Moritella profunda sp. nov. and Moritella abyssi sp. nov., two psychropiezophilic organisms isolated from deep Atlantic sediments

Ying Xu,1 Yuichi Nogi,2 Chiaki Kato,2 Ziyuan Liang,1 Hans-Jürgen Rüger,3 Daniel De Kegel1 and Nicolas Glansdorff1

1J. M. Wiame Research Institute for Microbiology, Free University of Brussels (VUB), and Flanders Inter-University Institute for Biotechnology, 1, ave E. Gryson, B-1070 Brussels, Belgium
2The DEEP STAR Group, Japan Marine Science and Technology Center, 2-15 Natsushima-machi, Yokosuka 237-0061, Japan
3Alfred Wegener Institut für Polar-und Meeresforschung, Am Handelshafen 12, D-27570 Bremerhaven, Germany

Strains 2674T (=LMG 21259T = JCM 11435T) and 2693T (=LMG 21258T = JCM 11436T) were isolated from Atlantic sediments at a temperature of 2°C and a depth of 2815 m off the West African coast. Polyphasic evidence indicates that the two strains belong to the genus Moritella and represent distinct species, for which the names Moritella profunda sp. nov. (for strain 2674T) and Moritella abyssi sp. nov. (for strain 2693T) are proposed. The moderate piezophily of the two organisms is intermediate between that of the type species, Moritella marina, which is not piezophilic, and Moritella yayanosii, an obligate piezophile. Both are strict psychrophiles with slightly different cardinal temperatures: at 0-1 MPa, maximal growth rates are observed at 2°C (M. profunda) and 4°C (M. abyssi) with maximum temperatures of 12°C (M. profunda) or 14°C (M. abyssi). The optimal pressure is lower than that at the site of isolation, and raising the temperature to 10°C makes the organisms more piezophilic.

Below 2000 m depth, the temperature of the ocean usually does not rise above 2–3°C (Yayanos, 1995). The deep sea is thus a habitat favourable to psychrophilic micro-organisms (highest growth temperature below 20°C; Morita, 1975). From the analysis of sediments collected from the tropical Atlantic off West Africa between 1500 and 4500 m depth, Rüger & Tan (1992) concluded that psychrophiles with maximum growth temperatures below 12°C were predominant among cultivable bacteria. Since the hydrostatic pressure increases by 0.1 MPa every 10 m down the water column, deep-sea resident organisms are expected to display various levels of piezophily or piezotolerance (Yayanos, 1995). Since the isolation of the first pure culture of a true piezophilic bacterium, collected at a depth of 5700 m (Yayanos et al., 1979), several psychropiezophilic species have been described (Kato et al., 1995, 1996; Nogi et al., 1998). Obligate piezophiles, i.e. unable to grow at atmospheric pressure, have been isolated from the deepest Pacific trenches (Yayanos, 1995; Kato et al., 2000; Bartlett, 2000). Most cultivable psychropiezophiles have been found to belong to a few genera of the γ-Proteobacteria, Colwellia, Moritella, Photobacterium and Shewanella (DeLong et al., 1997), and more recently, Psychromonas, a genus that also includes non-piezophilic organisms (Nogi et al., 2002, Xu et al., 2003).

Moritella is a recently described genus that presently comprises four species: the type, Moritella marina (formerly Vibrio marinus; Steven, 1990; Urakawa et al., 1998), which is non-piezophilic and psychrotolerant; Moritella japonica (Nogi et al., 1998), a moderate piezophile isolated from the Japan Trench at a depth of 6356 m; Moritella yayanosii, an obligate piezophile collected from the Challenger Deep in the Mariana Trench at a depth of 10 898 m (Nogi & Kato, 1999); and Moritella viscosa (Benediktsdóttir et al., 2000), originally described as Vibrio viscous (Lunder et al., 2000), a psychrotolerant marine pathogen of salmonids isolated from North Atlantic fish farms. From Atlantic sediments, we have isolated two novel psychophilic isolates (strains 2674T and 2693T) that represent novel species of Moritella.

Strains 2674T and 2693T were collected from the same station during the GEOTROPEX ’83 marine expedition at a depth of 2815 m in the Sierra Leone Rise region of the eastern tropical Atlantic (latitude 5°37’-0’N, longitude...
Samples were taken by means of a box-grab sampler and cultured on chilled sea-water agar plates as described by Xu et al. (2003). The temperature was 2 °C.

Details of the sampling and isolation procedure have been described by Rüger & Tan (1992).

Of the reference Moritella strains used in this study, M. marina ATCC 15381T, M. japonica JCM 10249T (Nogi et al., 1998) and M. yayanosii JCM 10263T (Nogi & Kato, 1999) were grown as described in the latter reference and M. viscosa NCIMB 13584T was grown according to Benediksdóttir et al. (2000). Sources of other reference strains, their maintenance and growth conditions were as described in Nogi et al. (1998) and Nogi & Kato (1999).

High-pressure cultivation and characterization of the novel isolates was carried out as described by Xu et al. (2003), with the exception that DNA–DNA hybridization was performed at 40 °C for 3 h.

The cells of both strains are Gram-negative, motile, do not form spores and present rather similar dimensions and morphologies (Fig. 1; see species descriptions below).

For strain 2674T under atmospheric pressure, the highest growth rate in Bacto Marine broth (Fig. 2) was recorded at 2 °C (about 5.5 h doubling time; no lower temperature was tested). Growth was observed up to 10–12 °C on all media but not at higher temperatures. Up to 8 °C, the final optical density (approx. 10^9 cells ml^{-1}) remained the same. Under identical conditions, strain 2693T showed the highest growth rate at 4–5 °C (Fig. 2); no growth was observed above 14 °C. Neither strain grew in the absence of NaCl.

Pressure affected the two strains differently. For strain 2674T at 6 °C, the growth-pressure profile peaked at 20–24 MPa, a lower pressure than at the place of isolation (28 MPa); at 10 °C, the optimal pressure was not markedly different but the degree of piezophily of the strain increased considerably (Fig. 3a). For strain 2693T, the optimum pressure was 19–20 MPa at 6 °C but, at 10 °C, the optimum was around 30 MPa (Fig. 3b). By comparing Figs 2 and 3, it can be noted that, under the more anaerobic conditions of the pressure cell, strain 2674T grew much better at 10 °C than in full aerobicism. The oxygen tolerance of this strain thus appears to be lower at the upper end of its temperature range.

Both strains are facultatively anaerobic, oxidase-positive, chemo-organotrophic and prototrophic (except for possible vitamin dependence, not tested). Their patterns of acid production from carbohydrates, tested according to Xu et al. (2003), were, however, different (see descriptions). Both are relatively oligotrophic: glucose or galactose at a concentration as low as 0.5 mg ml^{-1} already elicited good growth on minimal medium plates (Rüger, 1988) incubated at 4 °C.

Several carbon sources were used by both strains at a concentration of 1 g carbon l^{-1} under atmospheric pressure in minimal medium (Rüger, 1988), but the range was...
distinctly narrower for strain 2674^T (see descriptions). No gas was produced from nitrate, but both reduced nitrate to nitrite. Both strains proved to be sensitive to several antibiotics (as tested with Oxoid disks placed on sea-water agar plates) including the vibriostatic agent O/129. Salient properties of strains 2674^T and 2693^T and reference species are listed in Table 1 and in the descriptions below.

The G+C contents of both strains were similar to those of other *Moritella* species (Table 1). Moreover, comparative analysis of their complete 16S rRNA sequences with cognate sequences from various γ-Proteobacteria placed them in the *Moritella* cluster within a branch with a high bootstrap value (see Fig. 3 of Xu *et al.*, 2003). Between the two strains, there was 98-5 % identity. With respect to *M. marina*, *M. yayanosii*, *M. japonica* and *M. viscosa*, strain 2674^T exhibited 99-1, 98-9, 98-8 and 98-6 % identity, whereas strain 2693^T gave 98-6, 98-3, 98-7 and 98-5 % identity. In terms of similarity to other genera, the highest values were to species of *Shewanella* (93 % or less).

Reciprocal DNA–DNA hybridizations distinguished the two strains both from each other (55 % similarity) and from already described *Moritella* species; values were between 41-5 % for the pair 2674^T/*M. marina* and 60 % for the pair 2693^T/*M. yayanosii*. Other pairs gave values between 40 (*M. marina*/*M. yayanosii*) and 57-5 % (*M. viscosa/*M. yayanosii*).

**Fig. 3.** Growth response of strain 2674^T (a) and strain 2693^T (b) under different pressures at 6 (●) and 10 (○) °C. Growth rates were calculated as described in Fig. 2.

**Table 1.** Phenotypic comparison of *Moritella* species

<table>
<thead>
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<th>Characteristic</th>
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<td>Temperature (°C) at 0-1 MPa</td>
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<td>10</td>
<td>NG</td>
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</table>
The observed percentages thus remain below the value currently accepted to differentiate species within a particular genus (<70% binding; Wayne et al., 1987).

As in other Moritella species, major fatty acids of cells of both strains grown in Bacto Marine broth 2216 (see Nogi et al., 1998 for sample analysis) were C14:0, C16:0, C16:1 and C22:6 (docosahexaenoic acid; DHA). The occurrence of DHA is characteristic of the genus Moritella (DeLong et al., 1997; Kato et al., 1998). Similarly, the major isoprenoid quinone (Komagata & Suzuki, 1987; Nogi et al., 1998) was Q-8, as in other Moritella species (Nogi & Kato, 1999).

On the basis of the polyphasic analysis reported in this study, we propose to name strains 2674T and 2693T as Moritella profunda sp. nov. and Moritella abyssi sp. nov. on the basis of their origin (bottom sediments at a depth of 2800 m). As for other members of the Vibrionaceae, they are both facultatively anaerobic, capable of fermentative metabolism and oxidase-positive.

Considering their depth of isolation, it is no surprise that both are piezophilic and strictly psychrophilic; the term piezophilic appears more adequate than piezotolerant, since growth can actually be enhanced by increasing the hydrostatic pressure. M. profunda is more psychrophilic than M. abyssi (maximal growth rate at 2°C or possibly less compared with 4°C); both exhibit a relationship between the effects of temperature and pressure which has already been described for other psychropiezophilic species (see Kato et al., 1995; Yayanos, 1995) but also for thermopiezophiles (Martinsson et al., 1997): the optimal pressure is lower than at the place of isolation and increasing the temperature makes the strains more piezophilic. The shift of optimal pressure accompanying an increase in temperature from 6 to 10°C is particularly pronounced for M. abyssi (strain 2693T) whereas, for M. profunda (strain 2674T), for which 10°C is already close to the maximum temperature at 0-1 MPa (12°C), it is the range of pressures compatible with good growth that is extended considerably by such an increase. Partial compensation of the "gelling" effect of high pressure on membrane lipids could result from the increase in molecular mobility brought about by a rise in temperature (Martinsson et al., 1997). Alternatively, an increase in temperature could compensate for inhibition of enzyme activity due to pressure-induced compression. In a recent review on microbial adaptation to the psychropiezosphere (Glandsdorff & Xu, 2002) and in the accompanying paper (Xu et al., 2003), we have stressed that psychropiezophilic enzymes are molecular compromises between two conflicting requirements: efficient catalysis at low temperature requires enzyme flexibility (Gerday et al., 1997), whereas reduced compressibility and enhanced rigidity are necessary at high pressure (Gross & Jaenicke, 1994). The characterization of two novel psychophilic species of Moritella, which appear intermediate between surface dwellers and obligate piezophiles, is therefore of interest.

### Description of Moritella profunda sp. nov.

**Moritella profunda** (pro.fun’d’a. L. fem. adj. profunda from the deep).

Cells are Gram-negative, curved, relatively short to coccolid, non-sporulating rods, single or in pairs, 0-9-1-2 μm wide by 1-5-5-0 μm long, with rounded ends. Motile by means of a single, unsheathed, polar flagellum. On peptone/yeast extract/sea-water agar, colonies are smooth, colourless, flat and entire with an irregular margin. They become creamy-pink and pink on King's media A and B, respectively. Moderately halophilic: no growth is observed in the absence of NaCl but normal growth occurs with half-strength sea water. Strictly psychrophilic; with a temperature range from 2 (or lower, not tested) to 12°C under atmospheric pressure in Bacto Marine broth. The maximum growth rate is obtained at 2°C (possibly lower). The organism is piezophilic; growth in Bacto Marine broth is stimulated markedly by hydrostatic pressure, with a maximum of 20-24 MPa at 6°C and slightly higher at 10°C. At 6°C, the maximum pressure is between 50 and 60 MPa; it is increased considerably by raising the temperature to 10°C. At both extremes of the pressure domain, but mainly under high pressure, elongated forms may be observed. Facultatively anaerobic, prototrophic (possible vitamin dependency, not tested) chemo-organotroph capable of both fermentative and respiratory metabolism. Catalase, cytochrome oxidase, DNase and lipase tests are positive. Indole is not produced and arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase tests are negative. Nitrates are reduced to nitrite but no gas is produced. Acid is formed oxidatively and fermentatively from glucose (but no gas), D-fructose and D-galactose (weakly). No acid is produced from, adonitol, L-arabinose, cellobiose, dulcitol, glycerol, inositol, lactose, maltose, mannitol, D-mannose, D-raffinose, L-rhamnose, sorbitol, sucrose, D-trehalose and xylose.

Utilization of the following carbon sources is positive at both 6°C and slightly higher at 10°C: D-galactose, gluconate, α-D-glucose, ribose, citrate, pyruvate, glycerol, L-alanine, L-arginine and L-glutamate. Acetate is utilized after prolonged incubation for up to 6 weeks. The following carbon sources give negative results: L-arabinose, cellobiose, maltose, D-mannose, salicin, sucrose, D-trehalose, xylose, propionate, adipate, fumarate, succinate, β-hydroxybutyrate, lactate, mannitol, sorbitol, L-aspartate, L-histidine, L-ornithine, putrescine, p-hydroxybenzoate and quinate. Susceptible to disks (Oxoid) containing 10 μg O/129, 2 U penicillin G, 10 μg tetracycline, 10 μg chloramphenicol, 50 μg furazolidone and 300 U polymyxin B. The G+C content of the type strain is 41-4 mol%. The major isoprenoid quinone is Q-8. Predominant cellular fatty acids are C14:0, C14:1, C16:0, C16:1 and C22:6.

The type strain, strain 2674T (=LMG 21259T =JCM 11435T), was collected from the upper layer of deep Atlantic sediments at a depth of 2815 m off the West African coast.
Description of *Moritella abyssi* sp. nov.

*Moritella abyssi* (a.by’s:i. L. gen. n. *abyssi* from the abyss). Cells are Gram-negative, slightly curved, non-sporulating rods, single or in pairs, 0.8–1.2 μm wide by 2.0–7.0 μm long, with rounded ends. Motile by means of a single, unsheathed flagellum. On peptone/yeast extract/sea-water agar, colonies are smooth, punctiform, translucent, colourless, flat and entire with an irregular margin. On King’s medium A, colonies appear creamy. Moderately halophilic, no growth being observed in the absence of NaCl but normal growth in half-strength sea water. Strictly psychrophilic, with a temperature range from 2 (or lower) to 14 °C under atmospheric pressure in Bacto Marine broth. The maximum growth rate is obtained at 4–5 °C. Piezophilic: growth in Bacto Marine broth is stimulated by hydrostatic pressure, with a maximum at 19–20 MPa at 6 °C and 29–30 MPa at 10 °C. Elongated and irregular forms are often observed under atmospheric pressure. Facultatively anaerobic, prototrophic (possible vitamin dependency, not tested) chemo-organotroph capable of both fermentative and respiratory metabolism. Catalase, cytochrome oxidase, DNase, lipase and indole tests are positive. Arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase tests are negative. Nitrate is reduced to nitrite but no gas is produced. Acid is formed oxidatively and fermentatively from glucose (but no gas), from cellobiose, D-galactose, D-fructose, maltose and mannitol. No acid is produced from adonitol, L-arabinose, dulcitol, glycerol, inositol, D-glucose, maltose, D-trehalose or xylose. Utilization of the following carbon sources is positive within 4 weeks at 4 °C: cellobiose, D-galactose, gluconate, 2-D-glucone, maltose, ribose, salicin, fumarate, succinate, citrate, lactate, pyruvate, glycerol, mannitol, L-alanine, L-arginine, L-aspartate and L-glutamate. Positive after prolonged incubation for 6 weeks: acetate and β-hydroxybutyrate (weakly). Negative: L-arabinose, D-mannose, sucrose, D-trehalose, xylose, pro- pionate, adipate, sorbitol, L-histidine, L-ornithine, putrescine, p-hydroxybenzoate and quinate. Susceptible to disks (Oxoid) containing 10 μg O/129, 2 U penicillin G, 10 μg tetracycline, 10 μg chloramphenicol, 50 μg furazolidone and 300 U polymyxin B. The G+C content is 41.6 mol%. The major isoprenoid is Q-8. Predominant cellular fatty acids are C14 : 0, C14 : 1, C16 : 0, C16 : 1 and C22 : 6.

The type strain, strain 2693T ( = LMG 21258T = JCM 11436T), was collected from the upper layer of deep Atlantic sediments (2815 m) off the West African coast.

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References


