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Research Article

Identification of Lactic Acid Bacteria from Fermented Young Muskmelon (*Cucumis melon* Linn)

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Abstract

Fermentation has been used since ancient times as an easy method of vegetables' preservation, which also maintains and improves the nutritional and sensory properties of the final products. The lactic acid bacteria (LAB) involved in fermentation are generally recognized as safe. Lactic acid bacteria are a group of Gram positive, non-respiring, non- spore forming, cocci or rod, which produce lactic acid as the major end product of the fermentation of carbohydrate. This study isolated LAB from naturally-fermenting young muskmelon (*Cucumis melon* Linn) in 10% brine solution at pre-determined time intervals using the standard pour plating technique. A total of 18 isolates were chosen and purified by streak plating on MRS agar medium and incubated at 37˚C for 24-48h. Physiological and biochemical characterization of the isolates were carried out. Fermentation of carbohydrates was determined using the API 50 CHL System. Results of the study revealed that out of the three (3) strains of selected LAB strains; one strain was identified as *Lactobacillus brevis* while the two strains are *Pediococcus pentosaceus*. These LABS are potential inoculants for commercial production of fermented fruits and vegetables.

Keywords: Lactic Acid Bacteria; Fermentation; *Cucumis melon* Linn; *Lactobacillus brevis; Pediococcus pentosaceus*

Introduction

Fermentation is one of the oldest methods of food preservation. It is the process of conversion of sugars to acids by microorganisms [1]. Fermented vegetables are good appetizers, consumed by people of all ages and contain large amounts of lactobacilli which are important for the digestion of grains and vegetables, and have beneficial probiotic properties [2]. Fermented vegetables prepared by lactic acid bacteria (LAB) fermentation have unique flavor and beneficial health effects [3]. LAB in fermented vegetables help to enhance human nutrition by providing vitamins, minerals, and carbohydrates, and produce various aroma components, organic acids, enzymes, bacteriocins, and exopolysaccharides. These metabolic products impart some characteristic properties such as taste, texture and longer shelf life to the products [4]. LAB carries out detoxification of toxic compounds and degradation of mycotoxins in specific cases and therefore can reduce the health risk. Lactic acid bacteria are considered as safe additives and Generally Recognized as Safe (GRAS), useful to control the frequent development

of pathogens and spoilage microorganisms in food and feed [5]. The microorganisms involved in the fermentation of fermented vegetables have been identified as *Lactobacillus plantarum*, *L. brevis*, *Leuconostoc mesenteroides*, *Pediococcus cerevisiae*, *Pediococcus pentosaceus* and *Enterococcus faecalis* [3,6]. The LAB isolated from fermented young muskmelon (*Cucumis melon* Linn) differ from other microorganisms as they can tolerate considerably high concentration of salt and sugar. So far, there is no information about the lactic acid bacteria from brine-fermented young muskmelon (*Cucumis melon* Linn). Keeping this in view, the present study aimed to isolate and identify useful lactic acid bacteria from fermented young muskmelon (*Cucumis melon* Linn).

Materials and Methods

- **Samples:** The spontaneously fermenting young muskmelon (*Cucumis melon* Linn) in 10% brine solution; and samples were taken after 24 h and 3-5 days interval thereafter until 20 days for microbial analysis. The processing was conducted in food bioprocessing laboratory of Faculty of Agro-Industry, Royal University of Agriculture (RUA), Phnom Penh, Kingdom of Cambodia.
- **Isolation of Lactic Acid Bacteria (LAB):** Samples were taken at pre-determined time intervals for a period of 20 days. The standard dilution plating technique was carried out wherein 0.1ml of the appropriate dilutions of the sample was pour plated on de Man Rogosa and Sharpe (MRS) agar medium with 1% $CaCO_3$ and incubated at 37°C for 24-48 hrs [7]. Colonies were randomly selected based on their morphological appearance and streak plating was then used to purify the isolates. The individual bacterial colonies were stored in MRS agar stab at 4°C for further analysis.
- **Physiological and Biochemical characterization:** The purified isolates were further characterized on the basis of their biochemical tests i.e. tolerance at different NaCl concentrations (3%, 5%, 8%, 11%, and 14%); growth at different pHs (3.0, 4.0 and 5.0); and growth at different temperatures (10°C and 45°C).
- **Identification of Lactic Acid Bacteria:** Fermentation of carbohydrates was determined using API 50 CHL, a standardized system, consisting of 50 biochemical tests for the study of carbohydrate metabolism by microorganisms. API 50 CH is used in conjunction with API 50 CHL medium for the identification of Lactobacillus according to the manufacturer's instructions (Biomerieux, Marcy l' Etoile, France) [8]. Ten (10) ml of distilled water was dispensed into the incubation box with the strips placed in it, after the bacterial cultures had been introduced into the API 50 CHL system in API 50 CHL medium (5 ml), at concentration of 2 McFarland. The set-up of the system was then incubated at appropriate temperature of 35°C for 48 h, after which the wells were filled with the bacterial suspensions at the line mark with the addition of mineral oil. Bacterial strains were identified based on their ability for carbohydrates fermentation. Identification of isolates was carried out based on the color change of API strips reaction and recorded as + or –. Numerical profiles of strains were identified adding positive values in indicative table. Species designations were identified by evaluating with software identification Apiweb TM.

Results and Discussion

After proper selection of acid-forming bacteria (Figure 1) and purification (Figure 2), the isolates were subjected for physiological and biochemical tests. Results revealed that Lactic acid bacteria (LAB) isolates are all Gram-positive (+), non-spore forming, either rod- or cocci-shaped, catalase (-), oxidase (-), and acid-tolerant (Table 1). After proper characterization, the selected isolates were tested for their ability to ferment various carbohydrates using the API 50 CHL test kit. There are identified as*: Lactobacillus brevis 1, Pediococcus pentosaceus 1, and Pediococcus pentosaceus 1* (Table 2).

Figure 1: Plate showing clearing around colonies of acid-forming bacteria on MRS + $CaCO_3$ agar plate.

Figure 2: Plate showing discrete colonies of subculture LAB isolate.

Eighteen bacterial strains were isolated from different phases of the fermentation of young muskmelon (*cucumis melon* Linn), however only four bacterial strains have tolerance to high salt concentration and high temperature (Table 1); and these were characterized by the API 50 CHL System of identification (Table 2). The cultural characteristics of the isolates are shown in table 3. Three of the isolates were identified as *Lactobacillus brevis*, and two as *Pediococcus pentosaceus*.

Lactobacillus brevis is a species of lactic acid bacteria, all of which are Gram-positive, non-spore forming organisms whose main metabolic pathway involves fermenting hexose sugars to produce lactic acid. Along with other lactic acid-producing bacteria, *L. brevis* plays an integral role in the fermentation of certain foods such as sauerkraut and pickles and is likewise the most common cause of spoiled beer. In fact, in Germany at one point, more than half of beer spoilage incidents were due to *L. brevis* alone. *L. brevis* can be isolated from the food sources in inhabits, one example being kimchi [9].

Pediococcus pentosaceus are coccus shaped microbes, Gram-positive, non-motile, non-spore forming, and are categorized as a "lactic acid bacteria". *P. pentosaceus* are categorized as a "lactic acid bacteria" because the end product of its metabolism is lactic acid [10]. *P. pentosaceus*, like most lactic acid bacteria, are anaerobic and ferment sugars. Since the end product of metabolism is a kind of acid, *P. pentosaceus* are acid tolerant. They can be found in plant materials, ripened cheese, and a variety of processed meats [11]. *P. pentosaceus* is industrially important due to its ability as a starter culture to ferment foods such as various meats, vegetables, and cheeses [12]. *P. pentosaceus* bacteria is being cultured and researched for its ability to produce an antimicrobial agent (bacteriocins) as well as its use in food preservation [12]. *P. pentosaceus* can be cultured at 35 ^{0}C – 40 ^{0}C but are unable to grow at 50 ^{0}C. *P. pentosaceus* is able to grow in pH values between 4.5 and 8.0. The bacteria grow more stably at the more acidic pH range [11].

Code	Gram	Shape	Catalase	Oxidase	NaCl (%)					Temperature (°C)	
					3	5	8	11	14	10	45
2M5a	+	rod	_	_	***	***	***	***	*	**	**
3M7	+	coccus	_	_	***	***	***	***	*	**	**
7M5c	+	coccus	_	_	***	***	***	***	*	**	***
20M5	+	coccus	_	_	***	***	***	***	*	**	***

Table 1: Physiological, biochemical characteristics, and phenotypic properties of lactic acid bacteria isolated from brine-fermented muskmelon (*Cucumis melon* Linn).

Note: *, Minimum Growth; **, Moderate Growth; ***, Maximum Growth; –, No Growth

According to Tamang., *et al*. [20], LAB comprising of lactobacilli, pediococci and leuconostocs are the predominant microorganisms present in fermented leafy vegetables. This is in line with the work of Pederson and Albury [13] and Joshi and Sharma [14] that listed 5 species of lactic acid bacteria as important in the sauerkraut fermentation: *Streptococcus faecalis*, *Leuconostoc mesenteroides*, *Lac-*

No		2M5a	3M7	7M5c	20M5	No		2M5a	3M7	7M5c	20M5
1	0	+	+	+	+	26	ESC	+	+	+	+
2	GLY	-	-	-	+	27	SAL	+	+	+	-
3	ERY	-	-	-	-	28	CEL	+	+	+	-
4	DARA	-	-	-	-	29	MAL	+	+	+	+
5	LARA	+	+	+	-	30	LAC	-	-	-	-
6	RIB	+	+	+	-	31	MEL	-	-	-	-
7	DXYL	-	-	+	-	32	SAC	+	+	-	+
8	LXYL	-	-	-	-	33	TRE	+	+	+	-
9	ADO	-	-	-	-	34	INU	+	+	-	-
10	MDX	-	-	-	-	35	MLZ	-	-	-	-
11	GAL	+	+	+	-	36	RAF	-	-	-	-
12	GLU	+	+	+	+	37	AMD	-	-	-	-
13	FRU	+	+	+	+	38	GLYG	-	-	-	-
14	MNE	+	+	+	-	39	XLT	-	-	-	-
15	SBE	-	-	-	-	40	GEN	+	+	+	-
16	RHA	-	-	-	-	41	TUR	-	-	-	+
17	DUL	-	-	-	-	42	LYX	-	-	-	-
18	INO	-	-	-	-	43	TAG	+	+	-	-
19	MAN	-	-	-	-	44	DFUC	-	-	-	-
20	SOR	-	-	-	-	45	LFUC	-	-	-	-
21	MDM	-	-	-	-	46	DARL	-	-	-	-
22	MDG	-	-	-	-	46	LARL	-	-	-	-
23	NAG	+	+	+	+	48	GNT	+	-	-	-
24	AMY	+	+	+	-	49	2KG	-	-	+	-
25	ARB	+	+	+	-	50	5KG	-	-	-	-

Table 2: Phenotypic identification of lactic acid bacteria from fermenting young muskmelon *(Cucumis melon* Linn) using API 50 CHL gallery kit.

tobacillus brevis, *Pediococcus pentocaceus* and *Lactobacillus plantarum*.

Traditionally fermented foods, including fermented vegetables can be a rich source of new LAB strains, with interesting functional properties and with potential applications in food industry and health [21]. Sauerkraut, fermented cucumbers, and kimchi are the most studied lactic acid fermented vegetables mainly due to their commercial importance [15].

Fermentation of fruits and vegetables can occur "spontaneously" by the natural lactic bacterial surface microflora, such as

Colony Code	Species	ID (%)
2M5a	*Lactobacillus brevis 1*	98.7
3M7	*Pediococcus pentosaceus 1*	94.5
7M5c	*Pediococcus pentosaceus 1*	98.8
20M5	*Unknown*	

Table 3: Phenotypic identification of lactic acid bacteria from fermenting young muskmelon *(Cucumis melon* Linn) using API 50 CHL gallery kit.

Lactobacillus spp, *Leuconostoc* spp, and *Pediococcus* spp.; however, the use of starter culture such as *L. plantarum*, *L. rhamnosus*, *L. gasseri*, and *L. acidophilus* provides consistency and reliability of performance [16]. The present research thus investigated the LAB present at different phases of fermenting young muskmelon (*cucumis melon* Linn). The result of the study revealed that *Lactobacillus brevis*, and two as *Pediococcus pentosaceus* were the main LAB involved in the spontaneous fermentation of *Cucumis melon* Linn. This is in accordance with the work of [17], werein these LAB species were involved in the microbial dynamics of fermenting cucumber. These species particularly *Lactobacillus plantarum* and *Leuconostoc mesenteroides* are typical for spontaneous fermentation of vegetables [18]. *Consumis melon linn* interest for diverse fermented foods has increased in recent years because of the positive perception of their beneficial impact on health. Hence, there is an evident need to find novel methods and new food preservation agents from natural origins. Biopreservation, which refers to extending the shelf-life and enhancing the safety of foods using microorganisms or their metabolites, [19] would make these LAB as very good candidates as adjunct inocula [15].

Conclusion and Recommendation

The major lactic acid bacteria involved in the spontaneous fermentation of muskmelon (C*ucumis melon* Linn) are *Lactobacillus brevis* and *Pediococcus pentosaceus* strains. These lactic acid bacteria species can be exploited as pure starter cultures for the fermentation of other vegetables and fruits at the commercial scale.

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