

# *Pseudomonas karstica* sp. nov. and *Pseudomonas spelaei* sp. nov., isolated from calcite moonmilk deposits from caves

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## Abstract

A taxonomic study of two fluorescent *Pseudomonas* strains (HJ/4<sup>T</sup> and SJ/9/1<sup>T</sup>) isolated from calcite moonmilk samples obtained from two caves in the Moravian Karst in the Czech Republic was carried out. Results of initial 16S rRNA gene sequence analysis assigned both strains into the genus *Pseudomonas* and showed *Pseudomonas yamanorum* 8H1<sup>T</sup> as their closest neighbour with 99.8 and 99.7% 16S rRNA gene similarities to strains HJ/4<sup>T</sup> and SJ/9/1<sup>T</sup>, respectively. Subsequent sequence analysis of *rpoD*, *rpoB* and *gyrB* housekeeping genes confirmed the highest similarity of both isolates to *P. yamanorum* 8H1<sup>T</sup>, but phylogeny and sequences similarities implied that they are representatives of two novel species within the genus *Pseudomonas*. Further study comprising whole-genome sequencing followed by average nucleotide identity and digital DNA–DNA hybridization calculations, repetitive sequence-based PCR fingerprinting with the REP and ERIC primers, automated ribotyping with the *EcoRI* restriction endonuclease, cellular fatty acid analysis, quinone and polar lipid characterization, and extensive biotyping confirmed clear separation of both analysed strains from the remaining *Pseudomonas* species and showed that they represent two novel species within the genus *Pseudomonas* for which the names *Pseudomonas karstica* sp. nov. (type strain HJ/4<sup>T</sup>=CCM 7891<sup>T</sup>=LMG 27930<sup>T</sup>) and *Pseudomonas spelaei* sp. nov. (type strain SJ/9/1<sup>T</sup>=CCM 7893<sup>T</sup>=LMG 27931<sup>T</sup>) are suggested.

Members of the genus *Pseudomonas* are ubiquitous organisms inhabiting a wide variety of environments comprising waters, soils, plants, animals and humans. They are saprophytes involved in the decomposition of organic materials and remediation of xenobiotic compounds. *Pseudomonas* organisms play an important role in the food industry and the genus also contains recognised human, animal and plant pathogens [1–3]. Phylogenetically, the genus *Pseudomonas* is assigned in the phylum *Proteobacteria* within the class *Gammaproteobacteria* [4].

The present study describes a taxonomic investigation of two fluorescent *Pseudomonas* strains, HJ/4<sup>T</sup> (=CCM 7891<sup>T</sup>=LMG

27930<sup>T</sup>) and SJ/9/1<sup>T</sup> (=CCM 7893<sup>T</sup>=LMG 27931<sup>T</sup>), isolated from calcite moonmilk samples obtained from two caves in the Moravian Karst in the Czech Republic. Moonmilk is a generic term for a soft, wet, plastic, fine-grained speleothem with variable mineralogy present on ceilings, floors and walls of caves [5]. It consists of crystal aggregates of carbonate minerals, such as calcite and hydromagnesite, other minerals, such as aragonite and gypsum, and non-carbonate minerals, such as silicate, phosphate and sulfate [6]. Some types of moonmilk are assumed to be of microbial origin, created either by direct precipitation of calcite by micro-organisms or by forming a nucleation surface on which minerals precipitate

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**Keywords:** *Pseudomonas karstica*; *Pseudomonas spelaei*; sp. nov.; moonmilk; caves.

**Abbreviations:** ANI, average nucleotide identity; dDDH, digital DNA–DNA hybridization; FAME, fatty acid methyl ester; WGS, whole-genome sequencing.

The GenBank/ENA/DBJ accession numbers for the 16S rRNA, *rpoB* and *rpoD* gene sequences of *Pseudomonas karstica* HJ/4<sup>T</sup> (=CCM 7891<sup>T</sup>) are HQ844524, HQ844521 and HQ844517, respectively and those of *Pseudomonas spelaei* SJ/9/1<sup>T</sup> (=CCM 7893<sup>T</sup>) are HQ844525, HQ844523 and HQ844518, respectively. The whole-genome shotgun projects of *P. karstica* HJ/4<sup>T</sup> and *P. spelaei* SJ/9/1<sup>T</sup> have been deposited in GenBank/ENA/DBJ under the accession numbers WLY100000000 and WNNK000000000, respectively. The versions described in this paper are WLY101000000 and WNNK01000000, respectively.

One supplementary table and six supplementary figures are available with the online version of this article.