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

Morio Ishikawa, Kazuyuki Nakajima, Yuko Itamiya, Sayumi Furukawa, Yasushi Yamamoto and Kazuhide Yamasato

Department of Fermentation Science, Faculty of Applied Bio-Science, Tokyo University of Agriculture, 1-1 Sakuragaoka 1-chome, Setagaya-ku, Tokyo 156-8502, Japan

Eleven novel strains of marine-inhabiting lactic acid bacteria that were isolated from living and decaying marine organisms collected from a temperate area of Japan are described. The isolates were motile with peritrichous flagella and non-sporulating. They lacked catalase, quinones and cytochromes. Fermentation products from glucose were lactate, formate, acetate and ethanol. Lactate yield as percentage conversion from glucose was affected by the pH of the fermentation medium: ~55 % at the optimal growth pH of 8-0, greater than ~70 % at pH 7-0 and less than ~30 % at pH 9-0. The molar ratio of the other three products was the same at each cultivation pH, approximately 2 : 1 : 1. Carbohydrates and related compounds were aerobically metabolized to acetate and pyruvate as well as lactate. The isolates were slightly halophilic, highly halotolerant and alkaliphilic. The optimum NaCl concentration for growth was 2-0–3-0 % (w/v), with a range of 0–25-5 %. The optimum pH for growth was 8-0–9-5, with a range of 6-0–10-0. The G+C content of the DNA was 38-5–40-7 mol%. The isolates constituted two genomic species (DNA–DNA relatedness of less than 41 %) each characterized by sugar fermentation profiles. The cell-wall peptidoglycan of both phenotypes contained meso-diaminopimelic acid. The major cellular fatty acids were C16 : 0 and a-C13 : 0. Comparative sequence analysis of the 16S rRNA genes revealed that these isolates represent novel species constituting a phylogenetic unit outside the radiation of typical lactic acid bacteria and an independent line of descent within the group composed of the halophilic/halotolerant/alkaliphilic and/or alkalitolerant species in Bacillus rRNA group 1, with 94-8–95-1 % similarity to the genus Paraliobacillus, 93-7–94-1 % to the genus Gracilibacillus and 93-8–94-2 % to Virgibacillus marismortui. On the basis of possession of physiological and biochemical characteristics common to typical lactic acid bacteria within Bacillus rRNA group 1, chemotaxonomic characteristics and phylogenetic independence, a new genus and two species, Halolactibacillus halophilus gen. nov., sp. nov. and Halolactibacillus miurensis sp. nov., are proposed. The type strains are Halolactibacillus halophilus M2-2T (= DSM 17073T = IAM 15242T = NBRC 100868T = NRIC 0628T) (G+C content 40-2 mol%) and Halolactibacillus miurensis M23-1T (= DSM 17074T = IAM 15247T = NBRC 100873T = NRIC 0633T) (G+C content 38-5 mol%).

Abbreviations: HA group, halophilic/halotolerant/alkaliphilic and/or alkalitolerant group.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains M2-2T and M23-1T are AB196783 and AB196784, respectively.

A phylogenetic tree constructed using the maximum-likelihood method showing the relationships between the new isolates and other related bacteria, and tables detailing the products from glucose under aerobic and anaerobic cultivation conditions and the cellular fatty acid compositions of Halolactibacillus halophilus M2-2T and Halolactibacillus miurensis M23-1T and related taxa are available as supplementary material in IJSEM Online.
We previously reported the isolation, taxonomic characterization and phylogenetic position of a lactic acid bacterium named *Marinilactobacillus psychrotolerans* (Ishikawa et al., 2003b). It was isolated from marine organisms (living sponge, decaying alga and raw shellfish) and is slightly halophilic, highly halotolerant and alkaliphilic, growing preferably under the physico-chemical conditions found in sea water [total salt concentration, 3–2–3.8 % (w/v); pH 8.2–8.3 (surface)]. For this marine-inhabiting organism, we proposed the term ‘marine lactic acid bacterium’. In addition to the isolation and proposal of the genus *Marinilactobacillus*, a Gram-positive, spore-forming, facultatively anaerobic, halophilic, halotolerant, ‘slightly’ alkaliphilic and marine-inhabiting bacterium named *Paraliobacillus ryukyuensis* was isolated from a decaying marine alga (Ishikawa et al., 2002, 2003a). It produces lactate under anaerobic conditions and belongs to the phylogenetic group whose members have halophilic/halotolerant/alkaliphilic and/or alkali-tolerant properties (Garabito et al., 1997; Heyndrickx et al., 1999; Wainö et al., 1999; Lu et al., 2001; Zhilina et al., 2001, 2002; Yoon et al., 2002) within *Bacillus* rRNA group 1 (Ash et al., 1991) in the phyletic assemblage of bacteria classically defined as the genus *Bacillus*. Most of the member species of this phylogenetic group were isolated from saline or hypersaline environments: sediments of saline lakes, hypersaline soils, solar salterns, mud of deep-sea ridge and salt fields.

In the course of isolating a marine-inhabiting lactic acid bacterium, novel halophilic and alkaliphilic lactic acid bacteria belonging to this halophilic/halotolerant/alkaliphilic and/or alkali-tolerant group (henceforth referred to as the HA group) were isolated from decaying marine alga and a living sponge. Here we describe the isolation, taxonomic characterization and phylogenetic positions of the isolates, for which the names *Halolactibacillus halophilus* gen. nov., sp. nov. and *Halolactibacillus miurensis* sp. nov. are proposed.

The samples for the isolation of lactic acid bacteria were collected from Oura beach (35°08’N 139°40’E) on the Miura Peninsula in Kanagawa Prefecture, in the middle of the Japanese mainland, a temperate area, in July 1998. The samples were collected from the Japanese mainland, a temperate area, in July 1998. The samples for the isolation of lactic acid bacteria were collected from Oura beach (35°08’N 139°40’E) on the Miura Peninsula in Kanagawa Prefecture, in the middle of the Japanese mainland, a temperate area, in July 1998. The samples were collected from the Miura Peninsula in Kanagawa Prefecture, in the middle of the Japanese mainland, a temperate area, in July 1998. The samples were collected from the Miura Peninsula in Kanagawa Prefecture, in the middle of the Japanese mainland, a temperate area, in July 1998. The samples were collected from the Miura Peninsula in Kanagawa Prefecture, in the middle of the Japanese mainland, a temperate area, in July 1998.

Eleven bacterial strains were isolated from three kinds of decaying marine alga and a living sponge. Four isolates (M2-1, M2-2T, M2-3 and M2-4) were obtained from a decaying alga. One isolate, M9-1, was from another decaying alga. Another isolate, M13-1, was from a living sponge. Five isolates (M23-1T, M23-2, M23-3, M23-4 and M23-5) were from a third kind of alga.


For cultivation and taxonomic characterization, a 2.5 % NaCl GYPF broth, composed of 10 g glucose, 5 g yeast extract (Oriental Yeast), 5 g Polypeptone (Nippon Seiyaku), 5 g Extract Bonito (fish extract; Wako Pure Chemical), 1 g K2HPO4, 25 g NaCl, 1 g sodium thioglycolate, 5 ml salt solution ([mol l−1]: 40 mg MgSO4.7H2O, 2 mg MnSO4.4H2O and 2 mg FeSO4.7H2O) (Okada et al., 1992), and distilled water to 1000 ml, was used as the basal medium unless otherwise stated. The medium was adjusted to pH 8.5 and sterilized by filtration. When a larger cell mass was needed for cellular component analysis, the concentration of K2HPO4 in the 2.5 % NaCl GYPF broth was increased from 0.1 to 1 % to buffer the cultivation medium and improve growth (2.5 % NaCl GYPFK broth). The isolates were stored at 5 °C in this medium and transferred at 1-month intervals as described previously (Ishikawa et al., 2003b). The isolates were grown by standing cultivation at 30 °C unless otherwise stated. Anaerobic cultivation was conducted by using AnaeroPak- Kenki (CO2-generated; Mitsubishi Gas Chemical), as described previously (Ishikawa et al., 2003b). For cultivation of the reference strains the following media were used: *Marinilactibacillus psychrotolerans* M13-2T and *Paraliobacillus ryukyuensis* O15-7T, the same medium as for the isolates; *Amphibacillus xylanus* NRIC 1994T, 2.5 % NaCl GYPF broth without NaCl, pH 9.0; *Amphibacillus fermentum* DSM 13869T and *Amphibacillus tropicus* DSM 13870T, 2.5 % NaCl GYPF broth but without NaCl and salt solution, and addition of (1−1): 0.1 g MgCl2·6H2O, 0.2 g KCl, 50-4 g NaHCO3 and 63.6 g Na2CO3, pH 9.5; *G. halotolerans* DSM 11805T and *G. dipsosauri* DSM 11125T, marine broth 2216 (Difco) with addition of 10 g glucose l−1 and 5 g KCl l−1. All media were sterilized by filtration. *Marinilactibacillus psychrotolerans* M13-2T and *Paraliobacillus ryukyuensis* O15-7T were cultivated in the same way as the isolates. *Amphibacillus* species were grown by standing cultivation at 37 °C and *Gracilibacillus* species by shaking at 30 °C.

Cellular morphology, cultural characteristics, motility, Gram staining and flagellation were characterized as described previously (Ishikawa et al., 2002). Spore formation was examined microscopically for cultures grown at 37 °C.
on 2.5 % NaCl GYPF agar, marine agar 2216 with or without 5 p.p.m. Mn$^{2+}$ (MnSO$_4$), and 2 % NaCl yeast extract salts agar (pH 8.5) composed of 5 g yeast extract, 20 g NaCl, 5 g MgSO$_4$·7H$_2$O, 2 g CaCl$_2$, 1 g K$_2$HPO$_4$, 5 ml salt solution (see 2.5 % NaCl GYPF broth) and 15 g agar in 1 l distilled water, by using the method described previously (Ishikawa et al., 2002). Aerobic utilization of glucose was examined by using 2.5 % NaCl GCY broth, composed of 10 g glucose, 5 g Vitamin assay Casamino acids (Difco), 0.5 g yeast extract, 25 g NaCl, 1 g K$_2$HPO$_4$, 5 ml salt solution and distilled water in 1 l volume, as described previously (Ishikawa et al., 2002). Aerobic cultivation was conducted by shaking in a cotton-plugged, L-shaped test tube containing 5 ml of the medium, on a reciprocal shaker (125 strokes min$^{-1}$).

Catalase activity was examined by observing the evolution of bubbles upon addition of 3 % H$_2$O$_2$ solution to aerobically or anaerobically grown cells viewed under a stereoscopic microscope. Oxidase activity, growth behaviour as a function of oxygen concentration and sugar fermentation were studied as described previously (Ishikawa et al., 2003b). Production of acid was scored as positive when the titre was 0.1 M NaOH per 5 ml culture broth was ≥0.7 ml, as weakly positive when the titre was ≥0.5 ml and <0.7 ml, and as negative for titres <0.5 ml. Tests for nitrate reduction, and production of gas from glucose, ammonia from arginine and dextran from glucose were determined from the maximum specific growth rate, and pH. Growth optima for these physiological conditions and minimum concentrations of NaCl, growth temperature of NaCl concentration, pH and temperature, and maximum fermentation products from glucose consumed were anaerobically grown cells viewed under a stereoscopic microscope. Oxidase activity, growth behaviour as a function of oxygen concentration and sugar fermentation were studied as described previously (Ishikawa et al., 2002). Production of acid was scored as positive when the titre was 0.1 M NaOH per 5 ml culture broth was ≥0.7 ml, as weakly positive when the titre was ≥0.5 ml and <0.7 ml, and as negative for titres <0.5 ml. Tests for nitrate reduction, and production of gas from glucose, ammonia from arginine and dextran from glucose were conducted as described previously (Okada et al., 1992). Hydrolysis of DNA (DNase test agar; Difco), casein and gelatin (Difco) was tested using agar plate methods, as described by Gerhardt et al. (1994). Media used for the investigation of these conventional taxonomic features were supplemented with 2.5 % NaCl and adjusted to pH 8.5.

The following studies were conducted as described previously (Ishikawa et al., 2003b): growth optima and ranges of NaCl concentration, pH and temperature, and maximum and minimum concentrations of NaCl, growth temperature and pH. Growth optima for these physiological conditions were determined from the maximum specific growth rate, $\mu_{\text{max}}$ (h$^{-1}$).

Fermentation products from glucose consumed were analysed by HPLC as described previously (Ishikawa et al., 2003b). The amount of glucose was estimated by using the Somogyi method (Somogyi, 1945) or enzymically with glucose oxidase (Wako Pure Chemical). The isomeric forms of lactic acid produced were determined enzymically with D- and L-lactate dehydrogenases (Roche Diagnostics).

Products from glucose were investigated during aerobic and anaerobic cultivation. Aerobic cultivation was conducted as described above. AnaeroPack-Keep (not CO$_2$-generated; Mitsubishi Gas Chemical) was used for anaerobic cultivation. Products were analysed by HPLC as described previously (Ishikawa et al., 2003b). To investigate the effect of the initial pH of the anaerobic cultivation medium on the composition of products from glucose, cultivation was performed in 2.5 % NaCl GYPF broth buffered with 100 mM HEPES, with the initial pH adjusted to 7.0, 8.0 or 9.0. To minimize the effect of any decrease in pH during the fermentation, the products were analysed when the OD$_{660}$ of the culture reached about 0.20.

Analysis of fatty acids and detection of respiratory quinones were performed by using previously described methods (Ishikawa et al., 2003b). The presence of diaminopimelic acid was determined by TLC by using the method of Hasegawa et al. (1983), as described by Okada et al. (1992). The presence of cytochrome was determined by spectrophotometry using the method described by Ohama (1982) and Niimura et al. (1987). Amounts of 300 mg (dry weight) and 3 g (wet weight) of cells were used for the detection of respiratory quinones and cytochromes, respectively. Cells were cultivated aerobically by shaking.

DNA base composition was determined by analysing deoxyribonucleosides by reversed-phase HPLC (Tamaoka & Komagata, 1984). DNA–DNA hybridization was performed using the fluorometric method of Ezaki et al. (1989). For these experiments, total genomic DNA was prepared according to the combined methods of Marmur (1961) and Saito & Miura (1963).

Almost complete sequences of 16S rRNA genes were amplified by PCR using the primers 20F (5′-AGTTTGTATCG-GCTCA-3′, positions 10–26) and 1540R (5′-AAGGAGGT-GACTCAACGCA-3′, positions 1541–1522) (Escherichia coli numbering system; Brosius et al., 1978), as described previously (Yanagi & Yamasato, 1993; Ishikawa et al., 2003b). The amplified products were sequenced using an ABI PRISM BigDye Terminator Cycle Sequencing ready reaction kit and an ABI PRISM model 310 Genetic Analyzer (Perkin Elmer). The following five primers were used: 20F, 1540R, 350F (5′-CTACTTACCTCCATACCC-3′, positions 341–358), 800F (5′-GTAGTCCAGCGTAAAACGA-3′, positions 800–819) and 900R (5′-CGGCCGTACTCTCCAGCGG-3′, positions 989–879). Percentage similarities among the isolates, the members of the HA group and other typical lactic acid bacteria were calculated for aligned DNA sequences of 1452–1468 bases in length using the program GENETIX (SDC Software Development). Known sequences were retrieved from public databases and aligned with the newly determined sequences using the program CLUSTAL_X (Thompson et al., 1997). From the resulting multiple-sequence alignment, hypervariable regions at positions 66–103 (V1 region) and 1436–1456 (V5 region) were omitted to avoid analytical errors in constructing the tree. Unrooted phylogenetic trees were reconstructed by using the maximum-likelihood method of Felsenstein (1981), as implemented in the program fastDNAm (Olsen et al., 1984), and also from the $K_{\text{ape}}$ values (Kimura, 1980) by using the neighbour-joining method of Saitou & Nei (1987). The stability of the groupings was estimated by bootstrap analysis (1000 replications from the neighbour-joining dataset; Felsenstein, 1985).

All 11 newly isolated strains exhibited similar taxonomic features. Cultural and morphological characteristics and
other taxonomic features are given in the descriptions of the new genus and species. Cells and peritrichous flagella of strains M2-2T and M23-1T are shown in Fig. 1. Spores were not produced when the cells were cultivated on 2.5% NaCl GYPF agar, marine agar 2216 with or without 5 p.p.m. Mn²⁺ or 2% NaCl yeast extract salts agar. Catalase was negative for cells that were grown aerobically or anaerobically in 2.5% NaCl GYPF broth, on the same medium with the concentration of glucose reduced to 0-1%, or on 2.5% NaCl GYPF agar or 2% NaCl yeast extract salts agar.

Under aerobic conditions, no growth was observed in 2.5% NaCl GCY broth from which glucose was excluded. That is, in the stationary phase, the OD₆₆₀ of cultures grown in 2 NaCl GCY broth from which glucose was excluded. That is, on 2.5% Mn²⁺ NaCl GYPF agar, marine agar 2216 with or without 5 p.p.m.

Mn²⁺ or 2% NaCl yeast extract salts agar. Catalase was negative for cells that were grown aerobically or anaerobically in 2.5% NaCl GYPF broth, on the same medium with the concentration of glucose reduced to 0-1%, or on 2.5% NaCl GYPF agar or 2% NaCl yeast extract salts agar.

The optimum NaCl concentrations for growth were between 2-0% (0.34 M) and 3-0% (0.51 M) (1.5-3.0% for strains M9-1 and M23-5) for phenotype 1 and between 2.5% (0.43 M) and 3.0% for phenotype 2. The maximum specific growth rates, 𝜇ₘₐₓ (h⁻¹), of strain M2-2T, phenotype 1, were 0-18 in 0%, 0-22 in 0-5%, 0-30 in 1%0%, 0-46 in 1-5%, 0-48 in 2-0%, 0-54 in 2.5%, 0-40 in 3-0%, 0-40 in 3-75%, and 0-40 in 5-0% NaCl. Those of strain M23-1T, phenotype 2, were 0-40 in 0%, 0-44 in 0-5%, 0-56 in 1%0%, 0-56 in 1-5%, 0-56 in 2-0%, 0-70 in 2.5%, 0-60 in 3-0%, 0-48 in 3-75%, and 0-48 in 5-0% NaCl. The strains of phenotype 1 were able to grow between 0 and 23-5-24-0% (4-02-4-11 M) NaCl and those of phenotype 2 between 0 and 25-5% (4-36 M) NaCl.

Table 1. Profiles of sugar fermentation of the 11 new isolates

Fermentations were scored as: +, positive; –, negative; W, weakly positive (as defined in Methods). All 11 strains ferment D-glucose, D-fructose, D-galactose, D-mannose, D-cellulobiose, lactose, maltose, melibiose, sucrose, D-trehalose, D-raffinose, D-mannitol, starch, methyl a-D-glucoside, D-salicin and sodium gluconate, but do not ferment adonitol, myo-inositol, dulcitol or D-sorbitol.

<table>
<thead>
<tr>
<th>Carbon compound</th>
<th>Phenotype 1</th>
<th>Phenotype 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Arabinose</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>D-Arabinose</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>D-Ribose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Rhamnose</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>D-Melezitose</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Glycerol</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Inulin</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
The isolates were thus slightly halophilic (Kushner, 1978; Kushner & Kamekura, 1988) and highly halotolerant. These characteristics are the physiological features common to most members of the HA group. Other than the present isolates, only a few taxa of lactic acid bacteria that are characteristically halophilic and highly halotolerant (able to grow at NaCl concentrations >15%) have been described to date: *Tetragenococcus halophilus* and *Tetragenococcus muriaticus* isolated from salted foods (Iizuka & Yamasato, 1959; Satomi et al., 1997), *Marinilactibacillus psychrotolerans* isolated from marine organisms (Ishikawa et al., 2003b), and *Alkalibacterium olivapovliticus* isolated from alkaline edible-olive wash-water (Ntougias & Russell, 2001). *Weissella halotolerans*, which was isolated from meat products, has a salt tolerance of 14% (Kandler et al., 1983).

The initial medium pH that resulted in optimum growth was between 8.0 and 9.0 for phenotype 1 and 9.5 for phenotype 2. No growth was observed in media for which the initial pH was ≤6.0 or ≥10.0 for phenotype 1, or ≤5.5 or ≥10.5 for phenotype 2. For strain M2-2T, phenotype 1, the maximum specific growth rates (h⁻¹) were 0.38 at pH 7.0, 0.40 at pH 7.5, 0.42 at pH 8.0, 0.52 at pH 8.5, 0.50 at pH 9.0 and 0.14 at pH 9.5. For strain M23-1T, phenotype 2, they were 0.46 at pH 7.0, 0.46 at pH 7.5, 0.52 at pH 8.0, 0.46 at pH 8.5, 0.48 at pH 9.0, 0.68 at pH 9.5 and 0.40 at pH 10.0. The final pH of cultures in 2.5% NaCl GYPF broth reached 5.2–6.0, which was 0.5–1.3 pH units lower than the minimum pH required to initiate growth. According to Jones et al. (1994), alkaliphiles are organisms that grow optimally at pH values greater than 8. The isolates were alkaliphilic, as they grew optimally at pH values between 8.0 and 9.0 (phenotype 1) or 9.5 (phenotype 2).

Commonly occurring lactic acid bacteria are comparatively acid tolerant (their broth cultures attaining a final pH value <3.0) and grow optimally at neutral to slightly acid pH, such as pH 6.0. Although the isolates produce acid, their growth optima lie considerably to the alkaline side, and the pH has to be >6.0 for growth initiation, both of which are uncommon characteristics among typical lactic acid bacteria. *Marinilactibacillus psychrotolerans* (Ishikawa et al., 2003b) and *Alkalibacterium olivapovliticus* (Ntougias & Russell, 2001) are two other organisms that are not acid tolerant and not neutrophilic, but are alkaliphilic lactic acid bacteria. In the HA group, *Amphibacillus xylanus*, which was isolated from an alkaline compost of manure with grass and rice straw and has an energy metabolism conforming to that of the isolates (discussed later), is also alkaliphilic, growing optimally at pH 8.0–10.0 (Niimura et al., 1990). *Pediococcus urinaeaequi*, which was described as being isolated from horse urine, grows optimally at pH 8.5–9.0, as deduced from the amount of acid produced in buffered broth cultures that were started at different initial pH values (Nakagawa & Kitahara, 1959).

The strains reported here are marine inhabitants, as they were isolated from living and decaying marine organisms and had physiological properties consistent with the physico-chemical conditions found in sea water [total salt concentration 3.2–3.8% (w/v), pH 8.2–8.3 (surface)]. That is, they grow optimally in 2.0–3.0% NaCl (and can grow in 0–25.5% NaCl) and at pH 8.0–9.5 (and can grow at a pH range of 6.0–10.0). Isolation and taxonomic studies of lactic acid bacteria from marine environments are few to date and have generally been confined to isolates from cultured fish (Ringo & Gatesoupe, 1998; Gatesoupe, 1999). Recently, members of the genus *Marinilactibacillus* have been isolated: *Marinilactibacillus* sp. from coastal sub-seafloor sediment of the Okhotsk Sea (Inagaki et al., 2003) and *Marinilactibacillus piezotolerans* from deep sub-seafloor sediment of the Nankai Trough (Toppin et al., 2005). *Marinilactibacillus psychrotolerans*, which has been isolated from a decaying marine alga, a living sponge and a fresh shellfish, is a marine-inhabiting lactic acid bacterium with growth optima at 2.0–3.75% NaCl and pH 8.5–9.0 (Ishikawa et al., 2003b). For such organisms, Ishikawa et al. (2003b) proposed the term 'marine lactic acid bacteria'. Thus, the present isolates can be regarded as additional representatives of marine lactic acid bacteria on the basis of habitat, physiological properties and lactic acid fermentation (described later).

The optimum growth temperatures for the isolates were 30–37°C (phenotype 1) and 37–40°C (phenotype 2). The maximum specific growth rates (h⁻¹) of strain M2-2T, phenotype 1, were 0.48 at 25°C, 0.58 at 30°C, 0.60 at 37°C, 0.42 at 40°C and 0.06 at 42.5°C. Those of strain M23-1T, phenotype 2, were 0.62 at 25°C, 0.64 at 30°C, 0.74 at 37°C, 0.74 at 40°C and 0.18 at 42.5°C. Growth occurred between 5–10 and 40°C and between 5 (−1–8°C for strain M23-3) and 45°C for phenotypes 1 and 2, respectively.

The fermentation products of glucose and the effects of the initial pH of the fermentation medium were investigated. Under anaerobic cultivation in 2.5% NaCl GYPF broth, the isolates produced lactate in yields of 50–60% of the amount of glucose consumed, depending on the isolate. The other end-products were formate, acetate and ethanol in a molar ratio of approximately 2:1:1. No gas was produced. The l-isomer of lactate was 80–95% of the total lactate produced. The amount of lactate relative to that of the other three products was markedly affected by the initial pH of the fermentation medium. For the representative selected strains M2-2T (phenotype 1) and M23-1T (phenotype 2), the products from glucose in relation to the pH of the medium were determined by using buffered media. The lactate yield relative to glucose consumed by strain M2-2T at pH 8.0, 57%, increased to 75% at the more acidic pH 7.0, whereas it decreased remarkably to 22% at the more alkaline pH 9.0; that of strain M23-1T at suboptimum pH 9.0, 37%, increased at more acidic pH values, 57% at pH 8.0 and 65% at pH 7.0 (Table 2). For each of the initial cultivation pH values, the drop in pH value at the point of product analysis was ≤0.5 pH units. At all pH values, carbon recovery from glucose consumed was about 100%, and the molar ratios of formate, acetate and ethanol produced were generally approximately 2:1:1 (Table 2).
The similar alkaliphilic lactic acid bacteria *Marinilactibacillus psychrotolerans* and *Alkalibacterium olivapovliticus* likewise produce formate, acetate and ethanol in addition to lactate under anaerobic conditions, and their product ratios were similarly affected by the initial pH of the fermentation medium (Ishikawa et al., 2003b). Also, in the lactate-producing facultative anaerobes *Exiguobacterium aurantiacum* (which lies in the phylogenetic radiation of 'classical' Bacillus) and *Paraliobacillus ryukyuensis* (which belongs to the HA group), glucose fermentation responded similarly to pH changes in the cultivation medium (Gee et al., 1980; Collins et al., 1983; Ishikawa et al., 2002), as for the present isolates. Alternation of product composition in glucose fermentation (i.e., decreased lactate production and increased acetate production) would be active to produce formate, acetate and ethanol under their normal growth conditions.

Products from glucose under aerobic cultivation conditions were acetate, pyruvate and lactate, but formate and ethanol were not produced by the isolates or *Marinilactibacillus psychrotolerans* M13-2T (see Supplementary Table S1 in IJSEM Online). Carbon recovery was not balanced. This imbalance can be ascribed to CO₂ generation, if the metabolism of *Amphibacillus xylanus* lacks catalase, cytochromes and quinones. This bacterium has both an anaerobic pathway for glucose metabolism and an oxidative metabolic pathway that is not mediated by the respiratory chain (Niimura et al., 1989). Under anaerobic conditions, the end-products from glucose are formate, acetate and ethanol in a molar ratio of 2:1:1. Acetyl CoA and formate are formed from pyruvate, mediated by pyruvate-formate lyase. NADH produced through glycolysis is regenerated to NAD by aldehyde dehydrogenase and alcohol dehydrogenase. Under aerobic conditions, acetate and CO₂ are the end-products from glucose. Coupled with the conversion of pyruvate to acetyl CoA, NAD is reduced to NADH by pyruvate dehydrogenase and CO₂ is produced. NADH produced through glycolysis and pyruvate oxidation is regenerated to NAD by the NADH oxidase/peroxidase system, using O₂ as an electron acceptor. Consequently, ethanol production with generation of NADH does not occur under aerobic conditions. If we assume that

### Table 2. Effect of initial pH of the culture medium on the product balance of glucose fermentation by the representative strains M2-2T and M23-1T

<table>
<thead>
<tr>
<th>Strain</th>
<th>End-products [mol (mol glucose)⁻¹]</th>
<th>Lactate yield from consumed glucose (%)</th>
<th>Carbon recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lactate</td>
<td>Formate</td>
<td>Acetate</td>
</tr>
<tr>
<td>M2-2T pH 7</td>
<td>1-50</td>
<td>0-73</td>
<td>0-27</td>
</tr>
<tr>
<td>pH 8</td>
<td>1-13</td>
<td>0-81</td>
<td>0-37</td>
</tr>
<tr>
<td>pH 9</td>
<td>0-45</td>
<td>1-84</td>
<td>0-74</td>
</tr>
<tr>
<td>M23-1T pH 7</td>
<td>1-30</td>
<td>0-76</td>
<td>0-28</td>
</tr>
<tr>
<td>pH 8</td>
<td>1-13</td>
<td>0-81</td>
<td>0-45</td>
</tr>
<tr>
<td>pH 9</td>
<td>0-73</td>
<td>1-28</td>
<td>0-51</td>
</tr>
<tr>
<td>Marinilactibacillus psychrotolerans M13-2T⁺ pH 7</td>
<td>2-02</td>
<td>0-15</td>
<td>0-04</td>
</tr>
<tr>
<td>pH 8</td>
<td>1-50</td>
<td>0-52</td>
<td>0-20</td>
</tr>
<tr>
<td>pH 9</td>
<td>1-29</td>
<td>0-81</td>
<td>0-35</td>
</tr>
</tbody>
</table>

*Data from Ishikawa et al. (2003b).*
equimolar production of acetate and CO₂ also occurs in the isolates and in Marinilactibacillus psychrotolerans, carbon recovery under aerobic conditions can be calculated as 93–97 %.

The existence of the same oxidative pathway as that of Amphibacillus xylanus has been reported in several other species of lactic acid bacteria. For example, S. mutans has an oxidative pathway that is mediated by the NADH oxidase system (Fukui et al., 1988). Liu et al. (2002) concluded that Trichococcus floculiformis, which lacks cytochromes, also has this oxidative pathway, on the basis of an analysis of its metabolites and ATP production under air. Sakamoto & Komagata (1996) also reported NADH oxidase and NADH peroxidase activities and acetate production under aerobic conditions in the homofermentative lactic acid bacteria Lactobacillus, Pediococcus and Streptococcus, and in the heterofermentative lactic acid bacteria Leuconostoc. The isolates and Amphibacillus xylanus belong to the HA group in the phyletic radiation of 'classical' Bacillus, and Marinilactibacillus psychrotolerans and the lactic acid bacteria mentioned here belong to the radiation in which the usual lactic acid bacteria are located. All of these may have an identical oxidative metabolic pathway, even though they are located in different phyletic radiations. Thus, this metabolic system might be a common feature of aerotolerant fermentative bacteria.

The isolates possessed meso-diaminopimelic acid in the cell-wall peptidoglycan, as demonstrated by TLC. The peptidoglycan type of the genera belonging to the HA group is type A1γ, meso-diaminopimelic acid direct linkages, except for Halobacillus species and Filobacillus species. The cellular fatty acid compositions of the representative strains M2-2T (phenotype 1) and M23-1T (phenotype 2) were characterized by straight-chain, anteiso-branched saturated, iso-branched saturated and monounsaturated acids (see Supplementary Table S2 in IJSEM Online). The major cellular fatty acids were a-C₁₃:₀ and C₁₆:₀. Among the members of the HA group and other 'classical' Bacillus species that possess anteiso fatty acids as common features, possession of a-C₁₃:₀ as a major component is characteristic of the isolates (see Supplementary Table S2 in IJSEM Online).

Respiratory quinones and cytochromes were not present in strains M2-2T and M23-1T. Along with Amphibacillus xylanus, the absence of these respiratory components is characteristic among members of the HA group, which is composed of many aerobic and facultatively anaerobic genera and species.

The G+C contents of the DNA of the new isolates fell into narrow ranges: 39.6–40.7 mol% for phenotype 1 and 38.5–40.0 mol% for phenotype 2 (Table 3). The DNA–DNA relatedness values among the strains of phenotype 1

### Table 3. DNA base composition and DNA–DNA relatedness among the new isolates and related taxa

<table>
<thead>
<tr>
<th>Strain</th>
<th>DNA base composition (mol% G+C)*</th>
<th>Relative binding (%) of DNA from:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>M2-2T</td>
</tr>
<tr>
<td>Phenotype 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M2-1</td>
<td>39.6</td>
<td>84</td>
</tr>
<tr>
<td>M2-2T</td>
<td>40.2</td>
<td>100</td>
</tr>
<tr>
<td>M2-3</td>
<td>40.0</td>
<td>82</td>
</tr>
<tr>
<td>M2-4</td>
<td>40.2</td>
<td>87</td>
</tr>
<tr>
<td>M9-1</td>
<td>40.7</td>
<td>92</td>
</tr>
<tr>
<td>M13-1</td>
<td>40.5</td>
<td>85</td>
</tr>
<tr>
<td>Phenotype 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M23-1T</td>
<td>38.5</td>
<td>41</td>
</tr>
<tr>
<td>M23-2</td>
<td>39.2</td>
<td>ND</td>
</tr>
<tr>
<td>M23-3</td>
<td>39.7</td>
<td>ND</td>
</tr>
<tr>
<td>M23-4</td>
<td>38.7</td>
<td>ND</td>
</tr>
<tr>
<td>M23-5</td>
<td>40.0</td>
<td>ND</td>
</tr>
<tr>
<td>Paralibacillus ryukyuensis O15-7T</td>
<td>35.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4</td>
</tr>
<tr>
<td>Amphibacillus xylanus JCM 7361&lt;sup&gt;T&lt;/sup&gt;</td>
<td>36.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5</td>
</tr>
<tr>
<td>Amphibacillus fermentum DSM 13869&lt;sup&gt;T&lt;/sup&gt;</td>
<td>41.5&lt;sup&gt;h&lt;/sup&gt;</td>
<td>22</td>
</tr>
<tr>
<td>Amphibacillus tropicus DSM 13870&lt;sup&gt;T&lt;/sup&gt;</td>
<td>39.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24</td>
</tr>
<tr>
<td>G. halotolerans DSM 11805&lt;sup&gt;T&lt;/sup&gt;</td>
<td>39.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3</td>
</tr>
<tr>
<td>G. dipsosauri DSM 11125&lt;sup&gt;T&lt;/sup&gt;</td>
<td>35.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4</td>
</tr>
</tbody>
</table>

ND, Not determined.

*Data from: a, Ishikawa et al. (2002); b, Niimura et al. (1990); c, Zhilina et al. (2001); d, Wainø et al. (1999); e, Lawson et al. (1996).
were 82–92 % and those among the strains of phenotype 2 were 79–96 %. Isolates M2-2T, M9-1 and M13-1 of phenotype 1 showed low DNA–DNA relatedness values (35, 21 and 29 %, respectively) to M23-1 T from phenotype 2, and M23-1 T from phenotype 2 also showed low DNA–DNA relatedness (41 %) to M2-2T from phenotype 1 (Table 3). On the basis of their DNA–DNA relatedness, the isolates constitute two genomic species; furthermore, the strains within each genomic species correspond to the two phenotypes that were determined from the sugar fermentation profiles (Table 1). Levels of DNA–DNA relatedness between the two type strains and strains of the phylogenetically related genera *Amphibacillus*, *Gracilibacillus* and *Paraliobacillus* were 2–24 %, as low as levels between distinct species (Table 3).

Complete sequences of the 16S rRNA gene, 1491 bases in length and covering positions 41–1508 (*Escherichia coli* numbering system; Brosius et al., 1978), were determined for the type strain of each phenotype. The sequences were aligned and compared with the sequences of 29 species of related bacteria and lactic acid bacteria from public databases. The 16S rRNA gene sequence similarity value between M2-2T (phenotype 1) and M23-1 T (phenotype 2) was 99.1 %. Pairwise analysis revealed that the new isolates exhibited the highest similarity values to the genera *Paraliobacillus* (94.8–95.1 % similarity), *Amphibacillus* (92.9–94.3 %), *Gracilibacillus* (93.7–94.1 %) and *Virgibacillus marismortui* (93.8–94.2 %). A phylogenetic tree constructed by using the neighbour-joining method showed that the two phenotypes constitute an independent line of descent within the HA group in rRNA group 1 of the phylectic group classically defined as the genus *Bacillus*, and occupy a phylogenetic position that is closely related to the genera *Paraliobacillus*, *Gracilibacillus* and *Amphibacillus* (Fig. 2). This relationship between the isolates and these three genera was also found in the tree constructed using the maximum-likelihood algorithm (see Supplementary Fig. S1 in IJSEM Online).

The isolates possess all the essential characteristics of lactic acid bacteria that have been attributed to the most typical lactic acid bacteria, including production of lactic acid.

---

**Fig. 2.** Phylogenetic relationships between the new isolates and some other related bacteria, based on 16S rRNA gene sequences. *Exiguobacterium aurantiacum* NCDO 2321 T was used as an outgroup. The tree, constructed by using the neighbour-joining method, is based on comparison of approximately 1380 nucleotides. Bar, 0.01 *K*sub. Bootstrap values, expressed as a percentage of 1000 replications, are given at branching points; only values above 50 % are shown. *Bacillus* rRNA group 1 according to Ash et al. (1991) is shown.
through the Embden–Meyerhof pathway and lack of catalase, quinones, cytochromes and respiratory metabolism, but are novel lactic acid bacteria in terms of phylogeny. Typical lactic acid bacteria can be considered to have evolved retrogressively from facultative anaerobes as close ancestors. This assumption is supported by several findings. For example, *Streptococcus faecalis* (*Enterococcus faecalis*) has a haem-dependent cytochrome (Whittenbury, 1964; Bryan-Jones & Whittenbury, 1969). Whittenbury (1964) argued that its haem-dependent cytochrome may be a rudimentary respiratory system. In several lactic acid bacteria, enzymes involved in the TCA cycle, cytochromes, haem-dependent catalase and quinones, have been found (Whittenbury, 1964; Pritchard & Wimpenny, 1978; Morishita et al., 1999; Wang et al., 2000). The present isolates also could have evolved to lactic acid bacteria by following independent but similar evolutionary processes within the HA group, while retaining physiological characteristics consistent with the physico-chemical factors of salt concentration and pH that prevail in marine environments. This hypothetical consideration is supported by the close relationship between the new isolates and, on the one hand, *Paralibacillus ryukyuensis*, which has catalase, quinones and cytochromes but requires sugars for growth under both aerobic and anaerobic conditions and performs lactic acid fermentation, and, on the other, *Amphibacillus xylanus*, which lacks catalase, quinones and cytochromes and produces formate, acetate and ethanol via the Embden–Meyerhof pathway. The HA group should be an interesting phylogenetic group for studying the possible evolution of a common ancestor to divergent forms, as it is a compact cluster and includes aerobes, facultative anaerobes and lactic acid bacteria, rods and cocci, and has various behaviours in relation to salinity and pH.

The present strains that were isolated from decaying algae and a living sponge are marine-inhabiting lactic acid bacteria that are slightly halophilic, extremely halotolerant and alkaliophilic. The isolates constitute an independent phylogenetic lineage within the HA group, which is in rRNA group 1, one of the phyletic groups classically defined as the genus *Bacillus*. The HA group is composed of 11 genera, most of which inhabit saline to hypersaline environments. Each of the genera or species is characterized by diverse features: oxidative and/or fermentative metabolism, bacilli or cocci in cellular morphology, spore formation and combinations of halophilic, halotolerant, alkaliophilic and alkaliotolerant properties. The isolates differ from all members of the HA group in that they carry out aerobic and anaerobic energy metabolism (except for *Virgibacillus*, *Paralibacillus* and *Amphibacillus*, which ferment sugars). Similar to *Paralibacillus* and *Amphibacillus*, the present isolates also differ in that they require glucose (carbohydrates and related compounds) for growth even under aerobic conditions. They can be distinguished from the genus *Virgibacillus* by their lack of catalase and quinone and by their fatty acid compositions and from the genus *Paralibacillus* by their lack of catalase, quinones and cytochromes (the presence of cytochromes in *Paralibacillus* was confirmed in this study). Although they share the properties of lack of catalase, cytochrome and quinone, and comparable energy metabolism with *Amphibacillus xylanus*, they can be distinguished from it by their fatty acid compositions, lack of spore formation and the effects of salt concentration and pH on growth.

The isolates were distinguished from all members of the HA group by their combination of morphological, physiological, biochemical and chemotaxonomic features (Table 4). The isolates conformed to two genera in the group of typical lactic acid bacteria, *Marinilactibacillus* and *Alkalibacterium*, with respect to the phenotypic properties of cellular morphology, motility, halophilic and halotolerant properties and lactic acid fermentation pattern. However, they could be distinguished from these genera by the chemotaxonomic characteristics of peptidoglycan type and cellular fatty acid composition. The isolates could be distinguished from other homofermentative lactic acid rods (*Lactobacillus*, *Carnobacterium* and *Paralactobacillus*), depending on the species, by motility, cellular fatty acid composition, DNA base composition and halophilic and alkaliophilic properties. In conclusion, on the basis of the phenotypic features and phylogenetic independence described above, the novel isolates should be classified as a new genus composed of two novel species. We propose the name *Halolactibacillus halophilus* gen. nov., sp. nov. for phenotype 1 as the type species of this genus and *Halolactibacillus miurensis* sp. nov. for phenotype 2.

**Description of *Halolactibacillus gen. nov.***

*Halolactibacillus* [Ha.lo.lac’ti.ba.cil’lus. Gr. n. hals salt (loving); L. n. lac lactis milk; L. masc. n. bacillus stick, a small rod; N.L. masc. n. *Halolactibacillus* salt (loving) lactic acid rodlet].

Cells are Gram-positive, non-sporulating, straight rods, occurring singly, in pairs or in short chains, and elongated. Motile with peritrichous flagella. Catalase- and oxidase-negative. Negative for nitrate reduction, production of ammonia from l-arginine, production of dextran from sucrose and DNase. Weakly hydrolyse starch. Do not hydrolyse casein. Growth does not occur in the absence of sugars. Slightly halophilic and highly halotolerant; the optimum NaCl concentration for growth is 2–3 % (w/v), with a range of 0–25 % (w/v), Alkalophilic; the optimum pH for growth is 8–9–9.5, with a range of 6.0–10.0. Growth occurs between 5–10 and 40–45 0 C, with an optimum at 30–40 0 C. In anaerobic cultivation, l-lactic acid is the major end-product from glucose. Considerable amounts of formate, acetate and ethanol are produced in a molar ratio of approximately 2 : 1 : 1, without gas production. Carbohydrates and related compounds are aerobically metabolized to acetate and pyruvate, as well as lactate. The cell-wall peptidoglycan is meso-diaminopimelic acid. Major cellular fatty acids are α-C13 : 0 and C16 : 0. Respiratory quinones and cytochromes are absent. The G + C content of the DNA is
Table 4. Characteristics that distinguish the isolates from other members of the HA group in Bacillus rRNA group 1, *M. psychrotolerans* and *Alkalibacterium olivapovliticus*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spore formation</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anaerobic growth</td>
<td>+</td>
<td>(F)</td>
<td>+</td>
<td>(F)</td>
<td>−</td>
<td>+</td>
<td>(ANR)</td>
<td>+</td>
<td>(F)</td>
<td>+</td>
<td>(F)</td>
<td>+</td>
<td>(F)</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Catalase</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glucose requirement</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

**NaCl (%):**

Range: 0-25 to 5

Opt. pH:

Range: 6-10

Opt.

Range: 8-9.5

Major isoprenoid quinones

Peptidoglycan type

G+C content (mol%)

Major cellular fatty acids

Isolation source

Decaying marine algae, living sponge

Decaying marine algae

Surface mud, Great Salt Lake

Alkaline manure with grass and rice straw

Sediment, soda lake

Sediment, soda lake

Soils

Salt marsh soils

Solar salt

Mud, Iheya Ridge

Beach sediment

Decomposing system of kitchen refuse

Decaying marine algae, living sponge

Wash-waters of edible olives

*a*-C13:0, C16:0, i-C15:0, a-C15:0, a-C17:0, c-C17:0

**b**-C15:0, C16:0, C18:1

**d**-C14:0, C18:1

*Spore formation was not observed but culture survived heating.

†Produced in aerobic cultivation.

‡Optimum for the type strain.
38·5–40·7 mol%. The type species is Halolactibacillus halophilus. As determined by 16S rRNA gene sequence analysis, the genus Halolactibacillus is located within the phylogenetic group composed of halophilic/halotolerant/alkalophilic and/or alkali tolerant species in Bacillus rRNA group 1.

**Description of Halolactibacillus halophilus sp. nov.**

Halolactibacillus halophilus (ha.lo phi’lus. Gr. n. hals salt; Gr. adj. philos loving; N.L. masc. adj. halophilus salt loving).

This species has all the characteristics that define the genus. In addition, it has the characteristics described below. Deep colonies in 2·5 % NaCl glucose-yeast extract-peptone-fish extract agar medium are pale-yellow and lenticular, with diameters of 2–4 mm after 3 days at 30 °C. Surface colonies are round, convex, entire, pale-yellow and transparent, with diameters of 0·8–1·0 mm after 3 days at 30 °C. Cells are 0·6–0·9 × 3·6–4·5 μm, occurring singly, in pairs or in short chains. The optimum NaCl concentration for growth is 2·0–3·0 %, with a range of 0 to 23·5–24·0 %. The optimum pH for growth is 8·0–9·0, with a range of 6·5–9·5. Growth occurs between 5–10 and 40 °C, with an optimum at 30–37 °C. Lactic acid is the major fermentation product from glucose: 40–50 % of glucose consumed is converted to formate, acetate and ethanol. Lactate yield decreases at higher pH of the fermentation medium. The following carbohydrates and related compounds are fermented: L-arabinose, D-ribose, D-xyllose, D-glucose, D-fructose, D-galactose, D-mannose, D-cellulbiose, lactose, maltose, melibiose, sucrose, D-raffinose, D-salicin, D-trehalose, D-melezitose, D-mannitol, methyl α-D-glucoside, glycerol, starch and sodium gluconate. L-Arabinose, D-rhamnose, D-sorbitol, dulcitol, myo-inositol, adonitol and inulin are not fermented. The G+C content of the DNA is 38·5–40·0 mol%.

The type strain is strain M23–1T (= DSM 17074T = IAM 15247T = NBRC 100867T = NRIC 0633T). Isolated from decaying alga at Oura beach, Miura Peninsula, Kanagawa Prefecture, Japan. The G+C content of the type strain is 40·2 mol%.

**Description of Halolactibacillus miurensis sp. nov.**

Halolactibacillus miurensis (mi.u.ren’sis. N.L. masc. adj. miurensis from the Miura Peninsula, Japan, where the strains were isolated).

This species has all the characteristics that define the genus. In addition, it has the characteristics described below. Deep colonies in 2·5 % NaCl glucose-yeast extract-peptone-fish extract agar medium are pale-yellow and lenticular, with diameters of 2–4 mm after 3 days at 30 °C. Surface colonies are round, convex, entire, pale-yellow and transparent, with diameters of 1·0–1·5 mm after 3 days at 30 °C. Cells are 0·6–0·9 × 3·6–4·5 μm, occurring singly, in pairs or short chains. The optimum NaCl concentration for growth is 2·5–3·0 % (w/v), with a range of 0–25·5 %. The optimum pH for growth is 9·5, with a range of 6·0–6·5 to 10·0. Growth occurs at 5–45 °C, with an optimum at 37–40 °C. Lactic acid is the major fermentation product from glucose: 40–50 % of glucose consumed is converted to formate, acetate and ethanol. Lactate yield decreases at higher pH of the fermentation medium. The following carbohydrates and related compounds are fermented: L-arabinose, D-ribose, D-xyllose, D-glucose, D-fructose, D-galactose, D-mannose, D-cellulbiose, lactose, maltose, melibiose, sucrose, D-raffinose, D-salicin, D-trehalose, D-melezitose, D-mannitol, methyl α-D-glucoside, glycerol (weak), inulin, starch and sodium gluconate. L-Arabinose, D-rhamnose, D-sorbitol, dulcitol, myo-inositol and adonitol are not fermented. The G+C content of the DNA is 38·5–40·0 mol%.

The type strain is strain M23–1T (= DSM 17074T = IAM 15247T = NBRC 100867T = NRIC 0633T). Isolated from decaying alga at Oura beach, Miura Peninsula, Kanagawa Prefecture, Japan. The G+C content of the type strain is 38·5 mol%.

**Acknowledgements**

We are grateful to Masayuki Suzuki and Keiichi Goto, Mitsui Norin Co., Ltd for the fatty acid analysis, to Yoichi Niimura, Faculty of Applied Bio-Science, Tokyo University of Agriculture, for cytchrome analysis, and to Hajime Kanamori and Shihomi Ishizaki, Faculty of Applied Bio-Science, Tokyo University of Agriculture, for technical assistance.

**References**


Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in...
microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. Int J Sys Bacteriol 39, 224–229.


