Bosea eneeae sp. nov., *Bosea massiliensis* sp. nov. and *Bosea vestrisii* sp. nov., isolated from hospital water supplies, and emendation of the genus *Bosea* (Das *et al.* 1996)

Bernard La Scola,¹ Marie-Noëlle Mallet,¹ Patrick A. D. Grimont² and Didier Raoult¹

Correspondence

Didier Raoult
didier.raoult@
medecine.univ-mrs.fr

¹Unité des Rickettsies, CNRS UPRESA 6020, Faculté de Médecine, Université de la Méditerranée, 27 Boulevard Jean Moulin, 13385 Marseille Cedex 05, France

²Unité des Entérobactéries, Unité INSERM 199, Institut Pasteur, 75724, Paris Cedex 15, France

On the basis of phenotypic and DNA relatedness data, three novel species of the genus *Bosea* are proposed, *Bosea massiliensis* (63287^T = CIP 106336^T = CCUG 43117^T), *Bosea vestrisii* (34635^T = CIP 106340^T = CCUG 43114^T) and *Bosea eneeae* (34614^T = CIP 106338^T = CCUG 43111^T). The original description of the genus *Bosea* included thiosulphate oxidation as a phenotypic feature, when the sole and type species of the genus, *Bosea thiooxidans*, was proposed. The three novel species described herein were not able to oxidize thiosulphate; thus, it is proposed that this characteristic be removed from the description of the genus and considered as specific for *B. thiooxidans*. The novel species of the genus *Bosea* proposed here form a well-separated cluster in the *Bradyrhizobium* group of the α -2 subclass of the *Proteobacteria*, on the basis of 16S rDNA gene sequence analysis. However, 16S rDNA gene sequence analysis was not sufficient to delineate the species; hence, DNA–DNA relatedness and phenotypic data were also required. All of the novel species described in this study are fastidious bacteria isolated from a hospital water supply, using co-cultivation with amoebae. This group of bacteria are hypothesized to be a potential cause of nosocomial infections. For treatment of infections caused by these novel bacteria, doxycycline appears to be the sole antibacterial compound with a consistently low MIC value.

INTRODUCTION

Using an amoebal co-cultivation procedure, 68 strains of diverse α -*Proteobacteria* were isolated, including *Afipia* spp., from hospital water supplies (La Scola *et al.*, 2000). By amplification and sequencing of the 16S rRNA genes of these strains, 57 were found to be closely related to *Bosea thiooxidans*, a bacterium for which the genus and species is represented by a sole isolate, BI-42^T (DSM 9653^T), which was isolated from agricultural field soil during a study of chemolithoheterotrophic bacteria (Das *et al.*, 1996). On the basis of phenotypic and genotypic data, we propose that the bacteria recovered from the hospital water system be

assigned to the genus *Bosea* as three novel species, for which we propose the names *Bosea eneeae*, *Bosea vestrisii* and *Bosea massiliensis*. The characteristics of these novel species are described herein.

METHODS

Strains. The strains used in this study are listed in Table 1 and have been deposited in the Collection de Souches de l'Institut Pasteur (CIP, Paris, France) and the Culture Collection of the University of Göteborg (CCUG, Göteborg, Sweden). Isolation of these strains has been described previously (La Scola *et al.*, 2000). All strains were subcultured routinely on BCYE agar plates (bioMérieux) at 30 °C as described previously (La Scola *et al.*, 2000).

Phenotypic tests. The morphological properties of the strains were studied microscopically following Gram- and Gimenez-staining. Oxidase activity was detected using an *N,N*-dimethyl-*p*-phenylenediamine oxalate disc (Sanofi Diagnostics Pasteur). Catalase activity was detected by emulsifying a colony in 3% H₂O₂ and checking for the presence of microscopic bubbles. Other biochemical tests were performed by inoculation of API 20NE and API 50CH

The GenBank accession numbers for the 16S rRNA gene sequences of *B. vestrisii* 34635^T, *B. eneeae* 34614^T, *B. thiooxidans* BI-42^T and *B. massiliensis* 63287^T are AF288306, AF288300, AF508803 and AF288309, respectively.

Fatty acid composition data, antimicrobial susceptibility profiles and sequence similarity data for the strains described in this paper can be found as supplementary data in IJSEM Online (<http://ijs.sgmjournals.org>).

Table 1. Strains used in this study

Strain	Reference
<i>Bosea thiooxidans</i> BI-42 ^T (DSM 9653 ^T)	Das <i>et al.</i> (1996)
<i>Bosea massiliensis</i> 63287 ^T (CIP 106336 ^T =CCUG 43117 ^T)	La Scola <i>et al.</i> (2000)
<i>Bosea massiliensis</i> 34649 (CIP 106337=CCUG 43116)	La Scola <i>et al.</i> (2000)
<i>Bosea eneeae</i> 34614 ^T (CIP 106338 ^T =CCUG 43111 ^T)	La Scola <i>et al.</i> (2000)
<i>Bosea eneeae</i> 34617 (CIP 106342=CCUG 43112)	La Scola <i>et al.</i> (2000)
<i>Bosea vestrisii</i> 34635 ^T (CIP 106340 ^T =CCUG 43114 ^T)	La Scola <i>et al.</i> (2000)
<i>Bosea vestrisii</i> 34620 (CIP 106341=CCUG 43113)	La Scola <i>et al.</i> (2000)
<i>Bosea vestrisii</i> 63286 (CIP 106339=CCUG 43115)	La Scola <i>et al.</i> (2000)

strips (bioMérieux) according to the manufacturer's instructions and incubation at 30 °C. These strips were incubated for 7 and 15 days, respectively. The API 20NE strip tested for nitrate reduction, indole production, urease activity, glucose acidification, arginine dihydrolase activity, hydrolysis of gelatin and aesculin, β -galactosidase activity and assimilation of glucose, arabinose, mannose, mannitol, *N*-acetyl-D-glucosamine, maltose, gluconate, caprate, adipate, malate, citrate and phenylacetate. As the interpretation of arginine dihydrolase and gelatinase activities on this strip was difficult, assays for these activities were performed in ADH/ODC/LDC broth (Sanofi Diagnostics Pasteur) and on nutrient gelatin (Oxoid), respectively, according to the manufacturers' instructions with incubation at 30 °C for 7 days. H₂S production was tested using sodium thiosulphate as substrate (bioMérieux) and inoculating the H₂S test cup of the API 20E strip (bioMérieux) with bacteria suspended in nutrient broth (NB), with the omission of oil from the cup. *B. thiooxidans* was used as the control. The API 50CH system tested for any acidification of glycerol, erythritol, D-arabinose, L-arabinose, ribose, D-xylose, L-xylose, adonitol, methyl β -D-xyloside, galactose, D-glucose, D-fructose, D-mannose, L-sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl α -D-mannoside, methyl α -D-glucoside, *N*-acetyl-D-glucosamine, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, inulin, melezitose, D-raffinose, starch, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-keto-D-gluconate and 5-keto-D-gluconate. Growth and haemolysis were tested at 30 °C on Columbia agar with 5% sheep blood (bioMérieux), Columbia agar (bioMérieux) enriched with 0.2% yeast extract (Merck) and 5% sheep blood, MacConkey's agar (bioMérieux) and NB (Difco), alone or with 6% NaCl. Growth on BCYE agar was tested at 25, 30, 35, 37 and 42 °C. The presence of flagella was assessed by depositing bacteria that had been cultured on BCYE agar onto a Formvar film. The bacteria were then stained with a 0.33% solution of uranylacetate and examined using a JEOL JEM 1200 EX electron microscope.

Antimicrobial susceptibility testing. The strains were grown for 72–96 h on BCYE agar prior to testing. Antibiotic susceptibility testing was performed using a micro broth-dilution method in NB. For testing of susceptibility to cotrimoxazole, 5% lysed horse blood was added to the broth. The final inoculum for all broth tests contained between 1×10^5 and 5×10^5 c.f.u. ml⁻¹. The plates were incubated at 30 °C and read between 72 and 96 h. *Escherichia coli* (ATCC 25922) and *Enterococcus faecalis* (ATCC 29212) were used as controls.

Analysis of major proteins using SDS-PAGE. The strains were harvested after 5–7 days cultivation and suspended in deionized water in preparation for SDS-PAGE. SDS-PAGE was performed using the methods described by Laemmli (1970). Equal volumes

of antigen (titrated to 4 mg protein ml⁻¹) and sample buffer [0.0625 M Tris/HCl (pH 8.0), 2% SDS, 5% 2-mercaptoethanol, 10% (v/v) glycerol, 0.02% bromophenol blue] were separated electrophoretically through a 12% (w/v) resolving gel and a 5% (w/v) stacking gel, at a constant current (8–10 mA) at room temperature for 4 h in running buffer (25 mM Tris, 192 mM glycine, 0.1% SDS), using an electrophoretic cell (Mini Protein II; Bio-Rad).

16S rDNA sequence analysis. Amplification of the 16S rDNA genes of the three novel species described here has been described previously (La Scola *et al.*, 2000). The 16S rDNA nucleotide sequence of *B. thiooxidans* BI-42^T described by Das *et al.* (1996) is available under GenBank accession no. X81044. Due to discrepancies in the phylogenetic trees obtained by using this sequence, the sequence for BI-42^T was determined again and has been deposited in GenBank under accession no. AF508803. An evolutionary distance matrix, generated by using DNADIST, was determined using the assumptions of Kimura (1980). A dendrogram was inferred from the evolutionary distances using the neighbour-joining method (Saitou & Nei, 1987). Bootstrap replications were performed to estimate the reliability of each node of the dendrogram. The bootstrap values were obtained from 1000 randomly generated trees, using the SEQBOOT tool in the PHYLIP package (Felsenstein, 1989).

Analysis of DNA–DNA relatedness. DNA was extracted from the strains and purified as described by Brenner *et al.* (1972). The procedures for labelling DNA with tritium-labelled nucleotides and for hybridization (S1 nuclease treatment, trichloroacetic procedure) have been detailed elsewhere (Grimont *et al.*, 1980; Khammas *et al.*, 1989).

Analysis of DNA G+C content. Estimations were performed by HPLC, using a model 46200A system pump (Merck Clevenot). DNA was extracted as described by Ausubel *et al.* (1995). The denatured DNA solution was mixed with 100 μ l nuclease P1 solution (Boehringer) (0.1 mg ml⁻¹ in 40 mM sodium acetate buffer containing 2 mM ZnSO₄, pH 5.3) and incubated at 50 °C for 1 h. Then, 100 μ l bacterial alkaline phosphatase (Boehringer) solution (0.7 μ l of 2.4 U alkaline phosphatase ml⁻¹ in 99 μ l of 1 M Tris/HCl buffer) was added to the mixture and it was incubated at 37 °C for 1 h. An aliquot (5 μ l) of the hydrolysate was applied onto a Nucleosil 5C18 Lichrocart column (4 $\times$ 250 mm) (Merck). Elution was carried out at room temperature using a mixture of 0.2 M NH₄H₂PO₄ (pH 4.5) and acetonitrile (96:4, v/v). A flow rate of 1 ml min⁻¹ was used and absorbance was monitored at 270 nm. The calibration curve was obtained from a mixture of the four standard nucleotides (5 nmol ml⁻¹ in distilled water) (Sigma). After chromatography, the relative concentration of each nucleotide was calculated on the basis of the peak area in the HPLC elution profile and corrected as described by Tamaoka & Komagata (1984). Each determination was repeated five times.

Analysis of whole-cell fatty acid composition. Whole-cell fatty acids were analysed for the three novel species by GC using a 3- to 5-day-old culture of each strain grown on BCYE agar, as described by Miller & Berger (1985).

RESULTS AND DISCUSSION

The results of the biochemical reactions, which allowed differentiation of the strains, are given in Table 2. All analysed strains were Gram-negative, Gimenez-positive, oxidase-positive, catalase-positive (weak reactions) and motile by means of a single polar flagellum. All had urease activity, and grew in NB, on Columbia agar with 5 % sheep blood and 0.2 % yeast extract, and at 25, 30, 35 and 37 °C. Visible colonies were detectable after 48–72 h incubation at 30 °C on BCYE agar. All strains gave negative results in tests for arginine dihydrolase activity, aesculin and gelatin hydrolysis, β -galactosidase activity and caprate assimilation, acid production by fermentation or oxidation of substrates tested in the API 50CH system and thiosulphate oxidation. No strain grew in NB with 6 % NaCl. However, strains, even those of the same species, differed in their abilities to grow on Columbia agar with 5 % sheep blood, on MacConkey's agar and at 42 °C. There were also variations in the ability to reduce nitrate and assimilate D-glucose, gluconate, arabinose, D-mannose, mannitol, *N*-acetyl-D-glucosamine, maltose, adipate, malate, citrate and phenylacetate. *B. massiliensis* did not grow on MacConkey's agar. *B. thiooxidans* and *B. vestrisii* were the only species that

assimilated citrate, and *B. thiooxidans* was the only species that oxidized thiosulphate and which grew weakly at 42 °C. All strains of the same species yielded the same pattern of major protein bands upon SDS-PAGE analysis (Fig. 1). *B. massiliensis* was characterized by the presence of a 35 kDa protein band and *B. eneeae* by the presence of 32 and 20 kDa protein bands. The three isolates of *B. vestrisii* had indistinguishable protein profiles. All *Bosea* spp. tested possessed a large amount of C_{18:1 ω 7c} and also C_{16:1 ω 7c}, C_{16:0}, C_{16:0 3-OH}, C_{19:0 cyclo ω 8c} and C_{19:0 10 methyl} in their whole-cell fatty acids profiles (Table I, supplementary data; <http://ijs.sgmjournals.org>). *B. thiooxidans* was the only species possessing C_{15:0} and along with *B. massiliensis* the only species possessing C_{17:1 ω 8c}. *B. massiliensis* was characterized by a lack of C_{17:0 cyclo} and *B. eneeae* was characterized by a lack of C_{17:0}. Strains 34620 and 34635^T of *B. vestrisii* had similar profiles (except for the amount of C_{19:0 cyclo ω 8c} present), whereas the profile of *B. vestrisii* 63286 was considerably different. The MIC values for most antibacterials tested were found to be high. *B. massiliensis* was the most susceptible species and the only species with a low MIC value for ticarcillin (Table II, supplementary data; <http://ijs.sgmjournals.org>). All of the *Bosea* spp. tested were characterized by a high susceptibility to doxycycline, with MIC values of ≤ 0.5 mg l⁻¹, with the exception of *B. eneeae* 34614^T. Due to their proximity to immunocompromised patients and their fastidious nature, we can speculate that the novel species described here could be responsible for undiagnosed cases of hospital-acquired pulmonary infections,

Table 2. Biochemical reactions of *Bosea* spp.

Strains: 1, *B. thiooxidans*; 2, *B. massiliensis* 63287^T; 3, *B. massiliensis* 34649; 4, *B. vestrisii* 63286; 5, *B. vestrisii* 34635^T; 6, *B. vestrisii* 34620; 7, *B. eneeae* 34614^T; 8, *B. eneeae* 34617. +, Positive reaction; +^w, weak reaction; –, negative reaction.

Test	1	2	3	4	5	6	7	8
Growth on Columbia agar with 5 % sheep blood	+	–	+ ^w	+	+ ^w	+	+ ^w	+ ^w
Haemolysis on Columbia agar with 5 % sheep blood and 0.2 % yeast extract	+ ^w	+ ^w	+ ^w	+	+ ^w	+	+ ^w	+ ^w
Growth on MacConkey's agar	+	–	–	+	+	+	+ ^w	+
Growth at 42 °C	+ ^w	–	–	–	–	–	–	–
Thiosulphate oxidation	+	–	–	–	–	–	–	–
Nitrate reduction	+	+	–	–	–	+	–	–
Assimilation of:								
D-Glucose	+	–	–	–	+	+	–	–
Arabinose	+	–	–	–	+	+	–	–
Mannose	+	–	–	–	+	+	–	–
Mannitol	+	–	–	–	+	+	–	–
<i>N</i> -Acetyl-D-glucosamine	+	–	–	–	+	+	–	–
Maltose	+	–	–	–	+	+	–	–
Gluconate	+	+	–	+	+	+	+	–
Adipate	+	+	–	–	+	+	+	–
Malate	+	+	–	+	+	+	–	–
Citrate	+	–	–	+	+	+	–	–
Phenylacetate	+	–	–	+	–	–	–	–
DNA G+C content (mol%)	68.2	66.7	67.9	67.5	65.0	65.9	69.0	67.9

Fig. 1. Silver-stained SDS-PAGE image of whole-cell-protein preparations from *Bosea* spp. Lanes: 1, *B. massiliensis* 63287^T; 2, *B. massiliensis* 34649; 3, *B. eneeae* 34614^T; 4, *B. eneeae* 34617; 5, *B. vestrisii* 63286; 6, *B. vestrisii* 34635^T; 7, *B. vestrisii* 34620; 8, *B. thiooxidans* BI-42^T.

just as *Legionella* spp. are responsible for nosocomial pneumonia. If such a scenario were true, it is important to note the high susceptibility of the *Bosea* spp. to doxycycline.

The DNA G+C contents of the *Bosea* spp. ranged from 65 to 69 mol%. Phylogenetic relationships inferred from a comparison of the 16S rRNA gene sequences showed that the *Bosea* spp. and *Methylobacterium mesophilicum* formed a cluster that was separate from *Afpia* spp., *Nitrobacter* spp. and *Bradyrhizobium* spp. The *Bosea* spp. were also well-separated from *M. mesophilicum* (Fig. 2). Bootstrap values for the distributions of the four *Bosea* species were all greater than 800/1000. In spite of this clustering, we found in our first comparison of the 16S rDNA sequences of the *Bosea* spp. that the sequence similarity between *B. thiooxidans* BI-42^T and the three novel species of *Bosea*, using the sequence deposited by Das *et al.* (1996), was approximately 92%. A new determination of the 16S rDNA sequence of *B. thiooxidans* BI-42^T allowed us to discover that there is an error in the original sequence deposited in GenBank (accession no. X81044). In the original sequence, 50 bp were omitted (positions 277–331 of the new sequence; GenBank accession no. AF508803). Among the *Bosea* spp., sequence similarity values ranged from 98.32 to 99.86% (Table III, supplementary data; <http://ijs.sgmjournals.org>) using the corrected sequence. The most closely related species were found to be *B. eneeae* and *B. vestrisii*. DNA–DNA hybridization data confirmed that *B. thiooxidans*, *B. eneeae*,

Fig. 2. Dendrogram depicting the phylogenetic relationships between *Bosea* spp., within the *Bradyrhizobium* group of the α -Proteobacteria. The tree was derived from a comparison of 1381 bases of the 16S rDNA sequences. The support for each node of the tree, as determined by bootstrap resampling (1000 replications), is shown. Nucleotide accession numbers for the sequences used to construct this dendrogram are given in parentheses.

B. vestrisii and *B. massiliensis* were distinct species (Table 3). As described previously for several species, including *Bacillus* spp. (Ash *et al.*, 1991; Fox *et al.*, 1992), the study of 16S rDNA sequences alone may not be sensitive enough to discriminate confidently between groups of bacteria; therefore, these data must be accompanied by DNA–DNA hybridization data for accurate analyses to be made (Stackebrandt & Goebel, 1994). Indeed, species definition must be based on the results of DNA–DNA hybridization and phenotypic data (Wayne *et al.*, 1987; Grimont, 1998). Interestingly, the original description of the genus *Bosea* included thiosulphate oxidation, but this feature appears to be specific to *B. thiooxidans* and thus can be used to differentiate it from the novel species described herein. For the genus *Bosea*, the value of biochemical reactivity as a species identifier should be interpreted with caution, as different strains of the same species yielded different results. Only antibacterial susceptibility and SDS-PAGE analysis of proteins were found to be reliable phenotypic delineators for the *Bosea* spp. examined here. As the comparison of 16S

Table 3. DNA relatedness among *Bosea* spp.

Strains: 1, *B. thiooxidans* BI-42^T; 2, *B. massiliensis* 63287^T; 3, *B. enee* 34614^T; 4, *B. vestrisii* 34635^T; 5, *B. vestrisii* 63286. Relatedness values are shown as percentages; ΔT_m values (°C) are given in parentheses. ND, Not done.

Strain	1	2	3	4	5
<i>B. thiooxidans</i> BI-42 ^T	100	ND	7	ND	ND
<i>B. massiliensis</i> 63287 ^T	8	100	17	12	12
<i>B. massiliensis</i> 34649	14	71 (1.1)	15	12	14
<i>B. enee</i> 34614 ^T	16	14	100	42	38
<i>B. enee</i> 34617	11	12	79 (0.3)	43	39
<i>B. vestrisii</i> 34635 ^T	13	12	31	100	79
<i>B. vestrisii</i> 34620	13	13	35	100 (0.6)	78
<i>B. vestrisii</i> 63286	12	17	39	70 (0.7)	100

rDNA sequences is not sensitive enough for the reliable delineation of *Bosea* spp., comparison of sequences from a more divergent part of the genome, such as the *rrs-rrl* intergenic spacer, may be more suitable. This approach has already been used for other members of the α -*Proteobacteria*, including *Nitrobacter* spp. (Grundmann *et al.*, 2000) and *Bradyrhizobium* spp. (van Berkum & Fuhrmann, 2000).

Emended description of the genus *Bosea* (Das *et al.* 1996)

Bosea (Bos'e.a. N.L. gen. n. *Bosea* of J. C. Bose, the founder of the Bose Institute, where the type species *Bosea thiooxidans* was isolated).

Members of the genus are Gram-negative, but stain well with Gimenez stain, oxidase-positive, catalase-positive rods in the α -2 subgroup of the *Proteobacteria*. Motile by means of a single polar or subpolar flagellum. Grow on BCYE agar, in NB and weakly on Columbia agar with 5 % sheep blood, but not in NB containing 6 % NaCl. Colonies are smooth, mucoid, round and cream coloured. Grow well at 25 and 37 °C. Urease-positive and α -haemolytic on Columbia agar containing 5 % sheep blood and 0.2 % yeast extract. Negative in assays for arginine dihydrolase activity, aesculin and gelatin hydrolysis, β -galactosidase activity and maltose assimilation, and in assays for acid production by fermentation or oxidation of substrates tested in the API 50CH system, especially D-glucose, D-fructose, D-mannose and sucrose. Have high MIC values for penicillin and amoxicillin and low MIC values for doxycycline. The most abundant fatty acid is *cis*-octadec-9-anoic acid (C_{18:1ω7c}). DNA G+C content is 64.2–65 mol%. The type species is *Bosea thiooxidans*.

Description of *Bosea enee* sp. nov.

Bosea enee (e.ne'ae. N.L. gen. n. *eneae* of Enea, to honour Maryse Enea, a technician in the Unité des Rickettsies, for her many contributions to the isolation of obligate intracellular bacteria, especially *Rickettsiaceae*).

Exhibits all of the characteristics of the genus, growing on MacConkey's agar and Columbia agar with 5 % sheep blood, in co-cultivation with *Acanthamoeba polyphaga* in Page's amoebal saline, but not at 42 °C. Does not reduce nitrate or oxidize thiosulphate, and gives variable reactions for the assimilation of gluconate and adipate. Biochemical characteristics of use in differentiating this species from other species of *Bosea* are given in Table 2. The whole-cell fatty acids profile of the species is given in Table I (supplementary data). The antimicrobial susceptibility profile of the species is given in Table II (supplementary data) and is characterized only by the low MIC value for doxycycline. DNA G+C content is 67.9–69 mol%. Isolated from the water supply of the La Timone Hospital Centre (Marseilles, France). The type strain of *Bosea enee* is 34614^T (=CIP 106338^T =CCUG 43111^T).

Description of *Bosea massiliensis* sp. nov.

Bosea massiliensis (mas.si.li.en'sis. L. adj. *massiliensis* pertaining to Massilia, the ancient Roman name of Marseille, France, where the organism was isolated).

Exhibits all of the characteristics of the genus, growing well in co-cultivation with *Acanthamoeba polyphaga* in Page's amoebal saline, but does not grow at 42 °C or on MacConkey's agar. Does not oxidize thiosulphate and gives variable reactions for nitrate reduction and assimilation of gluconate, malate and adipate. Biochemical characteristics of use in differentiating this species from other species of *Bosea* are given in Table 2. The whole-cell fatty acids profile of the species is given in Table I (supplementary data). The antimicrobial susceptibility profile of the species is given in Table II (supplementary data) and is characterized by the lower MIC values to most antibiotics compared to other species of *Bosea*. DNA G+C content is 66.7–67.9 mol%. Isolated from the water supply of the La Timone Hospital Centre (Marseilles, France). The type strain of *Bosea massiliensis* is 63287^T (=CIP 106336^T =CCUG 43117^T).

Description of *Bosea vestrisii* sp. nov.

Bosea vestrisii (ves.tri'si.i. N.L. gen. n. *vestrisii* of Vestris, to honour Guy Vestris, a technician in the Unité des Rickettsies, for his many contributions to the isolation of obligate intracellular bacteria, especially *Rickettsiaceae*).

Exhibits all of the characteristics of the genus, growing on MacConkey's agar, Columbia agar with 5 % sheep blood and in co-cultivation with *Acanthamoeba polyphaga* in Page's amoebal saline, but not at 42 °C. Does not oxidize thiosulphate and gives variable reactions for nitrate reduction. Biochemical characteristics of use in differentiating this species from other species of *Bosea* are given in Table 2. The whole-cell fatty acids profile of the species is given in Table I (supplementary data). The antimicrobial susceptibility profile of the species is given in Table II (supplementary data) and is characterized by the low

MIC values for doxycycline and netilmicin. DNA G+C content is 65–67.5 mol%. Isolated from the water supply of the La Timone Hospital Centre (Marseilles, France). The type strain of *Bosea vestrisii* is 34635^T (=CIP 106340^T =CCUG 43114^T).

ACKNOWLEDGEMENTS

The authors are indebted to Lina Barrassi and Elisabeth Ageron for their technical help, and to Richard J. Birtles and Kelly Johnston for reviewing the manuscript.

REFERENCES

- Ash, C., Farrow, J. A., Dorsch, M., Stackebrandt, E. & Collins, M. D. (1991). Comparative analysis of *Bacillus anthracis*, *Bacillus cereus*, and related species on the basis of reverse transcriptase sequencing of 16S rRNA. *Int J Syst Bacteriol* **41**, 343–346.
- Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (editors) (1995). *Current Protocols in Molecular Biology*, pp. 2.2.1–2.4.5. New York: Wiley.
- Brenner, D. J., Fanning, G. R., Rake, A. V. & Johnson, K. E. (1972). Polynucleotide sequence divergence among strains of *Escherichia coli* and closely related organisms. *J Bacteriol* **109**, 953–965.
- Das, S. K., Mishra, A. K., Tindall, B. J., Rainey, F. A. & Stackebrandt, E. (1996). Oxidation of thiosulfate by a new bacterium, *Bosea thiooxidans* (strain BI-42) gen. nov., sp. nov.: analysis of phylogeny based on chemotaxonomy and 16S ribosomal DNA sequencing. *Int J Syst Bacteriol* **46**, 981–987.
- Felsenstein, J. (1989). PHYLIP – Phylogeny inference package (version 3.2). *Cladistics* **5**, 164–166.
- Fox, G. E., Wisotzkey, J. D. & Jurtshuk, P., Jr (1992). How close is close: 16S rRNA sequence identity may not be sufficient to guarantee species identity. *Int J Syst Bacteriol* **42**, 166–170.
- Grimont, P. A. D. (1998). Use of DNA reassociation in bacterial classification. *Can J Microbiol* **34**, 541–546.
- Grimont, P. A. D., Popoff, M. Y., Grimont, F., Coynault, C. & Lemelin, M. (1980). Reproducibility and correlation study of three deoxy-ribonucleic acid hybridization procedures. *Curr Microbiol* **4**, 325–330.
- Grundmann, G. L., Neyra, M. & Normand, P. (2000). High-resolution phylogenetic analysis of NO₂-oxidizing *Nitrobacter* species using the *rrs-rrl* IGS sequence and *rrl* genes. *Int J Syst Evol Microbiol* **50**, 1893–1898.
- Khammas, K. M., Ageron, E., Grimont, P. A. D. & Kaiser, P. (1989). *Azospirillum irakense* sp. nov., a nitrogen-fixing bacterium associated with rice roots and rhizosphere soil. *Res Microbiol* **140**, 679–693.
- Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- La Scola, B., Barrassi, L. & Raoult, D. (2000). Isolation of new fastidious α -Proteobacteria and *Afipia felis* from hospital water supplies by direct plating and amoebal co-culture procedures. *FEMS Microbiol Ecol* **34**, 129–137.
- Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.
- Miller, L. & Berger, T. (1985). Bacterial identification by gas chromatography of whole cell fatty acids. Avondale, PA: Hewlett-Packard.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- 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.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- van Berkum, P. & Fuhrmann, J. J. (2000). Evolutionary relationships among the soybean bradyrhizobia reconstructed from 16S rRNA gene and internally transcribed spacer region sequence divergence. *Int J Syst Evol Microbiol* **50**, 2165–2172.
- 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.