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SHORT COMMUNICATIONS

Bacteria of the Genus *Psychrobacter* Isolated from Water of the Black Sea

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The genus *Psychrobacter* and the related genera *Moraxella* and *Acinetobacter* of the family *Moraxellaceae* include gram-negative, oxidase-positive, nonfermenting, nonpigmented, nonmotile coccobacilli [1]. Bacteria of the genus *Psychrobacter* are halo- and psychrotolerant (some species are psychrophilic), which explains their wide distribution in marine and terrestrial environments. Psychrobacters dominate the ornithogenic soils of Antarctica. The genus *Psychrobacter* with the type species *P. immobilis* was first described in 1986 [2] and then was supplemented by the Antarctic species *P. urativorans, P. glacincola*, and *P. frigidicola* [3, 4]; the clinic isolate *P. phenylpyruvicus* [3]; the deep-sea species *P. pacifiensis,* isolated from the Japan Trench at a depth of 6000 m [5]; and the psychrophilic, halophilic species *P. submarinus* and *P. marincola,* isolated from marine environments [6].

The study of halophilic bacteria isolated from Black Sea water showed that they are gram-negative, nonmotile microorganisms, which were assigned to the genus *Psychrobacter* based on their phenotypic properties. This work was aimed at the molecular genetic analysis and the comprehensive taxonomic study of these microorganisms.

The strains under study were isolated from seawater samples collected in June 2000 within the reaches of the Karadag Nature Reserve in the Black Sea [7]. The isolates were studied for Gram staining; growth at different temperatures and NaCl concentrations; the ability to oxidize or ferment glucose (by the method of Baumann and Baumann [8]); the presence of dimethyl-*p*-phenylenediamine oxidase, arginine dihydrolase, lysine and ornithine decarboxylases, amylase, gelatinase, and lipase; the capability for denitrification and reduction of nitrate to nitrite; the requirement for sodium ions; and the ability to utilize 80 different compounds as sole carbon sources.

The isolates were examined in an EMB-100 electron microscope at a magnification of 12000–15000× by using specimens contrasted with 2% phosphotungstic acid.

The antibiotic sensitivity of the isolates to lincomycin, oleandomycin, polymyxin, streptomycin, erythromycin, tetracycline, cephalosporin, furadonin, aztreonam, rifampicin, chloramphenicol, nalidixic acid, and ciprofloxacin was studied by using agar medium B [8] and standard paper disks containing 10–30 μg of antibiotic.

16S rRNA gene sequence analysis was carried out with the universal primers Ant1fw: 5'-AGAGTTTGATCATGGCTCAG-3' (nucleotide positions 8–27); 16S5rev: 5'-TTACGCGGCTGCTGGCACG-3' (nucleotide positions 533–514); and LO231rev: 5'-GGTTTCCCCATTCGGAAATC-3' (nucleotide positions 247–227).

The amplification products were separated by horizontal electrophoresis in 1% agarose gel. Nucleotide sequences were determined with an automatic Perkin-Elmer ABI 272A sequenator and compared to the GenBank sequences with the aid of the BLAST program (http://www.ncbi.nlm.nih.gov).

For the 16S rRNA genes of the four isolates under study, gene regions containing from 448 to 456 nucleotides were sequenced. These regions turned out to be similar (by 97–98%) to the 16S rRNA gene sequences of the Antarctic species *P. glacincola* strains isolated from Antarctic sea ice at a depth of 350 m (the Emery ice shelf, located at 69° S, 78° E) [4].

The phenotypic properties of the isolates (aerobic, gram-negative, oxidase- and catalase-positive, nonpigmented, nonmotile, non-spore-forming coccobacilli) corresponded to the description of the genus *Psychrobacter* [2]. They did not contain arginine dihydrolase, lysine decarboxylase, or ornithine decarboxylase; did not require additional growth factors; grew well in simple mineral media; and could utilize butyrate, asparagine, glutamate, and proline as the sole sources of carbon and energy. The isolates were able to grow at 4 to 15°C and were tolerant to 6.5% NaCl.

The Black Sea isolates were found to be sensitive to nearly all the antibiotics tested, except for penicillin and lincomycin. The strain resistance to penicillin contradicts the description of the genus *Psychrobacter* [2] but is in agreement with the recent studies of the marine species of this genus [6].

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The isolates required Na^+ ions for growth and were tolerant to NaCl concentrations up to 10%. By comparison, the close psychrophilic species *P. glacincola* tolerates up to 15% NaCl.

The range of utilizable carbon sources included more than 20 compounds (D-glucose, sucrose, trehalose, cellobiose, fructose, dextrin, acetate, propionate, butyrate, lactate, fumarate, malate, sorbitol, ethanol, *m*-hydroxybenzoic acid, D-alanine, L-glutamate, asparagine, L-histidine, L-proline, and sarcosine). It should be noted that the close species *P. glacincola* is unable to utilize some of these carbon sources.

Like other psychrobacters, the Black Sea isolates are psychrotrophs, although they can grow at higher temperatures (5 to 28°C) than can *P. glacincola* (–18 to 22°C).

To conclude, the halophilic psychrobacters isolated from Black Sea water differ from the other species of the genus *Psychrobacter. P. glacincola*, which was isolated from a deep layer of Antarctic ice, is the most phenotypically and genetically close species to the Black Sea isolates. The 16S rRNA gene sequence similarity level between the Black Sea isolates and *P. glacincola* (97–98%) does not allow the isolates to be conclusively identified as members of this species. It is tempting to speculate that *P. glacincola* strains inhabited the world ocean several tens of millions of years ago, when it covered all the earth's surface. With the formation of the Black Sea and the ice cover of Antarctica, these strains radiated under the action of different environmental conditions (temperature, water salinity, etc.). This may account for the different levels of psychrotrophy and halophilicity of the Black Sea isolates and *P. glacincola* strains. DNA–DNA hybridization studies may show if these isolates belong to a new species of the genus *Psychrobacter.*

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REFERENCES

1. Rossau, R., Van Landschoot, A., Gillis, M., and De Ley, Taxonomy of *Moraxellaceae* fam. nov., a New Bacterial Family to Accommodate the Genera *Moraxella, Acinetobacter*, and *Psychrobacter* and Related Organisms, *Int. J. Syst. Bacteriol.*, 1991, vol. 41, pp. 310–319.

2. Juni, E. and Heym, G.A., *Psychrobacter immobilis* gen. nov., sp. nov. Genospecies Composed of Gram-Negative, Aerobic, Oxidase-Positive Coccobacilli, *Int. J. Syst. Bacteriol.*, 1986, vol. 36, pp. 388–391.

3. Bowman, J.P., Cavanagh, J., Austin, J., and Sanderson, K., Novel *Psychrobacter* Species from Antarctic Ornithogenic Soils, *Int. J. Syst. Bacteriol.*, 1996, vol. 46, no. 4, pp. 841–848.

4. Bowman, J.P., Nichols, D.S., and McMeekin, T.A., *Psychrobacter glacincola* sp. nov., a Halotolerant, Psychrophilic Bacterium Isolated from Antarctic Sea Ice, *Syst. Appl. Microbiol.*, 1997, vol. 20, pp. 209–215.

5. Maruyama, A., Honda, D., Yamamoto, H., Kitamura, K., and Higashihara, T., Phylogenetic Analysis of Psychophilic Bacteria Isolated from the Japan Trench, Including a Description of the Deep-Sea Species *Psychrobacter pacifiensis* sp. nov., *Int. J. Syst. Evol. Microbiol.*, 2000, vol. 50, pp. 835–846.

6. Romanenko, L., Schumann, P., Rohde, M., Lysenko, A., Mikhailov, V., and Stackerbrandt, E., *Psychrobacter submarinus* sp. nov. and *Psychrobacter marincola* sp. nov., Psychrophilic Halophiles from Marine Environments, *Int. J. Syst. Evol. Microbiol.*, 2002, vol. 52, pp. 1291–1297.

7. Smirnov, V.V., Onishchenko, O.M., and Kiprianova, O.A., Marine Gram-Negative Eubacteria Isolated from Black Sea Water, Mollusks, and Algae, *Mikrobiol. Zh.* (Kiev), 2001, vol. 64, no. 4, pp. 3–8.

8. Baumann, P. and Baumann, L., The Marine Gram-Negative Eubacteria, *The Prokaryotes: A Handbook on Habitats, Isolation, and Identification of Bacteria*, Starr, M.P. *et al.*, Eds., Berlin: Springer, 1986, vol. 2, pp. 1302–1331.