Alkalibacterium thalassium sp. nov., *Alkalibacterium pelagium* sp. nov., *Alkalibacterium putridalgicola* sp. nov. and *Alkalibacterium kapii* sp. nov., slightly halophilic and alkaliphilic marine lactic acid bacteria isolated from marine organisms and salted foods collected in Japan and Thailand

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We describe 10 new strains of marine lactic acid bacteria isolated from decaying marine algae, decaying seagrass, raw fish, salted fish and salted and fermented shrimp paste ('ka-pi') collected from a temperate area of Japan and Thailand. The isolates are Gram-positive and non-sporulating. They have motility with peritrichous flagella depending on the strains. They lack catalase and quinones. Under anaerobic conditions, lactate yields were 64–93 % of the glucose consumed; residual products were formate, acetate and ethanol with a molar ratio of approximately 2 : 1 : 1. The pH of the fermentation medium markedly affected the product composition; at higher pH, the yield of lactate decreased (15–48 % at pH 9.0) and yields of other products increased, retaining the molar ratio. Under aerobic conditions, acetate and lactate were produced from carbohydrates and related compounds. The isolates were slightly halophilic, highly halotolerant and alkaliphilic. The optimum NaCl concentration for growth ranged between 0.5 and 4.0 % (w/v), depending on the strain, with a growth range of between 0 and 17–21 % (11 % for one isolate). The optimum pH for growth ranged between 8.0 and 9.5, with a growth range of 6.0–11.0, depending on the strains. Comparative sequence analysis of the 16S rRNA genes revealed that the isolates occupied three phylogenetic positions within the genus *Alkalibacterium*, showing 97.1–99.8 % similarity to *Alkalibacterium indicireducens*. DNA–DNA hybridization values (<46 %) among the 10 isolates and phylogenetically related taxa resulted in the identification of four genomic species (designated groups GS1–GS4). The G + C contents of the DNA were 41.7 mol% (group GS1), 42.2 mol% (group GS2), 41.0–43.0 mol% (group GS3) and 38.4–39.4 mol% (group GS4). The cell-wall peptidoglycan was type A4 β , Orn–D–Asp, for three genomic species (groups GS1, GS2 and GS3), and type A4 β , Orn–D–Glu, for the remaining species (group GS4). The major components of cellular fatty acids were C_{16:0}, C_{16:1 ω 9c} and C_{18:1 ω 9c} (oleic acid). On the bases of phenotypic characteristics, genetic distinctiveness and phylogenetic affiliations, the four genomic species, groups GS1, GS2, GS3 and GS4, were classified as four novel species within the genus *Alkalibacterium*, for which the names *Alkalibacterium thalassium* sp. nov., *Alkalibacterium pelagium* sp. nov., *Alkalibacterium putridalgicola* sp. nov. and *Alkalibacterium kapii* sp. nov., respectively, are proposed. The respective type strains are T117-1-2^T (=DSM 19181^T=NBRC 103241^T=NRIC 0718^T), T143-1-1^T (=DSM 19183^T=NBRC 103242^T=NRIC 0719^T), T129-2-1^T (=DSM 19182^T=NBRC 103243^T=NRIC 0720^T) and T22-1-2^T (=DSM 19180^T=NBRC 103247^T=NRIC 0724^T).

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains T117-1-2^T, T143-1-1^T, T129-2-1^T and T22-1-2^T are AB294165, AB294166, AB294167 and AB294171, respectively.

Supplementary figures and tables are available with the online version of this paper.

The genus *Alkalibacterium* presently comprises four species: *Alkalibacterium olivapovliticus* (the type species; Ntougias & Russell, 2001), *Alkalibacterium psychrotolerans* (Yumoto *et al.*, 2004), *Alkalibacterium iburiense* (Nakajima *et al.*, 2005) and *Alkalibacterium indicireducens* (Yumoto *et al.*, 2008). These bacteria were isolated from artificial alkaline environments: *A. olivapovliticus* from the wash-water of edible olives, and the other three species from polygonum indigo fermentation liquor. All of the species possess alkaliphilic and halophilic properties: their optimum pH for growth is between 9 and 11.5, and the optimal NaCl concentrations are 3–5, 0–10 and 3–10 % (for each of three strains of *A. olivapovliticus*), 2–12 % (*A. psychrotolerans*), 3–13 % (*A. iburiense*) and 1–11 % (*A. indicireducens*). They produce lactic acid as the main product of glucose fermentation.

Previously, we isolated some slightly alkaliphilic and halophilic lactic acid bacteria from marine organisms. These organisms, members of the genera *Marinilactibacillus* (Ishikawa *et al.*, 2003) and *Halolactibacillus* (Ishikawa *et al.*, 2005), inhabit marine environments and grow preferably under physicochemical conditions found in seawater [total salt concentration, 3.2–3.8 % (w/v); pH, 8.2–8.3 (surface)]. For these organisms, we proposed the term 'marine lactic acid bacteria' (Ishikawa *et al.*, 2003). In the course of further isolation experiments of lactic acid bacteria from marine environments in the subtropical area of Japan and tropical Thailand, we isolated novel halophilic and alkaliphilic lactic acid bacteria, belonging phylogenetically to the genus *Alkalibacterium*, from decaying marine algae, decaying seagrass, raw fish, salted fish and a salted and fermented shrimp paste ('ka-pi'). Here we describe the taxonomic characterization of the isolates, for which the names *Alkalibacterium thalassium* sp. nov., *Alkalibacterium pelagium* sp. nov., *Alkalibacterium putridalgalicola* sp. nov. and *Alkalibacterium kapii* sp. nov. are proposed.

The samples for isolation of lactic acid bacteria were decaying algae that were collected from a foreshore site near the Oujima Islet (26° 07' N 127° 40' E) in Okinawa prefecture, a subtropical area of Japan, in September 1998, and living, raw or decaying algae and seagrass, shellfish, crabs and fish collected from a beach on Koh Samet (Samet Island; 12° 34' N 101° 27' E) in Rayong province and from a beach on Koh Chang (Chang Island; 12° 40' N 101° 17' E) in Trat province, Thailand, in November 1998. The samples of salted fish and salted and fermented shrimp paste ('ka-pi') were obtained from markets in Bangkok and Rayong, Thailand, in November 1998.

The isolates of lactic acid bacteria were obtained by successive enrichment and subsequent pour-plating following procedures described previously (Ishikawa *et al.*, 2003). For isolation from the samples collected in Thailand, a 7 % NaCl GYPF (glucose–yeast extract–peptone–fish extract) isolation broth for enrichment culture and a 7 % NaCl GYPF isolation agar (1.3 % agar) for pour-plating were used. The compositions of these

media were as described previously (Ishikawa *et al.*, 2003), except for pH. The final pH was adjusted to 9.0 by supplementing the media with 6.1 g Na₂CO₃ l⁻¹ and 8.9 g NaHCO₃ l⁻¹. The first enrichment culture was incubated anaerobically at 30 °C for 3 days. For the second enrichment culture, broth medium was inoculated from the first enrichment culture, whose pH had decreased to <7.0, and incubated anaerobically at 30 °C for 2 days. In isolation experiments using the samples collected from Okinawa, Japan, 12 % NaCl GYPFSK broth (pH 7.5) was used for the first enrichment, which was incubated for 21 days, and 18 % NaCl GYPFSK isolation broth was used for the second enrichment, which was incubated for 15 days; both enrichment cultures were grown at 25 °C in standing culture. The compositions of the media and the procedures for isolation were as described previously (Ishikawa *et al.*, 2003).

Ten strains of lactic acid bacteria used for study are described in Table 1, with sources, sites and dates of isolation. *A. olivapovliticus* NCIMB 13710^T, *A. psychrotolerans* JCM 12281^T, *A. iburiense* JCM 12662^T, *A. indicireducens* JCM 14232^T, *Marinilactibacillus psychrotolerans* M13-2^T and *Marinilactibacillus piezotolerans* JCM 12337^T were used as reference strains. The isolates and reference strains were grown on 2.5 % NaCl GYPF agar (pH 9.0; 1.3 % agar) or in 2.5 % NaCl GYPF broth (pH 9.0; Ishikawa *et al.*, 2003) at 30 °C unless described otherwise. Anaerobic cultivation was conducted by using AnaeroPack-Keep (not CO₂-generated; Mitsubishi Gas Chemical) as described previously (Ishikawa *et al.*, 2003).

The methods for characterization of conventional phenotypic features, growth responses to pH, NaCl concentration in the cultivation medium and temperature, analysis of fermentation products, chemotaxonomic analysis, determination of DNA G + C content and DNA–DNA relatedness and phylogenetic analysis were as described previously (Ishikawa *et al.*, 2003, 2005), except as noted below.

Flagellation was observed for cells grown on 75 % seawater-based Bacto tryptic soy agar (Difco) supplemented with 0.1 % (w/v) glucose (pH 9.0) at 25 °C anaerobically for 1 day. Antibiotic sensitivity was investigated by placing a filter-paper disc impregnated with antibiotic onto plates of 2.5 % NaCl GYPF agar. To investigate the effect of the initial pH of the anaerobic cultivation medium on the composition of the glucose-fermentation products, cultivation was performed in 2.5 % NaCl GYPF broth (1 % glucose), which was buffered by adding 100 mM Good's buffers: HEPES at pH 7.0 and CHES at pH 8.0 and 9.0. The composition of cellular fatty acids of representative strains (T117-1-2^T, T143-1-1^T, T129-2-1^T and T22-1-2^T) and reference strains (*A. iburiense* JCM 12662^T, *M. psychrotolerans* M13-2^T and *M. piezotolerans* JCM 12337^T) was analysed by using the Sherlock Microbial Identification system (version 5.0; MIDI) with a gas chromatograph (model HP6890; Hewlett Packard). Fatty acid methyl esters were prepared according to the

Table 1. List of isolates and their sources, sites and dates of isolation

Group*/strain	Isolation source	Collected at	First enrichment medium	Date of isolation
GS1 T117-1-2 ^T	Marine alga	Beach on Ko Samet (Samet Island), Rayong province, Thailand	7 % NaCl GYPF isolation broth (pH 9.0)	25 Nov 1998
GS2 T143-1-1 ^T	Decaying seagrass	Beach on Ko Chang (Chang Island), Trat province, Thailand	7 % NaCl GYPF isolation broth (pH 9.0)	26 Nov 1998
GS3 T129-2-1 ^T	Decaying marine alga	Beach on Ko Samet (Samet Island), Rayong province, Thailand	7 % NaCl GYPF isolation broth (pH 9.0)	25 Nov 1998
O26-12-1	Decaying marine alga	Foreshore site near Oujima Istet, Okinawa prefecture, Japan	12 % NaCl GYPFSK broth (pH 7.5)	5 Oct 1998
O26-12-2	Decaying marine alga	Foreshore site near Oujima Istet, Okinawa prefecture, Japan	12 % NaCl GYPFSK broth (pH 7.5)	5 Oct 1998
O26-12-3	Decaying marine alga	Foreshore site near Oujima Istet, Okinawa prefecture, Japan	12 % NaCl GYPFSK broth (pH 7.5)	5 Oct 1998
GS4 T22-1-2 ^T	Salted and fermented shrimp paste ('ka-pi')	Market in Bangkok, Thailand	7 % NaCl GYPF isolation broth (pH 9.0)	20 Nov 1998
T78-1-2	Salted and fermented shrimp paste ('ka-pi')	Market in the city of Royong, Rayong province, Thailand	7 % NaCl GYPF isolation broth (pH 9.0)	25 Nov 1998
T82-5-1	Salted fish	Market in the city of Royong, Rayong province, Thailand	7 % NaCl GYPF isolation broth (pH 9.0)	25 Nov 1998
T171-1-1	Raw fish	Beach on Ko Chang (Chang Island), Trat province, Thailand	7 % NaCl GYPF isolation broth (pH 9.0)	26 Nov 1998

*Based on the results of DNA–DNA relatedness.

manufacturer's instructions for the MIDI system. All of the tested strains were cultivated by using 2.5 % NaCl GYPF broth (pH 9.0) in standing culture for 1 day.

All 10 new isolates exhibited similar phenotypic features. Cells were Gram-positive and rod-shaped. They were motile with peritrichous flagella, except for strains T117-1-2^T and T22-1-2^T (Fig. 1). They did not produce spores or catalase. Cultural characteristics, cellular morphology, sensitivity to antibiotics and other conventional taxonomic features are given in the descriptions of the novel species.

The 10 isolates all required carbohydrates or related compounds for growth under aerobic, as well as anaerobic, conditions. Whilst the OD₆₆₀ of cultures grown aerobically in 2.5 % NaCl GCY broth (1 % glucose) was 0.3–0.9, that of cultures grown in 2.5 % NaCl GCY broth from which glucose was omitted was <0.1. Similarly, growth was poor in 2.5 % NaCl GYPF broth in which the glucose concentration was reduced from 1.0 to 0.1 %, and did not occur when glucose was omitted from the broth.

The 10 isolates were aerotolerant. When semi-solid 2.5 % NaCl GYPF medium (0.15 % agar) in a test tube was inoculated with a small amount of broth culture, mixed and incubated statically, the colonies that developed were uniform in both density and size throughout the medium column (about 6 cm in height). When cultivated on an

agar plate exposed to the atmosphere, the growth was less vigorous than that on an agar plate incubated anaerobically.

Nearly complete sequences of the 16S rRNA genes of the isolates were compared with those of lactic acid bacteria and other related bacteria from public databases. In the phylogenetic tree, the 10 isolates comprised three phylogenetic clusters within the radiation of the described and phylogenetically assigned species of the genus *Alkalibacterium* (Fig. 2; Supplementary Fig. S1, available in IJSEM Online).

DNA–DNA hybridization experiments showed that these 10 isolates constitute four genomic species. The DNA–DNA relatedness values among the isolates and type strains of phylogenetically related taxa are shown in Supplementary Table S1, available in IJSEM Online. No significant DNA–DNA relatedness was obtained between strains T117-1-2^T, T143-1-1^T and *A. indicireducens* JCM 14232^T, which were constituents of the same phylogenetic cluster (Fig. 2; Supplementary Fig. S1). Each of the other two phylogenetic clusters comprised a single genomic species. These four genomic species are designated groups GS1 (for T117-1-2^T), GS2 (for T143-1-1^T), GS3 (for T129-2-1^T, O26-12-1, O26-12-2 and O26-12-3) and GS4 (for T22-1-2^T, T78-1-2, T82-5-1 and T171-1-1). The G+C contents of the DNA of the isolates fell within the range

Fig. 1. Photomicrographs of a cell and peritrichous flagella of *A. pelagium* T143-1-1^T (a), *A. putridalgicola* T129-2-1^T (b) and *A. kapii* T78-1-2 (c) grown anaerobically at 25 °C for 1 day on 75 % seawater-based Bacto tryptic soy agar supplemented with 0.1 % (w/v) glucose (pH 9.0). Bars, 2 μm.

38.4–42.7 mol%; the values for each genomic species are shown in Supplementary Table S1. Levels of DNA–DNA relatedness among the type strains of the four genomic species and those of species of the genera *Alkalibacterium* and *Marinilactibacillus* were 3–46 % – as low as levels between distinct species (Supplementary Table S1).

16S rRNA gene sequence similarity values among the isolates and members of the genera *Alkalibacterium* and *Marinilactibacillus* are given in Supplementary Table S2, with the GenBank accession numbers of the isolates. Similarity between the four genomic species was 97.1–99.7 %. The similarity between groups GS1 and GS2 was 99.7 %. Groups GS1 and GS2 showed the highest similarity (99.7 and 99.8 %, respectively) to *A. indicireducens* A11^T, which was isolated from polygonum indigo fermentation

liquor (Yumoto *et al.*, 2008). The 16S rRNA gene sequences of isolates belonging to group GS3 were identical to each other and to that of *Alkalibacterium* sp. ARD M22. Their sequences were also 99.2 % similar to that of *Alkalibacterium* sp. ARD M12. These two uncharacterized strains (ARD M12 and ARD M22) were isolated from deep seawater (only the 16S rRNA gene sequences are available; GenBank accession nos AB167070 and AB167074, respectively). The members of group GS4 exhibited 98.1–98.2 % 16S rRNA gene sequence similarity between them and 98.0–98.2 % similarity to the members of group GS3 and *Alkalibacterium* sp. ARD M22. All of the four genomic species were related most closely to *A. indicireducens* among the four described species of the genus *Alkalibacterium*. The similarity values between *A. indicireducens* and each of the genomic species were 99.7 % (group GS1), 99.8 % (group GS2), 98.1–98.2 % (group GS3) and 97.1–97.2 % (group GS4).

The fermentation profiles of carbohydrates, sugar alcohols and related carbon compounds of the isolates are given in Table 2. The isolates utilized a fairly wide range of substrates, except for group GS4, which exhibited a rather limited utilization profile. All of the isolates utilized gluconate without gas production, and starch.

The isolates were slightly halophilic (Kushner, 1978; Kushner & Kamekura, 1988) and halotolerant. The optimum NaCl concentrations for growth were between 1.0 and 2.5 % for group GS1 (T117-1-2^T), between 0.5 and 1.5 % for group GS2 (T143-1-1^T), between 2.0 and 4.0 % for group GS3 and between 1.5 and 2.5 % for group GS4. The maximum specific growth rates of the type strains of the genomic species at each of the different NaCl concentrations tested are described in Supplementary Table S3, available in IJSEM Online. Strain T117-1-2^T (group GS1) was able to grow between 0 % (weak) and 11.0 % NaCl, strain T143-1-1^T between 0 and 17 %, members of group GS3 between 0 % (weak) and 18.0–20.0 % and those of group GS4 between 0 and 19–21 % NaCl.

The isolates were alkaliphiles according to the note of Jones *et al.* (1994): alkaliphiles are organisms that grow optimally at pH values >8. The optimum pH for growth was 9.0 for the single member of group GS1, between 9.0 and 9.5 for that of group GS2, between 8.0 and 9.0 for members of group GS3 and between 8.5 and 9.0 for those of group GS4. No growth was observed in media for which the initial pH was ≤6.5 or ≥11.5 for the single members of groups GS1 and GS2, ≤6.0 (≤5.5 for T129-2-1^T) or ≥10.5 for the members of group GS3 and ≤5.5–6.0 or ≥10.5 for those of group GS4. The maximum specific growth rates of the type strains of the genomic species are described in Supplementary Table S3.

M. psychrotolerans, *Halolactibacillus halophilus* and *Halolactibacillus miurensis* were isolated from marine organisms (decaying marine algae, a live sponge and a fresh shellfish, respectively) and are marine-inhabiting

Fig. 2. Phylogenetic relationships among the 10 new isolates and related bacteria. The tree, constructed by the neighbour-joining method, is based on a comparison of approximately 1400 nt. Bootstrap values, expressed as a percentage of 1000 replications, are given at branching points; only values >50% are shown. Bar, 0.01 K_{nuc} .

lactic acid bacteria with growth optima at 2.00–3.75% NaCl and pH 8.5–9.0, consistent with the physicochemical conditions of seawater (Ishikawa *et al.*, 2003, 2005). For these organisms, we previously proposed the term ‘marine lactic acid bacteria’ (Ishikawa *et al.*, 2003). As described later, the 10 present isolates are also lactic acid bacteria, producing lactic acid as the main product of glucose fermentation. Further, the isolates were marine inhabitants, isolated from marine organisms or salted foods produced from marine organisms and had halophilic and alkaliphilic properties consistent with those of seawater. These new isolates can be regarded as additional representatives of marine lactic acid bacteria within the phylogenetic radiation of typical lactic acid bacteria. Recently, new members of the genus *Alkalibacterium* have been isolated from other marine environments: *Alkalibacterium* spp. ARD M12 and ARD M22 from deep seawater potentially affected by a hydrothermal plume, Knipovich Ridge, Arctic Ocean (only the 16S rRNA gene sequences are available).

The optimum growth temperatures of the isolates were 37 °C (groups GS1 and GS2), 37–40 °C (group GS3) and 25–37 °C (group GS4). The maximum specific growth rates of the type strains of the four genomic species at 25.0–

42.5 °C, near their temperature optima, are shown in Supplementary Table S3. The ranges of temperature for growth were 10.0–42.5 °C (group GS1), 10.0–47.5 °C (group GS2), –1.8 to 40–45 °C (group GS3) and 5–10 to 40.0–42.5 °C (group GS4). The members of group GS3 grew well at lower temperatures, in terms of maximum growth attained, as did *M. psychrotolerans* (Ishikawa *et al.*, 2003), although growth rates were low. OD₆₆₀ values at –1.8 °C were 0.4–0.5 (20 days) and those at 30–37 °C (i.e. optimum temperature) were 1.0–1.2 (20–24 h).

Fermentation products from glucose were analysed for anaerobic cultures grown in 2.5% NaCl GYPF broth. Lactate, formate, acetate and ethanol were produced without generation of gas. The yield of lactate per glucose molecule consumed was 64–93%, depending on the isolate. The relative amounts of formate, acetate and ethanol (molar ratio) were approximately 2:1:1. The major optical isomer of lactate was the L-type for groups GS2, GS3 and GS4, with a relative ratio of 59–82% of the total lactate produced, whereas group GS1 produced solely D-lactate. The effect of the initial pH of the fermentation medium on the yield of lactate produced (mol glucose consumed)^{–1} was investigated by using buffered medium

Table 2. Profiles of sugar fermentation of the 10 new isolates

All 10 strains fermented D-ribose, D-glucose, D-fructose, D-mannose, maltose, sucrose, trehalose, starch and sodium gluconate and did not ferment L-rhamnose, melibiose, adonitol, *myo*-inositol or dulcitol. Fermentation was scored as positive (+) when the titre of 0.1 M NaOH per 5 ml culture broth was ≥ 0.7 ml, as weakly positive (w) when the titre was ≥ 0.5 ml and < 0.7 ml, and as negative (–) for titres < 0.5 ml.

Carbon compound	Group/strain									
	GS1	GS2	GS3				GS4			
	T117-1-2 ^T	T143-1-1 ^T	T129-2-1 ^T	O26-12-1	O26-12-2	O26-12-3	T22-1-2 ^T	T78-1-2	T82-5-1	T171-1-1
L-Arabinose	—	+	+	+	+	+	—	—	—	—
D-Arabinose	—	—	—	—	—	—	—	—	—	+
D-Xylose	—	+	+	+	+	+	—	—	—	—
D-Galactose	+	+	+	—	—	—	—	—	—	—
Cellobiose	+	+	+	+	+	+	—	+	+	+
Lactose	w	+	w	+	+	+	—	—	—	—
Raffinose	w	w	+	+	+	+	—	—	—	—
D-Salicin	+	+	+	+	+	+	—	—	—	—
Melezitose	—	—	—	+	+	+	—	—	—	—
D-Mannitol	—	—	—	+	+	+	—	—	—	—
D-Sorbitol	—	—	—	+	+	+	+	—	—	—
α-Methyl-D-glucoside	—	—	—	—	+	+	—	—	—	—
Glycerol	w	—	—	+	w	+	—	—	+	w
Inulin	—	—	+	—	+	+	—	—	—	—

for the representative strains T117-1-2^T (group GS1), T143-1-1^T (group GS2), T129-2-1^T (group GS3) and T22-1-2^T (group GS4). To minimize the effect of a pH change that might occur during cultivation, product analysis was performed when cultures were at an early stage of growth (i.e. OD₆₆₀ of approx. 0.2). The initial pH markedly affected the yield of lactate (Table 3). As the initial acidity increased, the yield of lactate from glucose increased; in the case of T143-1-1^T (group GS2), the yield of 48% at pH 9 increased to 87% at pH 7, whereas the yields of the other three products decreased, retaining the relative molar ratio among them. Except for *M. piezotolerans* (which produced similar amounts of lactic acid regardless of pH), the reference strains (type strains of *M. psychrotolerans*, *A. olivapovliticus*, *A. psychrotolerans* and *A. iburiense*) exhibited essentially the same inverse relationship of lactate yield with regard to the initial pH value of the medium. As has been discussed for the lactic acid bacteria *Marinilactibacillus* and *Halolactibacillus* and for *A. olivapovliticus* (Ishikawa *et al.*, 2003, 2005), we deduced that the new isolates and described species of the genus *Alkalibacterium* possessed the Embden–Meyerhof pathway and the pyruvate-associated enzymes lactate dehydrogenase and pyruvate–formate lyase, whose relative activities were affected by the pH value of the fermentation medium. Several homofermentative lactic acid bacteria produce formate, acetate and ethanol in a relative molar ratio of 2:1:1 with production of lactate from glucose fermentation; these organisms also showed decreased production of

lactate, while inversely increasing production of the other three products, in media with alkaline pH or limited glucose (Gunsalus & Niven, 1942; Carlsson & Griffith, 1974; Rhee & Park, 1980). These cultivation conditions are considered to affect the relative activities of lactate dehydrogenase and pyruvate–formate lyase, thereby altering the balance between lactate and other products (Carlsson & Griffith, 1974; Yamada & Carlsson, 1975; Axelsson, 1993).

The 10 new isolates grew aerobically, although they lack catalase and respiratory quinones (see later section). The type strains of each genomic species, T117-1-2^T (group GS1), T143-1-1^T (group GS2), T129-2-1^T (group GS3) and T22-1-2^T (group GS4), produced acetate and lactate from glucose under aerobic conditions, but did not generate formate or ethanol. The type strains of *A. olivapovliticus*, *A. psychrotolerans*, *A. iburiense* and *M. psychrotolerans* behaved similarly under these conditions; *M. piezotolerans* JCM 12337^T produced only acetate (Table 4).

The behaviour of the isolates under aerobic conditions described above is similar to that of *Amphibacillus xylanus* (Niimura *et al.*, 1990). *Amphibacillus xylanus* produces formate, acetate and ethanol with a molar ratio of 2:1:1 from pyruvate generated through the Embden–Meyerhof pathway under anaerobic conditions. Although lacking catalase, cytochromes and quinones and possessing an anaerobic pathway, *Amphibacillus xylanus* has an aerobic pathway that is not mediated by the respiratory chain,

Table 3. Effect of initial pH of culture medium on the product balance of glucose fermentation by the type strains of each genomic species and phylogenetically related species

Strain	Initial pH	End products [mol (mol glucose) ⁻¹]				Lactate yield from consumed glucose (%)	Carbon recovery (%)
		Lactate	Formate	Acetate	Ethanol		
T117-1-2 ^T (group GS1)	7	1.06	0.69	0.50	0.54	53	99
	8	0.81	0.98	0.59	0.52	40	93
	9	0.30	1.84	0.92	0.81	15	103
T143-1-1 ^T (group GS2)	7	1.71	0.76	0.24	0.33	87	119
	8	1.20	0.99	0.44	0.29	60	101
	9	0.95	0.77	0.43	0.50	48	92
T129-2-1 ^T (group GS3)	7	1.77	0.27	0.10	0.06	88	98
	8	1.03	1.45	0.69	0.43	51	112
	9	0.96	1.56	0.77	0.40	48	113
T22-1-2 ^T (group GS4)	7	1.76	0.32	0.14	0.11	88	101
	8	1.04	1.12	0.52	0.30	52	98
	9	0.93	1.38	0.65	0.39	47	104
<i>A. olivapovliticus</i> NCIMB 13710 ^{T*}	8	1.35	0.97	0.44	0.52	67	116
	9	1.01	1.21	0.53	0.52	50	106
	10	0.78	1.49	0.65	0.75	39	111
<i>A. psychrotolerans</i> JCM 12281 ^T	8	0.82	1.21	0.63	0.49	41	98
	9	0.51	1.20	0.62	0.78	25	92
	10	0.30	1.74	1.13	0.77	15	108
<i>A. iburiense</i> JCM 12662 ^T	8	0.54	1.63	5.57	7.98	27	109
	9	0.52	1.46	0.67	0.79	26	98
	10	0.20	2.43	0.61	0.90	10	100
<i>M. psychrotolerans</i> M13-2 ^{T*}	7	2.02	0.15	0.04	0.05	101	107
	8	1.50	0.52	0.20	0.19	75	97
	9	1.29	0.81	0.35	0.20	66	98
<i>M. piezotolerans</i> JCM 12337 ^T	7	1.07	0.72	0.43	0.39	54	93
	8	1.07	0.83	0.51	0.44	54	99
	9	1.06	0.77	0.52	0.37	53	95

*Data from Ishikawa *et al.* (2003).

producing only acetate and CO₂ from glucose (Niimura *et al.*, 1989). Oxidative pathways similar to that of *Amphibacillus xylanus* have been reported for several other lactic acid bacteria, including *Streptococcus mutans*, *Lactobacillus* spp., *Streptococcus* spp., *Leuconostoc* spp., *Trichococcus flocculiformis*, *M. psychrotolerans*, *H. halophilus* and *H. miurensis* (Fukui *et al.*, 1988; Sakamoto & Komagata, 1996; Liu *et al.*, 2002; Ishikawa *et al.*, 2005). As these reports suggest, the four genomic species described here, as well as *A. olivapovliticus*, *A. psychrotolerans*, *A. iburiense* and *M. piezotolerans*, probably have a system for aerobic metabolism of glucose similar to that present in *Amphibacillus xylanus*, *M. psychrotolerans*, *Halolactibacillus* spp. and other lactic acid bacteria.

meso-Diaminopimelic acid was not associated with the cell-wall peptidoglycans of the new isolates as determined by TLC. The purified cell walls of strains T117-1-2^T (group GS1), T143-1-1^T (group GS2) and T129-2-1^T (group GS3) contained aspartic acid, glutamic acid, alanine and ornithine in a molar ratio of 0.43–0.54:1:1.9–2.3:0.95–

0.98. In comparison, strain T22-1-2^T (group GS4) contained glutamic acid, alanine and ornithine in a molar ratio of 1:1.2:0.7. From these results, it was inferred that strains T117-1-2^T (group GS1), T143-1-1^T (group GS2) and T129-2-1^T (group GS3) contained peptidoglycan type A4β, Orn–D–Asp, which is the cell-wall type of *A. olivapovliticus* (Ishikawa *et al.*, 2003) and differs from those of *A. psychrotolerans* and *A. indicireducens* [type A4α, Lys (Orn)–D–Asp] (Nakajima *et al.*, 2005; Yumoto *et al.*, 2008) and *A. iburiense* (type A4α, Lys–D–Asp) (Yumoto *et al.*, 2004). Strain T22-1-2^T (group GS4) had peptidoglycan type A4β, Orn–D–Glu, which is the cell-wall type of *M. psychrotolerans* (Ishikawa *et al.*, 2003) and differs from those of the other three genomic species and the four described species of the genus *Alkalibacterium*. Thus, the genus *Alkalibacterium* comprises three types of species with regard to cell-wall type of peptidoglycan.

The major cellular fatty acids of strains T117-1-2^T (group GS1), T143-1-1^T (group GS2), T129-2-1^T (group GS3) and T22-1-2^T (group GS4) were straight-chain saturated and

Table 4. Products from glucose under aerobic cultivation of the type strains of each genomic species and phylogenetically related species

Formate and ethanol were not detected (ND) for any of the strains.

Strain	Glucose consumed (mM)	Products (mM)		Carbon recovery (%)*
		Lactate	Acetate	
T117-1-2 ^T (group GS1)	12.6	9.8	16.5	104
T143-1-1 ^T (group GS2)	15.4	9.1	19.7	93
T129-2-1 ^T (group GS3)	21.6	35.7	8.5	102
T22-1-2 ^T (group GS4)	13.6	17.0	12.9	110
<i>A. olivapovlitis</i> NCIMB 13710 ^T	15.5	14.4	18.2	105
<i>A. psychrotolerans</i> JCM 12281 ^T	15.0	16.1	15.1	104
<i>A. iburiense</i> JCM 12662 ^T	22.1	17.7	25.3	97
<i>M. psychrotolerans</i> M13-2 ^T †	33.2	21.5	40.0	93
<i>M. piezotolerans</i> JCM 12337 ^T	7.8	ND	15.7	101

*Carbon recovery was calculated on the assumption that acetate and CO₂ were produced in equimolar amounts.

†Data from Ishikawa *et al.* (2005)

monounsaturated, even-carbon-numbered types (Supplementary Table S4, available in IJSEM Online), primarily C_{16:0}, C_{16:1}ω9c and C_{18:1}ω9c (oleic acid).

Respiratory quinones were not present in strains T117-1-2^T (group GS1), T143-1-1^T (group GS2), T129-2-1^T (group GS3) or T22-1-2^T (group GS4).

All four genomic species were similar to those of the genera *Alkalibacterium* and *Marinilactibacillus* in the following characteristics: cellular morphology, various biochemical features, halophilic/halotolerant and alkaliphilic/alkalitolerant properties, products from glucose under aerobic and anaerobic conditions, effect of pH of medium on fermentation products and the G+C content of the DNA. Although sharing important taxonomic features, the four novel genomic species, the four described species of the genus *Alkalibacterium* and the two described species of the sister genus *Marinilactibacillus* can be distinguished from each other by a combination of features of the sugar-fermentation pattern, sensitivity to antibiotics, growth responses to NaCl concentration, pH and temperature, ability to produce ammonia from arginine, peptidoglycan type and G+C content of the DNA (Table 5).

Phylogenetically, the described four species of the genus *Alkalibacterium* and the four genomic species described in the present study compose an independent cluster separate from the cluster of the genus *Marinilactibacillus* in phylogenetic trees reconstructed by both the neighbour-joining and the maximum-likelihood methods (Fig. 2; Supplementary Fig. S1, available in IJSEM Online).

In conclusion, on the basis of the phenotypic features and phylogenetic and genetic independence that we have described, the isolates should be classified as belonging to four novel species of the genus *Alkalibacterium*. We propose the names *Alkalibacterium thalassium* sp. nov.

for group GS1, *Alkalibacterium pelagium* sp. nov. for group GS2, *Alkalibacterium putridalgalicola* sp. nov. for group GS3 and *Alkalibacterium kapii* sp. nov. for group GS4.

Description of *Alkalibacterium thalassium* sp. nov.

Alkalibacterium thalassium [tha.las'si.um. N.L. neut. adj. *thalassium* (from Gr. adj. *thalassios* -a -on) of/from the sea].

Cells are Gram-positive, non-sporulating, non-motile, straight rods measuring 0.6–1.4 × 3.6–9.0 µm, occurring singly, in pairs or in short chains. Deep colonies in 2.5% NaCl GYPF agar medium are pale yellow, opaque and lenticular, with diameters of 1.0–2.0 mm after 3 days at 30 °C. Surface colonies are round, convex, entire, pale yellow and transparent, with diameters of 0.5–1.0 mm after 3 days at 30 °C. Aerotolerant. Grows evenly throughout a column of semi-solid agar medium. Catalase- and oxidase-negative. Quinones are not detected. Negative for nitrate reduction, gelatin liquefaction and production of ammonia from L-arginine. Slightly halophilic; the optimum NaCl concentration for growth is 1.0–2.5% (w/v), with a range of 0.0–11.0% (w/v). Alkaliphilic; the optimum pH for growth is 9.0, with a range of 7.0–11.0. Growth occurs between 10 and 42.5 °C, with an optimum temperature of 37 °C. Lactic acid is the major fermentation product from D-glucose; other products are formate, acetate and ethanol with a molar ratio of approximately 2:1:1, without gas formation. Lactate yield decreases at higher pH of the fermentation medium (analysed for the type strain). D-Glucose is metabolized aerobically to acetate in addition to lactate (analysed for the type strain). The fermentation profile of carbohydrates, sugar alcohols and related carbon compounds is given in Table 2. Not inhibited by chloramphenicol, kanamycin or trimethoprim; inhibited by ampicillin. The peptidoglycan is A4β, Orn-D-Asp type. The major cellular fatty acids are C_{16:0}, C_{16:1}ω9c and

C_{18:1}ω₉c (oleic acid). The G+C content of the DNA of strain T117-1-2^T is 41.7 mol%.

The type strain is T117-1-2^T (=DSM 19181^T=NBRC 103241^T=NRIC 0718^T), which was isolated from a decaying marine alga collected at the seashore of Ko Samet (Samet Island), Rayong province, Thailand.

Description of *Alkalibacterium pelagium* sp. nov.

Alkalibacterium pelagium (pe.la'gi.um. N.L. neut. adj. *pelagium* of the sea, marine).

Cells are Gram-positive, non-sporulating, straight rods measuring 0.3–0.9 × 1.8–7.2 μm, occurring singly, in pairs or in short chains. Motile with peritrichous flagella. Deep colonies in 2.5 % NaCl GYPF agar medium are pale yellow, opaque and lenticular, with diameters of 1.0–2.0 mm after 3 days at 30 °C. Surface colonies are round, convex, entire, pale yellow and transparent, with diameters of 0.5–1.0 mm after 3 days at 30 °C. Aerotolerant. Grows evenly throughout a column of semi-solid agar medium. Catalase- and oxidase-negative. Quinones are not detected. Negative for nitrate reduction, gelatin liquefaction and production of ammonia from L-arginine. Slightly halophilic and highly halotolerant; the optimum NaCl concentration for growth is 0.5–1.5 % (w/v) with a range of 0.0–17.0 % (w/v). Alkaliphilic; the optimum pH for growth is 9.0–9.5, with a range of 7.0–11.0. Growth occurs between 10 and 47.5 °C, with an optimum temperature of 37 °C. Lactic acid is the major fermentation product from D-glucose; other products are formate, acetate and ethanol with a molar ratio of approximately 2 : 1 : 1, without gas formation. Lactate yield decreases at higher pH of the fermentation medium (analysed for the type strain). D-Glucose is metabolized aerobically to acetate in addition to lactate (analysed for the type strain). The fermentation profile of carbohydrates, sugar alcohols and related carbon compounds is given in Table 2. Not inhibited by chloramphenicol or kanamycin; inhibited by ampicillin and trimethoprim. The peptidoglycan is A4β, Orn–D-Asp type. The major cellular fatty acids are C_{16:0}, C_{16:1}ω₉c and C_{18:1}ω₉c (oleic acid). The G+C content of the DNA of strain T143-1-1^T is 42.2 mol%.

The type strain is T143-1-1^T (=DSM 19183^T=NBRC 103242^T=NRIC 0719^T), which was isolated from decaying seagrass collected at the seashore of Ko Chang (Chang Island), Trat province, Thailand.

Description of *Alkalibacterium putridalgicola* sp. nov.

Alkalibacterium putridalgicola [pu.tri.dal.gi'co.la. L. adj. *putridus* rotten, decayed; L. fem. n. *alga* seaweed; L. suff. -cola (from L. n. *incola*) dweller; N.L. n. *putridalgicola* dweller on putrid marine algae].

Cells are Gram-positive, non-sporulating, straight rods measuring 0.6–0.9 × 2.7–8.0 μm, occurring singly, in pairs or in short chains. Motile with peritrichous flagella. Deep

colonies in 2.5 % NaCl GYPF agar medium are pale yellow, opaque and lenticular, with diameters of 1.0–2.0 mm after 3 days at 30 °C. Surface colonies are round, convex, entire, pale yellow and transparent, with diameters of 0.5–1.0 mm after 3 days at 30 °C. Aerotolerant. Grows evenly throughout a column of semi-solid agar medium. Catalase- and oxidase-negative. Quinones are not detected (analysed for the type strain). Negative for nitrate reduction and gelatin liquefaction. Ammonia is produced from L-arginine. Slightly halophilic and highly halotolerant; the optimum NaCl concentration for growth is 2.0–4.0 % (w/v), with a range of 0.0 to 18.0–20.0 % (w/v). Alkaliphilic; the optimum pH for growth is 8.0–9.0, with a range of 6.5–10.0. Growth occurs between –1.8 and 40–45 °C, with an optimum temperature of 37–40 °C. Lactic acid is the major fermentation product from D-glucose; other products are formate, acetate and ethanol with a molar ratio of approximately 2 : 1 : 1, without gas formation. Lactate yield decreases at higher pH of the fermentation medium (analysed for the type strain). D-Glucose is metabolized aerobically to acetate in addition to lactate (analysed for the type strain). The fermentation profile of carbohydrates, sugar alcohols and related carbon compounds is given in Table 2. Not inhibited by chloramphenicol or kanamycin; inhibited by ampicillin and trimethoprim. The peptidoglycan type is A4β, Orn–D-Asp (analysed for the type strain). The major cellular fatty acids are C_{16:0}, C_{16:1}ω₉c and C_{18:1}ω₉c (oleic acid) (analysed for the type strain). The G+C content of the DNA is 41.0–43.0 mol% (42.5 mol% for the type strain).

The type strain is T129-2-1^T (=DSM 19182^T=NBRC 103243^T=NRIC 0720^T), which was isolated from a decaying marine alga collected at the seashore of Ko Samet (Samet Island), Rayong province, Thailand. Reference strains O26-12-1, O26-12-2 and O26-12-3 were also isolated from decaying marine algae.

Description of *Alkalibacterium kapii* sp. nov.

Alkalibacterium kapii [ka'pi.i. N.L. n. *kapium* ka-pi (a fermented shrimp paste in Thailand); N.L. gen. n. *kapii* of ka-pi].

Cells are Gram-positive, non-sporulating, straight rods measuring 0.6–1.1 × 1.8–3.6 μm, occurring singly, in pairs or in short chains. Motile with peritrichous flagella except for strain T22-1-2^T. Deep colonies in 2.5 % NaCl GYPF agar medium are creamy yellow, opaque and lenticular, with diameters of 1.0–2.0 mm after 3 days at 30 °C. Surface colonies are round, convex, entire, creamy yellow and transparent, with diameters of 0.5–1.0 mm after 3 days at 30 °C. Aerotolerant. Grows evenly throughout a column of semi-solid agar medium. Catalase- and oxidase-negative. Quinones are not detected (analysed for the type strain). Negative for nitrate reduction, gelatin liquefaction and production of ammonia from L-arginine. Slightly halophilic; the optimum NaCl concentration for growth is 1.5–2.5 % (w/v), with a range of 0.0 to 19.0–21.0 % (w/v). Alkaliphilic; the optimum pH for growth is 8.5–9.0, with a

Table 5. Differential characteristics of the novel *Alkalibacterium* species and phylogenetically related species

Taxa: 1, *Alkalibacterium thalassium* sp. nov.; 2, *Alkalibacterium pelagium* sp. nov.; 3, *Alkalibacterium putridalgicola* sp. nov.; 4, *Alkalibacterium kapii* sp. nov.; 5, *A. olivapovliticus* (Ntougias & Russell, 2001); 6, *A. psychrotolerans* (Yumoto *et al.*, 2004); 7, *A. iburiense* (Nakajima *et al.*, 2005); 8, *A. indicireducens* (Yumoto *et al.*, 2008); 9, *M. psychrotolerans* (Ishikawa *et al.*, 2003); 10, *M. piezotolerans* (Toffin *et al.*, 2005). +, Positive; –, negative; w, weak; v, variable reaction; R, resistant to antibiotic; S, susceptible to antibiotic; WS, weakly susceptible to antibiotic; ND, no data; Asp, aspartic acid; Glu, glutamic acid; Lys, lysine; Orn, ornithine.

Characteristic	1	2	3	4	5	6	7	8	9	10
Motility	–	+	+	+	+	+	+	+	+	–
Flagella	–	Peritrichous	Peritrichous	Peritrichous	Peritrichous*	Peritrichous	Peritrichous	Peritrichous	Peritrichous	–
Colour of colonies	Pale yellow	Pale yellow	Pale yellow	Creamy yellow	Yellowish-white*	Pale white	Pale white	Pale white	Pale yellow	Yellowish-white*
NaCl (%)										
Range	0–11	0–17	0 to 18–20	0 to 19–21	0–15	0–17	0 to 14–16	0–15, 0–14	0 to 17–20.5	0–12.0
Optimum	1–2.5	0.5–1.5	2–4	1.5–2.5	3–5	2–12	3–13	1–11	2–3.75	1.0–2.0
pH										
Range	7–11	7–11	6.0–6.5 to 10.0	6.5–10.0	8.5–10.8	9–12	9–12	9–12.3	6.0–10.0	5.5–10.0
Optimum	9	9.0–9.5	8–9	8.5–9.0	9.0–10.2	9.5–10.5	9.5–10.5	9.5–11.5	8.5–9.0	7.0–8.0
Growth temperature (°C)										
Range	10–42.5	10–47.5	–1.8 to 40–45	5–10 to 40–42.5	4 to 35–37	5–45	5–45	15–40, 18–37	–1.8 to 40–45	4–50
Optimum	37	37	37–40	25–37	27–32	34	30–37	20–30	37–40	37–40
Fermentation of sugars										
L-Arabinose	–	+	+	–	–	+	+	ND	+ or w	–
D-Xylose	–	+	+	–	–	+	v	–	+	+
Sucrose	+	+	+	+	+	–	v	w	+	+
Lactose	w	+	+	–	–	–	+	ND	+ or w	–*
Raffinose	w	w	+	–	–	–	–	–	v	–*
D-Salicin	+	+	+	–	+	+	+	ND	v	+
Antibiotic sensitivity										
Ampicillin (10 and 25 µg)	S	S	S	S	S	S*	S	S	S*	S*
Chloramphenicol (2 µg)	R	R	R	R	S	WS*	R	R	R*	R*
Kanamycin (2 and 10 µg)	R	R	R	R	S	R	R	R	R*	R*
Trimethoprim (10 and 25 µg)	R	S	S	R	S	S*	R	R	R*	R*
NH ₃ from L-arginine	–	–	+	–	w*	w*	w*	ND	w	w*
Peptidoglycan type	A4β, Orn–D–Asp	A4β, Orn–D–Asp	A4β, Orn–D–Asp	A4β, Orn–D–Glu	A4β, Orn–D–Asp	A4α, Lys (Orn)– D–Asp	A4α, Lys–D–Asp	A4α, Lys (Orn)– D–Asp	A4β, Orn–D–Glu	ND

Table 5. cont.

Characteristic	1	2	3	4	5	6	7	8	9	10
Major cellular fatty acids	C _{16:0} C _{16:1} ω9c C _{18:1} ω9c 41.7	C _{16:0} C _{16:1} ω9c C _{18:1} ω9c 42.2	C _{16:0} C _{16:1} ω9c C _{18:1} ω9c 41.0–43.0	C _{16:0} C _{16:1} ω9c C _{18:1} ω9c 38.4–39.4	C _{16:0} C _{16:1} ω9c C _{18:1} ω9c 39.7 ± 1.0	C _{16:0} C _{16:1} ω9c C _{18:1} ω9c 42.6–43.2	C _{16:0} C _{16:1} ω9c C _{18:1} ω9c* 40.6 (39.2*)	C _{16:0} C _{16:1} ω9c C _{18:1} ω9c 47.0–47.8	C _{16:0} C _{16:1} ω9c C _{18:1} ω9c* 34.6–36.2	C _{16:0} C _{16:1} ω9c C _{18:1} ω9c* 42.0 (36.6*)
DNA G + C content (mol%)										

*Determined in this study for the type strains.

†A photomicrograph of a cell and peritrichous flagella is shown in Supplementary Fig. S2, available in IJSEM Online.

‡Cultivated anaerobically on 2.5% NaCl GYPF agar.

§Optimum values for the type strain.

range of 6.0–6.5 to 10.0. Growth occurs between 5–10 and 40–42.5 °C, with an optimum temperature of 25–37 °C. Lactic acid is the major fermentation product from D-glucose; other products are formate, acetate and ethanol with a molar ratio of approximately 2:1:1, without gas formation. Lactate yield decreases at higher pH of the fermentation medium (analysed for the type strain). Carbohydrates and related compounds are metabolized aerobically to acetate in addition to lactate (analysed for the type strain). The fermentation profile of carbohydrates, sugar alcohols and related carbon compounds is given in Table 2. Not inhibited by chloramphenicol, kanamycin or trimethoprim; inhibited by ampicillin. The peptidoglycan type is A4β, Orn-D-Glu (analysed for the type strain). The major cellular fatty acids are C_{16:0}, C_{16:1}ω9c and C_{18:1}ω9c (oleic acid) (analysed for the type strain). The G + C content of the DNA is 38.4–39.4 mol% (39.4 mol% for the type strain).

The type strain is T22-1-2^T (=DSM 19180^T=NBRC 103247^T=NRIC 0724^T), which was isolated from 'ka-pi' (a salted and fermented shrimp paste) obtained at a market near Bangkok, Thailand. Reference strains T78-1-2, T82-5-1 and T171-1-1 were isolated from 'ka-pi', salted fish and raw fish.

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References

- Axelsson, L. T. (1993). Lactic acid bacteria: classification and physiology. In *Lactic Acid Bacteria*, pp. 1–63. Edited by S. Salminen & A. von Wright. New York: Marcel Dekker.
- Carlsson, J. & Griffith, C. J. (1974). Fermentation products and bacterial yields in glucose-limited and nitrogen-limited cultures of streptococci. *Arch Oral Biol* **19**, 1105–1109.
- Fukui, K., Kato, K., Komada, T., Ohta, H., Shimamoto, T. & Shimono, T. (1988). Kinetic study of a change in intracellular ATP level associated with aerobic catabolism of ethanol by *Streptococcus mutans*. *J Bacteriol* **170**, 4589–4593.
- Gunsalus, I. C. & Niven, C. F., Jr (1942). The effect of pH on the lactic acid fermentation. *J Biol Chem* **145**, 131–136.
- Ishikawa, M., Nakajima, K., Yanagi, M., Yamamoto, Y. & Yamasato, K. (2003). *Marinilactibacillus psychrotolerans* gen. nov., sp. nov., a halophilic and alkaliphilic marine lactic acid bacterium isolated from marine organisms in temperate and subtropical areas of Japan. *Int J Syst Evol Microbiol* **53**, 711–720.
- Ishikawa, M., Nakajima, K., Itamiya, Y., Furukawa, S., Yamamoto, Y. & Yamasato, K. (2005). *Halolactibacillus halophilus* gen. nov., sp. nov. and *Halolactibacillus miurensis* sp. nov., halophilic and alkaliphilic marine lactic acid bacteria constituting a phylogenetic lineage in *Bacillus* rRNA group 1. *Int J Syst Evol Microbiol* **55**, 2427–2439.
- Jones, B. E., Grant, W. D., Collins, N. C. & Mwatha, W. E. (1994). Alkaliphiles: diversity and identification. In *Bacterial Diversity and Systematics*, pp. 195–230. Edited by F. G. Priest, A. Ramos-Cormenzana & B. J. Tindall. New York: Plenum Press.

- Kushner, D. J. (1978).** Life in high salt and solute concentrations: halophilic bacteria. In *Microbial Life in Extreme Environments*, pp. 318–346. Edited by D. J. Kushner. London: Academic Press.
- Kushner, D. J. & Kamekura, M. (1988).** Physiology of halophilic eubacteria. In *Halophilic Bacteria*, vol. I, pp. 109–140. Edited by F. Rodríguez-Valera. Boca Raton, FL: CRC Press.
- Liu, J.-R., Tanner, R. S., Schumamm, P., Weiss, N., McKenzie, C. A., Janssen, P. H., Seviour, E. M., Lawson, P. A., Allen, T. D. & Seviour, R. J. (2002).** Emended description of the genus *Trichococcus*, description of *Trichococcus collinsii* sp. nov., and reclassification of *Lactosphaera pasteurii* as *Trichococcus pasteurii* comb. nov. and *Ruminococcus palustris* as *Trichococcus palustris* comb. nov. in the low-G+C Gram-positive bacteria. *Int J Syst Evol Microbiol* **52**, 1113–1126.
- Nakajima, K., Hirota, K., Nodasaka, Y. & Yumoto, I. (2005).** *Alkalibacterium iburiense* sp. nov., an obligate alkaliphile that reduces an indigo dye. *Int J Syst Evol Microbiol* **55**, 1525–1530.
- Niimura, Y., Koh, E., Uchimura, T., Ohara, N. & Kozaki, M. (1989).** Aerobic and anaerobic metabolism in a facultative anaerobe Ep01 lacking cytochrome, quinone and catalase. *FEMS Microbiol Lett* **61**, 79–84.
- Niimura, Y., Koh, E., Yanagida, F., Suzuki, K., Komagata, K. & Kozaki, M. (1990).** *Amphibacillus xylanus* gen. nov., sp. nov., a facultatively anaerobic sporeforming xylan-digesting bacterium which lacks cytochrome, quinone, and catalase. *Int J Syst Bacteriol* **40**, 297–301.
- Ntougias, S. & Russell, N. (2001).** *Alkalibacterium olivoapovlenticus* gen. nov., sp. nov., a new obligately alkaliphilic bacterium isolated from edible-olive wash-waters. *Int J Syst Evol Microbiol* **51**, 1161–1170.
- Rhee, S. K. & Park, M. Y. (1980).** Effect of environmental pH on fermentation balance of *Lactobacillus bulgaricus*. *J Bacteriol* **144**, 217–221.
- Sakamoto, M. & Komagata, K. (1996).** Aerobic growth of and activities of NADH oxidase and NADH peroxidase in lactic acid bacteria. *J Ferment Bioeng* **82**, 210–216.
- Toffin, L., Zink, K., Kato, C., Pignet, P., Bidault, A., Bienvenu, N., Birrien, J.-L. & Prieur, D. (2005).** *Marinilactibacillus piezotolerans* sp. nov., a novel marine lactic acid bacterium isolated from deep sub-seafloor sediment of the Nankai Trough. *Int J Syst Evol Microbiol* **55**, 345–351.
- Yamada, T. & Carlsson, J. (1975).** Regulation of lactate dehydrogenase and change of fermentation products in streptococci. *J Bacteriol* **124**, 55–61.
- Yumoto, I., Hirota, K., Nodasaka, Y., Yokota, Y., Hoshino, T. & Nakajima, K. (2004).** *Alkalibacterium psychrotolerans* sp. nov., a psychrotolerant obligate alkaliphile that reduces indigo dye. *Int J Syst Evol Microbiol* **54**, 2379–2383.
- Yumoto, I., Hirota, K., Nodasaka, Y., Tokiwa, Y. & Nakajima, K. (2008).** *Alkalibacterium indicireducens* sp. nov., an obligate alkaliphile that reduces indigo dye. *Int J Syst Evol Microbiol* **58**, 901–905.