EFFICACY OF LACTIC ACID BACTERIA FROM EDIBLE FRUITS AGAINST ANTIBIOTICS AND PATHOGENIC BACTERIA

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Abstract

Lactic Acid Bacteria (LAB) were isolated from fresh fruits samples of carambola, pomegranate, guava, papaya, lime, banana and pitahaya by using DeMan Rogos Sharpe (MRS) agar medium in the Microbiology Laboratory, Department of Botany, University of Mandalay during September 2017 to January 2018. The ten bacterial strains, PPTG 1 − 10, were observed and identified based on their colony morphology and biochemical tests. The most number of three LAB strains were observed in papaya with PPTG 4, 5 and 6, followed by two strains in pitayana with PPTG 9 and PPTG 10. The isolated LAB strains PPTG 1, PPTG 5 and PPTG 7 were confirmed as *Lactobacillus* spp.; PPTG 2 and PPTG 8 as *Pediococcus* spp.; and PPTG 3, PPTG 4, PPTG 6, PPTG 9 and PPTG 10 as *Leuconostoc* spp. According to the study on the abilities of antibiotic resistance, *Lactobacillus* sp. (PPTG 1) strain was resistent to ampacillin and amoxicillin; *Leuconostoc* sp. (PPTG 4) strain was resistent to ampacillin, amoxicillin, chloramphenicol and erythromycin; *Lactobacillus* sp. (PPTG 5) strain was resistent to tetracycline, ampacillin, amoxicillin, chloramphenicol and erythromycin; *Leuconostoc* sp. (PPTG 9) strain was resistent to tetracycline, ampacillin and amoxicillin; and *Leuconostoc* sp. (PPTG 10) strain was resistent to ampacillin and amoxicillin. The LABs possessing the antibiotic activities were tested for antimicrobial properties at Biotechnology Research Department, Kyaukse. *Lactobacillus* sp. (PPTG 1) isolated from carambola strain showed maximum zone of inhibition against *Staphylococcus aureus* with 19 mm, a zone of inhibition against *Escherichia coli* with 12 mm, *Bacillus cereus* with 11 mm. The physiological test on five strains showed that they can grow between pH 4.5 and pH 7.2 and salt tolerance was up to 6% NaCl. Therefore, among the study fruits the carambola is the most suitable one to be eaten for the health of human beings. Because of the most LAB strains were observed in papaya, it was also suitable to be consumed in daily life.

Keywords: LABs, edible fruits, antibiotic properties

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Introduction

Microorganisms play an essential role in the food production. The fermentations by Lactic acid bacteria (LAB) were involved for thousands of years in food and are one of the most ancient preservation techniques. The first signs of LAB utilizations date back to 6000BC, describing the fermentation of milk and fermentation of meat 1500BC and vegetable products 300BC. Vegetables and fruits are fundamental sources of water-soluble vitamins (vitamin C and group B vitamins), provitamin A, phytosterols, dietary fibres, minerals and phytochemicals for the human diet. Scientific evidences encouraged the consumption of vegetables and fruits to prevent chronic pathologies such as hypertension, coronary heart diseases and the risk of stroke (Saif 2016).

Lactic acid bacteria (LAB) have been extensively studied for their commercial potential, food preservation and health benefits. Industrial importance of LAB is based on their ability to ferment sugars readily into different metabolites and provide an effective method for preserving fermented food products. These bacteria are genetically diverse group of bacteria encompassing widely recognized genera which include: *Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus* and *Weissella*. Some authors include the genus Bifidobacterium because of its probiotic role (Emerenini *et al*. 2013).

Abubakr & Al-Adiwish (2017) stated that LABs possess antimicrobial activities and Patel *et al.* (2012) studied that antibiotic resistance or drug resistance can be defined as the ability of bacteria. On account of this, the present study was carried out for the partial fulfillment of isolation, characterization and identification of lactic acid bacteria from selected edible fruits and their efficacy against antibiotics, and antimicrobial properties.

LABs are non-pathogenic bacteria, technologically suitable for industrial processes and their capacity to produce antimicrobial compounds makes them beneficial for health. Obtaining genetically stable strains to be

used in probiotic products has been a concern for researchers in the field. Despite their human origin, recent studies have described novel sources for isolating LAB with potential probiotic benefits, such as wild type fruits and fermented vegetables. However, numerous lactobacilli were found to be abundant in the pollen, suggesting their role in suppressing the growth of molds and other spoilage organisms (Benavides *et al*. 2016).

Most probiotic bacteria are LAB, and among them, *Lactobacillus* is the most common genera. Probiotics are non-pathogenic microorganisms, which exert a positive health benefit on the host when ingested in an adequate amount. The majority of the commercialized and most studied probiotics have been isolated from dairy products and human gastrointestinal tract. Although, dairy foods are recognized to be the best vehicle for the delivery of viable probiotics to the human gut, the increasing number of individuals with lactose intolerance, dyslipidemia, and vegetarianism reinforces the importance of the development of non-dairy probiotic products such as fruit juices (Peres *et al*. 2012).

Raw fruit and their byproducts possess intrinsic physicochemical parameters that resemble those of the human gastrointestinal tract for some traits, such as the acidic environment and presence of anti-nutritional factors (tannins and phenols). The natural adaptation to the intrinsic characteristics of fruit may help fruit-originating bacteria to survive during the processing and storage of fruit-based probiotic formulations as well as in the human stomach (Garcia *et al*. 2016). Martins *et al*. (2016) stated that the variety of fruits ready to eat are containing also the probiotics of high functionality. Mozzi *et al*. (2010) informeded that the probiotic potential of lactic acid bacteria and their role in inhibition of pathogenic organisms have opened new horizons in the fields of medical sciences and food biotechnology.

Hnin Ei Phyu (2012) studied the isolation and characterization of *Lactobacillus* species from yogurt. Khine Zin Mar Thein (2014) studied the isolation and preliminary identification of acetic acid bacteria from honey.

However, the efficacy of lactic acid bacteria from edible fruits against antibiotics and pathogenic bacteria had not been conducted at the Department of Botany, University of Mandalay. Therefore, the present study was carried out for the partial fulfillment of efficacy of lactic acid bacteria from edible fruits against antibiotics and pathogenic bacteria.

The aim and objectives of this research are to isolate the Lactic Acid Bacteria (LAB), to identify by using their morphology and biochemical characteristics, to investigate the antibiotic resistance and the antipathogenic-bacterial activities of the isolated strains.

Materials and Methods

Lactic acid bacteria (LAB) were isolated from 7 samples of fresh fruits (carambola, pomegranate, guava, papaya, lime, banana and pitahaya) obtained from local markets during September 2017 to January 2018.

Preparation of Media and Chemicals Used for Isolation of Bacteria, and physiological and biochemical methods for identification of isolated strains were followed to Breed *et al.* (1957), Cruickshank *et al.* (1968), Speck (1976), Dickey & Kelman (1988), Atlas (1993), Sharma (2007) and Naeem *et al*. (2012). All the experiments were performed at Department of Botany, University of Mandalay. Growth Response of the selected strains at different pH and NaCl concentration were also studied according to Khalil & Anwar (2016).

Antibiotic resistance and antibacterial activity for LAB were carried out at the Biotechnology Research Department, Kyaukse, according to Sukumar & Ghosh (2010).

Results

Morphology of Isolated Lactic Acid Bacteria

The lactic acid bacteria were isolated from edible fruits (carambola, pomegranate, guava, papaya, lime, banana and pitahaya) by using De Man, Rogosa and Sharpe agar (MRS) medium. Ten different kinds of lactic acid

bacterial strains were observed from edible fruits. The lactic acid bacterial strains were named as PPTG 1, PPTG 2, PPTG 3, PPTG 4, PPTG 5, PPTG 6, PPTG 7, PPTG 8, PPTG 9 and PPTG 10. The individual colonies were sub-cultured on the separate media plates to find out the difference in shape, colour and colony formation.

Lactobacillus sp., *Pediococcus* sp. and *Leuconostoc* sp., were isolated from edible fruits. The colonial and microscopic morphology of isolated lactic acid bacteria were presented in Table 1 & 2.

Antibiotic Resistance Test for Lactic Acid Bacteria

Lactobacillus sp. (PPTG 1) strain was resistant to ampacillin and amoxicillin, *Leuconostoc* sp. (PPTG 4) strain was resistant to ampacillin, amoxicillin, chloramphenicol and erythromycin, *Lactobacillus* sp. (PPTG 5) strain was resistant to tetracycline, ampacillin, amoxicillin, chloramphenicol and erythromycin, *Leuconostoc* sp. (PPTG 9) strain was resistant to tetracycline, ampacillin and amoxicillin and *Leuconostoc* sp. (PPTG 10) strain was resistant to ampacillin and amoxicillin. *Pediococcus* sp. (PPTG 2), *Leuconostoc* sp. (PPTG 3), *Leuconostoc* sp. (PPTG 6), *Lactobacillus* sp. (PPTG 7) and *Pediococcus* sp. (PPTG 8) were non-resistant to antibiotics (Table 3).

Table 1. Morphological Characterization of Isolated Bacteria

Cell Morphology	Shape	Size (μm)	colour	Gram staining	Endospore forming	Motility
Lactobacillus sp. (PPTG 1)	Short rod	0.1-0.5 by 1.0-5.0	white	+	–	–
Pediococcus sp. (PPTG 2)	coccus	0.3-0.7	Pale yellow	+	–	–
Leuconostoc sp. (PPTG 3)	spherical	0.3-0.9	yellow	+	–	–

Cell Morphology	Shape	Size (μm)	colour	Gram staining	Endospore forming	Motility
Leuconostoc sp. (PPTG 4)	spherical	0.5-1.0	creamy	+	_	_
Lactobacillus sp. (PPTG 5)	rod	0.7-1.9 by 2.0-6.0	creamy	+	_	_
Leuconostoc sp. (PPTG 6)	spherical	0.2-0.5	yellow	+	_	+
Lactobacillus sp. (PPTG 7)	rod	0.5-1.0 by 2.0-8.0	yellow	+	_	_
Pediococcus sp. (PPTG 8)	coccus	0.1-0.4	white	+	_	_
Leuconostoc sp. (PPTG 9)	coccus	0.3-0.8	white	+	_	_
Leuconostoc sp. (PPTG 10)	coccus	0.2-1.0	Pale yellow	+	_	_

+ = Positive reaction
– = Negative reaction

Table 2. Biochemical Tests for Characterization of Isolated Bacteria

Biochemical Character-istics	Cit. Utiliz. Test	Cata-lase Test	Oxi-dase Test	Fermentation					
				Glu	Suc	Lac	Dex	Man	Mal
Lactobacillus sp. (PPTG 1)	+	+	+	+AG	+AG	_	+AG	+AG	_
Pediococcus sp. (PPTG 2)	_	+	_	+AG	+AG	+AG	+AG	_	+AG
Leuconostoc sp. (PPTG 3)	_	+	_	+AG	+AG	_	+AG	+AG	_
Leuconostoc sp. (PPTG 4)	+	+	+	+AG	+AG	+AG	+AG	+AG	+AG

Biochemical Characteristics	Cit. Utiliz. Test	Catalase Test	Oxidase Test	Fermentation					
				Glu	Suc	Lac	Dex	Man	Mal
Lactobacillus sp. (PPTG 5)	+	+	_	+AG	+AG	+AG	+AG	+AG	+AG
Leuconostoc sp. (PPTG 6)	_	+	_	+AG	+AG	_	+AG	+AG	+AG
Lactobacillus sp. (PPTG 7)	_	+	+	+AG	+AG	_	+AG	+AG	+AG
Pediococcus sp. (PPTG 8)	_	+	_	+AG	+AG	_	_	_	+A
Leuconostoc sp. (PPTG 9)	+	+	_	+AG	+AG	+AG	+AG	+AG	+AG
Leuconostoc sp. (PPTG 10)	+	+	_	+AG	+AG	+AG	+AG	+AG	+AG

+ = Positive reaction, + A = Acid

– = Negative reaction, + AG = Acid with gas

Table 3. Antibiotic Resistance Test for Lactic Acid Bacteria

LABs	Diameter of Inhibition Zone (mm)				
	Tetracycline	Ampacillin	Chloramphenicol	Amoxicillin	Erytho mycin
Lactobacillus sp. (PPTG 1)	–	71.08, 38.48	–	62.97, 41.16	–
Pediococcus sp. (PPTG 2)	–	–	–	–	–
Leuconostoc sp. (PPTG 3)	–	–	–	–	–
Leuconostoc sp. (PPTG 4)	–	28.54, 23.07	14.76, 18.48	23.85, 25.82	17.63
Lactobacillus sp. (PPTG 5)	60.55, 37.45	39.77, 32.24	59.65, 40.73	35.21, 32.49	44.52, 31.38
Leuconostoc sp. (PPTG 6)	–	–	–	–	–

LABs	Diameter of Inhibition Zone (mm)				
	Tetra-cycline	**Ampacillin**	**Chloram-phenicol**	**Amoxicillin**	**Erythomycin**
Lactobacillus sp. (PPTG 7)	–	–	–	–	–
Pediococcus sp. (PPTG 8)	–	–	–	–	–
Leuconostoc sp. (PPTG 9)	16.12	20.11, 21.23	–	24.14, 29.68	–
Leuconostoc sp. (PPTG 10)	–	33.46, 24.74	–	29.68, 26.99	–

– = non resistance to antibiotics

Antibacterial Activity Test for Lactic Acid Bacteria

Lactobacillus sp. (PPTG 1) strain showed maximum zone of inhibition against *Staphylococcus aureus*, average zone of inhibition against *Escherichia coli*, minimum zone of inhibition against *Bacillus cereus* and did not show any zone of inhibition on *Enterococcus faecalis*. *Leuconostoc* sp. (PPTG 4) strain showed zone of inhibition against *Staphylococcus aureus* and did not show any zone of inhibition on *Bacillus cereus*, *Escherichia coli*, *Enterococcus faecalis*. *Lactobacillus* sp. (PPTG 5) strain did not show any zone of inhibition. *Leuconostoc* sp. (PPTG 9) strain showed zone of inhibition against *Staphylococcus aureus* and did not show any zone of inhibition on *Bacillus cereus*, *Escherichia coli*, *Enterococcus faecalis*. *Leuconostoc* sp. (PPTG 10) strain showed zone of inhibition against *Staphylococcus aureus* and did not show any zone of inhibition on *Bacillus cereus*, *Escherichia coli*, *Enterococcus faecalis*. Tetracycline hydrochloride control showed zone of inhibition against *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis* (Table 4).

Table 4. Antibacterial Activity Test for Lactic Acid Bacteria

LABs	Diameter of Inhibition Zone (mm)			
	Bacillus cereus	*Escherichia coli*	*Staphylococcus aureus*	*Enterococcus faecalis*
Lactobacillus sp. (PPTG 1)	11	12	19	–
Leuconostoc sp. (PPTG 4)	–	–	11	–
Lactobacillus sp. (PPTG 5)	–	–	–	–
Leuconostoc sp. (PPTG 9)	–	–	9	–
Leuconostoc sp. (PPTG 10)	–	–	9	–
Tetracycline hydrochloride	23	27	13	16

– = non inhibition zone of pathogenic bacteria

Growth Responses of Selected LAB strains

The isolated LAB strains such as *Lactobacillus* sp. (PPTG 1), *Leuconostoc* sp. (PPTG 4), *Lactobacillus* sp. (PPTG 5) and *Leuconostoc* sp. (PPTG 10) possessed the antibiotic properties. Their growth responses were studied at the various pH of 4.5, 6.5 and 7.2. All strains can survive at all study pH level. According to the study on resistance from NaCl 1% to NaCl 6% also all the strains survived in concentration. But *Lactobacillus* sp (PPTG 1) showed the more resistance compared to the remaining 4 strains (Table 5).

Table 5. Physiological Tests for Bacteria possessing antibacterial activity

LABs	Growth at different pH			Growth at different NaCl (%)					
	4.5	6.5	7.2	1	2	3	4	5	6
Lactobacillus sp. (PPTG 1)	+	++	+++	+++	+++	+++	++	++	++
Leuconostoc sp. (PPTG 4)	+	++	+++	+++	+++	+++	++	+	+
Lactobacillus sp. (PPTG 5)	+	++	+++	+++	+++	+++	++	+	+
Leuconostoc sp. (PPTG 9)	+	++	+++	+++	+++	+++	+++	+	+
Leuconostoc sp. (PPTG 10)	+	++	+++	+++	+++	+++	+++	+	+

+ = Positive reaction – = Negative reaction

Discussion and Conclusion

The present study deals with isolation, identification of lactic acid bacteria from selective edible fruits and their efficacy resistance to antibiotics and anti-microbial properties. Seven samples of fresh fruits (carambola, pomegranate, guava, papaya, lime, banana and pitahaya) were obtained from local markets. Ten strains of LABs were isolated from this study and they were relatively named as PPTG 1 to PPTG 10.

Naeem *et al.* (2012) observed that the LABs can be isolated from *Carica papaya* (Papaya), *Musa* spp. (Banana), *Psidium guajava* (Guava) and *Punica granatum* (Pomegranate). In the present study also LABs can be isolated from these species.

Fijan (2014) mentioned that *Lactobacillus* includes the major part of the lactic acid bacteria (LAB) group (including *Lactobacillus*, *Lactococcus*, *Enterococcus*, *Oenococcus*, *Pediococcus*, *Streptococcus* and *Leuconostoc* species) that can convert hexose sugars to lactic acid thus producing an acid environment which inhibits the growth of several species of harmful bacteria. Some *Lactobacilli* are used for the production of yogurt, cheese, sauerkraut, pickles, sourdough, wine and other fermented products. Breed *et al*. (1958) stated that *Pediococcus* are the dominant microbial population on forage crops and silage. Ali (2011) mentioned that the genus *Leuconostoc* have long been known and applied by humans for making different food stuffs. For many centuries, they have been an effective form of natural preservation. Therefore, the resulting of *Lactobacillus* spp., *Pediococcus* spp. and *Leuconostoc* spp. are valuable for the resulting of noble strains for further studies.

According to the present study, *Lactobacillus* sp. (PPTG 1) strain was resistant to ampacillin and amoxicillin; *Pediococcus* sp. (PPTG 2) strain and *Leuconostoc* sp. (PPTG 3) strain were non-resistant to tested antibiotics; *Leuconostoc* sp. (PPTG 4) strain was resistant to ampacillin, amoxicillin, chloramphenicol and erythromycin; *Lactobacillus* sp. (PPTG 5) strain was resistant to tetracycline, ampacillin, amoxicillin, chloramphenicol and erythromycin; *Leuconostoc* sp. (PPTG 6) strain, *Lactobacillus* sp. (PPTG 7)

strain, *Pediococcus* sp. (PPTG 8) strain and *Leuconostoc* sp. (PPTG 9) strain was resistant to tetracycline, ampacillin and amoxicillin, and *Leuconostoc* sp. (PPTG 10) strain was resistant to ampacillin and amoxicillin. These finding are in related with some previous findings of Naeem *et al.* (2012) and Saranya & Hemashenpagam (2011).

Naeem *et al.* (2012) stated that all 15 isolates LAB strains were tested for antibiotic resistance and their susceptibility and resistance against 10 available antibiotics. Saranya & Hemashenpagam (2011) mentioned that Rifampicin, Ketoconazole, Novobiocin, Fluconazole, Gentamycin, Amphotericin, and Chloramphenicol were used to determine antibiotic resistance of lactobacilli strains. The resistances were determined according to the zone formation.

In this present study, *Lactobacillus* sp. (PPTG 1) strain showed maximum zone of inhibition against *Staphylococcus aureus*, average zone of inhibition against *Escherichia coli*, minimum zone of inhibition against *Bacillus cereus* and did not show any zone of inhibition on *Enterococcus faecalis*. *Leuconostoc* sp. (PPTG 4) strain showed zone of inhibition against *Staphylococcus aureus* and did not show any zone of inhibition on *Bacillus cereus*, *Escherichia coli*, *Enterococcus faecalis*. *Lactobacillus* sp. (PPTG 5) strain did not show any zone of inhibition. *Leuconostoc* sp. (PPTG 9) strain showed zone of inhibition against *Staphylococcus aureus* and did not show any zone of inhibition on *Bacillus cereus*, *Escherichia coli*, *Enterococcus faecalis*. *Leuconostoc* sp. (PPTG 10) strain showed zone of inhibition against *Staphylococcus aureus* and did not show any zone of inhibition on *Bacillus cereus*, *Escherichia coli*, *Enterococcus faecalis*. Tetracycline hydrochloride control showed zone of inhibition against *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis.* Therefore, *Lactobacillus* sp. (PPTG 1) is nearly as good as control in antibacterial activity, especially on *Staphylococcus aureus* with up to 19 mm in diameter of inhibition.

Saranya & Hemashenpagam (2011) mentioned that the LAB inhibited all the pathogenic bacteria. The activity of LAB on some gram positive and negative pathogenic bacteria such as *E.coli, Pseudomonas aeroginosa, Klebsiella pneumonia, Staphylococcus aureus* and *Bacillus cereus* and the inhibition zones were in the range of 1.4 to 2.8 cm. The largest zone of inhibition was produced by *L. plantarum* (13mm) against *S. aureus.* In the present study also *Lactobacillus* sp. (PPTG 1) is also possessing antibacterial activity, especially on *Staphylococcus aureus* with up to 19 mm *Staphylococcus aureus* with 19 mm, average zone of inhibition against *Escherichia coli* with 12 mm, minimum inhibition against *Bacillus cereus* with 11 mm. Therefore, the present finding is valuable as previous findings of Saranya & Hemashenpagam (2011).

The isolated LAB produced antimicrobial compounds to varying degree, the increase in the production of lactic acid with time have been attributed to lowered pH which permit the growth of LAB. Garcia *et al.* (2016) stated that the five tested *Lactobacillus* strains displayed the capability to inhibit pathogenic bacteria, including *E. coli*, *L. monocytogenes*, *Salmonella enteritidis*, *Salmonella typhimurium* and *Staphyloccus aureus* in the potato agar or well diffusion assays. Therefore, the present findings agreed with Garcia *et al.* (2016) in possessing the antimicrobial effect on *E. coli* and *S. aureus*.

Owing to the considerable economic importance of LAB, many researchers are now actively working on these bacteria using an array of genetic tools. In the last few decades probiotic potential of LAB and their role in inhibition of pathogenic organisms has opened new horizons in the fields of medicinal sciences and food biotechnology (Naeem *et al*. 2012).

Recent researchers reported that the isolation and identification of the isolate of LAB from different fruits had shown good proteolytic activity and probiotic properties. Martins *et al.* (2016) stated that banana and guava has considerable amounts of prebiotic substrates, which can contribute the viability of persistence of *Lactobacillus rhamnosus* and it has potential to

serve as a probiotic carrier. Previous studies indicated that probiotic culture produces acids that promote the reduction of pH, creating condition unfavourable to microbial growth and also on the growth of pathogens. The presence of such bacteria in raw fruits satisfies the nutritive and microbial profile of fruits to be a healthy food. Garcia *et al*. (2016) stated that the natural adaptation to the intrinsic characteristics of fruit may help fruit-originating bacteria to survive during the processing. The presence of LAB in fruits, according to present study, may promote the health of human beings.

In the present study the most number of LABs were observed in papaya fruit with three isolated strains such as *Leuconostoc* sp. (PPTG4), *Lactobacillus* sp (PPTG 5) and *Leuconostoc* sp (PPTG 6). Because of this finding, papaya is very suitable to be consumed in daily life for the health. The pitahaya fruit also endophytically possess two strains of LAB, *Leuconostoc* spp. (PPTG 9 and PPTG 10). The remaining fruits possess one strain of LAB each. Acording to the study on antipathogenic bacteria properties of LABs, *Lactobacillus* sp. (PPTG 1) strain isolated from carambola showed zone of inhibition against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus cereus*. Therefore, the carambola fruit is also very suitable for the health. Moreover, the selected LAB strains revealed the following desirable probiotic related properties of growth on various pH level and NaCl concentration.

In conclusion, the LAB strains can be isolated from freshly edible fruits and they have noble properties of antibiotic sensitivity, antimicrobial properties, especially on pathogenic bacteria, and also the stains having the ability of growth in different pH and NaCl concentration. The fruits are very important to be consumed not only for their nutritional properties but also as the providers of the beneficial probiotic strains inside the bodies.

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