Characterization of *Pediococcus pentosaceus* and *Pediococcus acidilactici* Strains and Replacement of the Type Strain of *P. acidilactici* with the Proposed Neotype DSM 20284

Request for an Opinion

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Levels of DNA relatedness were determined for 16 strains of *Pediococcus pentosaceus* and *Pediococcus acidilactici*, and two distinct groups were recognized. The DNA base compositions of the members of one group ranged from 37.4 to 38.7 mol% guanine plus cytosine and the DNA base compositions of the members of the other group ranged from 42.7 to 43.5 mol% guanine plus cytosine. The phenotypic characteristics after growth at 50°C and acid production from maltose correlated with the levels of DNA relatedness and DNA base compositions. Strains in the first group exhibited high levels of DNA relatedness to the type strain of *P. pentosaceus* and were identified as *P. pentosaceus* strains. Strains in the second group exhibited high levels of DNA relatedness to authentic reference strains of *P. acidilactici* but did not exhibit relatedness to *P. acidilactici* JCM 5885^T (= NCDO 1859^T) (T = type strain). The taxonomic standing of the type strain of *P. acidilactici* was examined, and strains in the second group were identified as *P. acidilactici*. In addition, we request the replacement of the type strain *P. acidilactici* with the proposed neotype DSM 20284 (= JCM 8797 = NRIC 0115).

Pediococcus strains are widely distributed in beverages, fermented foods, and dairy products (7, 11, 16). *Pediococcus pentosaceus* and *Pediococcus acidilactici* were not clearly separated in early studies, and the characteristics given sometimes included characteristics common to both species (5). Recently, a fluorometric DNA-DNA hybridization procedure has been reported to be useful for the identification of bacteria (5, 17). In this study we determined levels of DNA relatedness, DNA base compositions, and phenotypic characteristics for named strains of *P. pentosaceus* and *P. acidilactici* obtained from culture collections and strains isolated from fermented foods in Thailand. In this paper we request an opinion to replace the type strain of *P. acidilactici*, strain JCM 5885 (= NCDO 1859), with proposed neotype strain DSM 20284 (= JCM 8797 = NRIC 0115).

The strains which we studied and their sources are shown in Table 1. Glucose-yeast extract-peptone medium (17) was used as the basal medium throughout this study. Phenotypic characteristics and fermentation types were determined as described previously (16, 17). Vitamin requirements were determined by the method of Kihara and Snell (10), with some modifications. The isomers of lactic acid produced were determined enzymatically by the method of Okada et al. (13). DNA base compositions and levels of DNA relatedness were determined by methods described previously (5, 14, 15).

The named strains of *P. pentosaceus* and *P. acidilactici* studied shared common characteristics, as described below (16; this study). The cells are gram-positive cocci, are nonmotile, do not form spores, and occur in pairs and

tetrads. The strains split glucose fermentatively and are facultatively anaerobic. These bacteria are negative for catalase activity, gelatin hydrolysis, nitrate reduction, slime formation from sucrose, citrate utilization, acid production and reduction of litmus milk, and growth in glucose-yeast extract-peptone broth containing 10 and 12% NaCl. They grow at pH 4.5 to 8.2, at 20 to 42°C, and in glucose-yeast extract-peptone broth containing 4% NaCl. Pseudocatalase activity is found in some strains of *P. pentosaceus*. All of the strains tested hydrolyze esculin. A few strains hydrolyze arginine. These strains produce acid from D-ribose, D-fructose, D-glucose, D-mannose, and cellobiose, but fail to produce acid from α -methylglucoside, melezitose, glycerol, D-mannitol, and D-sorbitol. They do not produce gas from glucose. All of the strains tested require niacin and calcium pantothenate for growth, but do not require *p*-aminobenzoic acid. Strains of *P. acidilactici* require biotin for growth. Variable characteristics are shown in Table 2. Strains N31, N287, N37, N197, KM2, and DSM 20284 utilize glucose homofermentatively (16; this study). Strains N31, N287, N295, N37, N182, N197, KM2, KM19-2, C7, DSM 20284, and N53 produce DL-lactic acid from glucose (16, 19; this study).

The DNA base compositions range from 37.4 to 38.7 mol% and from 42.7 to 43.5 mol% guanine plus cytosine for strains of *P. pentosaceus* and strains of *P. acidilactici*, respectively (Table 3). On the basis of levels of DNA relatedness, the strains studied were separated into two groups. Strains NRIC 0094, NRIC 1102, N31, N287, N295, N37, N182, N197, KM2, and KM19-2 exhibited high levels of homology (more than 77%) with *P. pentosaceus* JCM 5890^T (T = type strain) and *P. acidilactici* JCM 5885^T. Consequently, they were identified as members of *P. pentosaceus* (20). Strains

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TABLE 1. Designations and isolation sources of *P. pentosaceus* and *P. acidilactici* strains

Strain ^a	Other designation(s) ^a	Sources
<i>P. pentosaceus</i> JCM 5890 ^T	ATCC 33316, ^T NCDO 990 ^T	Beer yeast
<i>P. pentosaceus</i> NRIC 0094		
<i>P. pentosaceus</i> NRIC 1102	ATCC 8081	Dairy product
<i>P. pentosaceus</i> N31	JCM 2023, TISTR 414	Pla-som (fermented fish)
<i>P. pentosaceus</i> N287	JCM 2031	Som-fug (fish cake)
<i>P. pentosaceus</i> N295	TISTR 423	Som-fug (fish cake)
<i>P. pentosaceus</i> N37	JCM 2024, TISTR 415	Phak-sian-dong (pickle)
<i>P. pentosaceus</i> N182	JCM 2028	Nham (fermented pork)
<i>P. pentosaceus</i> N197	JCM 2029	Sai-krog-prieo (sour pork sausage)
<i>P. pentosaceus</i> KM2	NRIC 0122	Khaomak (sweetened rice)
<i>P. pentosaceus</i> KM19-2	NRIC 0123	Khaomak (sweetened rice)
<i>P. acidilactici</i> JCM 5885 ^T	ATCC 33314, ^T NCDO 1859 ^T	Sake mash ^b
<i>P. acidilactici</i> NRIC 1087	ATCC 8042	
<i>P. acidilactici</i> DSM 20284	JCM 8797	Barley ^c
<i>P. acidilactici</i> N53	JCM 2032, TISTR 424	Nham (fermented pork)
<i>P. acidilactici</i> C7	NRIC 0124	Khanom-jeen (fermented rice noodle)

^a ATCC, American Type Culture Collection, Rockville, Md; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany; JCM, Japan Collection of Microorganisms, RIKEN, Saitama, Japan; NCDO, National Collection of Dairy Organisms, Reading, England; NRIC, Culture Collection Center, Tokyo University of Agriculture, Tokyo, Japan; TISTR, Thailand Institute of Scientific and Technological Research, Bangkok, Thailand.

^b See reference 12.

^c See references 1 and 2.

N53 and C7 exhibited high levels of homology (more than 83%) with *P. acidilactici* NRIC 1087 and DSM 20284 (below, strains in the second group, on the basis of levels of DNA relatedness, are referred to as members of the *P. acidilactici* group). *P. acidilactici* JCM 5885^T exhibited high levels of homology (more than 79%) with *P. pentosaceus* strains, but

low levels of homology with *P. acidilactici* NRIC 1087 and DSM 20284 (Table 3). The strains tested belonging to both species have been reported to contain straight-chain fatty acids C_{18:1} and C_{16:0} as the dominant fatty acids, together with C_{14:0}, C_{16:1}, and C_{18:0} fatty acids (18). No quinones have been found (18).

TABLE 2. Characteristics of *P. pentosaceus* and *P. acidilactici* strains^a

Characteristic	<i>P. pentosaceus</i>		<i>P. acidilactici</i>				
	10 Strains	Strain JCM 5890 ^T	Strain JCM 5885 ^T	Strain NRIC 1087	Strain DSM 20284	Strain N53	Strain C7
Pseudocatalase	+(−,3) ^b	+	+	−	+	−	−
Arginine hydrolysis	−(w,4)	−	−	+	+	+	+
Growth at:							
45°C	−(+,4)	−	+	+	+	+	+
48°C	−	−	−	+	+	+	+
50°C	−	−	−	+	+	+	+
Growth at pH 4.2	+(−,1)	+	+	+	+	+	+
Growth in the presence of 6.5% NaCl	+	+	+	+	−	−	−
Growth in the presence of 8% NaCl	+(−,4)	−	+	−	−	−	−
Acid produced from:							
L-Arabinose	+(−,5)	+	+	+	+	+	+
D-Galactose	+(−,1)	+	+	+	+	+	+
Inulin	−(+,2)	−	−	−	−	−	−
Lactose	−(w,1)	−	+	−	−	−	−
Maltose	+(−,1)	+	+	−	−	−	−
Melibiose	−(+,2)	+	−	−	−	−	−
Raffinose	−	w	−	−	−	−	−
L-Rhamnose	+(+,2)	−	−	+	−	−	−
Salicin	+(−,3)	+	+	+	+	+	+
Sucrose	−(+,3)	+	−	−	−	−	−
Trehalose	+(w,1;−,3)	+	+	+	+	−	−
D-Xylose	+(−,5)	w	+	+	+	+	+
Vitamin requirements							
Riboflavin	−(+,2)	−	−	−	−	−	−
Biotin	−(+,4)	−	−	+	+	+	+
Folic acid	−(+,5)	−	−	−	−	−	−
Pyridoxine	−(+,1)	−	−	−	−	−	−

^a Data from reference 16 and this study.

^b +, positive reaction or vitamin required for growth; −, negative reaction or vitamin not required for growth; w, weak reaction. The data in parentheses indicate the numbers of strains which have the reactions indicated.

TABLE 3. DNA base compositions and levels of DNA relatedness of *P. pentosaceus* and *P. acidilactici* strains

Strain	G+C content (mol%)	% Homology with the following labeled strains ^a			
		JCM 5890 ^T	JCM 5885 ^T	NRIC 1087	DSM 20284
<i>P. pentosaceus</i> strains					
JCM 5890 ^T	37.8 ^b	100	97	2	3
NRIC 0094	38.5	103	93	19	16
NRIC 1102	38.0	82	92	16	16
N31	38.1 ^b	99	79	8	13
N287	38.3	92	83	8	— ^c
N295	37.7 ^b	109	103	8	12
N37	37.5 ^b	79	80	8	14
N182	37.4 ^b	90	87	8	16
N197	37.6	100	85	10	19
KM2	38.3	77	85	31	16
KM19-2	38.7	94	116	13	28
<i>P. acidilactici</i> strains					
JCM 5885 ^T	37.8 ^b	—	100	1	2
NRIC 1087	43.4	9	17	100	96
DSM 20284	43.5	6	2	86	100
N53	42.8 ^b	11	13	92	93
C7	42.7	9	10	108	83

^a Hybridization was performed at 45°C for 3 h.^b Data from reference 18.^c —, not determined.

P. pentosaceus and *P. acidilactici* strains seem to be similar in their phenotypic and chemosystematic characteristics (1, 6–8, 11, 18, 19). In 1974, Garvie (6) proposed *P. acidilactici* NCDO 1859^T (= JCM 5885^T) as the neotype strain of this species on the basis of phenotypic characteristics. According to her descriptions, this strain seemed to be differentiated from *P. pentosaceus* NCDO 990^T only on the basis of growth at 45°C. Later, Back and Stackebrandt (2) and Dellaglio et al. (3) described the separation of *P. pentosaceus* and *P. acidilactici* strains on the basis of DNA-DNA hybridization data, and strain NCDO 1859^T was considered a strain of *P. pentosaceus*. Our data agree with the data of Back and Stackebrandt (2) and Dellaglio et al. (3). In 1986, Garvie (8) requested that the previous neotype strain, strain NCDO 1859, be rejected and replaced with strain DSM 20284 as the neotype strain. She referred to growth at 50°C and fermentation of maltose as criteria for separating the two species, but did not provide evidence from her own new experimental data. The Judicial Commission of the International Committee on Systematic Bacteriology denied her request because there is no clear phenotypic distinction between the two genospecies (9). This decision was based on the recommendation of the Ad Hoc Committee on Reconciliation of Approaches to Bacterial Systematics (20).

Request for an opinion to replace type strain NCDO 1859 (= JCM 5885) of *P. acidilactici* with DSM 20284. Our results showed that the *P. pentosaceus* strains which we studied did not grow at 50°C and that a few strains grew at 45°C. Most strains produced acid from maltose; one strain did not produce acid from maltose. The guanine-plus-cytosine contents of the DNAs of the *P. pentosaceus* strains were 4 to 5 mol% lower than the guanine-plus-cytosine contents of the DNAs of the *P. acidilactici* group strains, as reported previously (7, 11) (Table 3). In contrast, the *P. acidilactici* group strains grew at 50°C and did not produce acid from maltose (Table 2). Thus, growth at 50°C is still useful for separating these two species, as Nakagawa and Kitahara (12), Kitahara (11), Back (1), and Back and Stackebrandt (2) reported previously. In addition, acid production from mal-

tose is a good marker for separation. The fluorometric DNA-DNA hybridization data from this study confirmed that these two species should be differentiated. Garvie (6) used growth at 45°C as a criterion to separate the two species, but some strains of *P. pentosaceus* grew at 45°C (Table 2). This might produce confusion in the separation of the two species. Inevitable confusion will arise if NCDO 1859 is the type strain of *P. acidilactici*. Therefore, we request that type strain NCDO 1859 be replaced with a typical strain of *P. acidilactici*, as Garvie requested (8). Strain DSM 20284 shares phenotypic, DNA base composition, and DNA relatedness characteristics with authentic strains belonging to the *P. acidilactici* group and was previously proposed as a neotype strain of *P. acidilactici* by Garvie in 1986 (8). On the basis of these facts, we request that type strain NCDO 1859 be replaced with *P. acidilactici* DSM 20284.

Description of *P. acidilactici* neotype strain DSM 20284.

Strain DSM 20284 (= JCM 8797 = NRIC 0115) has the characteristics described above and shown in Table 2. These characteristics agree well with those in the standard description of the species (7). The guanine-plus-cytosine content of the DNA is 43.5 mol% (Table 3), as determined by high-performance liquid chromatography. This strain was isolated from barley (1). (DSM 20284 is Back's strain S213C in the 1989 Deutsche Sammlung von Mikroorganismen und Zellkulturen *Catalogue of Strains* [4], but the designation should be B213c on the basis of Back's original paper [1]).

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