Legionella taurinensis* sp. nov., a new species antigenically similar to *Legionella spiritensis

François Lo Presti,¹ Serge Riffard,¹ Helene Meugnier,¹
Monique Reyrolle,¹ Yves Lasne,² Patrick A. D. Grimont,³
Francine Grimont,³ François Vandenesch,¹ Jerome Etienne,¹
Jean Fleurette¹ and Jean Freney¹

Author for correspondence: François Lo Presti. Tel: +33 478 77 86 57. Fax: +33 478 77 86 58.
e-mail: derba@laennec.univ-lyon1.fr

¹ Centre National de
Référence des *Legionella*
UPRES EA1655, Faculté de
Médecine R.T.H. Laennec,
69372 Lyon cedex 08,
France

² Laboratoire des
Radioisotopes et de
Biochimie Moléculaire,
Hôpital Edouard Herriot,
69437 Lyon cedex 03,
France

³ Unité des Entérobactéries,
Institut Pasteur, 75724
Paris cedex 15, France

A group of 42 *Legionella*-like organisms reacting specifically with *Legionella spiritensis* serogroup 1 antisera were collected throughout Europe by the Centre National de Référence (French National Reference Centre) for *Legionella*. This group of isolates differed somewhat from *L. spiritensis* in terms of biochemical reactions, ubiquinone content and protein profile. The latter two analyses revealed that one of these *L. spiritensis*-like isolates, Turin I no. 1^T, was highly related, but not identical to any of the red autofluorescent species of *Legionella*. In fact, this strain was the first of these particular isolates recognized to emit a red autofluorescence when exposed to UV light. Profile analysis of randomly amplified polymorphic DNA established that the red autofluorescent *L. spiritensis*-like isolates constituted a homogeneous group distinct from *Legionella rubrilucens* and *Legionella erythra*. DNA–DNA hybridization studies involving the use of S1 nuclease confirmed that the indicated group of isolates are a new species of *Legionella*, for which the name *Legionella taurinensis* is proposed with strain Turin I no. 1^T (deposited as ATCC 700508^T) as the type strain.

Keywords: *Legionella taurinensis* sp. nov., taxonomy, identification

INTRODUCTION

Bacteria of the family *Legionellaceae* are usual inhabitants of both natural and man-made aquatic habitats (Fliermans, 1996; Fliermans *et al.*, 1981; Stout *et al.*, 1985). Their survival in these environments is highly dependent on their ability to invade and multiply within a large diversity of protozoa (Fields, 1996; Rowbotham, 1980). Since the description of this family in 1979 (Brenner *et al.*, 1979), 42 species represented by 64 serogroups have been described including *Legionella lytica*, a species not yet culturable on buffered charcoal-yeast extract (BCYE) media (Benson *et al.*, 1996; Hookey *et al.*, 1996). Other strains of *Legionella*-like amoebal pathogens (LLAP) that are not yet culturable could presumably add five more species to the genus *Legionella* (Adeleke *et al.*, 1996). Twenty-

five of the *Legionella* species were initially described on the isolation of a single unidentified strain and some of them are still represented (to our knowledge) by the original strain only (Benson *et al.*, 1991, 1996; Bornstein *et al.*, 1989; Brenner *et al.*, 1985; Dennis *et al.*, 1993; Edelstein *et al.*, 1982; Herwaldt *et al.*, 1984; Thacker *et al.*, 1988, 1989, 1992; Verma *et al.*, 1992; Wilkinson *et al.*, 1987, 1988). *Legionella pneumophila* remains the most frequently isolated species from patients, although eighteen other species, including *L. lytica*, have also been isolated in these instances from pneumonia patients (Hookey *et al.*, 1996; Lo Presti *et al.*, 1997). Some species, such as *Legionella parisiensis*, *Legionella gormanii* and *Legionella jordanis* were first obtained as environmental specimens before being recognized as human pathogens.

During several recent investigations of hospital and community water in Europe, 42 isolates reacting exclusively with antisera raised against *L. spiritensis* serogroup 1 were collected by the Centre National de Référence des *Legionella* (French National Reference Centre for *Legionella*). However, in contrast to *L.*

Abbreviations: DFA, direct immunofluorescence assay; LLAP, *Legionella*-like amoebal pathogens; RAPD, randomly amplified polymorphic DNA.

The GenBank accession number for the sequence reported in this paper is AF037597.

spiritensis, some of these isolates displayed red auto-fluorescence under longwave UV light. This unusual feature stimulated the characterization of this group of organisms. Analysis of phenotypic and genotypic characteristics presented in this report have led to the description of this group as a new species, *Legionella taurinensis*.

METHODS

Isolation procedure. Water samples (1 l) were collected from six European countries (Czech Republic, France, Italy, Portugal, Spain and Switzerland), concentrated by continuous centrifugation and/or filtration, and either untreated or treated by acidification according to previously described procedures (Bornstein *et al.*, 1989). All samples were cultured on BCYE agar supplemented with 0.1% α -ketoglutarate (BCYE α) and BCYE α supplemented with glycine, vancomycin and colistin (GVC).

Bacterial strains. Forty-two isolates of *Legionella* (Table 1) were obtained from the different sites. In addition, the type strains of 41 *Legionella* species (Table 2) were used in this study; these strains fell into 63 serogroups. Several potential new species, including '*Legionella donaldsonii*' (strain 'Glasgow' 86/35784; gift from T. G. Harrison, Colindale, UK) (Hookey *et al.*, 1996), which has not yet been formally described, were also tested (Table 2). As amoebal cultures were not available, one validly published species, *L. lytica*, could not be used for the DNA hybridization studies.

Cultural and biochemical tests. The type strains and the isolates were cultured on BCYE α or GVC when needed. Requirement for cysteine was tested by its omission when preparing the BCYE α media. The cultures were examined for autofluorescence using Wood's light (366 nm). Tests for catalase, oxidase, β -lactamase, hippurate hydrolysis, urease, glucose fermentation, gelatinase, nitrate reduction and the presence of flagella were done as previously described (Brenner *et al.*, 1985). Browning of tyrosine-supplemented agar was considered positive if the browning produced around the colonies was larger on tyrosine-supplemented agar than any browning produced around the colonies of the same strain on tyrosine-free agar after 3 d growth (Vickers & Yu, 1984). The colour of the colonies was also noted on DGVC agar plates, which is a GVC media modified by the addition of dyes (bromocresol purple and bromothymol

blue) allowing a visual differentiation between *Legionella micdadei*, *Legionella maceachernii* (colonies are blue) and the other species of *Legionella* (colonies are green) (Vickers *et al.*, 1987).

Direct immunofluorescence assays. The 42 *Legionella* isolates collected from different sites were identified by performing direct immunofluorescence assays (DFA) using 59 hyperimmune rabbit antisera prepared as previously described (Cherry *et al.*, 1978). Antisera were not available for four species: *Legionella* genomospecies 1, *Legionella lansingensis*, *Legionella shakespearei* and *Legionella waltersii*. Conversely, all of the 63 serogroup reference strains were tested by DFA using an antiserum prepared against strain Turin I no. 1^T, the first red autofluorescent isolate, designated as the type strain (ATCC 700508^T) of a group of such isolates referred to as cluster 'Turin'.

Cell wall fatty acids, isoprenoid quinone composition and protein profile analysis. Cell wall fatty acids and isoprenoid quinones were assayed as previously described (Lambert & Moss, 1989) for bacteria grown on BCYE α agar plates for 48–96 h at 37 °C. The protein electrophoretic patterns of 11 isolates (Turin I no. 1^T, Turin I no. 2, Turin II no. 180, Turin II no. 195, Madrid IV no. 1, Châlons/Saône eau 1 colonie 25, Hôtel-Dieu 2 B3, Lausanne 15 no. 6, St-Etienne III no. 1, Lisbonne III A5 and Toulouse 26 no. 14) were compared between isolates and with other species, namely *Legionella erythra* (both serogroups), *L. spiritensis* serogroup 1 and *Legionella rubrilucens*, after SDS-PAGE analysis according to a previously described technique (Bornstein *et al.*, 1989).

Genotypic characterization. DNA was extracted by the boiling water method (Gomez-Lus *et al.*, 1993) and purified by phenol-chloroform-isoamyl alcohol (25:24:1), followed by ethanol precipitation. Randomly amplified polymorphic DNA (RAPD) analysis was performed with primer SK2 (5' CGGCGGCGGCGG 3') (Meunier & Grimont, 1993) as previously described (Lo Presti *et al.*, 1997).

16S rDNA amplification and sequencing. DNA amplification of the 16S rRNA gene of the strain Turin I no. 1^T was performed with oligonucleotide primers L16A1 (5' AGA GTT GGA TCC TGG CTC AG 3') and L16r1488 (5' GAC TTC ACC CCA GTC ATG AA 3'), corresponding to positions 8–27 and 1488–1508, respectively, in the *Escherichia coli* numbering system (Brosius *et al.*, 1978). Sequencing was done by the dideoxy chain-termination method (Sanger *et al.*, 1977) under previously described conditions (Lo Presti

Table 1. *L. taurinensis* strains

Origin	No. of isolates	Strain
Czech Republic	1	BM-750
France	24	Châlons/Saône eau 1 colonie 25, Châlons/Saône eau 1 colonie 26, Croix-Rousse 2 C9, Croix-Rousse 2 C13, Gap I B 41, Gap I B42, Hôtel-Dieu 2 B3, Hôtel-Dieu 2 B4, Hôtel-Dieu 2 B12, Henry-Gabrielle I eau 1 C7, Henry-Gabrielle I eau 3 C7, Henry-Gabrielle I eau 3 C10, IB 39 no. 6, Moulins I C1, Moulins I D3, Moulins I D5, Moulins I D6, Mercure 96 I eau 8 no. 7, Mercure 96 I eau 8 no. 8, St-Etienne III no. 1, Toulouse 26 no. 14, Toulouse 26 no. 17, Toulouse 28 no. 7, Toulouse 28 no. 8
Italy	4	Turin I no. 1 ^T (ATCC 700508 ^T), Turin I no. 2, Turin II no. 180, Turin II no. 195
Portugal	8	Lisbonne III A5, Portugal A12, Portugal A13, Portugal A14, Portugal A16, Portugal A17, Portugal A19, Portugal A20
Spain	2	Madrid I no. 4, Madrid IV no. 1
Switzerland	3	Genève 10 no. 1, Lausanne 15 no. 6, Lausanne 96 V no. 1

Table 2. 16S rRNA similarity and DNA relatedness of strain Turin I no. 1^T

Source of unlabelled DNA (<i>Legionella</i> strain)	Relatedness (%) to labelled DNA from:			Similarity (%) to 16S rDNA gene of Turin I no. 1 ^T
	Turin I no. 1 ^T (ATCC 700508 ^T)		<i>L. rubrilucens</i> (ATCC 35304 ^T)	
	60 °C*	$\Delta T_m^\dagger$		
Turin I no. 1 ^T ‡	100	0.0	54	100.0
Turin I no. 2‡	100	0.0	ND	
Lisbonne III A5‡	95	0.0	ND	
Madrid IV no. 1‡	78	2.5	56	
<i>L. rubrilucens</i> (ATCC 35304 ^T)	64	7.5	100	99.03–99.1
<i>L. erythra</i> serogroup 2 (LC 217)	47			
<i>L. erythra</i> serogroup 1 (ATCC 35303 ^T)	36			98.44
<i>L. spiritensis</i> serogroup 2 (NCTC 12082)	25			
<i>L. spiritensis</i> serogroup 1 (ATCC 35249 ^T)	19			95.8
<i>L. cherrii</i> (ATCC 35252 ^T)	18			92.4
<i>L. birminghamensis</i> (ATCC 43702 ^T)	16			95.1
‘ <i>L. donaldsonii</i> ’ (strain LC 878)	15			95.0
<i>L. jordanis</i> (ATCC 33623 ^T)	15			94.1
<i>L. pneumophila</i> serogroup 1 (ATCC 33152 ^T)	14			95.0
<i>L. israelensis</i> (ATCC 43119 ^T)	13			92.7
<i>L. micdadei</i> (ATCC 33218 ^T)	13			94.6
<i>L. geestiana</i> (ATCC 49504 ^T)	9			92.4
<i>L. feeleei</i> serogroup 1 (ATCC 35072 ^T)	8			95.3
<i>L. maceachernii</i> (ATCC 35300 ^T)	8			93.9
<i>L. adelaidensis</i> (ATCC 49625 ^T)	7			94.1
<i>L. bozemanii</i> serogroup 1 (ATCC 33217 ^T)	7			94.7
<i>L. brunensis</i> (ATCC 43878 ^T)	7			95.2
<i>L. londiniensis</i> (ATCC 49505 ^T)	7			93.6
<i>L. nautarum</i> (ATCC 49506 ^T)	7			94.3
<i>L. hackeliae</i> serogroup 1 (ATCC 35250 ^T)	6			95.0
<i>L. longbeachae</i> serogroup 1 (ATCC 33462 ^T)	6			94.7
<i>L. oakridgensis</i> (ATCC 33761 ^T)	6			93.7
<i>L. fairfieldensis</i> (ATCC 49588 ^T)	5			95.7
<i>L. lansingensis</i> (ATCC 49751 ^T)	5			93.0
<i>L. anisa</i> (ATCC 35292 ^T)	4			94.1
Other <i>Legionella</i> species§	<3			<95.3
LLAP strains	ND			<95.0

* Reactions were done at least in duplicate at 60 °C.

† Divergence from the T_m of Turin I no. 1^T (ATCC 700508^T), calculated to the nearest 0.5%. Reactions were done in triplicate.

‡ Strains of the cluster 'Turin'.

§ Other *Legionella* species: *Legionella cincinnatiensis*, ATCC 43753^T; *Legionella dumoffii*, ATCC 33279^T; *Legionella genomospecies* 1, ATCC 51913^T; *Legionella gormanii*, ATCC 33297^T; *Legionella gratiana*, ATCC 49413^T; *Legionella jamestowniensis*, ATCC 35298^T; *Legionella moravica*, ATCC 43877^T; *Legionella parisiensis*, ATCC 35299^T; *Legionella quateirensis*, ATCC 49507^T; *Legionella quinlivanii* serogroup 1, ATCC 43830^T; *Legionella sainthelensi* serogroup 1, ATCC 35248^T; *Legionella santacrucis*, ATCC 35301^T; *Legionella shakespearei*, ATCC 49655^T; *Legionella steigerwaltii*, ATCC 35302^T; *Legionella tucsonensis*, ATCC 49180^T; *Legionella wadsworthii*, ATCC 33877^T; *L. waltersii*, ATCC 51914^T; *Legionella worsleiensis*, ATCC 49508^T; strain Greoux 11 D13, ATCC 700509; and strain IB V no. 2, ATCC 700511.

ND, Not determined.

et al., 1997), and the sequence (GenBank no. AF037597) was compared with those previously published (Adeleke *et al.*, 1996; Birtles *et al.*, 1996; Fry *et al.*, 1991; Hookey *et al.*, 1996) and available in the DDBJ, EMBL and GenBank databases with the gapped BLAST program (Altschul *et al.*, 1997) via the Internet (<http://www.ncbi.nlm.nih.gov>).

DNA studies. DNA was extracted and purified as previously described (Brenner *et al.*, 1982) and the native DNA was labelled by nick-translation with ³H-labelled nucleotides (Amersham). DNA–DNA hybridization with all species (Table 2) was performed by the standard S1 nuclease method (Grimont *et al.*, 1980). The thermal stability of heterologous

hybrids was calculated by a standard method (Crosa *et al.*, 1973).

The G + C (mol%) content of DNA from strain Turin I no. 1^T was determined by HPLC of nucleotides after hydrolysis of the DNA by P1 nuclease (Kaneko *et al.*, 1986) and also spectrophotometrically by the thermal denaturation method (Marmur & Doty, 1962).

RESULTS

Cultural and biochemical characterization

The 42 strains of the 'Turin' cluster of *Legionella* collected from hospital and community water in Europe grew on BCYE α agar and GVC medium, but not on blood agar or BCYE α lacking supplemented L-cysteine. The type strain, Turin I no. 1^T, was a Gram-negative rod with a single polar flagellum. Physiological tests performed on the cluster were positive for gelatin liquefaction and the presence of flagella (100% of isolates), catalase (93%), β -lactamase (98%) and oxidase (76%). Negative reactions were obtained for urease, carbohydrate fermentation and nitrate reduction. The majority of isolates (90%) did not hydrolyse hippurate. No brown pigment was produced on tyrosine-supplemented media by the majority of these strains (96%) when using the 3 d growth criteria. After 10 d growth, 67% of the isolates remained negative while half of the positive strains were detected as only weakly positive. Including the type strain (at the first isolation), 67% of the strains exhibited red autofluorescence under UV light whereas the other isolates were not autofluorescent or produced only a weak red autofluorescence after two subcultures. However, this autofluorescence was often lost after several passages on agar media; this situation is similar to that observed for the bluish-white autofluorescent species of *Legionella* (unpublished observations). All the isolates also gave rise to yellow fluorescence of the growth medium. Colonies produced a greenish colour on DGVC medium. Strain Madrid IV no. 1 turned out to be the most divergent strain of the cluster in terms of biochemical reactions (e.g. oxidase-negative, production of brown pigment on tyrosine-supplemented media).

DFA

Isolate Turin I no. 1^T and the 41 other members of the 'Turin' cluster gave a 3+ to 4+ fluorescence with *L. spiritensis* serogroup 1 antisera. Antisera developed against the Turin I no. 1^T isolate gave a 4+ fluorescence with the homologous strain and 3+ with the *L. spiritensis* type strain. The cross-reaction could not be abolished by absorption with *L. spiritensis*.

Cellular fatty acids and ubiquinone composition

The Turin I no. 1^T isolate and members of this group contain the 14-methylpentadecanoic acid (i16:0) which accounts for more than 20% of the fatty acid composition; this is followed by 10–20% 12-methyltetradecanoate (a15:0), *cis*-9-hexadecenoate (16:1)

and hexadecanoate (16:0). The branched-chain fatty acid 14-methylhexadecanoate (a17:0) accounts for 10% and the i14:0 for 3%; only minor quantities of 15:1, heptadecanoate (17:0) and octadecanoate (18:0) were found. In comparison, the Madrid IV no. 1 isolate, the most divergent strain of this cluster, had less a15:0 (1%) and more a17:0 (21%). The ubiquinone profiles of 'Turin' cluster isolates were dominated by the presence of quinone Q-12. Ubiquinones Q-11 and Q-13 each accounted for approximately 20% of the amount of Q-12.

Protein profiling

Protein profiles revealed 90–95% homology for the 11 isolates of cluster 'Turin' tested and a maximum of 86% homology with *L. rubrilucens*, 83% with *L. erythra* and 79% with *L. spiritensis*.

RAPD analysis

Strains of the cluster 'Turin' showed a unique pattern by RAPD analysis that differed from those obtained for all the 41 other *Legionella* species tested (data not shown).

Sequencing of the 16S rRNA genes

The sequence of the 16S rRNA gene of the strain Turin I no. 1^T (GenBank no. AF037597) was 99.10% identical to sequence X73398 of *L. rubrilucens*, which was the closest sequence, 99.03% identical to sequence Z32643 of *L. rubrilucens*, 98.44% identical to sequence M36027 of *L. erythra* serogroup 1 and only 95.8% identical to the 16S rRNA gene of *L. spiritensis* serogroup 1. The sequence was less than 95% related to each of the 16S rDNA sequences of the LLAP strains (including *L. lytica*).

DNA hybridization

³H-labelled DNA from strain Turin I no. 1^T was hybridized with unlabelled DNAs from type strains of the other *Legionella* species and three strains of the same cluster (Table 2). Table 2 shows that there was $\geq 78\%$ relatedness between each of the four strains of the cluster 'Turin' with a maximum of 2.5 °C divergence in thermal stability of the hybrids. In optimal reactions performed at 60 °C, labelled DNA from the strain Turin I no. 1^T was 64% related to the type strain of *L. rubrilucens*. The divergence in thermal stability of this hybrid was 7.5 °C. In the reciprocal reaction, the level of DNA relatedness between the type strain of *L. rubrilucens* and strain Turin I no. 1^T fell to 54%. The DNA relatedness obtained with Madrid IV no. 1, the most divergent strain of the cluster 'Turin', was 56% when it was hybridized to labelled DNA from *L. rubrilucens*.

G + C content

The G + C content of strain Turin I no. 1^T was 46 mol% by each of the two methods used.

DISCUSSION

The red autofluorescence exhibited by the majority of the isolates of the 'Turin' cluster under UV light did not affirm the match with the phenotypic characteristics of *L. spiritensis* with which the cluster appeared to show a potentially strong antigenic relationship. Further biochemical characterization of the 'Turin' cluster isolates revealed that a marked difference existed between the isolates and *L. spiritensis*, *L. rubrilucens* and *L. erythra*, in that the isolates of the cluster did not produce the brown pigment on tyrosine-supplemented media. The major fatty acids of the type strain, Turin I no. 1^T, comprised those with 16 carbon atoms, which is the case for the red autofluorescent species and *L. spiritensis*; however, the isolates of the 'Turin' cluster had more n16:0 than *L. erythra*, *L. rubrilucens* and *L. spiritensis*, and less i16:0 than *L. rubrilucens*. Since the differentiation of all these species by fatty acid composition varies according to growth medium, this feature would appear to be more useful for ascribing unknown isolates to groups of species (Wilkinson *et al.*, 1990). The ubiquinone composition of the 'Turin' cluster clearly differentiated it from *L. spiritensis*, which contains ubiquinone Q-13 as major ubiquinone and not quinone Q-12.

Sequencing of the 16S rRNA gene has proved to be a useful tool for assigning an unidentified isolate to the nearest species. Sequence homologies of this gene between the two described red autofluorescent *Legionella* species (*L. rubrilucens* and *L. erythra*) were 99.4% (Hookey *et al.*, 1996). Comparison of the 16S rRNA gene sequence of the type strain, Turin I no. 1^T, with those of the *L. rubrilucens* and *L. erythra* species, revealed high similarities (99.1 and 98.4%, respectively). This raised the possibility that strain Turin I no. 1^T was a subspecies or a new serogroup of *L. rubrilucens*. DNA hybridization ruled out this possibility by showing that strain Turin I no. 1^T constitutes a distinct species. It should be noted that DNA hybridization was not possible with the five LLAP groups (since total DNA was not available), but their 16S rDNA sequence had less than 95% homology with Turin I no. 1^T making it very unlikely that strain Turin I no. 1^T belonged to any of the LLAP groups (Stackebrandt & Goebel, 1994).

Recently, the RAPD technique has been used for the identification of a newly described clinical strain, *L. parisiensis*. This approach showed that species-specific profiles could be obtained for the bluish-white autofluorescent *Legionella* species (Lo Presti *et al.*, 1997). By applying the RAPD technique to the red autofluorescent species and to the 'Turin' cluster, a specific profile for the newly proposed species was produced. This specific profile yielded no common bands with the species-specific profile of *L. rubrilucens*, indicating that the 'Turin' cluster was a homogeneous group differing in genotype from *L. rubrilucens*.

Ultimately, the genetic definition of a species for the 'Turin' cluster (Wayne *et al.*, 1987) was fulfilled by the data obtained from whole DNA-DNA hybridization studies, which manifested a homogeneous group with more than 70% DNA relatedness between the four tested strains and less than 5 °C difference between them in thermal stability (Table 2). Moreover, this cluster has less than 70% relatedness at the DNA level to the closest species, *L. rubrilucens*, and the thermal stability of the hybrid of these two species differs by more than 7.5 °C. Consequently, both results confirm that the 'Turin' cluster forms the 43rd *Legionella* species. Thus, the confidence attained in differentiating this new species according to phenotypes from other *Legionella* species (Table 3) presents the opportunity to formalize the name (Benson *et al.*, 1996; Ursing *et al.*, 1995).

Description of *Legionella taurinensis* sp. nov.

Legionella taurinensis (tau.ri.nen'sis. M.L. adj. *taurinensis* pertaining to Turin, Italy, whose classical Latin name was Augusta Taurinorum)

This bacterium is a Gram-negative rod with a single polar flagellum. It gives positive reactions for catalase, oxidase, gelatinase and the presence of β -lactamase. Negative reactions are recorded for urease, nitrate reduction, acid production from D-glucose, browning of tyrosine agar and hippurate hydrolysis. Some of the strains autofluoresce red (at 365 nm). DFA results exhibit strong cross-reaction with *L. spiritensis*. This species has ubiquinone Q-12 as the major component. All the strains of this species have been isolated from water samples throughout Europe. The type strain of

Table 3. Phenotypic comparison of *L. taurinensis* and three other species

<i>Legionella</i> species	Oxidase	Tyrosine	Fluorescence under UV light	DFA	Major ubiquinone
<i>L. taurinensis</i>	+	—	—/Red (68%)	<i>L. spiritensis</i> serogroup 1	Q-12
<i>L. spiritensis</i> serogroup 1	+	+	—	<i>L. spiritensis</i> serogroup 1	Q-13
<i>L. spiritensis</i> serogroup 2	—	+	—	<i>L. spiritensis</i> serogroup 2	Q-13
<i>L. rubrilucens</i>	—	+	Red	<i>L. rubrilucens</i>	Q-12
<i>L. erythra</i> serogroup 1	+	+	Red	<i>L. erythra</i> serogroup 1	Q-12
<i>L. erythra</i> serogroup 2	Variable	+	Red	<i>L. rubrilucens</i>	Q-12

L. taurinensis is Turin I no. 1^T (= ATCC 700508^T), which was isolated from a humidifier in the city of Turin (Italy). It has a G+C content of 46 mol %.

ACKNOWLEDGEMENTS

We are grateful to Maddalena Castellani Pastoris (Istituto Superiore di Sanita, Roma, Italy) and Hans G. Trüper (University of Bonn, Bonn, Germany) for their help in designating the name of the new species, and to Morris Goldner (University of Laval, Canada) for editing the manuscript. We are also grateful to D. Blanc (CHU Vaudois, Lausanne, Switzerland), V. Drasar (Czech National Legionella Reference Laboratory, Vyskov, Czech Republic), M. Marques (Hospital of Santa Cruz, Carnaxide, Portugal) and C. Pelaz (Laboratorio de Legionella, Majadahonda, Spain) who provided us with some of the strains used in this study.

REFERENCES

- Adeleke, A., Pruckler, J., Benson, R. F., Rowbotham, T., Halablab, M. & Fields, B. (1996). *Legionella*-like amoebal pathogens – phylogenetic status and possible role in respiratory disease. *Emerg Infect Dis* **2**, 225–230.
- Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. L. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.
- Benson, R. F., Thacker, W. L., Lanser, J. A., Sangster, N., Mayberry, W. R. & Brenner, D. J. (1991). *Legionella adelaidensis*, a new species isolated from cooling tower water. *J Clin Microbiol* **29**, 1004–1006.
- Benson, R. F., Thacker, W. L., Daneshvar, M. I. & Brenner, D. J. (1996). *Legionella waltersii* sp. nov. and an unnamed *Legionella* genomospecies isolated from water in Australia. *Int J Syst Bacteriol* **46**, 631–634.
- Birtles, R. J., Rowbotham, T. J., Raoult, D. & Harrison, T. G. (1996). Phylogenetic diversity of intra-amoebal legionellae as revealed by 16S rRNA gene sequence comparison. *Microbiology* **142**, 3525–3530.
- Bornstein, N., Marmet, D., Surgot, M. & 9 other authors (1989). *Legionella gratiana* sp. nov. isolated from french spa water. *Res Microbiol* **140**, 541–552.
- Brenner, D. J., Steigerwalt, A. G. & McDade, J. E. (1979). Classification of the Legionnaires' disease bacterium: *Legionella pneumophila*, genus novum, species nova, of the family Legionellaceae, family nova. *Ann Intern Med* **90**, 656–658.
- Brenner, D. J., McWhorter, A. C., Leete Knutson, J. K. & Steigerwalt, A. G. (1982). *Escherichia vulneris*: a new species of Enterobacteriaceae associated with human wounds. *J Clin Microbiol* **15**, 1133–1140.
- Brenner, D. J., Steigerwalt, A. G., Gorman, G. W. & 13 other authors (1985). Ten new species of *Legionella*. *Int J Syst Bacteriol* **35**, 50–59.
- Brosius, J., Palmer, M. L., Kennedy, P. J. & Noller, H. F. (1978). Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. *Proc Natl Acad Sci USA* **75**, 4801–4805.
- Cherry, W. B., Pittman, B., Harris, P. P., Hebert, G. A., Thomason, B. M., Thacker, L. & Weaver, R. E. (1978). Detection of Legionnaires' disease bacteria by direct immunofluorescent staining. *J Clin Microbiol* **8**, 329–338.
- Crosa, J. H., Brenner, D. J. & Falkow, S. (1973). Use of single-strand specific nuclease for analysis of bacterial and plasmid deoxyribonucleic acid homo- and hetero-duplexes. *J Bacteriol* **115**, 904–911.
- Dennis, P. J., Brenner, D. J., Thacker, W. L., Wait, R., Vesey, G., Steigerwalt, A. G. & Benson, R. F. (1993). Five new *Legionella* species isolated from water. *Int J Syst Bacteriol* **43**, 329–337.
- Edelstein, P. H., Brenner, D. J., Moss, C. W., Steigerwalt, A. G., Francis, E. M. & George, W. L. (1982). *Legionella wadsworthii* species nova: a cause of human pneumonia. *Ann Intern Med* **97**, 809–813.
- Fields, B. S. (1996). The molecular ecology of legionellae. *Trends Microbiol* **4**, 286–290.
- Fliermans, C. B. (1996). Ecology of *Legionella*: from data to knowledge with a little wisdom. *Microb Ecol* **32**, 203–228.
- Fliermans, C. B., Cherry, W. B., Orrison, L. H., Smith, S. J., Tison, D. L. & Pope, D. H. (1981). Ecological distribution of *Legionella pneumophila*. *Appl Environ Microbiol* **41**, 9–16.
- Fry, N. K., Warwick, S., Saunders, N. A. & Embley, T. M. (1991). The use of 16S ribosomal RNA analysis to investigate the phylogeny of the family Legionellaceae. *J Gen Microbiol* **137**, 1215–1222.
- Gomez-Lus, P., Fields, B. S., Benson, R. F., Martin, W. T., O'Connor, S. P. & Black, C. M. (1993). Comparison of arbitrarily primed polymerase chain reaction, ribotyping, and monoclonal antibody analysis for subtyping *Legionella pneumophila* serogroup 1. *J Clin Microbiol* **31**, 1940–1942.
- Grimont, P. A. D., Popoff, M. Y., Grimont, F., Coynault, C. & Lemelin, M. (1980). Reproducibility and correlation study of three deoxyribonucleic-acid hybridization procedures. *Curr Microbiol* **4**, 325–330.
- Herwaldt, L. A., Gorman, G. W., McGrath, T. & 12 other authors (1984). A new *Legionella* species, *Legionella feeleei* species nova, causes Pontiac fever in an automobile plant. *Ann Intern Med* **100**, 333–338.
- Hookey, J. V., Saunders, N. A., Fry, N. K., Birtles, R. J. & Harrison, T. G. (1996). Phylogeny of Legionellaceae based on small-subunit ribosomal DNA sequences and proposal of *Legionella lytica* comb. nov. for *Legionella*-like amoebal pathogens. *Int J Syst Bacteriol* **46**, 526–531.
- Kaneko, T., Katoh, K., Fujimoto, M., Kumagai, M., Tamoka, J. & Katayama-Fujimura, Y. (1986). Determination of the nucleotide composition of a deoxyribonucleic acid by high-performance liquid chromatography of its enzymatic hydrolysate: a review. *J Microbiol Methods* **4**, 229–240.
- Lambert, M. A. & Moss, C. W. (1989). Cellular fatty acid compositions and isoprenoid quinone contents of 23 *Legionella* species. *J Clin Microbiol* **27**, 465–473.
- Lo Presti, F., Riffard, S., Vandenesch, F., Reyrolle, M., Ronco, E., Ichai, P. & Etienne, J. (1997). The first clinical isolate of *Legionella parisiensis*, from a liver transplant patient with pneumonia. *J Clin Microbiol* **35**, 1706–1709.
- Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- Meunier, J.-R. & Grimont, P. A. D. (1993). Factors affecting reproducibility of random amplified polymorphic DNA fingerprinting. *Res Microbiol* **144**, 373–379.
- Rowbotham, T. J. (1980). Preliminary report on the pathogenicity of *Legionella pneumophila* for freshwater and soil amoebae. *J Clin Pathol* **33**, 1179–1183.
- Sanger, F., Nicklen, S. & Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* **74**, 5463–5467.

- Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Stout, J. E., Yu, V. L. & Best, M. G. (1985). Ecology of *Legionella pneumophila* within water distribution systems. *Appl Environ Microbiol* **49**, 221–228.
- Thacker, W. L., Benson, R. F., Staneck, J. L., Vincent, S. R., Mayberry, W. R., Brenner, D. J. & Wilkinson, H. W. (1988). *Legionella cincinnatiensis* sp. nov. isolated from a patient with pneumonia. *J Clin Microbiol* **26**, 418–420.
- Thacker, W. L., Benson, R. F., Schiffman, R. B., Pugh, E., Steigerwalt, A. G., Mayberry, W. R., Brenner, D. J. & Wilkinson, H. W. (1989). *Legionella tucsonensis* sp. nov. isolated from a renal transplant recipient. *J Clin Microbiol* **27**, 1831–1834.
- Thacker, W. L., Dyke, J. W., Benson, R. F. & 7 other authors (1992). *Legionella lansingensis* sp. nov. isolated from a patient with pneumonia and underlying chronic lymphocytic leukemia. *J Clin Microbiol* **30**, 2398–2401.
- Ursing, J. B., Rossello-Mora, R. A., Garcia-Valdés, E. & Lalucat, J. (1995). Taxonomic note: a pragmatic approach to the nomenclature of phenotypically similar genomic groups. *Int J Syst Bacteriol* **45**, 604.
- Verma, U. K., Brenner, D. J., Thacker, W. L. & 7 other authors (1992). *Legionella shakespearei* sp. nov., isolated from cooling tower water. *Int J Syst Bacteriol* **42**, 404–407.
- Vickers, R. M. & Yu, V. L. (1984). Clinical laboratory differentiation of *Legionellaceae* family members with pigment production and fluorescence on media supplemented with aromatic substrates. *J Clin Microbiol* **19**, 583–587.
- Vickers, R. M., Stout, J. E., Yu, V. L. & Rihs, J. D. (1987). Manual of culture methodology for *Legionella*. *Semin Respir Infect* **2**, 274–279.
- Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 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.
- Wilkinson, H. W., Thacker, W. L., Benson, R. F., Polt, S. S., Brookings, E., Mayberry, W. R., Brenner, D. J., Gilley, R. G. & Kirklin, J. K. (1987). *Legionella birminghamensis* sp. nov. isolated from a cardiac transplant recipient. *J Clin Microbiol* **25**, 2120–2122.
- Wilkinson, H. W., Drasar, V., Thacker, W. L., Benson, R. F., Schlinder, J., Potuznikova, B., Mayberry, W. R. & Brenner, D. J. (1988). *Legionella moravica* sp. nov. and *Legionella brunensis* sp. nov. isolated from cooling-tower water. *Ann Inst Pasteur Microbiol* **139**, 393–402.
- Wilkinson, I. J., Sangster, N., Ratcliff, R. M., Mugg, P. A., Davos, D. E. & Lanser, J. A. (1990). Problems associated with identification of *Legionella* species from the environment and isolation of six possible new species. *Appl Environ Microbiol* **56**, 796–802.