Classification of organisms previously reported as the SP and Stewart–Letscher groups, with descriptions of *Necropsobacter* gen. nov. and of *Necropsobacter rosorum* sp. nov. for organisms of the SP group

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To allow classification of bacteria previously reported as the SP group and the Stewart–Letscher group, 35 isolates from rodents (21), rabbits (eight), a dog and humans (five) were phenotypically and genotypically characterized. Comparison of partial *rpoB* sequences showed that 34 of the isolates were closely related, demonstrating at least 97.4 % similarity. 16S rRNA gene sequence comparison of 20 selected isolates confirmed the monophyly of the SP group and revealed 98.5 %–100 % similarity between isolates. A BLAST search using the 16S rRNA gene sequences showed that the highest similarity outside the SP group was 95.5 % to an unclassified rat isolate. The single strain, P625, representing the Stewart–Letscher group showed the highest 16S rRNA gene similarity (94.9–95.5 %) to members of the SP group. *recN* gene sequence analysis of 11 representative strains resulted in similarities of 97–100 % among the SP group strains, which showed 80 % sequence similarity to the Stewart–Letscher group strain. Sequence similarity values based on the *recN* gene, indicative for whole genome similarity, showed the SP group being clearly separated from established genera, whereas the Stewart–Letscher group strain was associated with the SP group. A new genus, *Necropsobacter* gen. nov., with only one species, *Necropsobacter rosorum* sp. nov., is proposed to include all members of the SP group. The new genus can be separated from existing genera of the family *Pasteurellaceae* by at least three phenotypic characters. The most characteristic properties of the new genus are that haemolysis is not observed on bovine blood agar, positive reactions are observed in the porphyrin test, acid is produced from (+)-L-arabinose, (+)-D-xylose, dulcitol, (+)-D-galactose, (+)-D-mannose, maltose and melibiose, and negative reactions are observed for symbiotic growth, urease, ornithine decarboxylase and indole. Previous publications have documented that both ubiquinones and demethylmenaquinone were produced by the proposed type strain of the new genus, Michel A/76^T, and that the major polyamine of representative strains (type strain not included) of the genus is 1,3-diaminopropane, spermidine is present in moderate amounts and putrescine and spermine are detectable only in minor amounts. The major fatty acids of strain Michel A/76^T are C_{14:0}, C_{16:0}, C_{16:1ω7c} and summed feature C_{14:0} 3-OH/iso-C_{16:1} I. This fatty acid profile is typical for members of the family *Pasteurellaceae*. The G + C content of DNA of strain Michel A/76^T was estimated to be 52.5 mol% in a previous investigation. The type strain is P709^T (=Michel A/76^T =CCUG 28028^T =CIP 110147^T =CCM 7802^T).

Abbreviations: MR, methyl red; SL, Stewart–Letscher.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences determined in this study are GU951545, GU966646–GU966656 and GU565606–GU565613. Those for the partial *rpoB* gene sequences are GU966657, GU966658 and GU565614–GU565621, and those for the *recN* gene sequences are GU565622–GU565632.

Two supplementary tables and two supplementary figures are available with the online version of this paper.

At the time of writing, the family *Pasteurellaceae* includes 15 genera with validly published names, with *Chelonobacter* and *Basfia* most recently described (Christensen & Bisgaard, 2008; Gregersen *et al.*, 2009; Kuhnert *et al.*, 2010). In this study, we report the features of a group of organisms mainly isolated from clinically affected rodents, and suggest that this group be classified in a new genus.

Organisms tentatively named the SP group were first reported by Frederiksen (1981), the name referring to an abbreviation for 'Sektionsprotocol' (*sectio* L. 'an act of cutting' protocol), the original notes made on a necropsy, an experiment or a case of disease. Isolates reported included five strains from guinea pigs, two human isolates from blood and faeces, respectively, and an isolate from guinea pig previously reported by Stewart & Letscher (1976). An organism (Michel A/76^T; Supplementary Table S1, available in IJSEM Online) sharing the phenotypic characters of the SP group, isolated from pneumonic lung tissue of a guinea pig after a spontaneous death, had previously been reported by Mannheim *et al.* (1978). Organisms isolated from the lung, middle ear and intestine of guinea pigs, previously reported as *Avibacterium gallinarum*, were subsequently reclassified with the SP group by Boot & Bisgaard (1995). Finally, Stahel *et al.* (2009) reported on a group of organisms from various lesions in rabbits which demonstrated 99% 16S rRNA sequence similarity to strain CCUG 28028^T (=Michel A/76^T). However, reference was not given to the SP group. Frederiksen (1981), Aldová *et al.* (1992) and Lion *et al.* (1995) reported on human isolates and suggested that attention should be paid to these organisms also in medical laboratories.

The G+C content of DNA of strain Michel A/76^T was estimated to be 52.5 mol% (T_m) (Mannheim *et al.*, 1978). In addition, phenotypic characters excluded it from the family *Pasteurellaceae* according to Mannheim *et al.* (1978). DNA–DNA hybridization demonstrated that human isolates and isolates from guinea pigs should be classified in the same species, while the isolate of Stewart & Letscher (1976) represented a different species of the same genus (Ursing, 1981). rRNA cistron similarities of taxa belonging to the family *Pasteurellaceae*, however, showed that strain Michel A/76^T grouped at the common root for rRNA branches of *Pasteurella multocida*, *Actinobacillus lignieresii*, *Haemophilus influenzae* and *Aggregatibacter aphrophilus*, while a human isolate (P602; Supplementary Table S1) was located at the common root for the above-mentioned rRNA branches and *Aggregatibacter actinomycetemcomitans* (De Ley *et al.*, 1990). Only minor differences were observed in polyamine patterns of members of the SP group and the organisms reported by Stewart & Letscher (1976), and members of the SP and Stewart–Letscher (SL) groups also demonstrated polyamine relationships with other taxa of the family *Pasteurellaceae* (Busse *et al.*, 1997). Using an ELISA, Boot *et al.* (1995) demonstrated that the SP group comprised a distinct serological group, different from taxons 5 and 6 of Bisgaard. Finally, a 16S rRNA gene

sequence-based phylogeny showed that strain Michel A/76^T clustered with the rodent cluster (15) (Olsen *et al.*, 2005).

In this study, we report the phenotypic and phylogenetic features of the SP group, and, based upon low genetic similarity to other genera of the family *Pasteurellaceae* and unique phenotype, we suggest the SP group be classified as *Necropsobacter rosorum*, gen. nov., sp. nov.

A total of 35 isolates from rodents (21), rabbits (eight), a dog and humans (five) (Supplementary Table S1) were investigated. Phenotypic characterization was carried out as reported previously (Bisgaard *et al.*, 1991; Christensen *et al.*, 2007).

Partial *rpoB* sequences of all 35 isolates investigated (Supplementary Table S1) were determined according to Mollet *et al.* (1997), covering the region 509–680 (*Escherichia coli* positions) of the deduced protein sequence as reported previously (Angen *et al.*, 2003; Korczak *et al.*, 2004). Sequencing of the 16S rRNA gene of 20 strains (Supplementary Table S1) was performed according to previous reports (Christensen *et al.*, 2002; Kuhnert *et al.*, 2002; Angen *et al.*, 2003). BLAST search (Altschul *et al.*, 1997) was performed in GenBank (Benson *et al.*, 2010). Pairwise comparisons for similarity were performed by the program WATER included in EMBOSS (Rice *et al.*, 2000). Multiple alignment was performed by using CLUSTAL X (Thompson *et al.*, 1997). Phylogenetic trees were drawn using MEGA4 (Tamura *et al.*, 2007). Maximum-likelihood analysis including bootstrap analysis was performed by using fastDNaml (Felsenstein 1995; Olsen *et al.*, 1994) on a Linux compatible server. The analysis was run with a transition/transversion ratio of 1.3.

Of the 35 isolates, 34 had highly related partial *rpoB* gene sequences and formed a monophyletic group with 100% bootstrap support (Supplementary Fig. S1). From this group, 31 isolates including the type strain had almost identical *rpoB* gene sequences whereas three strains (P602, K423-6 and ORG1719_03) showed a minimum of 97.4% (ORG1719_03) *rpoB* gene sequence similarity to the type strain. The highest BLAST search similarity in GenBank was obtained with the group of 10 identical sequences already published (acc. nos EF579885–EF579894; Stahel *et al.*, 2009) (Supplementary Table S1). All other sequences were distantly related, the closest value of 85% *rpoB* gene sequence similarity being to strain YU-ZK-12-LC (acc. no. GU167914) labelled as *Gallibacterium anatis*. Strain P625, representing the SL group, was only distantly related to the SP group described above (86.4% similarity to the type strain) and other members of the family *Pasteurellaceae*. A comprehensive analysis of the use of *rpoB* gene sequences for the phylogeny of the family *Pasteurellaceae* was reported by Korczak *et al.* (2004). In general, the *rpoB* gene provided higher resolution than the 16S rRNA gene. Estimated similarities of partial *rpoB* gene sequences within the family were more than 77%, and generally more than 87% within a genus (Korczak & Kuhnert, 2008). However, it should be remembered that the 85–90% similarity

between genera of the family *Pasteurellaceae* at the partial *rpoB* gene sequence level has also been observed for very different bacteria, such as members of the family *Streptococcaceae*, when only a short region is compared (Christensen & Bisgaard, 2010).

Of the 20 isolates selected for 16S rRNA gene sequence analysis, 19 formed a monophyletic group with 100 % bootstrap support (Fig. 1), confirming the monophyly observed for the *rpoB* gene (Supplementary Fig. S1). Sequence similarities of 98.5 (P1514–P602)–100 % were observed between isolates, including the 10 published sequences. For comparison, the type strains of the type species of existing genera of the family *Pasteurellaceae* were included, except for *Haemophilus influenzae*, for which the whole genomic sequence of strain Rd KW20 was used. The 16S rRNA gene sequence of the reference strain [*Pasteurella*] *pneumotropica* biovar Heyl was also included, as well as type strains of [*P.*] *pneumotropica* and [*Actinobacillus*] *muris*. The group of 20 isolates investigated was only distantly related to other members of the family *Pasteurellaceae*, including the taxa [*P.*] *pneumotropica* and [*Actinobacillus*] *muris*, known to be associated with rodents. A BLAST search with 16S rRNA gene sequences showed highest similarity to isolate V6 from a rat (acc. no. AB279579), with 95.5 % similarity to strain P981. The

single strain, P625, representing the SL group, showed 94.9–95.5 % similarity to the SP group. Since similarities of 16S rRNA gene sequences within the family *Pasteurellaceae* are greater than 89 % and within a genus are generally greater than 93 % (Korczak & Kuhnert, 2008) and mostly below approximately 95 % (Christensen *et al.*, 2007), the SP group clearly represents a distinct genus from the 16S rRNA perspective, for which the name *Necropsobacter* gen. nov. is proposed.

All strains of the SP group as well as strain P625 of the SL group showed a characteristic 16S rRNA nucleotide signature with three deletions in the region 203–206 (*E. coli* *rrnB* positions) and three in the region 213–214 compared with the type strain of *P. multocida* (acc. no. AY078999).

The 11 most divergent isolates based on 16S rRNA and *rpoB* gene sequence analyses were further investigated by *recN* gene sequencing. The *recN* gene has proved useful in delineating genetic relatedness of strains as otherwise determined by DNA–DNA hybridization (Kuhnert & Korczak, 2006). Primers *recN*_first-L (5'-ATGCTTANY-CAWCTYACKATYAATMATTTC-3') and *recN*_first-R (5'-CCRCTAATYCCMACATCNACYTCATC-3') were used for amplification of 1.4 kb of the 1.7 kb gene at 50 °C annealing temperature. PCR products were sequenced with

Fig. 1. Phylogenetic relationships between the isolates investigated and existing members of the family *Pasteurellaceae* based on maximum-likelihood analysis of near full-length 16S rRNA sequences. Support for monophyletic groups by bootstrap analysis is indicated as numbers out of 100. DDBJ/EMBL/GenBank accession numbers are in parentheses. Strains in bold have been sequenced in the present investigation and the strains enclosed in square brackets share sequence with the preceding strain at the same branch with reference to Supplementary Table S1. Bar, evolutionary distance of 0.01 (represents sequence variation considering the models for nucleotide substitution and tree-shape used in the maximum-likelihood analysis).

the same primers as well as the two internal primers *recN*-1 (5'-GTAAGTATTGATGCGCTGCTG-3') and *recN*-2 (5'-CAGCAGCGCATCAATACTTAC-3') according to the protocol of Kuhnert & Korczak (2006). The phylogenetic analysis showed that the 11 strains form a distinct branch separated from known taxa (Supplementary Fig. S2). The majority of SP group isolates showed identical *recN* sequences with the designated type strain Michel A/76^T while the other strains demonstrated more than 97.0% similarity. The single strain, P625, of the SL group differed from the strains of the SP group, only sharing 80% sequence similarity with them.

When genetic relatedness was calculated based on the formula of Zeigler (2003), all strains of the SP group had similarity values of at least 0.89 with strain Michel A/76^T. When compared with the SL group strain, P625, this value was 0.56 (Table 1). Comparison of strain Michel A/76^T with strains representing 15 genera with validly published names indicated low similarity values. The highest value observed was 0.15 with *Avibacterium gallinarum*, consistent with the phylogenetic analysis (Supplementary Fig. S2). These values are clearly below the value of 0.4 normally indicating the threshold between genera. On the other hand, the close genetic and phylogenetic relationship observed between the SL and SP groups could argue for a common genus. However, more strains of the SL group need to be included to determine its taxonomic status. The *recN* gene sequences can further be used as an approximation for the mol% DNA G+C content (Kuhnert & Korczak, 2006): strains from the SP group showed 53.7–53.2 mol% G+C, the type strain, Michel A/76^T, showed 53.2 mol% G+C and the single SL

group strain, P625, only showed 48.3 mol% G+C. By the spectrophotometric method, the G+C content of DNA (*T_m*) for strain Michel A/76^T was estimated to be 52.5 mol% (Mannheim *et al.*, 1978) in correspondence within one per cent to the value estimated based on the *recN* gene sequence. The DNA G+C content of strain Michel A/76^T is the highest reported for genera of the family *Pasteurellaceae*, ranging from 36–47 mol% for the genus *Actinobacillus* (Olsen & Møller, 2005) to 44–47 mol% for the genera *Avibacterium* and *Chelonobacter* (Blackall *et al.*, 2005; Gregersen *et al.*, 2009).

All strains included in the SP group tested positive for catalase and oxidase, and a fermentative reaction in Hugh and Leifson medium with (+)-D-glucose was observed. The strains stained Gram-negative and were coccoid or pleomorphic rods. Motility at 22 and 37 °C was not observed. Common positive phenotypic tests included porphyrin, methyl red (MR) at 37 °C, nitrate reduction, phosphatase, and growth on MacConkey agar. Production of acid was observed from the carbohydrates listed in the genus and species descriptions. Positive reactions were observed for ONPG (*o*-nitrophenyl-D-galactopyranoside), α -glucosidase (PNPG), α -galactosidase, β -xylosidase (ONPX) and gas production from glucose. Negative reactions were observed for symbiotic growth, β -haemolysis, citrate (Simmons), urease, indole and pigment formation, and the characteristics listed in the genus and species descriptions.

Reactions observed were in accordance with those previously reported by Mannheim *et al.* (1978), Frederiksen (1981), Aldová *et al.* (1992) and Stahel *et al.* (2009). Stahel *et al.* (2009), however, reported on two atypical isolates. Differences in MR at 37 °C and production of acid from dulcitol, (+)-L-rhamnose and lactose separate the SP group from the organisms of Stewart & Letscher (1976). Phenotypical results obtained for all strains investigated were in accordance with those reported for the family *Pasteurellaceae* (Olsen *et al.*, 2005).

Only differences in haemolysis and production of acid from (+)-L-rhamnose and (–)-D-mannitol separate the SP group from the genus *Gallibacterium*, while differences in oxidase, MR at 37 °C, and (+)-L-arabinose separate the SP group from the genus *Aggregatibacter* (Table 2). However, contrary to the SP group, the genus *Aggregatibacter* demonstrates major variations in phenotypic characters, limiting the number of characters for separation. MR at 37 °C, and production of acid from (+)-L-rhamnose, (+)-xylose and dulcitol separate the SP group from the genus *Avibacterium*, while differences in acid production from dulcitol and trehalose and the reaction with α -glucosidase (PNPG) separate the SP group from the genus *Volucrobacter*. Differences in three or more characters also separate the SP group from the remaining genera of the family *Pasteurellaceae* (Table 2).

The ability to produce ubiquinones in addition to demethylmenaquinones characterizes major groups of the

Table 1. Genetic similarity of strain CCUG 28028^T to type species of genera of the family *Pasteurellaceae* based on *recN* sequences

Strain	Similarity to strain CCUG 28028 ^T
SL group P625	0.56
<i>Avibacterium gallinarum</i> NCTC 11188 ^T	0.15
<i>Aggregatibacter actinomycetemcomitans</i> HK1651	0.14
<i>Basfia succiniciproducens</i> DSM 22022 ^T	0.14
<i>Pasteurella multocida</i> CCUG 17976 ^T (=NCTC 10322 ^T)	0.09
<i>Histophilus somni</i> 8025 ^T	0.13
<i>Lonepinella koalarum</i> ATCC 700131 ^T	0.11
<i>Haemophilus influenzae</i> Rd KW20	0.10
<i>Volucrobacter psittacidia</i> JEO101	0.01
<i>Actinobacillus lignieresii</i> NCTC 4189 ^T	0.02
<i>Bibersteinia trehalosi</i> NCTC 10370 ^T	0.00
<i>Chelonobacter oris</i> 1662 ^T	0.00
<i>Gallibacterium anatis</i> CCUG 15563 ^T	0.00
<i>Mannheimia haemolytica</i> NCTC 9380 ^T	0.00
<i>Nicotella semolina</i> CCUG 43639 ^T	0.00
<i>Phocoenobacter uteri</i> NCTC 12872 ^T	0.00

Table 2. Key characters for differentiation of the genera within the family *Pasteurellaceae* most closely related to the genus *Necropsobacter* gen. nov.

Genera: 1, *Necropsobacter* gen. nov., sp. nov. (this investigation); 2, *Actinobacillus sensu stricto* (Christensen & Bisgaard, 2004); 3, *Pasteurella sensu stricto* (Christensen & Bisgaard, 2006); 4, *Haemophilus sensu stricto* (includes *H. influenzae*, *H. haemolyticus* and *H. aegyptius* – results for *H. parainfluenzae* and *H. pittmaniae* are given in brackets) (Nørskov-Lauritsen *et al.*, 2005); 5, *Mannheimia* (Angen *et al.*, 1999); 6, *Lonepinella* (Osawa *et al.*, 1995); 7, *Phococobacter* (Foster *et al.*, 2000); 8, *Gallibacterium* (Bisgaard *et al.*, 2009); 9, *Volucrobacter* (Christensen *et al.*, 2004); 10, *Histophilus* (Angen *et al.*, 2003); 11, *Avibacterium* (Blackall *et al.*, 2005; Bisgaard *et al.*, 2007); 12, *Nicoletella* (Kuhnert *et al.*, 2004); 13, *Bibersteinia* (Blackall *et al.*, 2007); 14, *Aggregatibacter* (Nørskov-Lauritsen & Kilian, 2006); 15, *Chelonobacter* (Gregersen *et al.*, 2009); 16, *Basfia* (Kuhnert *et al.*, 2010). Characters are scored as: +, 90 % or more of strains positive within 1–2 days; (+), 90 % or more of strains positive within 3–14 days; –, fewer than 10 % of strains positive within 14 days; v, 11–89 % of strains positive; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Catalase	+	v	+	v	v	–	–	v	v	–	v	+	v	v	+	–
Oxidase	+	v	+	+	v	–	+	v	v	+	+	+	v	–	+	+
β -Haemolysis	–	v	–	v	v	–	–	+	–	v	–	–	v	–	+	–
Methyl red test	+	–	–	ND	–	–	ND	+	+	ND	–	ND	–	–	ND	+
Urease	–	+	–*	+ [v]	–	–	–	v	–	–	–	+	–	–	–	–
Indole production	–	–	+	v	v	+	–	v	–	+	–	–	–	–	–	–
Acid production from:																
(+) -L-Arabinose	+	v	–	–	v	ND	ND	v	v	ND	v	–	–	–	+	–
(+) -D-Xylose	+	v	v	v	+	ND	–	v	v	–	–	–	–	v	+	+
Dulcitol	+	–	v	–	–	ND	ND	v	–	ND	–	–	–	ND	+	–
(+) -D-Galactose	+	v	+	+/(+)	v	ND	ND	+	+/(+)	–	v	–	–	v	ND	+
(+) -L-Rhamnose	+	–	–	–	v	ND	ND	–	–	ND	–	ND	ND	ND	ND	–
Maltose	+	+	–	+	v	ND	–	v	v	–	v	–	+	+	+	+
Trehalose	+	v	v	–	–	–	–	v	–	–	v	–	+	v	+	+
(–) -D-Mannitol	–	v	v	–	+	–	–	+	–	ND	v	–	+	v	+	+
(+) -D-Mannose	+	v	+	–[+]	–	+	–	+	+	ND	+	–	+	v	+	+
α -Glucosidase (PNPG)	+	v	v	–	–	–	ND	v	–	ND	v	ND	v	v	–	–

*Discrepant results: *Pasteurella dagmatis* positive.

family *Pasteurellaceae* (Mannheim, 1981). Both ubiquinones and demethylmenaquinones were produced by strain Michel A/76^T according to Mannheim *et al.* (1978), underlining the classification in the family *Pasteurellaceae* (Olsen *et al.*, 2005). Polyamines were investigated by Busse *et al.* (1997), who showed that the major polyamine of representative strains (not the type strain) of the SP group is 1,3-diaminopropane, spermidine is present in moderate amounts and putrescine and spermine are detectable only in minor amounts. Fatty acids of strain CCUG 28028^T were investigated by Culture Collection, University of Göteborg (CCUG) (Supplementary Table S2), who showed the major fatty acids to be C_{14:0}, C_{16:0}, C_{16:1} ω 7c and summed feature C_{14:0} 3-OH/iso-C_{16:1} I. This profile is typical for the type strains of the type species of the eight genera *Haemophilus*, *Gallibacterium*, *Histophilus*, *Volucrobacter*, *Nicoletella*, *Chelonobacter*, *Bibersteinia* and *Aggregatibacter* whose data are available from CCUG (Supplementary Table S2). The data are also in agreement with the data of Mutters *et al.* (1993) for members of the family *Pasteurellaceae* known at that time. The exception was that they reported C_{16:1} ω 9c to be a major fatty acid, whereas CCUG found C_{16:1} ω 7c to constitute the majority of fatty acids in members of the family *Pasteurellaceae*. Assuming that data for CCUG 28028^T can be compared to the other data at CCUG, small

differences in proportions of C_{12:0} existed between this strain and the type strains of the type species of the genera *Histophilus* and *Chelonobacter*. C_{18:1} ω 7c/12t/9t was not detected in CCUG 28028^T but was found in minor amounts in the type strains of the type species of six other genera of the family *Pasteurellaceae*. Strain CCUG 28028^T showed a fatty acid profile typical of the family *Pasteurellaceae* with limited or no possibility to distinguish the proposed genus from the existing genera of this family.

Results obtained from phenotypic and genotypic tests demonstrated good agreement. The monophyly of the new genus, *Necropsobacter* gen. nov., was clearly indicated by 16S rRNA, *rpoB* and *recN* gene sequence analysis. Furthermore, the proposed genus, *Necropsobacter* gen. nov., fulfils the suggested minimal standards for description of members of the family *Pasteurellaceae*, including the number of strains investigated (Christensen *et al.*, 2007). The authors desist from classification of the Stewart–Letscher group, since more strains are needed to demonstrate the possible divergence within this group. The high phenotypic and genetic homogeneity among the strains belonging to the SP group indicates the existence of a novel species within the genus *Necropsobacter* gen. nov., for which the name *Necropsobacter rosorum* sp. nov. is

proposed. The genetic and phenotypic homogeneity of the species was demonstrated by more than 97.4 and 97.0 % similarity in *rpoB* and *recN* gene sequences, respectively, and identical phenotypes for all isolates characterized.

Description of *Necropsobacter* gen. nov.

Necropsobacter (Ne.crop.so.bac'ter. Gr. n. *nekros* dead body, corpse; Gr. n. *opsis* appearance, view; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Necropsobacter* a rod isolated from an autopsy).

Cells are non-motile at 22 and 37 °C, Gram-stain-negative, coccoid or pleomorphic rods. Colonies on bovine blood agar are regular, circular and slightly raised with an entire margin. Haemolysis is not observed on bovine blood agar. Positive for catalase and oxidase, and show a fermentative reaction in Hugh and Leifson medium with (+)-D-glucose. Positive for the porphyrin test, nitrate reduction, MR at 37 °C and phosphatase. Produce acid from (+)-L-arabinose, (+)-L-rhamnose, (+)-D-xylose, dulcitol, (+)-D-galactose, (+)-D-mannose, maltose, trehalose and melibiose. A reaction is observed for α -glucosidase (PNPG). Negative for symbiotic growth, urease, ornithine decarboxylase, indole and gas from (+)-D-glucose as well as production of acids from (-)-D-mannitol and the β -glucuronidase (PGUA) reaction. Both ubiquinones and demethylmenaquinones are produced by the type strain of the type species according to Mannheim *et al.* (1978). The G + C content of DNA from the type strain of the type species is estimated to be 52.5 mol% according to Mannheim *et al.* (1978). Major fatty acids are C_{14:0}, C_{16:0}, C_{16:1 ω 7c} and summed feature C_{14:0} 3-OH/iso-C_{16:1} I; minor fatty acids are listed in Supplementary Table S2. The type species is *Necropsobacter rosorum*.

Description of *Necropsobacter rosorum* sp. nov.

Necropsobacter rosorum (ro.so'rum. L. n. *rosor* -oris a rodent; L. gen. pl. n. *rosorum* of rodents).

Displays the following properties in addition to those given in the genus description. Colonies have a diameter of approximately 2.0 mm after 24 h of incubation at 37 °C. The surface of the colonies is smooth, shiny and opaque with a greyish tinge. Colonies have an unguent-like consistency and do not adhere to the agar surface. Growth on MacConkey agar is positive. Production of acid is observed from glycerol (late), (+)-D-arabitol, (-)-D-ribose, (-)-D-fructose, (-)-L-fucose, (+)-D-glucose, lactose (late), sucrose, raffinose and dextrin. Positive reactions for ONPG, α -galactosidase and β -xylosidase (ONPX). Negative reactions for citrate (Simmons), acid from mucic acid, malonate base, H₂S/TSI, KCN growth, Voges-Proskauer at 37 °C, nitrate gas, arginine dehydrolase, lysine decarboxylase, phenylalanine deaminase, gelatinase, Tweens 20 and 80, and pigment formation. Acid is not produced from meso-erythritol, adonitol, xylitol, (-)-L-xylose, myo-inositol, (-)-D-sorbitol, (+)-D-fucose, (-)-L-sorbose, cellobiose, (+)-melezitose, (+)-D-glycogen, inulin, aesculin, amygdalin, arbutin,

gentiobiose, salicin or (+)-turanose. Negative reactions are also observed for β -glucosidase (NPG), α -fucosidase (ONPF) and α -mannosidase. Strains reported have mainly been isolated from lesions in laboratory rodents, rabbits and human beings.

The type strain P709^T (=Michel A/76^T =CCUG 28028^T =CIP 110147^T =CCM 7802^T) was isolated from pneumonic lesions of a guinea pig.

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