**Limnohabitans australis** sp. nov., isolated from a freshwater pond, and emended description of the genus *Limnohabitans*

Martin W. Hahn,1 Vojtěch Kasalický,2,3 Jan Jezbera,1,2 Ulrike Brandt1 and Karel Šimek2,3

Correspondence
Martin W. Hahn
martin.hahn@oeaw.ac.at

1Institute for Limnology, Austrian Academy of Sciences, Mondseestrassse 9, A-5310 Mondsee, Austria
2Biology Centre of the Academy of Sciences ČR, v.v.i., Institute of Hydrobiology, Na Sádkách 7, 37005 České Budějovice, Czech Republic
3Faculty of Science, University of South Bohemia, Branišovská 31, 37005 České Budějovice, Czech Republic

A chemo-organotrophic, aerobic, non-motile strain, MWH-BRAZ-DAM2DT, isolated from a freshwater pond in Brazil, was characterized phenotypically, phylogenetically and chemotaxonomically. Phylogenetic analysis of 16S rRNA gene sequences indicated affiliation of the strain with the genus *Limnohabitans* (**Comamonadaceae, Betaproteobacteria**). 16S rRNA gene sequence similarities between the isolate and *Limnohabitans curvus* MWH-C5T, representing the type species of the genus, and the type strains of *Limnohabitans parvus* and *Limnohabitans planktonicus* were 98.2, 96.5 and 97.0 %, respectively. DNA–DNA reassociation analyses with DNA of the type strains of all three previously described *Limnohabitans* species revealed similarity values in the range 26.2–44.6 %. The predominant fatty acids of the isolate were C\(_{16}:1\)_v/\(_{7}\)c/C\(_{16}:0\), C\(_{12}:0\) and C\(_{8}:0\) 3-OH, the major quinone was ubiquinone Q-8 and the DNA G+C content was 55.8 mol%. The isolate could be discriminated from the type strains of the three *Limnohabitans* species by several phenotypic traits including differences in the utilization of several carbon sources. Based on the phylogeny of the isolate and its differences from the three most closely related species, the isolate represents a novel species for which the name *Limnohabitans australis* sp. nov. is proposed. The type strain is MWH-BRAZ-DAM2DT (\(^=\)DSM 21646\(^T\)=CCUG 56719\(^T\)).
dilution-acclimatization method and NSY medium (Hahn et al., 2004, 2005). Strain MWH-BRAZ-DAM2DT grew on a variety of solidified complex media including Luria-Bertani agar (Difco BD), Casitone agar (Difco BD), R2A agar (Remel) and NSY agar (Hahn et al., 2004), forming unpigmented, smooth, convex colonies.

Carbon source utilization tests and other phenotypic characterizations were performed as described previously (Hahn et al., 2009). Briefly, growth enabled by utilization of specific substrates was determined by comparison of optical density established in liquid one-tenth-strength NSY medium (0.3 g l\(^{-1}\)) with and without the respective test substrate (0.5 g l\(^{-1}\)). Optical density differences of <10%, 10–50% and >50% of the optical density established on medium without test substrate were scored after 10 days of growth as no utilization (−), weak utilization (W) and good utilization (+), respectively.

Analysis of the phylogenetic position of the novel isolate was performed by 16S rRNA gene sequence analysis as described previously (Hahn et al., 2009).

### Table 1. Phenotypic traits of strain MWH-BRAZ-DAM2DT (Limnohabitans australis sp. nov.) and other members of the genus Limnohabitans

| Characteristic | 1 | 2 | 3 | 4 |
|---------------|---|---|---|---|
| Cell morphology | Curved rods | Curved rods | Short rods | Rods |
| Cell length (μm) | 1.0–1.7 | 1.0–1.5 | 0.6 | 0.9 |
| Cell width (μm) | 0.4–0.5 | 0.4–0.5 | 0.3 | 0.3 |
| Growth temperature | Minimum (°C) | 12 (w) | 4 | 4 (w) | 4 |
| Maximum (°C) | 36 | 34 | 34 | 34 |
| Maximum NaCl concentration (%) | 0.2 | 0.5 | 0.5 | 0.5 |
| Catalase | – | + | + | + |
| Utilization of: | | | | |
| Ethanol | – | w | – | w |
| Glycerol | – | – | w | + |
| Glyoxylic acid | w | – | – | w |
| Glycolate | – | – | – | w |
| Acetate | + | + | – | + |
| Propionate | – | w | – | – |
| Pyruvate | + | + | w | + |
| DL-Malate | – | + | + | + |
| Malonate | w | – | – | – |
| Oxaloacetate | – | – | – | + |
| Succinate | + | + | w | + |
| Fumarate | w | + | w | + |
| Citrate | – | + | + | + |
| L-Glutamate | – | – | + | + |
| L-Glutamine | – | – | w | + |
| L-Histidine | – | – | – | + |
| L-Phenylalanine | – | – | – | + |
| L-Proline | – | – | + | + |
| L-Serine | – | – | – | + |
| L-Tryptophan | – | – | + | w |
| D-Ribose | – | w | – | – |
| D-Glucose | w | + | + | + |
| D-Galactose | – | w | – | – |
| D-Mannose | – | + | w | + |
| Sucrose | – | w | – | – |
| D-Glucuronate | + | + | – | – |
| DNA G+C content (mol%) | 55.8 | 55.5 | 59.9 | 59.9 |

Strains: 1, *Limnohabitans australis* sp. nov. strain MWH-BRAZ-DAM2DT; 2, *L. curvus* strain MWH-C5T (Hahn et al., 2010); 3, *L. parvus* strain II-B4T (Kasalický et al., 2010); 4, *L. planktonicus* strain II-D5T (Kasalický et al., 2010). Substrate utilization tests were performed for all four strains under the same conditions. All four strains grew under anoxic conditions, were oxidase-positive, non-motile and unpigmented, and possessed ubiquinone Q-8 as major quinone. Furthermore, all strains were positive for utilization of D-glycerate, butyrate and α-ketoglutarate and none of the four strains utilized oxalate, DL-lactate, L-arginine, L-sorbose, N-acetylglucosamine, L-carnitine, betaine or spermidine. −, Negative; +, positive; w, weakly positive.
described previously (Hahn et al., 2009). Determination of the DNA G+C content, analysis of major respiratory lipoquinones and DNA–DNA reassociation experiments, to ascertain whether the novel isolate belongs to a previously described *Limnohabitans* species (Wayne et al., 1987), were all carried out by the Identification Service and B. J. Tindall, DSMZ, Braunschweig, Germany. Fatty acid profiles were characterized by using the MIS Sherlock automatic identification system (MIDI) and the Sherlock Aerobic Bacterial Database (TSBA60) as described by Greenblatt et al. (1999). Biomass of duplicate cultures obtained by growing the strain in NSY medium (3 g l⁻¹) for 2 days at 21 °C was analysed. Results of the phenotypic and chemotaxonomic investigations are presented in Tables 1 and 2.

BLAST searches (Altschul et al., 1990) against the database with the 16S rRNA gene sequence of the novel isolate in December 2009 resulted in five and 81 hits with >99 % and >97 % sequence similarities, respectively. Thirteen out of the 81 hits represented cultivated strains, whereas the majority represented uncultivated bacteria. No cultivated strains were among the hits with >99 % sequence similarity. This group was represented by environmental sequences obtained from two estuary systems (Delaware and Chesapeake Bays, USA), as well as from Ipswich River, MA, USA (Shaw et al., 2008; Crump & Hobbie, 2005). A phylogenetic analysis of the relationship of cultivated *Limnohabitans* strain and environmental sequences representing the so-called ‘Rhodoferax sp. BAL47 cluster’ was presented previously (Hahn et al., 2010).

Phylogenetic analyses with sequence sets representing the most closely related recognized species by using the neighbour-joining (NJ) and the maximum-likelihood (ML) methods consistently revealed the affiliation of strain MWH-BRAZ-DAM2Dᵀ with the genus *Limnohabitans* (Fig. 1). The 16S rRNA gene sequence of the isolate possessed sequence similarities of 98.2, 97.0 and 96.5 % to those of the type strains of *Limnohabitans curvus*, *Limnohabitans parvus* and *Limnohabitans planktonicus*, respectively. DNA–DNA reassociation analyses with DNA of the type strains of the three previously described *Limnohabitans* species resulted in similarity values (mean values of duplicate measurements) of 26.2 % (*L. parvus*), 30.0 % (*L. planktonicus*) and 44.6 % (*L. curvus*). The duplicate measurements performed for each of the three pairings differed by 2.6 % (*L. curvus*) to 5.0 % (*L. planktonicus*). The predominant fatty acids of the isolate were C₁₆:₁,7c/δ6c (73.5 %), C₈:₀ 3-ΟΗ (8.2 %), C₁₆:₀ (7.7 %) and C₁₂:₀ (7.4 %). The major quinone was ubiquinone Q-8 and the DNA G+C content was 55.8 mol% (Tables 1 and 2).

Strain MWH-BRAZ-DAM2Dᵀ can be distinguished from the type strain of *L. curvus* (Hahn et al., 2010) by its ability to utilize malonate and its inability to utilize ethanol, propionate, malate, citrate, D-ribose, D-galactose, D-mannose and sucrose, as well as by a lower maximum NaCl concentration that supports growth and differences in the minimum and maximum growth temperatures (Table 1). In addition, the two strains differ in the presence of minor fatty acid compounds (Table 2). Differential traits that separate strain MWH-BRAZ-DAM2Dᵀ from all three previously described *Limnohabitans* species (Hahn et al., 2010; Kasalicky et al., 2010) are utilization of malonate and no utilization of malate or citrate, as well as the absence of the minor fatty acids C₁₆:₁,7c/δ6c, C₁₈:₁,7c and C₁₈:₁,9c, a catalase-negative reaction, a lower minimum NaCl concentration that supports growth and higher minimum and maximum growth temperatures (Tables 1 and 2). Differences in thermal adaptation may reflect differences in adaptation to local climate conditions at the sites of origin of the four strains (subtropical versus temperate climate) and may not represent a trait shared by all members of the proposed species *L. australis* sp. nov. (Hahn & Pöckl, 2005).

The phylogenetic analysis, as well as several phenotypic and chemotaxonomic similarities, suggest that strain MWH-BRAZ-DAM2Dᵀ belongs to the genus *Limnohabitans*. The results of the DNA–DNA reassociation analyses demonstrate that the strain does not belong to one of the previously described *Limnohabitans* species when the recommendation of a threshold value of 70 % DNA–DNA similarity for delineation of prokaryotic species (Wayne et al., 1987) is

### Table 2. Whole-cell fatty acid composition of *Limnohabitans australis* sp. nov. and other members of the genus *Limnohabitans*

| Fatty acid | 1   | 2   | 3   | 4   |
|-----------|-----|-----|-----|-----|
| C₈:₀ 3-ΟΗ | 8.2 | 2.7 | 1.0 | 0.7 |
| C₁₀:₀ 3-ΟΗ | ND  | ND  | ND  | 1.5 |
| C₁₂:₀ | 7.4 | 4.5 | 3.6 | 2.9 |
| C₁₂:₀ 3-ΟΗ | ND  | ND  | 1.8 | ND  |
| C₁₄:₀ | 1.0 | 1.0 | 0.4 | 0.5 |
| C₁₄:₁0₅c | 0.6 | 0.4 | 0.2 | 0.2 |
| C₁₅:₁0₆c | ND  | ND  | 1.3 | ND  |
| C₁₆:₀ | 7.7 | 14.0 | 15.0 | 19.5 |
| C₁₆:₁0₅c | ND  | 0.2 | 0.5 | 0.7 |
| C₁₆:₁0₇c/C₁₆:₁0₆c | 73.5 | 76.7 | 66.4 | 62.4 |
| C₁₇:₀ | ND  | ND  | 1.3 | ND  |
| C₁₇:₀ Cyclo | ND  | ND  | ND  | 0.7 |
| C₁₇:₁0₆c | ND  | ND  | 2.6 | ND  |
| C₁₈:₀ | ND  | 0.3 | 0.5 | 0.3 |
| 11-Me-C₁₈:₁0₇c | ND  | 0.3 | ND  | 1.3 |
| C₁₈:₁0₇c/C₁₈:₁0₆c | 1.7 | 1.8 | 5.3 | 8.9 |
| C₁₈:₁0₉c | ND  | 0.2 | 0.3 | 0.5 |
considered. Therefore, it is proposed that the novel species *Limnohabitans australis* sp. nov. be established to accommodate strain MWH-BRAZ-DAM2D⁺.

**Emended description of the genus *Limnohabitans***

Hahn et al. 2010 emend. Kasalický et al. 2010

The description of the genus *Limnohabitans* is as given previously (Hahn et al., 2010; Kasalický et al., 2010), but with the following amendment. Members of the genus can be catalase-positive or catalase-negative.

**Description of *Limnohabitans australis* sp. nov.**

*Limnohabitans australis* (aus. tra’lis. L. masc. adj. australis southern, relating to the region in which the organism was isolated).

Cells are curved rods, 1.0–1.7 μm in length and 0.4–0.5 μm in width. Chemo-organotrophic, aerobic, facultatively anaerobic, oxidase-positive and catalase-negative. Colonies grown on NSY agar are unpigmented, circular and convex in width. Chemo-organotrophic, aerobic, facultatively anaerobic, oxidase-positive and catalase-negative. Colonies

The type strain is MWH-BRAZ-DAM2D⁺ (=DSM 21646T =CCUG 56719T), isolated from Monjolinho Pond, São Carlos, Brazil. The DNA G+C value of the type strain is 55.8 mol%.

**Acknowledgements**

D. Elhottová and J. Petrásek are acknowledged for determination of fatty acid profiles supported by the project ASCR-ISB no. AV0Z 60660521. The DSMZ, Braunschweig, Germany, is acknowledged for chemotaxonomic analyses and for DNA–DNA reassociation experiments. This study was supported by the Czech–Austrian KONTAKT project MEB 060702/CZ 05/2007 (granted to K.-S. and M. W. H.), by the Austrian Science Fund (FWF) project P19853 (granted to M. W. H.), by the Grant Agency of the Czech Republic under research grant 206/08/0015 (granted to K. S.) and by the institutional project of the ASCR no. AV0Z 60170517.

**References**

Altschul, S., Gish, W., Miller, W., Myers, E. & Lipman, D. (1990). Basic local alignment search tool. J Mol Biol 215, 403–410.

Crump, B. C. & Hobbie, J. E. (2005). Synchrony and seasonality in bacterioplankton communities of two temperate rivers. Limnol Oceanogr 50, 1718–1729.

Greenblatt, C. L., Davis, A., Clement, B. G., Kitts, C. L., Cox, T. & Cano, R. J. (1999). Diversity of microorganisms isolated from amber. Microb Ecol 38, 58–68.

Hahn, M. W. & Pöckl, M. (2005). Ecotypes of planktonic *Actinobacteria* with identical 16S rRNA genes adapted to thermal niches in temperate, subtropical, and tropical freshwater habitats. Appl Environ Microbiol 71, 766–773.

Hahn, M. W., Studier, P., Wu, Q. L. & Pöckl, M. (2004). The filtration-acclimatization method for isolation of an important fraction of the not readily cultivable bacteria. J Microbiol Methods 57, 379–390.

Hahn, M. W., Pöckl, M. & Wu, Q. L. (2005). Low intraspecific diversity in a *Polynucleobacter* subcluster population numerically dominating bacterioplankton of a freshwater pond. Appl Environ Microbiol 71, 4539–4547.

Hahn, M. W., Lang, E., Brandt, U., Wu, Q. L. & Scheuerl, T. (2009). Emended description of the genus *Polynucleobacter* and the species *P. necessarius* and proposal of two subspecies, *P. necessarius* subsp. necessarius subsp. nov. and *P. necessarius* subsp. asymbionticus subsp. nov. Int J Syst Evol Microbiol 59, 2002–2009.

Hahn, M. W., Kasalický, V., Jezbera, J., Brandt, U., Jezberová, J. & Šimek, K. (2010). *Limnohabitans curvus* gen. nov., sp. nov., a planktonic bacterium isolated from a freshwater lake. Int J Syst Evol Microbiol 60, 1358–1365.

Kasalický, V., Jezbera, J., Šimek, K. & Hahn, M. W. (2010). *Limnohabitans planktonicus* sp. nov. and *Limnohabitans parvus* sp. nov., planktonic betaproteobacteria isolated from a freshwater pond. *Int J Syst Evol Microbiol* 60, 879–885.
reservoir, and emended description of the genus *Limnohabitans*. *Int J Syst Evol Microbiol* 60, 2710–2714.

Percent, S. F., Frischer, M. E., Vescio, P. A., Duffy, E. B., Milano, V., McLellan, M., Stevens, B. M., Boylen, C. W. & Nierzwicki-Bauer, S. A. (2008). Bacterial community structure of acid-impacted lakes: what controls diversity? *Appl Environ Microbiol* 74, 1856–1868.

Shaw, A. K., Halpern, A. L., Beeson, K., Tran, B., Venter, J. C. & Martiny, J. B. (2008). It’s all relative: ranking the diversity of aquatic bacterial communities. *Environ Microbiol* 10, 2200–2210.

Šimek, K., Pernthaler, J., Weinbauer, M. G., Horňák, K., Dolan, J. R., Nedoma, J., Mašín, M. & Amann, R. (2001). Changes in bacterial community composition and dynamics and viral mortality rates associated with enhanced flagellate grazing in a mesoeutrophic reservoir. *Appl Environ Microbiol* 67, 2723–2733.

Šimek, K., Horňák, K., Jezbera, J., Nedoma, J., Vrba, J., Straškrábová, V., Macek, M., Dolan, J. R. & Hahn, M. W. (2006). Maximum growth rates and possible life strategies of different bacterioplankton groups in relation to phosphorus availability in a freshwater reservoir. *Environ Microbiol* 8, 1613–1624.

Šimek, K., Kasalický, V., Jezbera, J., Jezberová, J., Hejzlar, J. & Hahn, M. W. (2010). Broad habitat range of the phylogenetically narrow R-BT065 cluster representing a core group of the betaproteobacterial genus *Limnohabitans*. *Appl Environ Microbiol* 76, 631–639.

Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* 37, 463–464.

Zwart, G., Crump, B. C., Kamst-van Agterveld, M. P., Hagen, F. & Han, S.-K. (2002). Typical freshwater bacteria: an analysis of available 16S rRNA gene sequences from plankton of lakes and rivers. *Aquat Microb Ecol* 28, 141–155.