Aminobacter ciceronei sp. nov. and *Aminobacter lissarensis* sp. nov., isolated from various terrestrial environments

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The bacterial strains IMB-1^T and CC495^T, which are capable of growth on methyl chloride (CH_3Cl, chloromethane) and methyl bromide (CH_3Br, bromomethane), were isolated from agricultural soil in California fumigated with CH_3Br, and woodland soil in Northern Ireland, respectively. Two pesticide-/herbicide-degrading bacteria, strains ER2 and C147, were isolated from agricultural soil in Canada. Strain ER2 degrades *N*-methyl carbamate insecticides, and strain C147 degrades triazine herbicides widely used in agriculture. On the basis of their morphological, physiological and genotypic characteristics, these four strains are considered to represent two novel species of the genus *Aminobacter*, for which the names *Aminobacter ciceronei* sp. nov. (type strain IMB-1^T = ATCC 202197^T = CIP 108660^T = CCUG 50580^T; strains ER2 and C147) and *Aminobacter lissarensis* sp. nov. (type strain CC495^T = NCIMB 13798^T = CIP 108661^T = CCUG 50579^T) are proposed.

The genus *Aminobacter* was proposed following the transfer of *Pseudomonas aminovorans* den Dooren de Jong 1926 to *Aminobacter aminovorans* (Urakami *et al.*, 1992). At the same time, two novel species were described, *Aminobacter aganoensis* and *Aminobacter niigataensis* (Urakami *et al.*, 1992). More recently, the closely related species *Chelatobacter heintzii* (Auling *et al.*, 1993) was also shown to be a member of the genus *Aminobacter*, as a later heterotypic (formerly subjective) synonym of *Aminobacter aminovorans* (Kämpfer *et al.*, 2002).

The facultatively methylotrophic strain IMB-1^T was isolated from CH_3Br-fumigated soil collected in Irvine, CA, USA (Connell Hancock *et al.*, 1998; Miller *et al.*, 1997), and was initially phylogenetically characterized based on 16S rRNA gene sequence analysis as being closely related to members of the genus *Rhizobium* in the '*Alphaproteobacteria*'. Strain IMB-1^T was able to grow on C_1 compounds such as CH_3Cl, CH_3Br, CH_3I and methylated amines as sole carbon and energy sources but was not able to grow on CH_3F. Growth also occurred on glucose, acetate and pyruvate; some growth was observed with low levels of methanol (Connell Hancock *et al.*, 1998). No growth or oxidation was observed with methane, formate, propyl iodide, dibromomethane, dichloromethane or difluoromethane (Connell Hancock *et al.*, 1998; Miller *et al.*, 1997; Schaefer & Oremland, 1999). Oxidation of CH_3Br in soil was greatly enhanced by addition of CH_3Br-grown cells of strain IMB-1^T to the soil (Connell Hancock *et al.*, 1998).

The facultatively methylotrophic strain CC495^T was isolated from the soil of a beech woodland at Lissara House, near

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains IMB-1^T, ER2, C147 and CC495^T are AF034798, L20802, AF246220 and AF107722, respectively.

Crossgar, County Down, Northern Ireland. Phylogenetic analysis of its 16S rRNA gene sequence indicated that, as with strain IMB-1^T, it was closely associated with the genus *Rhizobium* (Coulter *et al.*, 1999). Strain CC495^T, in the presence of cyanocobalamin, was able to grow on CH_3Cl and CH_3Br as sole carbon and energy sources but was not able to utilize either CH_3I or CH_3F. However, oxidation of CH_3I by CH_3Cl-grown cell suspensions was observed. Growth also occurred on methylamine, for which no supplementation of the medium by cyanocobalamin was required (Coulter *et al.*, 1999). The C_1 compounds methanol, methane, formaldehyde, formate, methane thiol and dichloromethane did not act as growth substrates, although formate, formaldehyde and methane thiol were oxidized by CH_3Cl-grown cell suspensions. Strain CC495^T was able to utilize glucose, pyruvate and glycerol as sole carbon and energy sources, but not veratrate or syringate (Coulter *et al.*, 1999). Under microaerophilic or anaerobic conditions, suspensions of CH_3Cl-grown cells of strain CC495^T catalysed the transhalogenation of the halomethanes CH_3Cl, CH_3Br and CH_3I, i.e. the exchange of various halide ions with the halomethanes (Harper *et al.*, 2000). Several other bacteria have also been isolated that utilize methyl halides as sole sources of carbon (Doronina *et al.*, 1996; Goodwin *et al.*, 1997). These strains have been designated *Hyphomicrobium chloromethanicum* CM2^T (McDonald *et al.*, 2001), *Methylobacterium chloromethanicum* CM4^T (McDonald *et al.*, 2001) and *Leisingera methylohalidivorans* MB2^T (Schaefer *et al.*, 2002).

The facultatively methylotrophic strain ER2 was isolated from an agricultural soil in Canada. This strain rapidly degraded the aryl *N*-methyl carbamate insecticide carbofuran (Topp *et al.*, 1993) and was initially characterized phylogenetically as being closely related to members of the methylotrophic bacteria. Strain ER2 is able to utilize several *N*-methyl carbamate insecticides as sole sources of carbon and nitrogen. The atrazine-degrading bacterial strain C147 was isolated from farm soil in Canada. It rapidly degraded the herbicide atrazine (Topp *et al.*, 2000). Strain C147 is able to utilize atrazine and other *S*-triazine herbicides as sole sources of carbon and nitrogen. Here we report the physiological characteristics, fatty acid composition and phylogenetic characterization (based on 16S rRNA gene sequence analysis and DNA–DNA hybridization) for strains IMB-1^T, CC495^T, ER2 and C147.

The complete 16S rRNA gene sequences (McDonald *et al.*, 1997) from strains IMB-1^T, CC495^T, ER2 and C147 were aligned, using the ARB program (Ludwig *et al.*, 2004), to representative organisms from *Aminobacter* and related genera, and their phylogenetic positions were determined using the DNADIST, DNAML, DNAPARS and SEQBOOT programs of the PHYLIP package (Felsenstein, 1993). Phylogenetic dendrograms were constructed from the distance data using the Fitch–Margoliash method and the dendrograms were drawn using TreeView version 1.5 (Page, 1996). DNA–DNA hybridization was carried out using the two methods of Huß *et al.* (1983) and Kämpfer *et al.* (2002). The two methods gave comparable results, with the exception of hybridization of *A. aminovorans* and strain CC495^T, for which repeat hybridizations gave widely differing values (20·8–72·0 %) in reciprocal hybridizations. These differences in reciprocal hybridization had already been detected in previous studies (Urakami *et al.*, 1992; Kämpfer *et al.*, 2002). This may be due to the presence of plasmids in CC495^T; however, no plasmids have been detected in this strain to date.

Phenotypic characterization and fatty acid analysis were carried out as described by Kämpfer *et al.* (1999, 2002), and indicated that IMB-1^T, ER2 and C147 were very similar at this level of characterization (Tables 1 and 2).

Phylogenetic analysis of the 16S rRNA gene sequences of strains IMB-1^T, CC495^T, ER2 and C147 (Fig. 1) showed them to be located within the genus *Aminobacter* (Urakami *et al.*, 1992), as supported by bootstrap values. The 16S rRNA gene of strain IMB-1^T had high sequence similarity with *A. aminovorans* DSM 7048^T (99·6 %), *A. aganoensis* DSM 7051^T (99·6 %), *A. niigataensis* DSM 7050^T (99·6 %), C147 (99·6 %) and ER2 (97·8 %). Analysis of the 16S rRNA gene sequence of strain CC495^T showed it to be most closely related to *A. aminovorans* (99·3 %), *A. aganoensis* (99·2 %) and *A. niigataensis* (99·2 %). The 16S rRNA gene of strain ER2 had high sequence similarity with *A. aganoensis* DSM 7051^T (97·9 %), *A. niigataensis* DSM 7050^T (97·9 %), IMB-1^T (97·8 %), CC495^T (97·8 %), C147 (97·8 %) and *A. aminovorans* DSM 7048^T (97·7 %). Analysis of the 16S rRNA gene sequence of strain C147 showed it to be most closely related to *A. aganoensis* DSM 7051^T (99·6 %), *A. niigataensis* DSM 7050^T (99·6 %), IMB-1^T (99·6 %), *A. aminovorans* DSM 7048^T (99·5 %) and CC495^T (98·9 %).

The previously characterized strains of *Aminobacter* (*A. aminovorans* DSM 7048^T, *A. niigataensis* DSM 7050^T and *A. aganoensis* DSM 7051^T) and strains ER2 (Topp *et al.*, 1993) and C147 (Topp *et al.*, 2000) were tested for their ability to grow on CH_3Cl or CH_3Br, and then screened by Southern probing and PCR for the presence of genes that code for enzymes involved in degradation of methyl halides (McDonald *et al.*, 2002). However, all strains tested negative, indicating that strains IMB-1^T and CC495^T are distinct from the other *Aminobacter* species, representing the only species able to grow on CH_3Cl or CH_3Br. The previously characterized *Aminobacter* species (*A. aminovorans*, *A. niigataensis*, *A. aganoensis*) and IMB-1^T and CC495^T were tested for the ability to degrade atrazine or carbofuran by HPLC analysis of cell suspensions incubated in mineral salts medium. Degradation was tested with the substrate as sole carbon and nitrogen sources in the absence of glucose, or as a sole nitrogen source in the presence of glucose, which supports growth as determined by turbidity. No degradation was detected for any of the strains tested. PCR amplification of genomic DNA using gene-specific primers also indicated that none of the strains possessed the genes *atzA* (atrazine chlorohydrolase) (de Souza *et al.*, 1996) or *mcd*

Table 1. Physiological characteristics of the type strains of *Aminobacter* species

+, Positive; −, negative; (+) weakly positive; pNP, *para*-nitrophenyl; pNA, *para*-nitroanilide. Test results given in the table were read after 72 h of incubation at 30 °C. All seven strains were positive for the following: utilization of *N*-acetyl-D-glucosamine, L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, D-mannose, D-maltose, D-ribose, D-xylose, i-inositol, D-mannitol, D-sorbitol, acetate, 4-aminobutyrate, DL-3-hydroxybutyrate, DL-lactate, oxoglutarate, L-alanine, L-aspartate, L-histidine, L-leucine, L-ornithine, L-proline and L-serine, and hydrolysis of bis-pNP-phosphate, pNP-phenyl phosphonate, L-alanine-pNA and L-proline-pNA. All seven strains were negative for the following: utilization of *p*-arbutin, α-D-melibiose, salicin, adonitol, maltitol, putrescine, *cis*-aconitate, *trans*-aconitate, adipate, azelate, citrate, fumarate, itaconate, mesaconate, suberate, L-phenylalanine, 3-hydroxybenzoate and phenylacetate, hydrolysis of aesculin, pNP β-D-galactopyranoside, pNP β-D-glucuronide, 2-deoxythymidine-5′-pNP phosphate and L-glutamate γ-3-carboxy-pNA and acid production from lactose, adonitol, rhamnose, methyl D-glucoside, erythritol and melibiose.

Test	IMB-1^T	ER2	C147	CC495^T	*A. aminovorans* DSM 7048^T	*A. niigataensis* DSM 7050^T	*A. aganoensis* DSM 7051^T
Utilization of:							
Gluconate	(+)	−	−	−	−	−	−
L-Rhamnose	+	+	+	+	+	+	−
Sucrose	+	−	−	+	+*	+*	+*
D-Trehalose	+	−	−	+	−	+*	+*
Propionate	+	+	+	+	+	−	+
Glutarate	+	+	+	+	+	−	+
L-Malate	−	−	−	−	−	−	+
Pyruvate	+	+	+	+	−	+	+
β-Alanine	+	(+)	(+)	+	−	+	+
L-Tryptophan	+	−	−	+	−	−	−
4-Hydroxybenzoate	−	−	−	−	−	+	+
Hydrolysis of:							
pNP α-D-glucopyranoside	(+)	(+)	(+)	−	+	+	+
pNP β-D-glucopyranoside	−	−	−	−	(+)	(+)	(+)
pNP phosphorylcholine	(+)	−	−	−	+	−	(+)
Acid produced from:†							
Glucose	(+)	−	−	(+)	(+)*	(+)*	(+)*
Sucrose	−	−	−	(+)	(+)*	(+)*	(+)*
D-Mannitol	(+)	−	−	(+)	(+)*	(+)*	(+)*
Dulcitol	−	−	−	−	(+)	(+)	−
Salicin	−	−	−	−	(+)	(+)	−
Inositol	−	−	−	−	−	(+)*	(+)*
Sorbitol	−	−	−	(+)	−	(+)*	(+)*
L-Arabinose	(+)	−	−	(+)	(+)*	(+)	−*
Raffinose	−	−	−	−	(+)	−	−
Maltose	(+)	−	−	−	(+)	(+)*	(+)*
D-Xylose	(+)	−	(+)	(+)	(+)*	(+)*	(+)*
Trehalose	−	−	−	−	(+)*	(+)*	−*
Cellobiose	−	−	−	−	(+)	−	−
D-Arabitol	−	−	−	(+)	(+)	(+)*	(+)*
D-Mannose	(+)	−	−	(+)	(+)	(+)	(+)

*Data are in line with those published by Urakami *et al.* (1992).

†Acid formation from carbohydrates in most cases was very weak (even after prolonged incubation). These tests cannot be recommended for differentiation.

(methylcarbamate hydrolase) (Tomasek & Karns, 1989). These genes were found in strains ER2 and C147, respectively, and are widely found in other Gram-negative bacteria that degrade atrazine or carbofuran. Therefore, there is no evidence for the degradation of atrazine or carbofuran by any of the other characterized *Aminobacter* species. This ability of strains ER2 and C147 to degrade atrazine or carbofuran is therefore unique among the *Aminobacter* species.

The result of genotypic and phenotypic investigations justify the proposal of two novel *Aminobacter* species.

Table 2. Major fatty acid compositions of type strains of species of the genus *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. 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. Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 4 contains 16 : 1ω7*c* and/or 15 : 0 iso 2-OH; summed feature 7 contains 18 : 1ω7*c*, 18 : 1ω9*t* and/or 18 : 1ω12*t*. Unknown fatty acids have no name listed in the peak library file of the MIDI system and therefore cannot be identified.

Compound	IMB-1^T	ER2	C147	CC495^T	*A. aminovorans* DSM 7058^T	*A. niigataensis* DSM 7050^T	*A. aganoensis* DSM 7051^T
Saturated fatty acids:							
9 : 0	1·1				1·0		
16 : 0	5·7	6·8	6·5	8·9	4·9	6·0	6·2
17 : 0	1·0		0·7		0·7		
18 : 0	2·9	5·0	4·4	3·3	1·5	2·0	2·0
20 : 0	0·3	0·7	0·5				
Unsaturated fatty acids:							
15 : 1ω8*c*							1·3
17 : 1ω8*c*	0·6				0·7		
17 : 1ω6*c*							
11-Methyl 18 : 1ω7*t*	18·9	14·3	13·4	10·5			
19 : 1ω12*t*					1·3		
20 : 0ω6,9*c*	0·6						
20 : 1ω9*t*	0·6	1·0	0·9		0·8	0·7	
Branched fatty acids:							
15 : 0i						3·1	2·9
17 : 0i	2·4	2·9	2·4	2·4	1·0	3·0	4·8
19 : 0i			0·5				
Hydroxy fatty acids:							
12 : 0 3-OH	0·4	0·4	0·3	0·3	0·3	0·4	0·4
15 : 0i 3-OH							
Summed features:							
Summed feature 4	0·5				0·5	1·0	1·1
Summed feature 7	59·1	52·7	54·7	69·8	46·4	48·6	64·8
Cyclopropane acids:							
17 : 0 cyclo					0·5		
19 : 0 cyclo ω8*c*	5·6	15·3	14·3	5·0	13·7	10·3	4·7
Unknown 14·966	1·0	0·8	0·9		0·7		
Unknown 18·081					24·8	21·0	10·9
Unknown 18·597					0·5	1·8	0·8
Unknown 18·804					0·4	0·7	

Description of *Aminobacter ciceronei* sp. nov.

Aminobacter ciceronei (cic.er.one'i. N.L. gen. n. *ciceronei* of Cicerone, named after Professor Ralph Cicerone, an American atmospheric chemist who has made many seminal contributions to our understanding of the chemistry of atmospheric trace gases, with particular reference to his work on the biogeochemistry of methyl halides).

Gram-negative, rod-shaped cells. Cells are 0·6 μm in diameter and 1·3 μm long. Cells are motile and non-pigmented. Growth is aerobic. Grows on CH_3Br, CH_3Cl, CH_3I and methylamine as sole carbon and energy sources. Further physiological features are given in Table 1. The main fatty acids are $C_{16:0}$ and $C_{18:1}$ (see Table 2). Optimum temperature for growth is 28–30 °C. Optimum pH for growth is 6·5–7·5. G+C content of the DNA is 62·0–63·7 mol%. Levels of DNA–DNA relatedness to representatives of the genus *Aminobacter* are indicated in Table 3.

The type strain, IMB-1^T (=ATCC 202197^T=CIP 108660^T =CCUG 50580^T), was isolated from CH_3Br-fumigated agricultural soil at Irvine, CA, USA. Strain ER2 was isolated from soil from Prince Edward Island (Canada) that was enriched with carbofuran-degrading micro-organisms by perfusing it continuously for 4 months with distilled water containing 100 mg carbofuran l^{-1}; strain C147 was isolated from a loam soil from a site near Ottawa, Ontario, Canada.

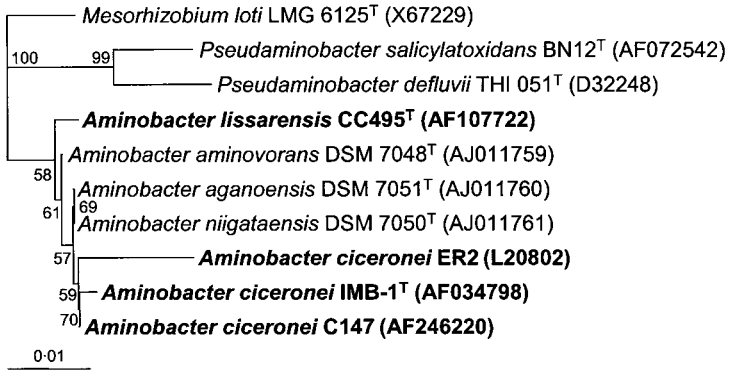

Fig. 1. Phylogenetic analysis of the 16S rRNA gene sequences of *Aminobacter ciceronei* sp. nov. strains IMB-1^T, ER2 and C147, *Aminobacter lissarensis* sp. nov. CC495^T, other *Aminobacter* strains and related genera. The dendrogram shows the results of an analysis in which DNADIST was used. Bootstrap values greater than 50% derived from 100 replicates are also shown. Bar, 1% sequence divergence, as determined by measuring the lengths of the horizontal lines connecting any two species.

Description of *Aminobacter lissarensis* sp. nov.

Aminobacter lissarensis (liss.ar.en′sis. N.L. masc. adj. *lissarensis* pertaining to Lissara House in Northern Ireland, where the type strain was isolated).

Gram-negative, rod-shaped cells, 0·5–0·6 μm in diameter and 1·3–1·5 μm long. Cells are motile and faintly pink-pigmented. Growth is aerobic. Grows on CH_3Cl and CH_3Br as sole carbon and energy sources in the presence of cyanocobalamin (1 mg l^{-1}). Methyl amine, glucose, pyruvate and glycerol also act as growth substrates without a requirement for supplementation of the medium with cyanocobalamin. C_1-compound assimilation is via the serine pathway. Further physiological features are given in Table 1. The main fatty acid is $C_{18:1}$. Optimum temperature for growth is 25 °C. Optimum pH for growth is 6·7–7·2. G+C content of the DNA is 62·5 mol%. Levels of DNA–DNA hybridization of the type strain to representatives of the genus *Aminobacter* are indicated in Table 3.

The type strain, CC495^T (=NCIMB 13798^T=CIP 108661^T=CCUG 50579^T), was isolated from an unpolluted beech woodland soil in County Down, Northern Ireland.

Table 3. DNA–DNA hybridization values between members of the genera *Aminobacter*

Mean values of at least two hybridizations are given. ND, Not determined.

Source of unlabelled DNA	Source of labelled DNA						
	1	2	3	4	5	6	7
1. Strain IMB-1^T	100	87·7	99·0	44·3	47·7	37·2	17·9
2. Strain ER2	>100	100	55·7	ND	38·3	48·7	13·0
3. Strain C147	80·0	ND	100	ND	34·3	40·2	37·4
4. Strain CC495^T	ND	ND	ND	100	20·8	49·5	31·8
5. *A. aminovorans* DSM 7048^T	33·0	43·8	56·6	ND	100	45·0*	58·0*
6. *A. aganoensis* DSM 7051^T	20·3	35·8	32·9	28·6	55*	100	60·0*
7. *A. niigataensis* DSM 7050^T	14·7	37·0	32·9	16·4	ND	66*	100

*Data from Kämpfer *et al.* (2002).

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