PEDIOCOCCUS SPECIES AND RELATED BACTERIA FOUND IN FERMENTED FOODS AND RELATED MATERIALS IN THAILAND

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(Received November 14, 1983)

Tetrad-forming cocci were isolated from 17 kinds of fermented fish, meat, vegetables, and other materials in Thailand. Out of 58 strains identified, 22 were *Pediococcus pentosaceus* Mees, 2 were *Pediococcus acidilactici* Lindner, 26 were *Pediococcus halophilus* Mees, 4 were *Pediococcus* sp., and 4 were “*Tetracoccus*” sp. They were widely distributed in fermented products in Thailand, and play roles in souring and ripening.

The tetrad-forming bacteria mainly described as *Pediococcus* strains are found in a wide variety of beverages and fermented foods. These bacteria produce lactic acid from sugars. Spoilage of beer by such bacteria is reported (1). On the other hand, they play important roles in souring and ripening fermented foods; cucumber pickles (2, 3), shoyu (soy-sauce) (4, 5), miso (fermented soy bean paste) (6), nam-pla (fermented fish sauce in Thailand) (7), and burong dalag (fermented fish in the Philippines) (8). A great many fermented fish, meat, and vegetable foods are known in Thailand (9, 10), but little has been reported on the microorganisms involved. This paper deals with the identification of *Pediococcus* strains and related bacteria isolated mainly from these fermented foods.

MATERIALS AND METHODS

Isolation of bacteria. The tetrad-forming bacteria were isolated from the fermented foods obtained at the markets in Thailand, and from horse urine and animal feces by agar plate culture after 3 to 5 days' incubation at 30°. The medium (GYP-CaCO₃) was composed of 10.0 g, glucose; 10.0 g, yeast extract (Difco); 10.0 g, peptone (Difco); 10.0 g, CaCO₃; 12.0 g, agar; and 1,000 ml, distilled

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Table 1. The representative strains of tetrad-forming cocci and their isolation sources.

Strain	Other designations	Source	Identification in this study	Remarks
Ph93	JCM 2017	Fermented products Ching-chung (fermented small fish) Hoi-malangpu-dong (pickled mussel) Kapi (fish or shrimp paste) Kung-chom (fermented shrimps)	<i>P. halophilus</i>	
Ph47	TISTR 433		<i>P. halophilus</i>	
C01			" <i>Tetracoccus</i> " sp.	catalase +
Ph58	JCM 2014		<i>P. halophilus</i>	
Ph59	TISTR 434	"	<i>P. halophilus</i>	
Ph134		"	<i>P. halophilus</i>	
Ph139		"	<i>P. halophilus</i>	
Ph72		Nam-budu (fermented fish in liquid form)	<i>P. halophilus</i>	
Ph78		"	" <i>Tetracoccus</i> " sp.	catalase +
Ph79		"	" <i>Tetracoccus</i> " sp.	catalase +
Ph88	TISTR 436		<i>P. halophilus</i>	
Ph45	TISTR 432	Nam-pla (fish sauce)	<i>P. halophilus</i>	
Ph27	TISTR 431	Pla-chom (fermented small fish)	<i>P. halophilus</i>	
Ph51	JCM 2022	"	" <i>Tetracoccus</i> " sp.	catalase +
Ph63	JCM 2015	"	<i>P. halophilus</i>	
Ph65	TISTR 435	"	<i>P. halophilus</i>	
Ph155	TISTR 439	"	<i>P. halophilus</i>	
Ph37		Pla-paeng-daeng (fermented fish)	<i>P. halophilus</i>	
Ph01	TISTR 429	Pla-ra (fermented fish)	<i>P. halophilus</i>	
Ph02		"	<i>P. halophilus</i>	
Ph08		"	<i>P. halophilus</i>	
Ph25	TISTR 430	"	<i>P. halophilus</i>	
Ph137	JCM 2019	"	<i>P. halophilus</i>	
Ph144	JCM 2020	"	<i>P. halophilus</i>	
Ph160		"	<i>P. halophilus</i>	
N31	JCM 2023	Pla-som (fermented fish)	<i>P. pentosaceus</i>	pseudocatalase +
N199	JCM 2030	"	<i>P. pentosaceus</i>	pseudocatalase +
N247		"	<i>P. pentosaceus</i>	

N271		TISTR 421	"	<i>P. pentosaceus</i>	pseudocatalase +
N303			"	<i>P. pentosaceus</i>	
N287	JCM 2031		Som-fak (fish cake)	<i>P. pentosaceus</i>	
N295		TISTR 428	"	<i>P. pentosaceus</i>	pseudocatalase +
Ph103			Tai-pla (fermented fish entrails)	<i>P. halophilus</i>	
Ph120		TISTR 437	"	<i>P. halophilus</i>	
Ph122	JCM 2018		"	<i>P. halophilus</i>	
Ph130		TISTR 438	"	<i>P. halophilus</i>	
Ph133			"	<i>P. halophilus</i>	
N01		TISTR 413	Nham (fermented pork)	<i>P. pentosaceus</i>	pseudocatalase +
N38		TISTR 416	"	<i>P. pentosaceus</i>	pseudocatalase +
N53	JCM 2032	TISTR 424	"	<i>P. acidilactici</i>	
N54		TISTR 425	"	<i>P. acidilactici</i>	
N65			"	<i>P. pentosaceus</i>	pseudocatalase +
N78		TISTR 417	"	<i>P. pentosaceus</i>	pseudocatalase +
N182	JCM 2028		"	<i>P. pentosaceus</i>	pseudocatalase +
N256		TISTR 420	"	<i>P. pentosaceus</i>	pseudocatalase +
N115	JCM 2026		Sai-krog-prieo (sour pork sausage)	<i>P. pentosaceus</i>	
N147			"	<i>P. pentosaceus</i>	
N197	JCM 2029		"	<i>P. pentosaceus</i>	pseudocatalase +
N220			"	<i>P. pentosaceus</i>	
N257			"	<i>P. pentosaceus</i>	
N278		TISTR 422	"	<i>P. pentosaceus</i>	pseudocatalase +
N133	JCM 2027	TISTR 419	Sai-krog-prieo (sour beef sausage)	<i>P. pentosaceus</i>	
N86	JCM 2021	TISTR 426	Phak-gard-dong (pickled vegetables)	<i>P. pentosaceus</i>	
N37	JCM 2024	TISTR 415	Phak-sian-dong (pickled vegetables)	<i>Pediococcus</i> sp.	
			Other materials	<i>P. pentosaceus</i>	
N48			Pieces of foods in chicken esophagus	<i>P. pentosaceus</i>	pseudocatalase +
U01		TISTR 427	Horse urine	<i>Pediococcus</i> sp.	
U03			Horse urine	<i>Pediococcus</i> sp.	
U20			Rabbit dung	<i>Pediococcus</i> sp.	

JCM, Japan Collection of Microorganisms, Hiroshima, Wako, Saitama, Japan. TISTR, Thailand Institute of Scientific and Technological Research, Bangkok, Thailand.

Table 2. Reference strains received from culture collections.

Species	Strain designations
<i>Pediococcus acidilactici</i>	ATCC 8042
<i>Pediococcus acidilactici</i>	ATCC 8081
" <i>Pediococcus cerevisiae</i> "	TISTR 335←UQM 786
<i>Pediococcus halophilus</i>	TUA Ps 033←NISL 7127
<i>Pediococcus halophilus</i>	TISTR 333←DMKU B-120
<i>Pediococcus pentosaceus</i>	TUA Pc.←IFO 3891
" <i>Pediococcus soyae</i> "	TISTR 72←IAM 1688
" <i>Pediococcus urinae-equi</i> "	TISTR 394←IAM 1684
" <i>Tetracoccus soyae</i> "	TISTR 395←TUA F-2
<i>Leuconostoc mesenteroides</i>	TISTR 53←ATCC 10830

ATCC, American Type Culture Collection, Rockville, Maryland, U.S.A. DMKU, Department of Microbiology, Kesetsart University, Bangkok, Thailand. IAM, Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan. IFO, Institute for Fermentation, Osaka, Japan. NISL, Noda Industrial Science Laboratory, Noda, Chiba, Japan. TISTR, Thailand Institute of Scientific and Technological Research, Bangkok, Thailand. TUA, Tokyo University of Agriculture, Tokyo, Japan. UQM, Department of Microbiology, University of Queensland, Brisbane, Australia.

water; adjusted to pH 6.8. For the isolation of halophilic strains, 50.0 g of NaCl was added to 1,000 ml of GYP-CaCO₃ agar medium. Acid-forming bacteria were detected by the formation of colonies with clear zone dissolving CaCO₃. As colonies appeared they were stabbed in GYP-CaCO₃ agar, and tetrad-forming bacteria were observed microscopically. Throughout the study, no anaerobic culture technique was employed for the isolation. Working cultures were maintained on GYP-sodium acetate-mineral salts broth composed of 10.0 g, glucose; 10.0 g, yeast extract (Difco); 10.0 g, peptone (Difco), 10.0 g, sodium acetate; 200 mg, MgSO₄·7H₂O; 10 mg, MnSO₄·4H₂O; 10 mg, FeSO₄·7H₂O; 10 mg, NaCl; and 1,000 ml, distilled water; adjusted to pH 6.8. For the halophilic strains, 50.0 g of NaCl was added to 1,000 ml of GYP-sodium acetate-mineral salts broth. The cultures were maintained by stabbing into GYP-CaCO₃ agar and by lyophilization, and preserved at 4°. Names of samples in Thai are mainly cited from the references (9, 10), and difficulties are encountered in translation of Thai into English because some foods are produced in the limited local provinces and the processes are obscure.

Bacterial cultures. A total of 68 representative strains were used in this study. Strain designations and sources are shown in Tables 1 and 2. Named strains received from the culture collections were used as reference strains. Names which are not on the Approved Lists of Bacterial Names, 1980 (11) are enclosed in quotation marks.

Identification methods.

Morphological characteristics. Cell form, cell size, cell arrangement, and gram

stain were observed on the cells grown on GYP agar plate after 3 to 5 days' incubation at 30°. Hucker's modification was employed for gram stain (12). Motility was examined by the appearance of the stab culture in semi-solid medium composed of 10.0 g, glucose; 5.0 g, beef extract; 5.0 g, yeast extract (Difco); 0.5 ml, Tween 80; 0.04 g, Bromocresol Purple (B.C.P.); 1.5 g, agar; and 1,000 ml, distilled water; adjusted to pH 6.8 (13). Spore formation was observed in a gram stained specimen.

Cultural characteristics. The colonial appearance was observed by the use of GYP agar plate after 1, 3, and 5 days' incubation at 30°. Turbidity and final pH of broth culture were observed by using GYP-sodium acetate-mineral salts broth.

Production of acid from sugars. Medium composed of 4.0 g, yeast extract (Difco); 5.0 g, peptone (Difco); 5.0 g, sugar; 200 mg, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; 10 mg, $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$; 10 mg, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$; 10 mg, NaCl; and 1,000 ml, distilled water; adjusted to pH 6.8; and colored with Bromocresol Purple, was used to test for the production of acid from sugars. After 3 to 5 days' incubation, the change of the indicator was recorded. In some cases acid produced in 3 ml of broth was titrated with 0.05 N NaOH. Twenty-two sugars were employed. For halophilic bacteria, 50.0 g of NaCl was added to 1,000 ml of basal medium, and for the "*Pediococcus urinae-equi*" and related strains, the pH was adjusted to 7.2. Production of gas from glucose was tested by the method of GIBSON and ABD-EL-MALEK (14). Identification of lactic acid was determined by the method of DAWES *et al.* (15). Fermentation products from glucose were analysed by the method of Fehling and Lieman (16) to decide the type of lactic acid fermentation. Optical isomers of lactic acid were determined by the method of OKADA *et al.* (17).

Splitting of hydrogen peroxide. The production of catalase and pseudo-catalase involves the splitting of hydrogen peroxide. The strains were cultivated on GYP agar plate containing 0.05, 1.0 or 2.0 g of glucose, and on YTG agar medium composed of 0.5 g, glucose; 10.0 g, Tryptone (Difco); 5.0 g, yeast extract (Difco); 5.0 g, NaCl; 2.0 g, $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$; 15.0 g, agar; and 1,000 ml, distilled water (18). After 1 and 3 days' incubation at 30°, a 3% solution of hydrogen peroxide was flooded over the colonies appearing on the agar plates, and the bubbling of gas was observed. The result was confirmed by the benzidine test (19). When both hydrogen peroxide splitting activity and the benzidine test were positive, the culture was assumed to be catalase positive. When hydrogen peroxide splitting activity was positive and the benzidine test was negative, the culture was assumed to be catalase negative but pseudocatalase positive. The activity of the culture was also tested on heated blood agar.

Biochemical characteristics. The following biochemical characteristics were tested by the methods described in the references: Reaction to litmus milk and hydrolysis of gelatin (16); hydrolysis of aesculin and hippurate (20), hydrolysis of arginine (21), the cleavage of glucose (22), nitrate reduction (16); reduction of

tellurite, production of indole, MR-test, V-P reaction, utilization of citrate on Simmon citrate agar (23); slime formation on SYP medium composed of 10.0 g, sucrose; 10.0 g, yeast extract (Difco); 10.0 g, peptone (Difco); 15.0 g, agar; and 1,000 ml, distilled water; and oxidase test (24). Tolerance to Teepol was tested by the growth in GYP-sodium acetate-mineral salts medium containing 0.01, 0.02, or 0.05% Teepol, and by the growth in GYP-sodium acetate-mineral salts medium containing 8% of ethanol, and observed after 3 to 7 days' incubation at 30°.

Physiological characteristics. The relation to oxygen was tested by growth in semi-solid agar (13). The relation to temperature was tested by growth in GYP-sodium acetate-mineral salts broth incubated at 20°, 30°, 37°, 40°, 42°, 48°, and 50° for 3, 5 and 7 days. Effect of starting pH was detected by growth in GYP-sodium acetate-mineral salts broth adjusted to an initial pH of 3.7, 4.5, 5.0, 6.7, 7.0, 7.4, 8.2, 8.6, 9.0, or 9.2 with 1 N HCl and 1 N NaOH. Halophilic or halotolerant characteristics were determined by the growth in GYP-sodium acetate-mineral salts broth containing 0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, 16.0, 18.0, 20.0, or 22.0% of NaCl, and recorded after 3, 5, and 7 days at 30°. When the strains grew well in the presence of NaCl ranging from 4 to 12% and even at 18% of NaCl, these were termed to be halophilic. These strains are considered to require NaCl for growth. When the strains grew in the absence of NaCl but grew even at 18% NaCl, those were termed to be halotolerant. These strains are considered not to require NaCl for growth.

RESULTS

The tetrad-forming bacteria isolated, including the strains received from the culture collections, were divided into two major groups. Out of 67 strains, 62 strains were catalase negative and 5 strains were catalase positive. The strains tested produced cocci forms and occurred in tetrads, in pairs, and rarely singly. Cell size was approximately 0.4 to 1.0 μm in diameter. They were all gram positive, non-motile, and non-sporing. White, raised, and circular colonies appeared on GYP agar plate. Filiform growth was observed along stab lines on stab cultures.

In general, the catalase negative strains produced acid from a larger number of sugars than catalase positive strains did. The acidity of halophilic, catalase negative strains and catalase positive strains were much less than those of non-halophilic, catalase negative strains as shown in Tables 3 and 4. The main fermentation product from glucose by the isolates was lactic acid, and the production ratio of lactic acid to consumed glucose ranged from 75.10 to 99.74%, except for *Leuconostoc mesenteroides* TISTR 53 (55.50%) as shown in Table 5. On the basis of the results, the type of lactic acid fermentation was homotype. Further, DL+D isomers were produced by strains N78 and N86; and DL+L by the strains N53 and Ph01. Although the isolates were divided into the two major

Table 3. Acidity produced by the pseudocatalase positive and pseudocatalase negative pediococci in sugar media.

Strain	Days	Acidity ^a							
		L-Arabinose	D-Xylose	Fructose	Galactose	D-Glucose	Mannose	Sucrose	Basal medium
N01	3	0	0.05	1.00	2.60	2.40	1.40	1.80	0
	7	0.05	0.05	2.40	2.90	2.90	1.90	2.20	0
N31	3	0.05	0.05	0.90	0	1.80	1.90	1.30	0
	7	0.05	0.05	1.70	0	2.40	2.50	2.60	0
N38	3	3.60	2.40	2.60	2.20	3.00	3.00	0	0
	7	3.55	2.90	2.90	2.50	2.95	3.10	0	0
N53	3	3.40	2.50	1.90	2.10	2.20	2.65	0	0
	7	3.60	3.30	2.95	2.70	3.00	3.00	0	0
N256	3	3.60	0	2.70	2.80	2.70	2.40	0	0
	7	3.80	0	3.20	3.00	2.90	3.00	0	0
N271	3	0.05	0.10	0.90	0.10	2.40	2.10	0.05	0
	7	0.05	0.05	1.90	0.10	2.60	2.50	0.05	0
N295	3	0.05	0.05	2.30	3.20	3.10	3.00	3.20	0
	7	0.05	0.05	2.60	3.10	3.10	3.20	3.40	0

^a Acidity is expressed as ml of 1 N/20 NaOH consumed to neutralize 3 ml of culture broth incubated for 3 and 7 days.

Basal medium, free sugar.

Table 4. Acidity produced by the halophilic pediococci and the catalase positive tetracocci in sugar media.

Strain	Days	Acidity ^a							
		L-Arabinose	D-Xylose	Fructose	Galactose	D-Glucose	Mannose	Sucrose	Basal medium
Ph25	3	0.25	0.20	0.20	0.20	0.25	0.20	0.10	0.10
	7	0.30	0.20	0.30	0.25	0.30	0.25	0.20	NT
Ph27	3	0.15	0.20	0.25	0.20	0.25	0.20	0.20	0.10
	7	0.20	0.25	0.30	0.25	0.25	0.30	0.25	NT
Ph45	3	0.05	0.10	0.40	0.40	0.40	0.40	0.10	0
	7	0	0.10	0.45	0.45	0.60	0.50	0.25	NT
Ph51	3	0	0	0.50	0	0.60	0	0	0
	7	0	0	0.80	0	0.90	0	0	0
Ph79	3	0	0	0.70	0	0.50	0	0	0
	7	0	0	0.70	0	0.80	0	0	0
TUA Ps033	3	0	0.05	0.30	0.35	0.40	0.40	NT	NT
	7	0	0.05	0.35	0.35	0.45	0.45	0.15	0

^a Acidity is expressed as ml of 1 N/20 NaOH consumed to neutralize 3 ml of culture broth incubated for 3 and 7 days.

Basal medium, free sugar; NT, not tested.

Table 5. The production ratio of lactic acid to consumed glucose and optical isomers of lactic acid of the representative strains.

Strain	Consumed glucose (mg/ml)	Lactic acid produced (mg/ml)	Lactic acid produced (%)	Optical isomers of lactic acid
N01	7.9	7.2	91.14	
N31	9.03	7.65	84.72	
N78	6.39	4.80	75.10	DL+D
N256	8.93	8.10	90.71	
N271	6.87	6.64	96.65	
N53	7.9	7.88	99.74	DL+L
ATCC 8042	9.47	7.88	83.21	
Ph01	3.0	2.93	97.96	DL+L
Ph37	2.32	2.25	96.98	
Ph45	4.57	4.28	93.65	
Ph133	2.04	1.91	93.63	
Ph155	4.13	3.49	84.50	
TUA Ps033	4.2	3.38	80.48	
TISTR 72	3.77	3.04	80.48	
TISTR 333	2.04	1.80	88.24	
N86	4.37	3.71	84.90	DL+D
U01	4.20	3.71	88.33	
C01	2.20	1.80	81.82	
Ph51	3.44	2.59	75.29	
Ph78	4.67	3.83	82.01	
Ph79	4.50	3.71	82.44	
TISTR 395	4.57	3.71	81.18	
TISTR 53	9.73	5.40	55.50	

groups, some of the catalase negative strains showed the activity of pseudocatalase. Catalase positive strains reduced nitrate to nitrite. A large number of strains grew in medium containing 8% of alcohol. All the strains were presumed to be microaerophilic or facultatively anaerobic because they grew in stabbed semi-solid agar and cleaved glucose fermentatively. Out of 67 strains, 29 grew with NaCl at concentrations of more than 18‰, and they were regarded as halophilic.

Identification of the isolates

On the basis of characteristics mentioned above and described in Tables 6 and 7, tetrad-forming, catalase negative bacteria were included in the genus *Pediococcus* (25), and identified as *Pediococcus pentosaceus* Mees, *Pediococcus acidilactici* Lindner, *Pediococcus halophilus* Mees, and *Pediococcus* sp. Tetrad-forming catalase positive bacteria have not been studied enough to date to identify them correctly. Therefore, the present authors include these bacteria tentatively in "*Tetracoccus*" sp.

Pediococcus pentosaceus Mees Strains: N01, N31, N37, N38, N48, N65, N78, N115, N133, N147, N182, N197, N199, N220, N247, N256, N257, N271,

Table 6. The general characteristics of species of the genera *Pediococcus* and "*Tetracoccus*" based on the strains isolated from Thai fermented foods and related materials.

Characteristics	<i>P. pentosaceus</i> No. of strains 25	<i>P. acidilactici</i> 3	<i>P. halophilus</i> 29	<i>Pediococcus</i> sp. 5	" <i>Tetracoccus</i> " sp. 5
Cell form	cocci				
Cell size (μm)	0.4–1.0				
Cell arrangement	occurring singly, in pairs and in tetrads				
Gram reaction	+	+	+	+	+
Motility	—	—	—	—	—
Spore formation	—	—	—	—	—
Final pH in GYP broth	4.52–4.61	4.60–4.63	4.54–5.11	5.10–5.53	4.87–5.08
Gas from glucose	—	—	—	—	—
Catalase	—	—	—	—	+
Pseudocatalase	+(–10)	—	—	—	—
Benzidine test	—	—	—	—	+
Reaction in litmus milk:					
Reduction	—	—	—	—	—
Peptonization	—	—	—	—	—
Acid curd	—	—	—	—	–(+1)
Hydrolysis of gelatin	—	—	—	—	—
Hydrolysis of aesculin	+(–5)	—	—	—	—
Hydrolysis of arginine	–(+ ^w 2)	+	+(–14)	—	+(–1)
Hydrolysis of hippurate	—	—	—	+	—
Cleavage of glucose	+f	+f	+f	+f	+f
Nitrate reduction	—	—	—	—	+
Reduction of tellurite	NG	NG	NT	NT	NT
Indole	—	—	—	—	—
Methyl red test	+	+	+	+	+
Voges-Proskauer reaction	—	—	—	—	—
Utilization of citrate	—	—	NT	NT	—
Slime formation	—	—	NT	NT	—
Oxidase	—	—	—	—	—
Tolerance to Teepol:					
0.01%	+(–1)	+	+(–6)	NT	NT
0.02%	+(–7)	+	+(–9)	NT	NT
0.05%	–(+1, + ^w 1)	—	–(+4)	NT	NT
Tolerance to 8% ethanol	+(–1)	+	+	NT	NT
Relation to oxygen	M	M	M	M	F
Growth at 20°	+	+	+	+	+
Growth at 42°	+(–1)	+	–(+ ^w 8, NT1)	—	+
Growth at 48°	—	+	NT	NT	NT

Table 6. (Continued).

Characteristics	<i>P. pentosaceus</i>	<i>P. acidilactici</i>	<i>P. halophilus</i>	<i>Pediococcus</i> sp.	" <i>Tetracoccus</i> " sp.
	No. of strains 25	3	25	5	5
Growth at 50°	—	+	NT	NT	NT
Optimum temperature	30–37°	30–40°	30°	20–30°	30–37°
Growth at pH 3.7	—	—	NT	NT	NT
Growth at pH 4.5	+	+	NT	NT	NT
Growth at pH 5.0	+	+	—	—	+(–1)
Growth at pH 8.2	+(–7)	+	+	+	+
Growth at pH 8.6	—	—	+	+	+
Growth at pH 9.2	—	—	–(–5, NT4)	+	NT
Optimum pH	6.7–7.4	6.7–7.4	6.7–8.6	7.4–9.0	6.7–8.2
Tolerance to NaCl (%):					
Optimum	0–2	0–2	4–12	0–2	0–4
Maximum	10	8	22 or more	12	18
Halophilicity	—	—	+	—	—

+, positive; +^w, weakly positive; —, negative; NT, not tested.

The numbers in parentheses indicate the numbers of strains showing positive, weakly positive, negative characteristic, or characteristic not tested. NG, no growth; F, fermentative; M, microaerophilic.

N278, N287, N295, N303, ATCC 8081, TUA Pc., and TISTR 335.

The characteristics of these strains agreed with the description of the species (25, 26), but variations were found in production of pseudocatalase, hydrolysis of aesculin and arginine, tolerance to Teepol, tolerance to ethanol, growth at pH 8.2, growth at 42°, growth at 6 to 8% NaCl, and acid from sugars. Strain characteristics are shown in Table 8. The strains in this species were isolated from pla-som (fermented fish), som-fak (fish cake), nham (fermented pork), sai-krog-prieo (sour pork sausage and sour beef sausage), phak-gard-dong and phak-sian-dong (pickled vegetables), and pieces of food in chicken esophagus.

Pediococcus acidilactici Lindner Strains: N53, N54, and ATCC 8042.

The strains in this species grew even at 50°, hydrolysed arginine, but did not produce acid from maltose. Detailed characteristics are shown in Table 9. These strains were isolated from nham (fermented pork).

Pediococcus halophilus Mees Strains: Ph01, Ph02, Ph08, Ph25, Ph27, Ph37, Ph45, Ph47, Ph58, Ph59, Ph63, Ph65, Ph72, Ph88, Ph93, Ph103, Ph120, Ph122, Ph130, Ph133, Ph134, Ph137, Ph139, Ph144, Ph155, Ph160, TUA Ps033, TISTR 72, and TISTR 333.

Table 7. Acid formation from carbohydrates of the strains of *Pediococcus* sp. and "*Tetracoccus*" sp.

Carbohydrates	<i>P. pentosaceus</i> No. of strains 25	<i>P. acidilactici</i> 3	<i>P. halophilus</i> 29	<i>Pediococcus</i> sp. 5	" <i>Tetracoccus</i> " sp. 5
L-Arabinose	+(−9)	+	−(+3, + ^w 1)	+ ^w (+1)	−
D-Ribose	+	+	+(−2)	+ ^w (+2)	−
D-Xylose	−(+10)	+	+(+ ^w 1, −9)	+ ^w	−
Rhamnose	−(+3)	−(+1)	−	−	−
Fructose	+(+ ^w 1)	+	+	+	+
Galactose	+(−2)	−	+(+ ^w 2, −8)	+	−(+1)
D-Glucose	+	+	+	+	+
Mannose	+	+	+	+	−(+2)
α Methyl D-glucoside	−	−	+(−4)	−	−
Salicin	+(−6)	+(−1)	+(−4)	+	−
Lactose	−(NT1)	−	−	+(+ ^w 1)	−(+1)
Maltose	+(−1)	−	+(−3)	+	−(+1)
Sucrose	−(+10)	−	+(+ ^w 2, −2)	+	−(+1)
Trehalose	+(−6, NT1)	−(+1)	+(+ ^w 1, −3)	+	+(−1)
Melezitose	−	−	−(+6, + ^w 5)	−	−
Raffinose	−(+4)	−	−(+ ^w 1)	−(+2)	−
Dextrin	−	−	−(+1)	+ ^w (+1)	−
Inulin	−(+5)	−	−	+ ^w	−
Starch	−	−	−	−	−
Glycerol	−	−	−(+7, + ^w 1)	+(+ ^w 2)	+(−1)
Mannitol	−	−	−(+11)	+	+(−2)
Sorbitol	−	−	−(+12)	−(+1)	+(−2)

+, positive; +^w, weakly positive; −, negative; NT, not tested. The number in parentheses indicate the numbers of strains showing positive, weakly positive, negative characteristic, or characteristic not tested.

The strains in this species were halophilic and grew at 18% NaCl. They grew at pH 8.6 but not at pH 5.0. Some strains grew even at pH 9.2. Variations among the strains were found in hydrolysis of arginine, tolerance to Teepol, and production of acid from sugars. Detailed characteristics are shown in Table 10. The strains in this species were isolated from ching-chung (fermented small fish), hoi-malangpu-dong (pickled mussel), kung-chom (fermented shrimps), nam-budu (fermented fish in liquid form), nam-pla (fish sauce), pla-chom (fermented small fish), pla-paeng-daeng (fermented fish), pla-ra (fermented fish), and tai-pla (fermented fish entrails).

Table 8. Characteristics of 25 strains

Strain	Pseudocatalase	Hydrolysis of aesculin	Hydrolysis of arginine	Tolerance to Teepol:	0.01 %	0.02 %	0.05 %	Tolerance to 8% ethanol	Growth at pH 5.0	Growth at pH 8.2	Growth at 42°	Growth in 6% NaCl	Growth in 8% NaCl	L-Arabinose	D-Ribose
N01	+	-	+	⁴	+	-	-	+	+	-	+	+	+	-	+
N31	-	-	-		+	-	-	+	+	+	+	+	+	-	+
N37	-	-	-		+	+	-	+	+	+	+	+	+	+	+
N38	+	+	-		+	+	-	+	+	+	+	+	+	+	+
N48	+	+	-		+	+	-	+	+	+	+	+	+	-	+
N65	+	+	-		+	+	-	+	+	+	+	+	+	+	+
N78	+	+	-		+	-	-	+	+	-	+	+	-	+	+
N115	-	-	-		+	-	-	+	+	+	+	+	+	-	+
N133	-	+	-		+	+	-	+	+	+	+	+	-	-	+
N147	-	+	-		+	+	-	+	+	+	+	+	+	+	+
N182	+	+	-		+	+	-	+	+	-	+	+	-	-	+
N197	+	+	-		+	+	-	+	+	+	+	+	+	+	+
N199	+	+	-		+	+	+	⁵	+	+	+	+	+	+	+
N220	-	+	-		+	+	-	+	+	-	+	+	+	+	+
N247	+	+	-		+	+	-	+	+	+	+	+	+	+	+
N256	-	+	-		+	+	-	+	+	+	+	+	+	+	+
N257	-	+	-		+	+	-	+	+	+	+	+	+	+	+
N271	-	-	-		-	-	-	-	+	-	-	+	+	-	+
N278	+	+	-		+	+	-	+	+	+	+	+	+	+	+
N287	-	+	-		+	-	-	+	+	+	+	+	+	-	+
N295	+	+	-		+	+	+	+	+	+	+	+	+	-	+
N303	+	+	-		+	+	-	+	+	+	+	+	+	+	+
ATCC 8081	+	+	-		+	+	-	+	+	-	+	+	+	+	+
TUA Pc.	+	+	+	⁶	+	+	-	+	+	+	+	-	-	+	+
TISTR 335	+	+	-		+	-	-	+	+	-	+	-	-	+	+

+, positive; +^⁵, weakly positive; -, negative; NT, not tested.

Pediococcus sp. Strains: N86, U01, U03, U20, and TISTR 394.

The strains studied grew at pH 9.2 but not at pH 5.0, and they hydrolysed hippurate. They were not halophilic. Variation among the strains was found in production of acid from sugars. Out of 5 strains, 4 strains produced acid from lactose. Detailed characteristics are shown in Table 11. The characteristics of the isolates were almost identical with those of "*P. urinae-equi*" TISTR 394. However, this species is not on the Approved Lists of Bacterial Names, 1980 (11) and further characterization of this species has not been reported to date. Therefore, the present authors retain the naming of the isolates. The strains were

of *Pediococcus pentosaceus*.

	D-Xylose	Rhamnose	Fructose	Galactose	D-Glucose	Mannose	α Methyl D-glucoside	Salicin	Lactose	Maltose	Sucrose	Trehalose	Melezitose	Raffinose	Dextrin	Inulin	Starch	Glycerol	Mannitol	Sorbitol
1	-	-	+	+	+	+	-	-	-	+	+	+	-	-	-	+	-	-	-	-
2	-	-	+	+	+	+	-	-	-	+	+	-	-	-	-	+	-	-	-	-
3	+	+	+	+	+	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-
4	+	-	+	+	+	+	-	+	-	+	+	+	-	-	-	-	-	-	-	-
5	-	-	+	+	+	+	-	+	-	+	+	+	-	+	-	-	-	-	-	-
6	-	-	+	+	+	+	-	+	-	+	-	+	-	-	-	-	-	-	-	-
7	-	-	+	+	+	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-
8	-	+	+	+	+	+	-	+	-	+	-	+	-	-	-	-	-	-	-	-
9	-	-	+	+	+	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-
10	+	-	+	+	+	+	-	+	-	+	+	+	-	+	-	-	-	-	-	-
11	+	-	+	+	+	+	-	+	-	+	+	+	-	-	-	-	-	-	-	-
12	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	+	-	+	+	+	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-
14	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	-	+	+	+	+	+	-	+	-	+	+	+	-	-	-	+	-	-	-	-
16	+	-	+	+	+	+	-	+	-	+	+	+	-	-	-	+	-	-	-	-
17	+	-	+	+	+	+	-	-	NT	+	-	NT	-	-	-	-	-	-	-	-
18	+	-	+	+	+	+	-	+	-	+	+	+	-	-	-	-	-	-	-	-
19	-	-	+	+	+	+	-	+	-	+	-	+	-	-	-	-	-	-	-	-

isolated from phak-gard-dong (pickled vegetables), horse urine and rabbit dung.

"*Tetracoccus*" sp. Strains: CO1, Ph51, Ph78, Ph79, and TISTR 395.

The strains studied were catalase positive and reduce nitrate to nitrite. They were halotolerant, growing at a concentration of 18% of NaCl, but not requiring NaCl for growth. Variations among the strains were found in the curdling of litmus milk, hydrolysis of arginine, and production of acid from sugars. Detailed characteristics are shown in Table 12. The characteristics of the isolates were identical to those of "*Tetracoccus soyae*" TISTR 395 which was originally isolated from soy mash by UENO and OMATA (27). However, this species is not on the

Table 9. Characteristics of 3 strains

Strain	Pseudocatalase	Hydrolysis of aesculin	Hydrolysis of arginine	Tolerance to Teepol:	0.01%	0.02%	0.05%	Tolerance to 8% ethanol	Growth at pH 5.0	Growth at pH 8.2	Growth at 48°	Growth in 6% NaCl	Growth in 8% NaCl	L-Arabinose	D-Ribose
N53	—	—	+		+	+	—	+	+	+	+	+	+	+	+
N54	—	—	+		+	+	—	+	+	+	+	+	+	+	+
ATCC 8042	—	—	+		+	+	—	+	+	+	+	—	—	+	+

+, positive; +^w, weakly positive; —, negative

Approved Lists of Bacterial Names, 1980 (11), and it has not been characterized to date. Therefore, the present authors include these strains tentatively in the genus "*Tetracoccus*", and retain the naming of the strains.

DISCUSSION

Pediococcus strains are widely distributed in the fermented foods in Thailand, and the species are source specific. The halophilic strains were frequently isolated from the fermented products of fish, and the non-halophilic ones found in products of meat and vegetables, as shown in Table 1. This may be due to the concentration of NaCl in the foods. Some products of fermented fish seem to be similar to "shiokara" in Japan. On the contrary, *P. pentosaceus* strains were found in pla-som (fermented fish) which is produced under a low concentration of NaCl.

Recently, isolation of *Pediococcus* strains from nam-pla (fish sauce in Thailand) (7) was reported, and microflora in fermented foods in Asian countries have attracted interest of microbiologists (7). Further studies will make clear the function of the microorganisms involved.

As mentioned above, the catalase positive tetrad-forming cocci have not been differentiated from related genera at present. Taxonomic study of this group of bacteria is essential, and chemotaxonomic comparison would be useful for the differentiation of "*Tetracoccus*" from related genera. The strains of "*Tetracoccus*" have been isolated from shoyu mash (27), and they are presumed to play roles in making the shoyu (4, 5). In this study, such bacteria were also isolated from nam-budu (fermented fish in liquid form), pla-chom (fermented small fish), and kapi (fish or shrimp paste).

The authors wish to thank Prof. Dr. K. Komagata, Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan, for editing the manuscript; Prof. Dr. M. Kozaki, Dr. T. Toyoda and Dr. S. Okada, Laboratory of Applied Microbiology, Tokyo University of Agricul-

of *Pediococcus acidilactici*.

D-Xylose	Rhamnose	Fructose	Galactose	D-Glucose	Mannose	α Methyl D-glucoside	Salicin	Lactose	Maltose	Sucrose	Trehalose	Melezitose	Raffinose	Dextrin	Inulin	Starch	Glycerol	Mannitol	Sorbitol
+	-	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
+	+	+	+	+	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-

ture, Tokyo, Japan, for advice about methods, analysis of optical isomers of lactic acid, and supplying materials. We are also grateful to Miss Poonsook Atthasumpunna and Mrs. Wanchern Daengsubha, TISTR, Bangkhen, Bangkok 10900, Thailand, for providing the cultures.

This study was in part supported by the co-operation MIRCEN Research Grant (UNEP/UNESCO).

Table 10. Characteristics of 29 strains

Strain	Pseudocatalase	Hydrolysis of arginine	Hydrolysis of hippurate	Tolerance to Teepol:	0.01 %	0.02 %	0.05 %	Tolerance to 8% ethanol	Growth at pH 5.0	Growth at pH 8.2	Growth at pH 9.2	Growth at 42°	Growth in 18% NaCl	L-Arabinose	D-Ribose
Ph01	—	—	—	+	+	—	—	+	—	+	+	—	+	—	+
Ph02	—	—	—	—	—	—	—	+	—	+	—	—	+	—	—
Ph08	—	+	—	+	+	—	—	+	—	+	+	—	+	—	+
Ph25	—	—	—	+	+	—	—	+	—	+	+	—	+	+	+
Ph27	—	—	—	+	+	—	—	+	—	+	+	—	+	—	+
Ph37	—	—	—	+	+	—	—	+	—	+	+	—	+	+	+
Ph45	—	—	—	+	+	+	+	+	—	+	+	—	+	—	+
Ph47	—	+	—	+	+	+	+	+	—	+	+	—	+	—	+
Ph58	—	—	—	+	+	—	—	+	—	+	—	—	+	—	+
Ph59	—	—	—	+	+	—	—	+	—	+	+	—	+	—	+
Ph63	—	+	—	+	+	—	—	+	—	+	+	+	+	—	+
Ph65	—	—	—	+	+	+	+	+	—	+	+	+	+	—	+
Ph72	—	—	—	—	—	—	—	+	—	+	+	+	+	—	+
Ph88	—	+	—	—	—	—	—	+	—	+	NT	+	+	—	+
Ph93	—	—	—	+	—	—	—	+	—	+	NT	+	+	—	+
Ph103	—	+	—	+	—	—	—	+	—	+	NT	+	+	—	+
Ph120	—	+	—	—	+	—	—	+	—	+	+	+	+	—	+
Ph122	—	+	—	+	+	—	—	+	—	+	+	+	+	—	+
Ph130	—	+	—	+	+	—	—	+	—	+	NT	—	+	—	+
Ph133	—	+	—	—	—	—	—	+	—	+	—	—	+	—	+
Ph134	—	+	—	—	+	—	—	+	—	+	+	—	+	—	+
Ph137	—	—	—	+	+	—	—	+	—	+	+	—	+	+	+
Ph139	—	+	—	—	—	—	—	+	—	+	—	—	+	+	+
Ph144	—	+	—	+	+	+	+	+	—	+	+	+	+	—	+
Ph155	—	—	—	+	+	—	—	+	—	+	+	—	+	—	+
Ph160	—	+	—	—	—	—	—	+	—	+	—	—	+	—	+
TUA Ps033	—	+	—	+	+	—	—	+	—	+	+	+	+	—	+
TISTR 72	—	—	—	+	+	—	—	+	—	+	+	+	+	—	—
TISTR 333	—	+	—	+	—	—	—	+	—	+	+	NT	+	—	+

+, positive; +^w, weakly positive; —, negative; NT, not tested.

Table 11. Characteristics of 5 strains

Strain	Pseudocatalase	Hydrolysis of arginine	Hydrolysis of hippurate	Growth at pH 5.0	Growth at pH 8.2	Growth at 42°	Growth in 6% NaCl	Growth in 8% NaCl	Growth in 12% NaCl	L-Arabinose	D-Ribose
N86	—	—	+	—	+	—	+	+	+	+	+
U01	—	—	+	—	+	—	+	+	+	+	+
U03	—	—	+	—	+	—	+	+	+	+	+
U20	—	—	+	—	+	—	+	+	+	+	+
TISTR 394	—	—	+	—	+	—	+	+	+	+	+

+, positive; +^w, weakly positive; —, negative.

Table 12. Characteristics of 5 strains

Strain	Catalase	Hydrolysis of arginine	Hydrolysis of hippurate	Reaction in litmus milk:	Reduction	Peptonization	Acid curd	Growth at pH 5.0	Growth at pH 8.2	Growth at pH 42°	Growth in 18% NaCl	L-Arabinose	D-Ribose
C01	+	—	—	—	—	—	+	—	+	+	+	—	—
Ph51	+	+	—	—	—	—	—	+	+	+	+	—	—
Ph78	+	+	—	—	—	—	—	+	+	+	+	—	—
Ph79	+	+	—	—	—	—	—	+	+	+	+	—	—
TISTR 395	+	+	—	—	—	—	—	+	+	+	+	—	—

+, positive; —, negative.

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