NOTE

***Chelatobacter heintzii* (Auling *et al.* 1993) is a later subjective synonym of *Aminobacter aminovorans* (Urakami *et al.* 1992)**

¹ Institut für Angewandte Mikrobiologie, Justus-Liebig-Universität, Heinrich-Buff-Ring 26–32, D-35392 Giessen, Germany

² Department of Applied Chemistry and Microbiology, College of Agriculture and Forestry at the University of Helsinki, FIN 000140 Helsinki University, Finland

³ Institut für Mikrobiologie und Genetik, Universität Wien, Dr Bohrgasse 9, A-1030 Wien, Austria

⁴ Institut für Bakteriologie, Mykologie und Hygiene, Veterinärmedizinische Universität, Veterinärplatz 1, A-1210 Wien, Austria

Peter Kämpfer,¹ Alexander Neef,¹ Mirja S. Salkinoja-Salonen² and Hans-Jürgen Busse^{3,4}

Author for correspondence: Peter Kämpfer. Tel: +49 641 99 37352. Fax: +49 641 99 37359. e-mail: peter.kaempfer@agrar.uni-giessen.de

***Chelatobacter heintzii*, which was described as a nitrilotriacetate-utilizing organism, was re-investigated in order to clarify its taxonomic position. On the basis of 16S rDNA sequence comparisons, it is obvious that this species clusters phylogenetically with species of the genus *Aminobacter*. The results of investigations of the fatty acid patterns, polar lipid profiles, polyamine patterns and quinone system supported this placement. The substrate-utilization profiles and fatty acid patterns of four strains (belonging to two different genomovars) revealed homogeneous results and showed high levels of similarity to *Aminobacter aminovorans*. DNA–DNA similarity studies confirmed that both genomovars of *Chelatobacter heintzii* belong to *Aminobacter aminovorans*. It could be shown that all species of this group are highly interrelated. On the basis of these data and previously published results, it is obvious that *Chelatobacter heintzii* is a later subjective synonym of *Aminobacter aminovorans*.**

Keywords: *Chelatobacter heintzii*, *Aminobacter*, phylogeny, DNA–DNA similarity, phenotypic traits

In a study of nitriloacetate-utilizing organisms, Auling *et al.* (1993a) described two new genera, for which the names *Chelatococcus* and *Chelatobacter* were proposed. Two strains represented the only species of the genus *Chelatococcus*, namely *Chelatococcus asaccharovorans*. These organisms showed a coccoid cell morphology. Analysis of partial 16S rRNA sequences and 16S rRNA cataloguing showed that *Chelatococcus* was most closely related to *Rhodopseudomonas acidophila* (Auling *et al.*, 1993a).

Nine further strains were shown to belong, phylogenetically, to the *Agrobacterium–Rhizobium* branch of the α -*Proteobacteria*. These organisms were subjected to DNA–DNA similarity studies (Auling *et al.*, 1993a) and represented two similarity groups, A₁ and A₂ (genomovars), for which no differentiating phenotypes could be detected. For this reason, only one species, *Chelatobacter heintzii*, was proposed. The name *Chelatobacter* was validated in the same year (Auling *et al.*, 1993b). One year earlier, Urakami *et al.* (1992) had reported the investigation of some non-methane-, non-methanol- and non-methylamine-utilizing bacteria, among them organisms originally described as [*Pseudomonas*] *aminovorans*, which Green

& Gillis (1989) had already shown could be considered as misnamed. In a polyphasic taxonomic investigation, it was shown that the organisms under study represented a new genus, for which the name *Aminobacter* was proposed. Three species, *Aminobacter aminovorans*, *Aminobacter aganoensis* and *Aminobacter niigataensis*, were described which could be (clearly) differentiated from each other on the basis of DNA–DNA similarities as well as biochemical and physiological tests (Urakami *et al.*, 1992).

Although already discussed by Auling *et al.* (1993a), the phylogenetic (and also phenotypic) similarity of organisms belonging to the two genera (*Chelatobacter* and *Aminobacter*) became evident in a polyphasic study of Kämpfer *et al.* (1999), in which the genus *Pseudaminobacter* was described. In the latter study, the type strains of all three *Aminobacter* species and *Chelatobacter heintzii* DSM 6450 were included. The 16S rDNA sequences of *Aminobacter aganoensis* and *Aminobacter niigataensis* were identical across 1466 bp. Furthermore, similarities above 99.6% were found between the 16S rDNA sequences from the type strains of all three species of *Aminobacter* and that of *Chelatobacter heintzii* DSM 6450 (Kämpfer *et al.*,

Table 1. Major fatty acid compositions of type strains of species of the genera *Chelatobacter* and *Aminobacter*

Values are percentages of total fatty acids. All strains were grown on trypticase soy broth agar at 28 °C for 48 h prior to fatty acid analysis. Data for *Aminobacter aminovorans*, *Aminobacter aganoensis* and *Aminobacter niigataensis* are from Kämpfer *et al.* (1999).

Compound	<i>Chelatobacter heintzii</i> (4 strains)	<i>A. aminovorans</i> DSM 7048 ^T	<i>A. niigataensis</i> DSM 7050 ^T	<i>A. aganoensis</i> DSM 7051 ^T
Saturated fatty acids				
9:0	0.7–1.7	1.0		
16:0	6.3–8.4	4.9	6.0	6.2
17:0	0.7–2.4	0.7		
18:0	2.1–7.2	1.5	2.0	2.0
Unsaturated fatty acids*				
15:1 ω 8 <i>c</i>				1.3
17:1 ω 8 <i>c</i>	0.4–1.3	0.7		
19:1 ω 12 <i>t</i>		1.3		
20:1 ω 7 <i>t</i>	0.7–1.3	0.8	0.7	
Branched fatty acids				
15:0 <i>i</i>			3.1	2.9
17:0 <i>i</i>	2.6–6.9	1.0	3.0	4.8
Hydroxy fatty acids				
12:0 3OH	0.2–0.6	0.3	0.4	0.4
Summed features†				
Summed feature 4	0.4–1.6	0.5	1.0	1.1
Summed feature 7	35.7–46.2	46.4	48.6	64.8
Cyclopropane acids				
17:0 cyclo		0.5		
19:0 cyclo ω 8 <i>c</i>	4.8–14.7	13.7	10.3	4.7
Unknown fatty acids‡				
14:966	0.6–1.5	0.7		
18:081	11.3–24.5	24.8	21.0	10.9
18:597	0.5–1.2	0.5	1.8	0.8
18:804	0.8–1.3	0.4	0.7	

* For unsaturated fatty acids, the position(s) of the double bond is located by counting from the methyl (ω) of the carbon chain.

† Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 4 contained one or more of the following fatty acids: 16:1 ω 7*c* and 15:0 iso 2OH. Summed feature 7 contained one or more of the following isomers: 18:1 ω 7*c*, 18:1 ω 9*t* and/or 18:1 ω 12*t* (*cis* and *trans* isomers are indicated by the suffixes *c* and *t*).

‡ Unknown fatty acids have no names listed in the Peak library file of the MIDI system and therefore cannot be identified.

1999). These high values clearly demonstrated the strong generic relationships of members of the genera *Aminobacter* and *Chelatobacter* according to 16S rRNA sequence comparisons.

Representatives of both genera contained an ubiquinone with 10 isoprenoid units in the side-chain (ubiquinone Q-10) (Urakami *et al.*, 1992; Auling *et al.*, 1993a). With respect to the overall polar lipid patterns, the same profile and nearly the same amounts of polar lipids were detected. For all three type strains of *Aminobacter* species and the type strain of *Chelatobacter heintzii*, phosphatidylmonomethylethanolamine was the sole predominant polar lipid. Phosphatidylcholine, phosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylethanolamine, diphosphatidylglycerol and an unidentified phospholipid were present in minor amounts (Kämpfer *et al.*, 1999). All three *Aminobacter* species and *Chelatobacter heintzii* were

also very similar in their polyamine patterns, which were characterized by the predominant compound *sym*-homospermidine, large amounts of putrescine and small amounts of spermidine (Busse & Auling, 1988; Hamana & Kishimoto, 1996; Auling *et al.*, 1993a; Kämpfer *et al.*, 1999). Thus, the results of chemotaxonomic investigations supported the conclusions based on phylogenetic studies.

With respect to whole-cell fatty acid patterns, all of the type strains of *Aminobacter* species and *Chelatobacter heintzii* contained the fatty acids 16:0, 18:0, 17:0 iso, summed feature 4 (16:1 ω 7*c* and/or 15:0 iso 2OH), summed feature 7 (18:1 ω 7*c*, 18:1 ω 9*t* and/or 18:1 ω 12*t*) and an unknown fatty acid, not identified with the MIDI database (Table 1). These fatty acids have also been detected in previous studies and are characteristic of all validly described species of the genus *Aminobacter* (Urakami *et al.*, 1992). The presence of

Table 2. Differentiating characteristics for *Aminobacter* species

+, Positive; —, negative. The test results presented were read after 72 h incubation at 30 °C. All seven strains were positive for the following: utilization of *N*-acetyl D-glucosamine, L-arginine, L-asparagine, L-aspartate, β -alanine, betaine, butyrate, cadaverine, L-alanine pNA (pNA, *p*-nitroanilide), caprate, D-cellobiose, D-xylose, D-galactose, L-glycine, glutarate, D-glucose, glycerol, D-glutamate, D-glucarate, D-glucuronate, L-histidine, L-hydroxyproline, DL-3-hydroxybutyrate, isobutyrate, *iso*-inositol, L-alanine, DL-lactate, L-leucine, L-fucose, L-leucinamide, L-lyxose, L-valinamide, D-mannose, methyl α -D-mannoside, *m*-tartrate, L-ornithine, phenyl lactate, L-proline, quinate, α -D-raffinose, D-ribose, sorbate, L-serine, D-sorbitol, D-turanose and L-valine and hydrolysis of pNP α -D-glucopyranoside (pNP, *p*-nitrophenyl), bis-pNP phosphate, pNP phosphorylcholine and L-proline pNA. All seven strains were negative for the following: utilization of acetamidocaprinate, acetate, *N*-acetyl D-galactosamine, *trans*-aconitate, *cis*-aconitate, adipate, adonitol, DL-2-aminoadipate, DL-3-aminobutyrate, DL-aspartate, *p*-arbutin, azelate, citrate, L-citrulline, D-alanine, D-fucose, DL-isoleucine, *m*-erythritol, D-glucosamine, gluconate, 3-hydroxybenzoate, 4-hydroxybenzoate, itaconate, isovalerate, L-lysine, DL-malate, malonate, maleate, α -D-melibiose, mesaconate, methyl α -D-glucopyranoside, maltitol, D-mannitol, mucate, 2-oxoisocaproate, phenylacetate, L-phenylalanine, pimelate, putrescine, salicin, spermidine, spermine, suberate, succinate, L-tryptophan, tyramine and D-xylitol and hydrolysis of aesculin, pNP β -D-galactopyranoside, pNP β -D-glucuronide, 2-deoxythymidine-5'-pNP phosphonate and L-glutamate- γ -3-carboxy pNA.

Characteristic	<i>Chelatobacter heintzii</i> (4 strains)	<i>A. aminovorans</i> DSM 7048 ^T	<i>A. niigataensis</i> DSM 7050 ^T	<i>A. aganoensis</i> DSM 7051 ^T
Utilization of:				
Acetate	+	+	—	—
4-Aminobutyrate	+	+	—	+
D-Arabinose	—	—	+	—
L-Arabinose	+	+	—	+
D-Arabitol	+	+	—	—
D-Fructose	+	+	+	—
Fumarate	+	—	—	—
α -D-Galacturonate	—	—	—	+
D-Glucosaminic acid	+	+	—	—
Glycerophosphate	—	—	+	+
2-Hydroxyvalerate	+	+	+	—
Lactose	—	+	—	—
Lactulose	+	—	+	+
L-Malate	—	+	—	+
D-Maltose	—	+	+	+
Oxoglutarate	+	+	+	—
Propionate	+	+	—	+
Pyruvate	+	—	+	+
L-Rhamnose	+	+	+	—
D-Sucrose	—	+	+	+
D-Trehalose	—	+	+	+
Hydrolysis of:				
pNP β -D-glucopyranoside	+	—	+	+
pNP phenylphosphonate	+	+	+	—

the hydroxy fatty acid 12:0 3OH was confirmed for *Aminobacter* species. This fatty acid was also detected in *Chelatobacter heintzii* (Table 1).

In order to evaluate the intraspecific variation of fatty acid patterns within *Chelatobacter heintzii*, four strains were again studied; two of them represented genomovar 1 (subgroup A₁ of Auling *et al.*, 1993a) (ATCC 29600^T = DSM 10368^T, DSM 6450) and two represented genomovar 2 (subgroup A₂ of Auling *et al.*, 1993a) (DSM 6465 = TE6, DSM 6449 = TE8). The methods used are as described by Kämpfer *et al.* (1999). The results are listed in Table 1. No significant difference could be detected between the strains belonging to different genomovars of *Chelatobacter*

heintzii. From Table 1, it is obvious that the four strains of *Chelatobacter heintzii* showed high levels of similarity to the type strain of *Aminobacter aminovorans*.

Further physiological and biochemical tests, in addition to those reported, were performed with the four strains (Kämpfer *et al.*, 1999). Again, no significant differences could be found between the strains for 125 features. A large number of organic compounds can be used as sole sources of carbon (Table 2). Although some test results were different from those observed for *Aminobacter aminovorans*, the majority of the physiological test results were identical for *Aminobacter aminovorans* and *Chelatobacter heintzii*.

Table 3. Results from DNA–DNA hybridization studies

Source of unlabelled DNA	Genomic group	Source of labelled DNA						
		1	2	3	4	5	6	7
<i>Chelatobacter heintzii</i>								
1. DSM 10368 ^T	A ₁		103	86	70	68	39	75
2. DSM 6450 (= TE9)	A ₁	85		64	58	73	30	49
3. DSM 6449 (= TE8)	A ₂	80	71		102	81	44	76
4. DSM 6465 (= TE6)	A ₂	76	78	112		73	56	63
5. <i>Aminobacter aminovorans</i> DSM 7048 ^T		71	77	77	68		45	58
6. <i>Aminobacter aganoensis</i> DSM 7050 ^T		59	53	69	60	55		60
7. <i>Aminobacter niigataensis</i> DSM 7051 ^T		49	60	72	63		66	
8. <i>Sphingomonas pruni</i> IFO 15498 ^T		9	7	7	9	8	6	7
Pooled SD (%)		4	3	5	6	11	2	8

To provide further clear evidence for the similarity of *Chelatobacter heintzii* and *Aminobacter aminovorans*, DNA–DNA hybridization experiments were performed with all species of the genus *Aminobacter*. Strains were grown overnight in nutrient broth. DNA was isolated according to the protocol of Wisotzkey *et al.* (1990) and quality-controlled in a 1% agarose gel. Labelling of DNAs, hybridizations and data analysis were performed as described by Ziemke *et al.* (1998) except that, for nick translation, 2 µg DNA was labelled during a 3 h incubation at 15 °C. The results are displayed in Table 3. The similarity values among the different *Chelatobacter* strains varied between 58 and 112%. Differences were found between the genomic groups A₁ and A₂ described by Auling *et al.* (1993a), although they were less pronounced than in the earlier study (Table 3). A clear differentiation into two genomospecies is not indicated by the data.

Values between all *Chelatobacter* strains and *Aminobacter aminovorans* DSM 7048^T were between 68 and 81%. Interestingly, strain DSM 6449 also showed a high level of DNA–DNA similarity to the type strain of *Aminobacter niigataensis*, DSM 7050^T, in both reciprocal experiments (76 and 72%). The similarity values obtained for the four *Chelatobacter* strains displayed some variability. However, it is obvious that both genomovars, A₁ and A₂, are genomically more similar to *A. aminovorans* (68–77 and 68–81%, respectively) than to *Aminobacter niigataensis* (39–75 and 63–76%). The lowest DNA–DNA similarity values were measured against *Aminobacter aganoensis* (30–69%, *n* = 8). The relatively high DNA–DNA similarity values among the three *Aminobacter* species, previously found by Urakami *et al.* (1992), were confirmed. Also, the differences in reciprocal analyses found by Urakami *et al.* (1992) were detected. Pooled standard deviations for all of the hybridization experiments were between 2 and 11%.

In conclusion, the results from 16S rDNA sequencing, chemotaxonomic investigation (fatty acids, quinones, polyamines, polar lipids) and physiological characteri-

zation provide clear evidence that *Chelatobacter heintzii* is very similar to *Aminobacter aminovorans*. DNA–DNA hybridizations clearly show that representatives of both genomovars described by Auling *et al.* (1993a) share at least 70% DNA–DNA similarity with *Aminobacter aminovorans*.

These results, combined with previously published data, justify the proposal that *Chelatobacter heintzii* is a later subjective synonym of *Aminobacter aminovorans*.

Emended description of *Aminobacter* Urakami *et al.* 1992

(Am.i.no.bac'ter. N.L. n. *aminum* amine; N.L. n. *bacter* rod, staff; N.L. masc. n. *Aminobacter* amine rod).

The following description is based on the data of Urakami *et al.* (1992), Auling *et al.* (1993a) and Kämpfer *et al.* (1999) and the data presented in this paper.

Non-spore-forming, Gram-negative, rod-shaped organisms that have rounded ends and are 0.5–0.9 × 1.0–3.0 µm in size. Cells occur singly (rarely in pairs) and are motile. Colonies are white to light yellow. Cells grow abundantly in nutrient broth and peptone/yeast extract/glucose broth. No water-soluble fluorescent pigment is produced. Denitrification does not occur. Acids are produced from sugars oxidatively but not fermentatively. Growth factors are not required as essential supplements. Ammonia, nitrate, urea and peptone are utilized as nitrogen sources. Oxidase and catalase are produced. Aerobic. Metabolism is strictly respiratory and not fermentative. Good growth occurs between pH 6.0 and 8.0. Good growth occurs at 30 and 37 °C, but does not occur at 42 °C. The DNA base composition ranges from 62 to 64 mol% G + C. The cellular fatty acids include a large amount of straight-chain unsaturated 18:1 acid. The hydroxy acids include a large amount of 12:0 3OH. The ubiquinone

system is ubiquinone Q-10. The type species is *Aminobacter aminovorans*.

Emended description of *Aminobacter aminovorans* Urakami *et al.* 1992

The following description is based on the data of Urakami *et al.* (1992), Auling *et al.* (1993a) and the data presented in this paper. *Aminobacter aminovorans* has all of the characteristics that define the genus. In addition, it has the following characteristics.

Growth occurs in peptone water. Nitrate is not reduced to nitrite. The physiological properties are shown in Table 2. Further details can be obtained from Urakami *et al.* (1992) and Auling *et al.* (1993a). Some strains utilize monomethylamine, trimethylamine and trimethylamine-*N*-oxide; some are able to utilize EDTA. The type strain is strain JCM 7852^T (= NCIB 9039^T = ATCC 23314^T), which was isolated by den Dooren de Jong in 1926, from soil enrichments containing various amines.

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