Published in final edited form as:

Int J Syst Evol Microbiol. 2010 December ; 60(Pt 12): 2710–2714. doi:10.1099/ijms.0.018952-0.

***Limnohabitans planktonicus* sp. nov., and *Limnohabitans parvus* sp. nov., two novel planktonic *Betaproteobacteria* isolated from a freshwater reservoir**

Vojtěch Kasalický^{1,2}, Jan Jezbera¹, Karel Šimek^{1,2}, and Martin W. Hahn³

¹Biology Centre of the Academy of Sciences CR, v.v.i. Institute of Hydrobiology, Na Sádkách 7, 37005 České Budějovice, Czech Republic

²Faculty of Science, University of South Bohemia, Branišovská 31, 37005 České Budějovice, Czech Republic

³Institute for Limnology, Austrian Academy of Sciences, Mondseestrasse 9, A-5310 Mondsee Austria.

Abstract

Two strains II-B4^T and II-D5^T, isolated from the meso-eutrophic freshwater ímov Reservoir (Czech Republic) were phenotypic ally, phylogenetically and chemotaxonomically characterized. Both strains are chemoorganotrophic, facultatively anaerobic, rod-shaped, non-motiles with identical G+C contents of their DNA of 59.9 mol%. Their major polar lipids were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine, and their major fatty acids were C_{16:1}ω7c/C_{16:1}ω6c, C_{16:0}, C_{18:1}ω7c/C_{18:1}ω6c and C_{12:0}. Both strains contained Q-8 as the only respiratory quinone component. The 16S rRNA sequences of the two strains possess a similarity of 99.0%, however the DNA-DNA reassociation value was only 26.7%, and the strains can be discriminated from each other by several chemotaxonomic and biochemical traits. The phylogenetic analysis of the 16S rRNA gene sequences revealed the affiliation of both strains with the genus *Limnohabitans* within the family *Comamonadaceae*. The two investigated strains represent a narrow phylogenetic cluster (so-called R-BT065 cluster) formed by a large number of environmental sequences obtained from the pelagic zones of various freshwater habitats. We propose to place the two strains in separate new species within the genus *Limnohabitans*. The two proposed species are *Limnohabitans planktonicus* sp. nov. with the type strain II-D5^T (= DSM 21594^T = CIP 109844^T), and *Limnohabitans parvus* sp. nov. with the type strain II-B4^T (= DSM 21592^T = CIP 109845^T).

Betaproteobacteria frequently represent the numerically dominating group of bacterioplankton in a broad variety of freshwater habitats. Recent research demonstrated that only a few, phylogenetically narrow subgroups of the *Betaproteobacteria* are responsible for the ecological success of this group in freshwater habitats (e.g., Zwart *et al.*, 2002). The so-called R-BT065 cluster (Šimek *et al.*, 2001) represents such a phylogenetically defined group of abundant *Betaproteobacteria*. This cluster was defined based only on environmental sequences retrieved from freshwater habitats, and represents a subgroup of the so-called 'Rhodoferax sp. BAL47' cluster (Zwart *et al.*, 2002). Members of the R-BT065 cluster can be detected and quantified in environmental samples by a specific FISH probe (Šimek *et al.*, 2001). Application of this probe revealed that the targeted cells possess a

Correspondence: Vojtěch Kasalický, vojtech.ves@post.cz.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Limnohabitans planktonicus* sp. nov. strain II-D5^T and *Limnohabitans parvus* sp. nov. II-B4^T are FM165535 and FM165536, respectively

planktonic lifestyle, and revealed that this cluster comprises typically 5-30 % (maximum ~ 50 %) of total bacterioplankton in non-acidic stagnant freshwater habitats (Šimek *et al.*, 2001, 2005; Pérez and Sommaruga, 2006; Salcher *et al.*, 2008; Šimek *et al.*, unpub. data).

Ecological studies revealed that members of the R-BT065 cluster possess the ability to rapidly respond to changes in autochthonous (algal-derived) organic substrate supply or shifts in predation pressure by bacterivorous protists (Jezbera *et al.*, 2006, Šimek *et al.*, 2006, 2008, Horák *et al.*, 2008). Moreover, this cluster usually represents the fastest growing segment of bacterioplankton in lakes (e.g. Šimek *et al.*, 2006, Salcher *et al.*, 2008). Thus, this cluster of bacteria obviously plays an important role in organic matter processing and transformation in freshwater systems. So far, the so-called R-BT065 cluster is lacking validly described species. Here, we describe two strains affiliated with this cluster and propose to establish for them two new species in the genus *Limnohabitans* (Hahn *et al.*, in press).

The two strains, II-B4^T and II-D5^T, were isolated from the water column of Šimov Reservoir (Supplemental Material Table S1 in IJSEM Online) using the filtration-acclimatization method (Hahn *et al.*, 2004). Briefly, the whole water sample was filtered through a 0.8 µm polycarbonate membrane filter (Millipore) and was subsequently diluted with sterile medium in order to obtain cell concentrations suitable for inoculation of 24-well microplates with approximately 0.5 cell per well. The established cultures were acclimatized to growth in NSY medium by stepwise addition of increasing doses of NSY medium (Hahn *et al.*, 2004).

The isolated strains were routinely grown in NSY medium with strength of 3 g l⁻¹ either liquid or solidified with 1.5% (w/v) agar. Both strains were tested for growth on commercially available complex media. The strength of complex media and agar concentrations were adjusted to 3 g l⁻¹ and 1.5%, respectively. Due to inefficient growth of the investigated strains on media containing only a single carbon source, assimilation experiments were performed by comparison of optical density (OD) established in liquid one-tenth-strength NSY medium (0.3 g l⁻¹) with and without 0.5 g l⁻¹ test substance (pH of 7.2) as described previously (Hahn *et al.*, 2009). OD differences of <10%, of 10-50%, and of >50% of the OD established on the medium without test substance were scored as no assimilation, weak assimilation and assimilation, respectively. Growth at different temperatures (4, 6, 8, 10, 12, 15, 21, 34 and 36 °C), and growth under anoxic conditions in an anaerobic chamber were examined on NSY medium amended with 1.5% agar. NaCl tolerance was determined using NSY agar supplemented with different NaCl concentrations (0, 0.05, 0.075, 0.1, 0.15, 0.2, 0.3, 0.5, 1.0 and 1.5% w/v).

Gram-staining was performed as described by Hucker and Conn (1927). Catalase activity was tested by bubble formation in a 3% (v/v) H₂O₂ solution. Oxidase activity was determined by oxidation of 1% *p*-aminodimethylaniline oxalate. Cell morphologies of DAPI-stained cells from cultures grown for 3 days in 3 g l⁻¹ NSY medium at RT were inspected by an Olympus BX 60 microscope using the semiautomatic image analysis system LUCIA D (Lucia 3.52, Laboratory Imaging, Prague, Czech Republic).

The 16S rRNA genes of the two strains were sequenced and analyzed as described previously (Hahn *et al.*, 2009). Neighbour-Joining trees were calculated by using the software MEGA4 (Tamura *et al.*, 2007), and Maximum-Likelihood trees were generated by using the RaxML web server (Stamatakis *et al.*, 2008).

Cellular fatty acid (FA) contents of the strains were characterized by using the MIS Sherlock automatic identification System (MIDI, Inc., Newark, DE) and the Sherlock Aerobic Bacterial Database (TSBA60) as described by Greenblatt *et al.* (1999). For this analysis,

biomass of replicated cultures of each strain grown in NSY medium (3 g l^{-1}) for 2 days at 21°C was analyzed.

The determination of the G+C content of DNA, the analyses of major respiratory quinones, polar lipids, and the DNA–DNA hybridization of both strains were carried out by the Identification Service of the DSMZ and Dr. Brian J. Tindall, DSMZ, Braunschweig, Germany. For DNA–DNA reassociation experiments, DNA was isolated using a French pressure cell (Thermo Spectronic) and purified by chromatography on hydroxyapatite, as described by Cashion et al. (1977). DNA–DNA hybridization was carried out under optimal conditions for DNA–DNA reassociation as described by De Ley et al. (1970), with the modifications described by Huß et al. (1983), using a Cary 100 Bio UV/VIS spectrophotometer equipped with a Peltier-thermostated 6×6 multicell changer and a temperature controller with an *in situ* temperature probe (Varian). The base composition of the DNA of the strains was determined as described by Tóth et al. (2008).

Both strains, II-B4^T and II-D5^T, are Gram-negative, rod-shaped and non-motile bacteria. Both grew well on NSY medium, R2A agar (Remel), Standard Methods agar (Remel), Peptone agar (BD-Difco), Casitone agar (BD-Difco), solidified Luria–Bertani broth (BD-Difco), solidified Brain Heart Infusion (BD-Difco), and solidified Tryptic Soy Broth (BD-Difco) whereas no growth occurred on Löwenstein Medium Base (BD-Difco). Growth of both strains was observed in the temperature range $4\text{--}34^\circ\text{C}$. They grew well under aerobic conditions, whereas anaerobic growth was only weak. The results of the phenotypic and chemotaxonomic investigations are presented in Tables 1 and 2. The strain II-D5^T was able to utilize 19 (plus 4 weakly) of 37 tested substrates, whereas the strain II-B4^T responded with negative growth in more than half of tests (22 substrates). The major polar lipids of both strains were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine (Supplemental Material Figure S2 in IJSEM Online), and their major fatty acids were $\text{C}_{16:1}\omega 7\text{c}/\text{C}_{16:1}\omega 6\text{c}$, $\text{C}_{16:0}$, $\text{C}_{18:1}\omega 7\text{c}/\text{C}_{18:1}\omega 6\text{c}$ and $\text{C}_{12:0}$. Their major ubiquinone was Q-8, respectively, and they shared identical G+C contents of their DNA of 59.9 mol%.

The phylogenetic analyses of the 16S rRNA gene sequences by two independent algorithms resulted in trees with largely identical branching orders (Fig. 2). The two trees only differed in the phylogenetic positions of the taxa *Polaromonas*, *Caenimonas*, and *Variovorax*, which are more distantly related to the two investigated strains than the other reference taxa. Both phylogenetic reconstructions indicated the affiliation of strains II-D5^T and II-B4^T with the genus *Limnohabitans* within the family *Comamonadaceae*. Phylogenetic analyses with sequence sets including environmental sequences (data not shown) confirmed the affiliation of the two strains with the R-BT065 cluster (Šimek et al., 2001). Note that *L. curvus* does not belong to this important cluster of freshwater bacteria, thus the two investigated strains represent the first taxonomically described members of this phylogenetic cluster.

The affiliation of the two investigated strains to the genus *Limnohabitans* is also supported by several chemotaxonomic and phenotypic traits shared by the two strains and the type strain of the type species of the genus *Limnohabitans*. These traits include the major fatty acids and quinone compositions, low salinity tolerance, lack of pigmentation, lack of motility, and their inability to assimilate lactate.

At the opposite, the two investigated strains II-B4^T and II-D5^T can be discriminated from *L. curvus* by the presence or absence of minor fatty acids components, and some physiological and metabolic characteristics (Table 1). Furthermore, the two species can be differentiated from each other by their morphology and by several metabolic traits (see Table 1 for details), or by the absence or presence of seven minor fatty acids (Table 2). The strains

differ, for instance, in assimilation of acetate, L-histidine, oxaloacetate, and in the presence/absence of C_{17:0} and C_{17:1}ω6c fatty acids. Traits discriminating both strains from the type strain of *L. curvus* are, for example, assimilation of L-glutamate and L-proline, as well as, lack of assimilation of D-gluconate and propionate (Table 1).

Contrary to *L. curvus*, both newly described strains share a short diagnostic 16S rRNA gene sequence (*E. coli* positions 65–83) targeted by the FISH probe R-BT065 (5′-GTTGCCCCCTCTACCGTT-3′, Šimek *et al.*, 2001). Comparative analysis of the almost full-length 16S rRNA gene sequences (1411 bp) of the strains II-B4^T and II-D5^T revealed a sequence similarity of 99.0 % whereas the similarities with *L. curvus* were 96.8 and 96.7%, respectively. DNA-DNA reassociation experiments with DNA extracted from strains II-B4^T and II-D5^T resulted in a value of 26.7%, which clearly justifies the proposal to place the two strains in separate species (Wayne *et al.*, 1987).

Regarding to all explicit differences between the two strains and the type strain of *L. curvus*, as well as between the two strains, we propose to establish the two new species *Limnohabitans parvus* sp. nov. with the type strain II-B4^T, and *Limnohabitans planktonicus* sp. nov. with the type strain II-D5^T within the genus *Limnohabitans*.

Description of *Limnohabitans parvus* sp. nov.

Limnohabitans parvus [par'vus. L. masc. adj. *parvus* small, referring to the small cell size of the type strain].

Cells are Gram-negative, short rods, about 0.3 μm in diameter and about 0.6 μm length, non-motile, unpigmented, oxidase-positive and catalase-positive. Growth is evident at temperatures of 4–34 °C, well-growing under aerobic conditions, weak anaerobic growth. Good growth occurs on NSY, R2A, Standards Method Agar, TSB, LB Broth, Peptone, Brain Heart Infusion, and Casitone agar, not growing on Löwenstein Medium. 1–2 mm large colonies are formed within 2–5 days (RT). Growth occurs at a salinity range 0–0.5% NaCl (w/v). The only respiratory quinone is Q-8. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. The fatty acids profile is largely composed of C_{16:1}ω7c/C_{16:1}ω6c (66.4 %), C_{16:0} (15.0 %), C_{12:0} (3.6 %), and C_{18:1}ω7c/C_{18:1}ω6c (5.3 %). The major detected hydroxy-fatty acids are C_{8:0}-3OH (1.0 %) and C_{12:0}-3OH (1.8 %). The type strain is II-B4^T (= DSM 21592^T = CIP 109845^T). The type strain was isolated from the freshwater meso-eutrophic ímov Reservoir, Czech Republic, and possesses a free-living planktonic lifestyle.

Description of *Limnohabitans planktonicus* sp. nov.

Limnohabitans planktonicus [plank.to'ni.cus. N.L. masc. adj. (from Gr. adj. *planktos*) *planktonicus* living in the plankton, planktonic].

Cells are Gram-negative, rod-shaped, about 0.3–0.4 μm in diameter and about 0.9 μm in length, non-motile, unpigmented, oxidase-positive and catalase-positive. Growth is evident at temperatures of 4–34 °C, well-growing under aerobic conditions, weak anaerobic growth. Good growth occurs on NSY, R2A, Standards Method Agar, TSB, LB Broth, Peptone, Brain Heart Infusion, and Casitone agar, not growing on Löwenstein Medium. 1–2 mm large colonies are formed in 2–5 days (RT). Growth occurs at a salinity range 0–0.5% NaCl (w/v). The only respiratory quinone is Q-8. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. The fatty acids profile is largely composed of C_{16:1}ω7c/C_{16:1}ω6c (62.4 %), C_{16:0} (19.5 %), C_{18:1}ω7c/C_{18:1}ω6c (8.9 %), and C_{12:0} (2.9 %). The major detected hydroxyl-fatty acids were C_{8:0}-3OH (0.7 %) and C_{10:0}-3OH (1.5 %). The type strain is II-D5^T (= DSM 21594^T = CIP

109844^T). The type strain was isolated from the freshwater meso-eutrophic ímov Reservoir, Czech Republic, and possesses a free-living planktonic lifestyle.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

D. Elhottová and J. Petrásek are acknowledged for determination of fatty acids profiles supported by the project ASCR - ISB No. AV0Z 60660521. We wish to thank H. G. Trüper for etymological advice, and A. Hartmanová, R. Malá and U. Brandt for excellent technical assistance. The DSMZ, Braunschweig, Germany is acknowledged for chemotaxonomic analyses. This study was largely supported by the Grant Agency of the Czech Republic under research grants 206/08/0015 (granted to KS), by the Czech-Austrian KONTAKT project MEB 060602 / CZ 05-2007 (granted to KS and MWH), by the institutional project of the ASCR No. AV0Z 60170517, and by the Austrian Science Fund (FWF) project P19853 (granted to MWH). The authors also benefited from participation in ALTERnet (A Long-Term Biodiversity, Ecosystem and Awareness Research Network), an EU Network of Excellence (GOCE-CT-2003-505298).

Abbreviations

FISH	Fluorescent <i>in situ</i> hybridization
OD	Optical density
RT	room temperature

References

- Cashion P, Hodler-Franklin MA, McCully J, Franklin M. A rapid method for base ratio determination of bacterial DNA. *Anal Biochem.* 1977; 81:461–466. [PubMed: 907108]
- De Ley J, Cattoir H, Reynaerts A. The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem.* 1970; 12:133–142. [PubMed: 4984993]
- Greenblatt CL, Davis A, Clement BG, Kitts CL, Cox T, Cano RJ. Diversity of microorganisms isolated from amber. *Microbiol Ecol.* 1999; 38:58–68.
- Hahn MW, Stadler P, Wu QL, Pöckl M. The filtration-acclimatization-method for isolation of an important fraction of the not readily cultivable bacteria. *J Microb Meth.* 2004; 57:379–390.
- Hahn MW, Lang E, Brandt U, Wu QL, Scheuerl T. Emended description of the genus *Polynucleobacter* and the species *P. necessarius* and proposal of two subspecies, *P. necessarius* subspecies *necessarius* subsp. nov. and *P. necessarius* subsp. *asymbioticus* subsp. nov. *Int J Syst Evol Microbiol.* 2009; 59:2002–2009. [PubMed: 19567561]
- Hahn MW, Kasalický V, Jezbera J, Brandt U, Jezberová J, Šimek K. *Limnohabitans curvus* gen. nov., sp. nov., planktonic bacterium isolated from a freshwater lake. *Int J Syst Evol Microbiol.* (in press).
- Horák K, Jezbera J, Šimek K. Impact of *Microcystis aeruginosa* and flagellates on bacterial growth and activity in a eutrophic reservoir. *Aquat Microb Ecol.* 2008; 52:107–117.
- Hucker GJ, Conn HJ. Further studies on the methods of Gram staining. *NY Agric Exp. Stn Tech Bull.* 1927; 128
- HuB VAR, Festl H, Schleifer KH. Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol.* 1983; 4:184–192. [PubMed: 23194591]
- Jezbera J, Horák K, Šimek K. Prey selectivity of bacterivorous protists in different size fractions of reservoir water amended with nutrients. *Environ Microbiol.* 2006; 8:1330–1339. [PubMed: 16872397]
- Pérez MT, Sommaruga R. Differential effect of algal- and soil-derived dissolved organic matter on alpine lake bacterial community composition and activity. *Limnol Oceanogr.* 2006; 51:2527–2537.

- Salcher MM, Pernthaler J, Zeder M, Psenner R, Posch T. Spatio-temporal niche separation of planktonic *Betaproteobacteria* in an oligo-mesotrophic lake. *Environ Microbiol.* 2008; 10:2074–2086. [PubMed: 18430016]
- Šimek K, Pernthaler J, Weinbauer MG, Horák K, Dolan JR, Nedoma J, Mašín M, Amann R. Changes in bacterial community composition, dynamics and viral mortality rates associated with enhanced flagellate grazing in a meso-eutrophic reservoir. *Appl Environ Microbiol.* 2001; 67:2723–2733. [PubMed: 11375187]
- Šimek K, Horák K, Jezbera J, Mašín M, Nedoma J, Gasol JM, Schauer M. Influence of top-down and bottom-up manipulations on the R-BT065 subcluster of β -proteobacteria, an abundant group in bacterioplankton of a freshwater reservoir. *Appl Environ Microbiol.* 2005; 71:2381–2390. [PubMed: 15870325]
- Šimek K, Horák K, Jezbera J, Nedoma J, Vrba J, Straškrábová V, Macek M, Dolan JR, Hahn MW. Maximum growth rates and possible life strategies of different bacterioplankton groups in relation to phosphorus availability in a freshwater reservoir. *Environ Microbiol.* 2006; 8:1613–1624. [PubMed: 16913921]
- Šimek K, Horák K, Jezbera J, Nedoma J, Znachor P, Hejzlar J, Sedláčková J. Spatio-temporal patterns of bacterioplankton production and community composition related to phytoplankton composition and protistan bacterivory in a dam reservoir. *Aquat Microb Ecol.* 2008; 51:249–262.
- Stamatakis A, Hoover P, Rougemont J. A rapid bootstrap algorithm for the RAxML web-servers. *Syst Biol.* 2008; 57:758–771. [PubMed: 18853362]
- Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis, (MEGA) software version 4.0. *Molecular Biology and Evolution.* 2007; 24:1596–1599. [PubMed: 17488738]
- Tóth EM, Kéki Z, Homonnay ZG, Borsodi AK, Márialigeti K, Schumann P. *Nocardioides daphniae* sp. nov., isolated from *Daphnia cucullata*, (Crustacea: Cladocera). *Int J Syst Evol Microbiol.* 2008; 58:78–83. [PubMed: 18175687]
- Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, Krichevsky MI, Moore LH, Moore WEC, Murray RGE, Stackebrandt E, Starr MP, Trüper HG. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol.* 1987; 37:463–464.
- Zwart G, Crump BC, Kamst-van Agterveld MP, Hagen F, Han SK. Typical freshwater bacteria: an analysis of available 16S rRNA gene sequences from plankton of lakes and rivers. *Aquat Microb Ecol.* 2002; 28:141–155.

Fig. 1. Electron microscopy images illustrating the cell morphology and size of the type strains II-B4^T (A) and II-D5^T (B), respectively. Cells of a liquid culture were concentrated by centrifugation and fixed with 2.5% glutaraldehyde, post-fixed with OsO₄ and embedded with Spurr resin. Ultra-thin sections were counterstained with uranyl acetate and lead citrate. The image was obtained by transmission electron microscopy.

Fig. 2.

Neighbour-joining (NJ) tree based on almost complete 16S rRNA gene sequences, reconstructing the phylogenetic position of the two investigated strains. The NJ tree shared with a maximum likelihood (ML) tree (not shown) calculated with the same sequence set identical branching orders except of the branchings by *P. vacuolata*, *C. koreensis*, and *V. paradoxus*. Bootstrap values obtained by the NJ (first value) and the ML algorithm (second value) are shown. Nodes not reconstructed in the ML tree show instead of a bootstrap value a horizontal slash. Bar indicates 0.05 substitutions per nucleotide position

Table 1

Phenotypic differentiation characteristics of type strains II-B4^T and II-D5^T from each other and from *Limnohabitans curvus* strain MWH-C5^T. The substrate utilization tests were performed for all three strains under the same conditions. All three strains are catalase-positive, oxidase-positive, non-motile and unpigmented and share the following traits: low salinity tolerance (0.5%) assimilate (all '+') α -ketoglutarate, citrate, butyrate, D-glucose, D-glycerate, malate; no assimilation ('-') of L-arginine, betaine, L-carnitine, DL-lactate, malonate, N-acetyl-glucosamine, oxalate, L-sorbose and spermidine. '+', positive; 'w', weak; '-', negative assimilation. Data for *L. curvus* from Hahn *et al.* (in press).

	II-B4 ^T	II-D5 ^T	L. curvus
Cell morphology	short rods	rods	curved rods
Cell length (μm)	0.6 ± 0.14	0.9 ± 0.26	1-1.5
Cell width (μm)	0.3 ± 0.05	0.3 ± 0.07	0.4 - 0.5
Acetate	–	+	+
Ethanol	–	w	w
Fumarate	w	+	+
Galactose (D–)	–	–	w
Gluconate (D–)	–	–	+
Glutamate (L–)	+	+	–
Glutamine	w	+	–
Glycerol	w	+	–
Glycolate	–	w	–
Glyoxylate	–	w	–
Histidine (L–)	–	+	–
Mannose (D–)	w	+	+
Oxaloacetate	–	+	–
Phenylalanine (L–)	–	+	–
Proline (L–)	+	+	–
Propionate	–	–	w
Pyruvate	w	+	+
Ribose (D–)	–	–	w
Saccharose (D–)	–	–	w
Serine (L–)	–	+	–
Succinate	w	+	+
Tryptophan (L–)	+	w	–

Table 2

Cellular fatty acid composition of both strains II-B4^T and II-D5^T and *Limnohabitans curvus* strain MWH-C5^T. Values are percentages of the summed fatty acids; *nd* – not detected. For unsaturated fatty acids, the position of the double bond is located by counting from the methyl (ω) end of the carbon chain. *Cis* and *trans* isomers are indicated by the suffixes *c* and *t*, respectively. Data for *L. curvus* from Hahn *et al.* (in press).

	II-B4 ^T	II-D5 ^T	<i>L. curvus</i>
C _{8:0} -3OH	1.0	0.7	2.7
C _{10:0} -3OH	nd	1.5	nd
C _{12:0}	3.6	2.9	4.5
C _{12:0} -3OH	1.8	nd	nd
C _{14:0}	0.4	0.5	1.0
C _{14:1} ω 5c	0.2	0.2	0.4
C _{15:1} ω 6c	1.3	nd	nd
C _{16:0}	15.0	19.5	14.0
C _{16:1} ω 5c	0.5	0.7	0.2
C _{16:1} ω 7c/C _{16:1} ω 6c	66.4	62.4	76.7
C _{17:0}	1.3	nd	nd
C _{17:0} cyclo	nd	0.7	nd
C _{17:1} ω 6c	2.6	nd	nd
C _{18:0}	0.5	0.3	0.3
C _{18:1} ω 7c 11Me	nd	1.3	0.3
C _{18:1} ω 7c/C _{18:1} ω 6c	5.3	8.9	1.8
C _{18:1} ω 9c	0.3	0.5	0.2