Bordetella petrii* sp. nov., isolated from an anaerobic bioreactor, and emended description of the genus *Bordetella

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A novel *Bordetella* species was isolated from an anaerobic, dechlorinating bioreactor culture enriched from river sediment. The only strain, Se-1111R^T (= DSM 12804^T = CCUG 43448^T), for which the name *Bordetella petrii* is proposed, is designated the type strain of the novel species. Strain Se-1111R^T was isolated from the dechlorinating mixed culture due to its ability to anaerobically reduce selenate to elemental selenium. Comparative 16S rDNA sequence analysis showed a close relationship between Se-1111R^T and members of the genus *Bordetella* within the β -Proteobacteria. This close phylogenetic relatedness was also reflected in several metabolic properties of Se-1111R^T, including its incapacity to utilize carbohydrates, by the high G+C content (63.8 mol%) of its DNA and by the presence of Q-8 as the major isoprenoid quinone. DNA–DNA hybridization experiments with type strains of all species of the genus *Bordetella* and closely related species *Achromobacter xylosoxidans* subsp. *denitrificans* provided further evidence for the assignment of strain Se-1111R^T as a novel species of the genus *Bordetella*. This genus currently consists of seven aerobic species, all of which are known to occur in close pathogenic, opportunistic or possibly commensal relationships with various host organisms. *B. petrii* is the first member of this genus isolated from the environment and capable of anaerobic growth. The proposal of the novel species and an emended description of the genus *Bordetella* is presented.

Keywords: *Alcaligenaceae*, *Bordetella*, DNA–DNA relatedness, anaerobic selenate reduction, TCB-dechlorinating bioreactor

INTRODUCTION

The family *Alcaligenaceae* within the β -Proteobacteria (Woese *et al.*, 1984, 1985) comprises the genera *Bordetella* and *Alcaligenes*. Based on phenotypic and genotypic data, this family also includes the genus *Achromobacter* (Yabuuchi *et al.*, 1998). However, until now it has not been formally assigned to the family *Alcaligenaceae* and consequently the term *Alcaligenes–Achromobacter–Bordetella* complex will

be used throughout the text. *Bordetella* species occur exclusively in close association with humans and various warm-blooded animals, whereas *Achromobacter* and *Alcaligenes* species are either environmental or facultative pathogenic organisms (Pittman, 1984; De Ley *et al.*, 1986; Mandell *et al.*, 1987; Busse & Auling, 1992; Yabuuchi *et al.*, 1998).

Currently, the genus *Bordetella* comprises seven species. *Bordetella pertussis* and *Bordetella parapertussis* are aetiological agents of whooping cough (Hewlett, 1995; Matthews & Preston, 1997). Whereas *B. pertussis* is an obligate pathogen for humans, *B. parapertussis* strains are also found in sheep where they can cause chronic pneumonia. Human *B. parapertussis* isolates are highly clonal and appear to be distinct

Abbreviation: FBR, fluidized bed reactor.

The GenBank/EMBL accession numbers for the sequences of strain Se-1111R^T homologous to the *ompA* and *risA* genes of *B. avium* are AJ242599 and AJ242553, respectively.

from the sheep isolates (Yuk *et al.*, 1998). *Bordetella bronchiseptica* causes respiratory disease in various mammalian species including dogs, rodents, horses and pigs but only rarely in humans (Woolfrey & Moody, 1991; Gueirard *et al.*, 1994). Recent data obtained by different molecular genetic techniques, including DNA sequence analysis of virulence genes, comparative 16S rDNA analysis, multilocus enzyme electrophoresis and IS typing, demonstrated that *B. pertussis*, *B. parapertussis* and *B. bronchiseptica* were very closely related (Musser *et al.*, 1986; Arico *et al.*, 1987; Müller & Hildebrandt, 1993; van der Zee *et al.*, 1996, 1997). These data were in agreement with previous DNA–DNA hybridization studies and showed that this group should rather be considered to be subspecies or strains of a single species with different host adaptations than presenting different species (Kloos *et al.*, 1981; Weyant *et al.*, 1995).

Bordetella avium is a pathogen for birds causing severe problems in poultry, especially in turkeys, causing coryza or rhinotracheitis (Arp & Cheville, 1984; Gentry-Weeks *et al.*, 1992; Temple *et al.*, 1998). Similar to the species belonging to the *B. bronchiseptica* complex, *B. avium* exhibits a strong tropism for the ciliated epithelium of the upper respiratory tract (Arp & Cheville, 1984). Physiological and genetic evidence strongly supports the classification of *B. avium* as a distinct species (De Ley *et al.*, 1986; Weiss, 1992; Matthews & Preston, 1997). Recently, three new species were included in the genus *Bordetella*, *Bordetella hinzii*, *Bordetella holmesii* and *Bordetella trematum* (Vandamme *et al.*, 1995, 1996; Weyant *et al.*, 1995). *B. hinzii* is mainly found as a commensal of the respiratory tracts of fowl. It has also some pathogenic potential in immunocompromised humans (Cookson *et al.*, 1994; Vandamme *et al.*, 1995; Funke *et al.*, 1996). Only recently, *B. hinzii* was reported as the causative agent of fatal septicaemia (Kattar *et al.*, 2000). *B. holmesii* isolates, previously classified into the so-called CDC non-oxidizer group 2 (NO-2), were included into the genus *Bordetella* on the basis of genetic and chemotaxonomic analyses (Weyant *et al.*, 1995). It has been isolated repeatedly from blood of young adults and occasionally from sputum (Weyant *et al.*, 1995; Tang *et al.*, 1998). The most recently described member of the genus is *B. trematum*, which was isolated from ear infections and wounds of humans, but never from the respiratory tract. Little is known about the biology of this organism and its pathogenic significance (Vandamme *et al.*, 1996).

Most members of the genus *Alcaligenes* and *Achromobacter* are found in the environment. Some of them, e.g. *Alcaligenes faecalis* and *Achromobacter xylosoxidans*, are facultative pathogens. In contrast, the bordetellae are generally considered to occur exclusively in close association with various host organisms. Accordingly, the obligate human pathogen *B. pertussis* is extremely fastidious and sensitive to environmental conditions. Other *Bordetella* species are more resistant against detrimental growth conditions. For example, it

has been shown that *B. bronchiseptica* is able to survive for a prolonged period in buffered saline or lake water without the addition of nutrients (Porter *et al.*, 1991). However, as yet nothing is known about the occurrence of *Bordetella* species in the environment. The adaptation to a specific host organism may have caused the loss of genetic information resulting in the reduction of some metabolic properties of these species required for survival in the environment. In fact, recent genomic sequencing projects at the Sanger Centre, UK, indicate that the genome size of *B. pertussis* is significantly smaller than that of *B. bronchiseptica*. In accordance, it has been shown recently that among the factors lost by *B. pertussis* there are genes mainly involved in metabolic functions including biosynthesis of lipopolysaccharide, amino acid metabolism and degradation of aromatic compounds (Middendorf & Gross, 1999).

In this paper, a novel *Bordetella* strain, isolated from a mixed anaerobic, dechlorinating culture, which is able to reduce selenate to elemental selenium is described. Comparative 16S rDNA sequence analysis, DNA base composition, isoprenoid quinone content, DNA–DNA hybridization experiments and several metabolic properties suggest that this organism should be classified within the *Alcaligenes*–*Achromobacter*–*Bordetella* complex and assigned to the genus *Bordetella* as a novel species, for which the name *Bordetella petrii* is proposed. To our knowledge, *Bordetella petrii* is the first member of this genus isolated from the environment and capable of anaerobic growth.

METHODS

Reference strains. All bacterial strains used in this study are listed in Table 1. *B. pertussis*, *B. parapertussis*, *B. bronchiseptica* and *B. avium* strains were either grown on BG agar plates (Difco) or in SS liquid medium (Stainer & Scholte, 1970; Gentry-Weeks *et al.*, 1992).

Material, media and culture conditions. Samples were taken from a fluidized bed reactor (FBR) inoculated with an anaerobic, trichlorobenzene-dechlorinating consortium enriched from sediment of the River Saale near Jena, Germany (Selent, 1999). Within the FBR, the dechlorinating consortium was immobilized on polyurethane foam cubes. The microbial diversity of this dechlorinating consortium was investigated by both PCR-amplified 16S rDNA clone libraries and 16S rDNA sequence analysis of previously obtained cellular isolates (von Wintzingerode *et al.*, 1999; von Wintzingerode, 1999). For enrichment of anaerobic, selenate-reducing bacteria, foam cubes were removed from the bioreactor using a sterile tweezer and transferred to reduced RAMM medium (Shelton & Tiedje, 1984). After 7 d incubation at 30 °C under anaerobic conditions (GasPak anaerobic jars; Anaerogen), enrichment cultures were set up by transferring one foam cube to each Erlenmeyer flask containing 50 ml Se-medium (Macy *et al.*, 1989) which contained (l⁻¹): 2.2 g NaCl, 0.3 g KCl, 0.3 g NH₄Cl, 0.2 g KH₂PO₄, 0.15 g CaCl₂·2H₂O, 0.4 g MgCl₂·6H₂O, 0.6 g NaHCO₃, 3.78 g Na₂SeO₄, 3.78 g potassium acetate, 10 ml trace metal solution SL8, 10 ml vitamin solution and 800 µl methanol. After 5 d anaerobic incubation in anaerobic jars (Mart, CO₂, N₂, H₂ atmosphere), a red precipitate was

Table 1. Reference bacterial strains used in this study, and DNA–DNA reassociation rates among strain Se-1111R^T and closely related species

Species	Strain/other designation	Reassociation rate (%) with Se-1111R ^T
<i>Bordetella avium</i>	DSM 11332 ^T /ATCC 35086 ^T	26.4
<i>Bordetella bronchiseptica</i>	CCUG 219 ^T /ATCC 19395 ^T	22.9
<i>Bordetella bronchiseptica</i>	DSM 10303/ATCC 4617	22.5
<i>Bordetella bronchiseptica</i>	CCUG 7865/BB7865	—
<i>Bordetella hinzii</i>	DSM 11333 ^T /LMG 13501 ^T	33.4
<i>Bordetella holmesii</i>	CCUG 34073 ^T /ATCC 51541 ^T	18.5
<i>Bordetella parapertussis</i>	CCUG 413 ^T /ATCC 15311 ^T	35.1
<i>Bordetella parapertussis</i>	DSM 4922/—	—
<i>Bordetella pertussis</i>	DSM 5571 ^T /ATCC 9797 ^T	35.1
<i>Bordetella pertussis</i>	Tohama I/—	—
<i>Bordetella trematum</i>	DSM 11334 ^T /CCUG 32381 ^T	32.0
<i>Achromobacter xylosoxidans</i> subsp. <i>denitrificans</i>	DSM 30026 ^T /ATCC 15173 ^T	30.0

visible, which served as an inoculum for subcultures in modified Se-medium (Se-medium with 1.2 g NaCl l⁻¹ and additional 1–4 g yeast extract l⁻¹, 0.3 g Na₂SO₄ l⁻¹ and 2.02 g KNO₃ l⁻¹). A pure culture was achieved by repeated streaking on agar plates containing the above-mentioned medium (1.5 % agar, anaerobic incubation). Cells were stored at –70 °C in liquid growth medium supplemented with 30–50 % (v/v) glycerol.

Determination of anaerobic selenate reduction. Strain Se-1111R^T was grown anaerobically in cultures containing 10 ml basic medium (Se-medium without methanol, supplemented with 0.02 g KNO₃ l⁻¹ and 4 g yeast extract l⁻¹) at 30 °C for 30 d. An anaerobic culture grown in basic medium without selenate served as control. An aliquot of the culture (2.5 ml) was withdrawn for determination of whole-cell proteins using the DC protein assay kit (Bio-Rad) as suggested by the manufacturer. For determination of elemental selenium, an aliquot of the remaining culture (7.0 ml) was removed. Cells were collected by centrifugation (2500 g, 60 min) in FEP vials (tetrafluorethylene-perfluor-ethylene) and washed two times each with 8.5 ml PBS (138 mM NaCl, 2.7 mM KCl, 8.1 mM Na₂HPO₄, 1.8 mM KH₂PO₄, pH 7.4) to remove soluble selenium salts. The resulting cell pellet was lysed in 1.0 ml suprapure nitric acid (Merck) by pressurized digestion (2 h at 110 °C and 3 h at 160 °C). Selenium was determined by electrothermal atomic absorption spectrometry using a Perkin-Elmer 5100 spectrometer. To test for reproducibility all experiments were performed with four cultures in parallel (both selenate-containing cultures and controls).

Phenotypic tests. For phenotypic tests, cells of strain Se-1111R^T were grown aerobically on LB agar at 30 °C for 48 h. Growth and haemolysis were evaluated on MacConkey III agar plates (Oxoid), Columbia blood agar plates (Becton-Dickinson) and on BG agar plates containing 15 % (v/v) defibrinated horse blood (Difco), respectively. Reduction of tetrazolium was determined on LB agar plates supplemented with 75 mg 2,3,5-triphenyltetrazolium chloride (Sigma) l⁻¹. Motility was tested by both phase-contrast microscopy of overnight cultures grown in LB liquid media at 30 °C and by examining colony morphology on LB swarming agar (0.8 g NaCl l⁻¹, 0.3 %, w/v agar). Oxidase and catalase activities were tested with oxidase test strips (Merck) and by mixing

cells of a single colony with a 3 % (v/v) H₂O₂ solution on a microscopic slide, respectively.

The API 20NE, API 50CH, API 20A and API 20E were used as recommended by the manufacturer (bioMérieux). All tests were repeated at least twice.

16S rDNA sequencing and phylogenetic analyses. The nearly complete 16S rRNA genes (1523 bp) of the novel strain Se-1111R^T and reference strains *B. trematum* DSM 11334^T, *B. bronchiseptica* DSM 10303, *B. parapertussis* DSM 4922 and *Achromobacter xylosoxidans* subsp. *denitrificans* DSM 30026^T were determined. Small subunit rRNA genes were PCR-amplified from cell lysates using primers TPU1 (5'-AGAGTTTGATCMTGGCTCAG-3'; *Escherichia coli* positions 8–27) and RTU8 (5'-AAGGAGGTGATCCAN-CCRCA-3'; *E. coli* positions 1522–1541) (modified from Weisburg *et al.*, 1991) and were subsequently cloned into the pCR2.1 vector by TOPO TA cloning (Invitrogen). 16S rDNA inserts were sequenced using IR dye-labelled vector-specific M13 primers and 16S rDNA-specific primers and the ThermoSequenase Fluorescent Labelled Primer Cycle Sequencing kit (Amersham-Pharmacia). Sequencing reactions were analysed on an automated LICOR DNA-4000L sequencer (MWG-BIOTECH). Sequence alignment and phylogenetic analyses were performed by using the ARB software package (Ludwig & Strunk, 1997). A total of 1313 bases was used for phylogenetic analyses by using the shortest 16S rRNA sequence of the alignment as a filter (*Achromobacter ruhlandii*, EMBL accession no. AB010841). Uncertain sequence positions were excluded from phylogenetic analyses. Evolutionary trees were constructed by applying distance matrix (ARB, neighbour-joining with the correction of Jukes & Cantor, 1969), parsimony (ARB, PHYLIP, DNAPARS; Felsenstein, 1993) and compatibility criterion methods (ARB, PHYLIP, DNACOMP; Felsenstein, 1993). To provide confidence estimates for tree topologies, 100 bootstrapped replicate resamplings were performed.

DNA base composition, DNA–DNA hybridizations and isoprenoid quinone analysis. DNA was isolated as described by Cashion *et al.* (1977). The mean G + C value of the DNA was determined by HPLC (Mesbah *et al.*, 1989). The degrees of DNA–DNA binding were determined spectrometrically by the initial renaturation rate method (De Ley *et al.*, 1970) with modifications described by Huß *et al.* (1983) and Escara

& Hutton (1980). The renaturation of the nucleic acids was monitored using a Gilford spectrometer model 2600 equipped with a temperature programmer (Gilford model 2527-R). The renaturation ratios were calculated using the program TRANSFER.BAS (Jahnke, 1992). Isoprenoid quinones were extracted from 100 mg lyophilized cells and analysed by reverse-phase HPLC (Moss & Guerrant, 1983). All experiments were performed by the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, Braunschweig, Germany).

Southern blotting. Chromosomal DNA was prepared as described by Stibitz & Garletts (1992). The bacteria were grown in the appropriate medium at 37 °C to the exponential growth phase, harvested by centrifugation and resuspended in 10 mM Tris/HCl/20 mM NaCl, pH 7.2 to give a final concentration of 2×10^9 cells ml⁻¹. The suspension was mixed with an equal volume of 2% (w/v) low-melting-point agarose prepared in the same buffer, pipetted into casting moulds and allowed to cool. Solidified plugs were incubated in lysozyme buffer [10 mM Tris/HCl/50 mM NaCl/0.2% sodium deoxycholate/0.5% sodium *N*-laurylsarcosine/1 mg lysozyme (Merck) ml⁻¹, pH 7.2] with gentle agitation at 37 °C for 2 h. After washing at room temperature in 20 mM Tris/HCl/50 mM EDTA, pH 7.2, plugs were treated with proteinase K buffer [100 mM EDTA/1% sodium *N*-laurylsarcosine/0.2% sodium deoxycholate/2 mg proteinase K (Merck) ml⁻¹] at 50 °C overnight. Proteinase K was removed by washing at room temperature in 20 mM Tris/HCl/50 mM EDTA, pH 7.2 for at least 1 h. Samples were then equilibrated in TE buffer (10 mM Tris/HCl/1 mM EDTA, pH 8.0) and stored at 4 °C. For restriction enzyme digestion, slices of the plugs were incubated in 200 µl respective buffer at 50 °C for 1 h. Enzyme was then added and slices were incubated at 37 °C overnight. Reactions were stopped by addition of 20 µl 45% saccharose/100 mM EDTA containing 0.1% bromophenol blue. Plugs were then chilled on ice prior to loading onto a 1% agarose gel. Gels were run at 200 V in 0.5 × TBE buffer (1 × is 89 mM Tris-HCl, 89 mM borate, 0.2 mM EDTA, pH 8.0) at 14 °C in a contour-clamped homogeneous electric field DR III apparatus (Bio-Rad). Pulsed-field gels were blotted on a model 785 vacuum blotter (Bio-Rad) following the manufacturer's instructions. For hybridization, the ECL labelling and signal detection system was used (Amersham-Pharmacia). DNA fragments containing parts of the coding regions for the pertussis toxin, adenylate cyclase toxin, dermonecrotic toxin, filamentous haemagglutinin, pertactin, fimbriae (serotypes 2 and 3), the BvgAS two-component system, the Risa response regulator and the outer-membrane protein A were used as probes for the hybridization experiments (Klein, 1999).

PCR amplification and sequencing of putative coding regions. For PCR amplification of parts of the putative open reading frames of strain Se-1111R^T homologous to the *ompA* and *risA* genes of *B. avium*, the following primers were used which were designed according to the *B. avium* DNA sequences: *omp1e* (5'-CCCTCCAAATTCGCTCTGGCGCTT-3'), *omp2b* (5'-GTCCAGTTCGGCCTGGTCTACGTT-3'), *risa1c* (5'-AAAACACCACTCCTACCCGCAAG-A-3') and *risa2d* (5'-CCCAGGCCCCAGACGGTTTGG-ATG-3'). PCR products were purified using the QIAquick PCR purification kit (Qiagen) and sequenced using an automatic DNA sequencer model ABIPRISM 377 (PE Applied Biosystems). For comparative sequence analysis, the respective programs of the GCG software package were used (Devereux *et al.*, 1984).

RESULTS AND DISCUSSION

Cultural and morphological characteristics

Using culture conditions favouring anaerobic, respiratory selenate reduction, strain Se-1111R^T was isolated from a dechlorinating microbial consortium. It formed small, irregular, slightly red coloured colonies when grown under anaerobic conditions on selenate-containing medium. Phase-contrast microscopy of 48 h LB cultures revealed non-motile, Gram-negative, predominantly short or coccoid rods (0.6 µm in width and 1.5 µm in length) with rare longer and wider forms that sometimes occurred as chains. Electron microscopy showed the presence of fimbriae on the cell surface and the occurrence of bacteriophages in the preparation (Fig. 1).

Metabolic properties

The general metabolic properties of strain Se-1111R^T are characterized by its ability to grow under aerobic, microaerophilic and anaerobic conditions. With regard to its substrate spectrum, the novel isolate was an asaccharolytic, non-fermenting bacterium. The following characteristics were in common with all other members of the genus *Bordetella* (Vandamme *et al.*, 1996): aerobic and microaerobic growth at 37 °C; aerobic growth at 30 °C; assimilation of citrate; and catalase activity.

The following characteristics were negative for strain Se-1111R^T, as well as for all other members of the genus *Bordetella* (Vandamme *et al.*, 1996): production of acetylmethylcarbinol and indole; gelatin liquefaction; aesculin hydrolysis; and assimilation of D-

Fig. 1. Transmission electron micrograph (negatively stained) of a single cell of strain Se-1111R^T. Fimbriae are located on the cell surface. Arrows indicate bacteriophage tails (bar, 0.5 µm). An intact bacteriophage is shown in the inset (bar, 0.05 µm) (R. Lurz, MPI für Molekulare Genetik, Berlin, Germany).

Table 2. Phenotypic characteristics of strain Se-1111R^T and closely related species (Vandamme *et al.*, 1996)

Strains: 1, Se-1111R^T; 2, *B. avium*; 3, *B. hinzii*; 4, *B. holmesii*; 5, *B. trematum*; 6, *B. pertussis*; 7, *B. parapertussis*; 8, *B. bronchiseptica*; 9, *Achromobacter xylosoxidans* subsp. *denitrificans*. +, Positive; –, negative; ND, not determined; v, strain-dependent.

Characteristic	1	2	3	4	5	6	7	8	9
Growth on MacConkey agar	+	+	+	–	+	–	+	+	+
Motility	–	+	+	–	+	–	–	+	+
Fimbriae	+	+	ND	ND	ND	+	+	+	ND
Urease	–	–	–	–	–	–	+	+	–
Oxidase	+	+	+	–	–	+	–	+	+
Haemolysis	–	–	ND	–*	ND	+†	+†	+†	ND
Tetrazolium reduction	+	+	+	+	+	ND	+	+	+
Nitrate reduction (API 20NE)									
Formation of nitrite	–	–	–	–	v	–	–	+	+
Denitrification	+	–	–	–	v	–	–	–	–‡
Assimilation of:									
Caprate	–	–	+	–	–	–	–	v	+
D-Gluconate	+	–	–	–	–	–	–	–	–
L-Malate	+	+	+	–	+	–	–	v	+
Adipate	+	+	+	–	+	–	–	v	+
Phenylacetate	–	+	+	–	+	–	–	+	+

* Weyant *et al.* (1995).

† Weiss (1992).

‡ Positive with classical method.

glucose, D- and L-arabinose, mannose, mannitol, N-acetylglucosamine, maltose, glycerol, erythritol, ribose, L- and D-xylose, adonitol, rhamnose, methyl β -D-xyloside, inositol, sorbitol, methyl α -D-glucoside, amygdalin, arbutin, salicin, cellobiose, lactose, melibiose, saccharose, trehalose, inulin, melezitose, raffinose, starch, glycogen, xylitol and gentiobiose. Under anaerobic conditions, no growth occurred with the following substrates: glucose; mannitol; lactose; saccharose; maltose; xylose; salicin; arabinose; glycerol; cellobiose; mannose; melezitose; raffinose; sorbitol; rhamnose; or trehalose. All of the remaining characteristics are shown in Table 2.

Anaerobic selenate reduction

As judged from the amount of whole-cell protein, strain Se-1111R^T grew equally well under anaerobic conditions in medium containing nitrate and selenate and in medium containing only nitrate (333 μ g protein ml^{–1}). The ratio of elemental selenium formed to whole-cell protein was 200 fg selenium (μ g protein)^{–1}.

Phylogenetic classification

The nearly complete 16S rRNA gene (1523 bp) of strain Se-1111R^T was sequenced. Furthermore, the 16S rRNA gene sequence of the type strain of *B. trematum* (DSM 11334^T) was determined to include all

Bordetella species in phylogenetic analyses. Fig. 2 shows the phylogenetic relationship of strain Se-1111R^T within the *Alcaligenes–Achromobacter–Bordetella* complex, as determined by comparative 16S rRNA gene sequence analyses. With all treeing algorithms, strain Se-1111R^T constituted a separate branch and *Bordetella* spp. were its closest neighbours. Strain Se-1111R^T and *Bordetella* spp. were clearly separated from a cluster comprising all validly described *Achromobacter* spp., as well as several strains isolated from a soil microarthropod (strains R5, R6 and 151; Hoffmann *et al.*, 1998), the rhizosphere of rapeseed (strain 3-17; Bertrand *et al.*, 1996) and trichloroethylene-degrading soil isolate KP22 (Hanada *et al.*, 1998). Within the *Bordetella* cluster and the *Achromobacter* cluster, branching orders differed among the various algorithms. Despite variations in cluster-specific branching patterns and low bootstrap values, overall tree topologies were in agreement with recent studies, which showed that *Bordetella* spp. and members of the genus *Achromobacter* formed two closely related 16S rRNA clusters, clearly separated from *Alcaligenes* spp. (Yabuuchi *et al.*, 1998; Kattar *et al.*, 2000). It is therefore concluded that strain Se-1111R^T is phylogenetically affiliated to the genus *Bordetella*. Sequence similarities between strain Se-1111R^T and members of the genus *Bordetella* were 97.9–98.6% with the highest value to *B. bronchiseptica* and *B. parapertussis*. In contrast, sequence similarities to members of the genus *Achromobacter*

Fig. 2. Phylogenetic tree based on 1313 consecutive positions of the 16S rRNA gene of strain Se-1111R^T and members of the genera *Bordetella*, *Achromobacter* and *Alcaligenes* within the β -Proteobacteria. The dendrogram was generated using the neighbour-joining method with 100 bootstrap resamplings. Phylogenetic trees generated by the parsimony and the compatibility criterion methods showed identical overall tree topologies. Branching patterns within the *Bordetella* and the *Achromobacter* clusters changed among different algorithms. Numbers at branching points indicate bootstrap proportions of confidence in percentages (N, neighbour-joining; P, parsimony; C, compatibility). Only values $\geq 70\%$ are shown. *Zoogloea ramigera* ATCC 19544^T (EMBL accession no. D14254) was used as an outgroup. Bar, 10% estimated sequence divergence.

were lower (97.4–97.9%) with the highest value to *Achromobacter xylosoxidans* subsp. *xylosoxidans*.

DNA base composition and isoprenoid quinone analysis

The DNA G+C content of strain Se-1111R^T was 63.8 ± 0.5 mol%. The only isoprenoid quinone detectable was Q-8.

DNA–DNA hybridizations

DNA–DNA hybridization rates determined with the type strains of all *Bordetella* spp. and *Achromobacter xylosoxidans* subsp. *denitrificans* were all below the threshold value of 70% (Wayne *et al.*, 1987) indicating that strain Se-1111R^T represents a distinct species (Table 1).

Identification of open reading frames highly similar to genes encoded by various *Bordetella* spp.

To investigate the presence of well-characterized genes of various *Bordetella* species, hybridization experiments were performed with DNA fragments containing parts of the genes encoding pertussis toxin, filamentous haemagglutinin, fimbriae (serotypes 2 and

3), pertactin, dermonecrotic toxin, adenylate cyclase toxin, the BvgAS two-component system, the RisA response regulator and the OmpA outer-membrane protein. Positive results were obtained for all corresponding control organisms (e.g. *B. pertussis* and pertussis toxin gene probe). However, with strain Se-1111R^T strong and reproducible hybridization signals could be detected only for the *ompA* and *risA* genes (Fig. 3). To evaluate the degree of similarity of these DNA fragments to their *Bordetella* counterparts, parts of the coding regions of the putative *ompA* and *risA* genes were PCR-amplified using primers derived from the respective DNA sequences of *B. avium*. In both cases, PCR fragments of the expected length (461 and 445 bp, respectively) were obtained. While the *risA* genes of *B. pertussis*, *B. parapertussis*, *B. bronchiseptica* and *B. avium* are identical (Jungnitz *et al.*, 1998), the *risA*-like gene of strain Se-1111R^T contained several point mutations corresponding to a sequence divergence of 10.5%. However, all mutations found were conservative, encoding identical amino acid sequences. In the case of the *ompA* gene, strain Se-1111R^T exhibited 14.0% divergence to a consensus sequence derived from the aligned DNA sequences of Se-1111R^T, *B. avium*, *B. bronchiseptica*, and *B. pertussis*. In contrast, *B. avium*, *B. bronchiseptica* and *B. pertussis* showed 9.1, 7.1 and 6.3% divergence, respectively.

Fig. 3. Southern blots of *Xba*I- and *Spe*I-digested chromosomal DNA demonstrating the genomic distribution of the *risA* and *ompA* genes in various *Bordetella* strains and strain Se-1111R^T.

Taxonomic position of strain Se-1111R^T

Both biochemical and chemotaxonomic characteristics showed that strain Se-1111R^T is a member of the *Alcaligenes*–*Achromobacter*–*Bordetella* complex. Comparative 16S rRNA gene sequence analyses affiliated strain Se-1111R^T with *Bordetella* spp. and clearly separated it from closely related *Achromobacter* spp. Despite phenotypic differences between strain Se-1111R^T and *Bordetella* spp. (anaerobic growth, aerobic utilization of D-gluconate), it is concluded that strain Se-1111R^T does not represent a novel genus but is a member of the genus *Bordetella*. DNA–DNA hybridization values obtained with the type strains of all *Bordetella* spp. revealed that strain Se-1111R^T represents a novel species within this genus, proposed as *B. petrii*.

To our knowledge, *B. petrii* is the first species of this genus isolated from the environment and capable of anaerobic growth. As judged from Southern blot hybridization, *B. petrii* does not appear to have the BvgAS signal transduction system, which regulates the infectious cycle of *Bordetella* species, or any of the virulence-related genes tested for. However, as all other *Bordetella* species and many *Alcaligenes* and *Achromobacter* species are pathogenic or facultative

pathogenic organisms, the pathogenic potential of *B. petrii* in several infection models is in the process of being analysed.

Description of *Bordetella petrii* sp. nov.

Bordetella petrii [pe'trii. N.L. gen. n. *petrii* of Petri, named in honour of R. J. Petri, an early German microbiologist who developed the Petri-dish (Petri, 1887)].

B. petrii was isolated from an anaerobic, dechlorinating mixed culture which had been enriched from river sediment (Saale near Jena, Germany). Its pathogenic significance remains unknown. Cells are Gram-negative, non-spore-forming, non-motile and rod-shaped to circular. Cells are 0.4–0.7 µm wide to 1.0–2.8 µm in length. Cells possess fimbriae of different diameters. Colonies are of white-creamy colour and differ in size. Growth occurs under both aerobic and anaerobic conditions. Anaerobic growth is non-fermentative, possibly due to denitrification or to selenate reduction to elemental selenium. Some biochemical characteristics are described in the text and in Table 2. The major respiratory isoprenoid quinone is Q-8. The G+C content is 63.8 mol%. Strain Se-1111R^T is designated the type strain (= DSM 12804^T = CCUG 43448^T).

Emendation of description of the genus *Bordetella* (Skerman et al. 1980)

Based on the study of phenotypic and chemotaxonomic characteristics and comparative 16S rRNA gene sequence analysis of strain Se-1111R^T, the type strain of the species *Bordetella petrii*, the following emended description of the genus *Bordetella* is proposed.

Gram-negative, catalase-positive, asaccharolytic coccobacilli with an DNA G+C content of 60–69 mol%. Growth occurs strictly aerobically, facultatively anaerobically and non-fermentatively. One species is able to grow anaerobically under conditions favouring respiratory nitrate and selenate reduction. Species assimilate citrate under aerobic conditions, one species assimilates D-gluconate. Additional biochemical attributes are given in Results. The major respiratory isoprenoid quinone is Q-8. Strains were isolated from humans and warm-blooded animals, one strain was isolated from the environment. Based on comparative 16S rRNA gene sequence analysis, the species forms a distinct cluster separated from members of the genera *Achromobacter* and *Alcaligenes* within the β -Proteobacteria. The type species of the genus is *Bordetella pertussis*.

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