and bile salts in the upper GIT [53-54]. Bacteria inhabiting the intestinal tract must have intrinsic resistance mechanisms to cope with bile salts. Thus, they may survive under high acidity in the stomach and high concentration of bile components in the human GIT [57]. A study from [16] was the first to demonstrate probiotic potentials of lactic acid bacterium isolated from *tempoyak*, which are acid, and bile salts tolerance, anti- oxidative, antiproliferative effects, and remarkable adhesion on HT- 29 cells.

Besides, sodium chloride (NaCl) is one of the most extensively used additives in food manufacturing as it has a preservative and antimicrobial effect due to the reduction of water activity values. The results from [18] stated that LAB strains which are *Pediococcus acidilactici* TN1 (from *tapai nasi*) and *Lactobacillus farciminis* TY1 (from *tempoyak*) were able to grow at the wide range of NaCl concentrations

(0.5 - 5.0 %, w/v) and temperatures (28 - 70 ˚C). They also demonstrated a probiotic characteristic such as being able to degrade protein, acidify skim milk, endure 0.3% (w/v) of bile salts, and tolerate to phenol up to 0.5% (w/v).

Other bioactive properties of *tempoyak* are immunostimulatory and anti-mutagenic. Finding from [25] stated that *tempoyak* has immunostimulatory effects towards murine macrophage cell line RAW 264.7. Immunostimulatory properties possessed by *tempoyak* are mainly due to the fermentation process. There is a possibility that during the fermentation process, the bacteria produce specific metabolites that could potentially be responsible for the immunostimulatory properties found in *tempoyak*. However, further studies are needed to identify the specific compounds responsible for inducing immunostimulatory effects. As for the mutagenic properties, *tempoyak* is specifically possessed by *Lactobacillus plantarum* against sodium azide and 2-nitrofluorene. *Lactobacillus plantarum* has the potential to be a chemopreventive agent by binding the mutagens and supressing mutagenesis. Thus, *Lactobacillus plantarum* could be consider as a good candidate for functional food development as a supplement product to prevent development of colon cancer [16].

Moreover, a study about the probiotic properties of Malaysian *tempoyak* has been done [23]. The properties of seven isolates from species *Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus crispatus, Lactobacillus reuteri*, and *Lactobacillus pentosus* were observed. The study showed that the isolates were able to produce EPS and had great potency to exhibit high survivability to acid (pH 3.0), bile salts (0.3%) and in vitro model of gastrointestinal condition. These properties are observed because it mimics the environment in the human GIT as EPS has the prebiotic potential to positively affect the gastrointestinal microbiome. Before reaching the small intestine where the probiotics are living, the strains should have the ability to survive the harsh acidity environment in the stomach. Other properties observed are the antioxidant capacity, capability to reduce cholesterol, and inhibitory activity against pathogens. Generally, the seven *Lactobacillus* strains isolated from *tempoyak* are having the potential to be used as promising probiotics with functional merits since they perform good abilities in all those mentioned properties.

Recently, there is a growing interest in the application of starter microorganism, which demonstrates specific functions such as probiotic activity or reduction of toxic compounds. From study [30], *Staphylococcus simulans* PMRS35 isolated from *budu*, which efficiently produces lipase and protease in a high salted environment, was selected as an autochthonous starter for the development of flavor and aroma of the product. This strain did not exhibit decarboxylase activity. In addition, this strain displayed not only negative results towards pathogenic genes, hemolytic activity, and biofilm formation ability but also sensitivity to conventional antibiotics, which include chloramphenicol, vancomycin, erythromycin, tetracycline, and gentamycin. Most importantly, the isolate maintained its viability towards simulated GIT conditions with the survival rate of more than 80%. These results demonstrated that this species has the potential for use as a starter to improve the quality and the health functionality of fermented foods.

Microflora can be divided into two groups: LAB and non-LAB (including yeast and mould). Fermented foods exist in many cultures, and LAB or yeast is the common starter cultures used in food fermentation aimed at enhancing the texture and flavour of the desired products. *Kefir* milk is one of the excellent sources of probiotic yeast strains and could be used as a new yeast probiotic formulation or in food supplements. The research from [55] involved the isolation and identification of potential probiotic yeast strains in the *kefir* drink samples from Malaysia. The *Saccharomyces* and *Kodamaea* were found to be the major population in the *kefir* drink samples. The yeast strains found could be used as a new source of probiotic formulation such as in tablet or capsule form or can be incorporated in the supplement or functional food.

## COMMERCIALIZATION OF PROBIOTIC BASED FOOD: SYSTEMATIC APPROACH AND ASSESSMENT

The increasing research on science and innovation has shifted consumer awareness from conventional to functional foods that are more nutritious and healthier. For foods to be marketed as a functional, it has to meet certain conditions that include conformation and meeting food safety regulation of a particular country, or even to international country. Among emerging functional foods in the market, probiotics-based foods and beverages are considered as one of the future foods that are more prominent with wider acceptability among consumers, and demand for probiotic-based foods and beverages needs to be streamlined with proper guidelines, systematic approach and assessment of probiotic safety before it reaches to the consumers [59].

The first step for any potential probiotics for food application starts with the screening of microorganisms, which are isolation and identification. The identification of species or strain should be performed by using 16s RNA methods and verified with a combination of phenotypic and genotypic tests. For safety assessment, it is essential to substantiate the efficacy of identified microbes by screening probiotic potential of foods in vitro and potentially in vivo tests with human clinical trials. Normally characteristics such as tolerance to bile acid, gastric juice resistance, adherence to human epithelial cells, ability to reduce pathogen in the gut, antimicrobial activity, bile salt hydrolase activity, and safety assessment are conducted to elucidate the probiotic potential of a given microbe [60]. However, as evidence to certify given probiotics in food application, screening probiotic potential of foods prescribed or recommended by the Food and Agriculture Organization (FAO)/World Health Organization (WHO) working group includes the production of undesirable metabolites and their evaluation, presence of potential antimicrobial resistance factors, possible side effects in human clinical studies, epidemiological post-market assessment of adverse effects in consumers, hemolytic activity, and toxicity [61]. For *in vivo* animal studies, the expert working group encourages the results from in vitro to understand the probiotic mechanism and encourages animal studies to substantiate safety concerns, which are essential to evaluate efficacy. The animal studies' results need to be carefully interpreted, inferring to human physiology, pathology, anatomy, test group, and a range of other factors involved. Meanwhile, human studies in vivo involve the study of the effectiveness of either probiotic strain or such foods by conducting human trials. This study depends on the regulations, the type of probiotic microorganisms used in foods and beverages, and the studied sample population [59].

Labelling of probiotic-based foods and beverages with scientific evidence and facts is important and needs to be verified by regulating body or agency. The uses of the word "probiotic" must be meet specific criteria [61], which are specific claims such as health, nutritional, functional, and those beyond "contains probiotics" must be further substantiated, and the term probiotics are not applied to cultures associated with traditionally fermented foods with no evidence of a health benefit. Besides, the word probiotics can be used for the defined strains from human samples with adequate safety and efficacy evidence, and the microorganism species in the "probiotics" must be included after it has passed necessary clinical studies with beneficial effects on the host [23].

## CONCLUSION AND FUTURE PERSPECTIVES

Malaysian-traditional fermented foods have been produced and consumed since long ago and have become staples in the Malaysian diet. Its popularity remains until now and is seen to be potentially marketed as a natural prebiotic food that has health-promoting bacteria. The diversity of Malaysian traditional fermented foods has led to the discovery of local LAB strains, which showed the highest potential as natural probiotic strains. Future studies are recommended in using the meta-omics approach for profiling the local probiotic strains generated by Malaysian traditional fermented foods. Furthermore, a metabolomics study for the identification a wide range of metabolites including bioactive compounds associated with Malaysian traditional fermented foods could also be done.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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