Genus

Actinobacteria/Coriobacteriia/Eggerthellales/Eggerthellaceae/

Rubneribacter

Danylec et al. 2018[VP]

Melanie Huch, Dominic A. Stoll, and Nicolas Danylec, *Department of Safety and Quality of Fruit and Vegetables, Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Karlsruhe, Germany*

Edited by: Martha E. Trujillo, *University of Salamanca, Salamanca, Spain*

Rub.ne.ri.bac'ter. N.L. masc. n. *bacter*, a rod; N.L. masc. n. *Rubneribacter* a rod-shaped bacterium named after Max Rubner, a German medical doctor of the nineteenth century after whom the Max Rubner-Institut was named where the type strain was isolated.

The genus *Rubneribacter* is a member of the family *Eggerthellaceae* within the order *Eggerthellales*, class *Coriobacteriia*, and phylum Actinobacteria. Cells of the genus *Rubneribacter* are Gram-stain-positive, nonmotile, and rod shaped. The cell length is 0.98±0.20 µm, and the diameter is 0.30±0.02 µm. Cells occur singly or in short chains and do not produce endospores. Cells of *Rubneribacter badeniensis* grow on solid as well as in liquid media within 48–72 h at 37°C under strictly anaerobe conditions. Arginine is hydrolyzed. Cells do not produce catalase, indole, oxidase, or urease. Acid is not produced from sugars. The major cellular fatty acids are $C_{14:0}$ iso, $C_{15:0}$ anteiso, $C_{16:0}$ DMA, and one unidentified fatty acid methyl ester. The detected respiratory quinones are MK-7 (53%), MK-6 (25%), and MK-5 (21%). Polar lipid analysis determined eight glycolipids, three phospholipids, one phosphatidylglycerol, one diphosphatidylglycerol, and one unidentified lipid. The genome size is 3.36 Mb, and the DNA G+C content is 65.1 mol% (genome analysis). No plasmids have been isolated. The known habitat is the human gut.

DNA G+C content (mol%): 65.1 (genome analysis).

Type species: **Rubneribacter badeniensis** Danylec et al. 2018[VP].

Gram-stain-positive, strictly anaerobic, and **rod-shaped cells**. The length of the cells is 0.98±0.20 µm, and the diameter is 0.30±0.02 µm. Cells are nonmotile, occur singly or in short chains, and do not produce endospores. Visible cell growth on solid as well as in liquid media within 48–72 h at 37°C under strictly anaerobe conditions. **Arginine is hydrolyzed**. In addition, reactions of proline arylamidase, phenylalanine arylamidase, leucine arylamidase, tyrosine arylamidase, alanine arylamidase, glycine arylamidase, histidine arylamidase, and serine arylamidase are positive. Cells do not produce catalase, indole, oxidase, or urease. **Acid is not produced from sugars**. The major cellular fatty acids **are $C_{14:0}$ iso, $C_{15:0}$ anteiso, $C_{16:0}$ DMA, and one unidentified fatty acid methyl ester**. The major respiratory quinone is menaquinone (MK) **MK-7 (53%).** In addition, **MK-6 (25%)** and **MK-5 (21%)** are present. Polar lipid analysis determined eight glycolipids, three phospholipids, one phosphatidylglycerol, one diphosphatidylglycerol, and one unidentified lipid. The **genome size is 3.36 Mb,** and the DNA G+C content is 65.1 mol% (genome analysis).

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No plasmids have been isolated. The known habitat is the **human gut**. The secondary plant metabolite resveratrol is metabolized neither to dihydroresveratrol nor to lunularin. Member of the class *Coriobacteriia*, order *Eggerthellales*, and family *Eggerthellaceae*.

DNA G+C content (mol%): 65.1 (genome analysis).

Type species: **Rubneribacter badeniensis** Danylec et al. 2018[VP].

Number of species with validly published name: 1.

Family classification: The genus *Rubneribacter* is classified within the family *Eggerthellaceae*.

Further descriptive information

Cell morphology and ultrastructure

Cells of the type strain are Gram-stain-positive and non-motile. Cells are rod shaped (Figure 1). The cell length is 0.98 ± 0.20 µm, and the cell diameter is 0.30 ± 0.02 µm. Cells of *R. badeniensis* occur singly or in short chains. Cells do not produce endospores.

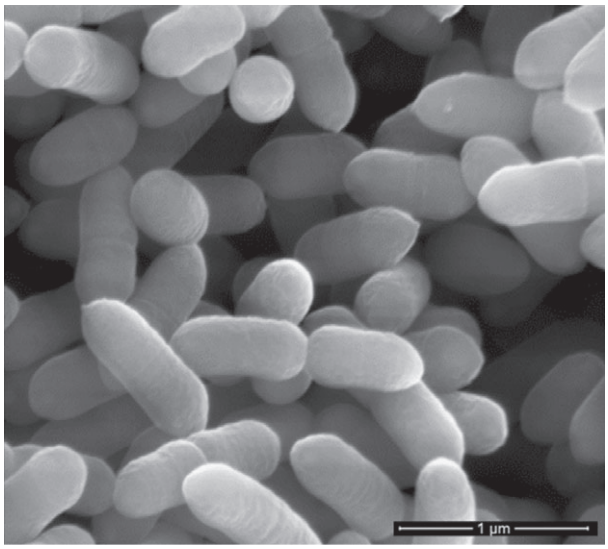
Colonial and cultural characteristics

On solid media, visible colonies of *R. badeniensis* appear within 48–72 h at 37°C under atmospheric conditions of $N_2/CO_2/H_2$ (80:10:10). Colonies of *R. badeniensis* are small (1–2 mm), circular, pale white, and semitranslucent. Liquid cultures of *R. badeniensis* display white turbidity and low optical densities similar to McFarland standard 0.5 within 48 h. Hemolysis does not occur on blood agar plates. Growth is promoted by the supplementation of heme solution (2.5 mg/l), vitamin K_1 solution (2 µg/ml), and arginine solution (2 g/l). *Rubneribacter badeniensis* tolerates 2% ox-bile (w/v).

Chemotaxonomic characteristics

Cells of the type strain *R. badeniensis* contain eight glycolipids, three phospholipids, one phosphatidylglycerol, one diphosphatidylglycerol, and one unidentified lipid. The predominant cellular fatty acids are $C_{14:0}$ iso, $C_{15:0}$ anteiso, $C_{16:0}$ DMA, and one unidentified fatty acid methyl ester. *Rubneribacter badeniensis* contains the respiratory quinones MK MK-7 (53%), MK-6 (25%), and MK-5 (21%). At the time of writing, within the family *Eggerthellaceae*, MK-5 was only described for *Ellagibacter isourolithinifaciens* and *Enteroscipio rubneri* (*Enteroscipio*) (Beltran et al., 2018; Danylec et al., 2018).

FIGURE 1. Scanning electron micrograph of the cells of *Rubneribacter badeniensis* ResAG-85[T]. Bar, 1 µm. (Dr. Melanie Huch.)



Genome features

The draft genome sequence has been determined for the type strain *R. badeniensis* ResAG-85[T]. The representative genome (PPEL00000000) consists of 201 contigs; the N50 is 28,746 bp; and the genome coverage is 164×. The genome size is 3,364,395 bp; and the G+C content is 65.1 mol%. A number of 2,557 predicted protein-coding genes were identified. No plasmid could be isolated. The genome was screened for antimicrobial resistance genes *in silico* using ResFinder 3.2 (Zankari et al., 2012). This screening predicted the tetracycline resistance gene *tet*(W).

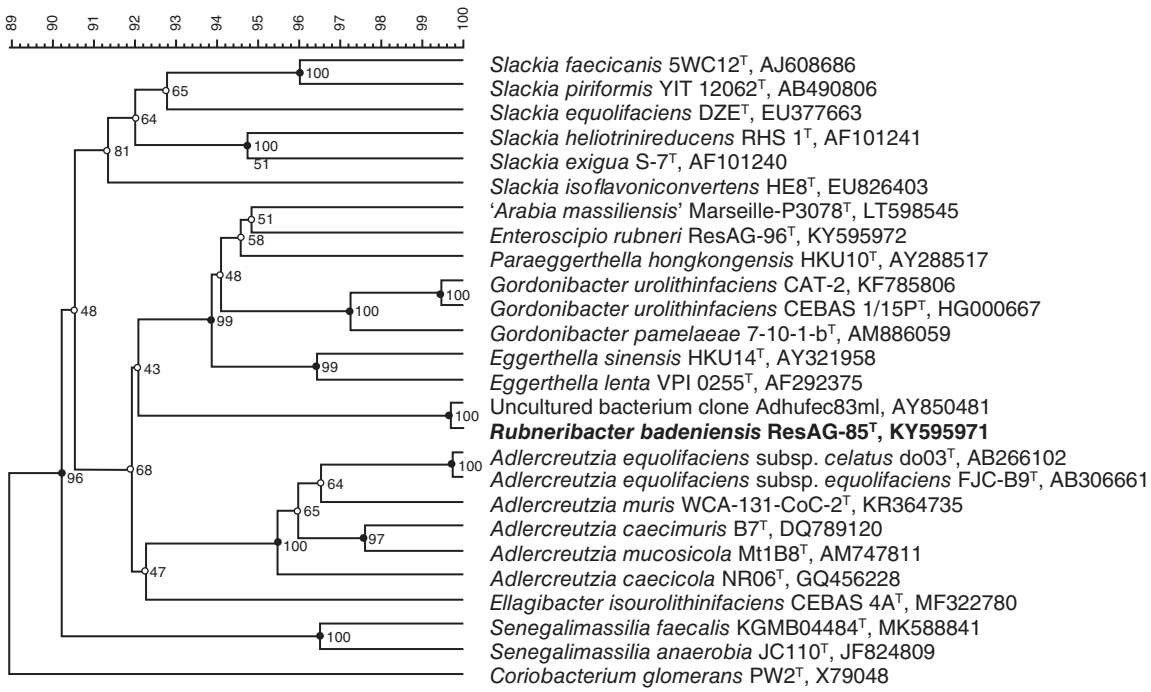
Enrichments and isolation procedures

Rubneribacter badeniensis was isolated from a human fecal sample using brain heart infusion broth supplemented with 0.5% yeast extract, 0.05% L-cysteine monohydrochloride, 1 mg/ml resazurin sodium salt, 2.5 mg/l heme solution, 2 µg/ml vitamin K_1 solution, 1 µg/ml ampicillin, 5 µg/ml colistin, 5 µg/ml chloramphenicol, 18 µg/ml cholic acid, and 80 µM *trans*-resveratrol under anaerobic conditions, that is, $N_2/CO_2/H_2$ (80:10:10) at 37°C.

Maintenance procedures

Strains can be maintained on blood agar plates and brain heart infusion agar plates as well as in brain heart infusion broth, incubated anaerobically at 37°C. Strains should be subcultured weekly. However, reactivation of Hungate cultures

FIGURE 2. Tree based on 16S rRNA genes showing the phylogenetic position of *Rubneribacter badeniensis* ResAG-85^T (KY595971) within the *Eggerthellaceae*. *Coriobacterium glomerans* PW2^T (X79048) (*Coriobacterium*) was used as an outgroup. The tree was built using multiple sequence-based alignment and UPGMA in BioNumerics 7.6 (Applied Maths, Sint-Martens-Latem, Belgium). Numbers at nodes indicate support for internal branches within the tree obtained by bootstrap analysis (percentages of 1,000 resamplings). Bar, percentage of 16S rRNA gene sequence similarity.



which were stored at room temperature was possible within two months postinoculation. For long-term storage, strains can be stored at −80°C in brain heart infusion supplemented with 10% glycerol and flushed with N_2/CO_2 (80:20).

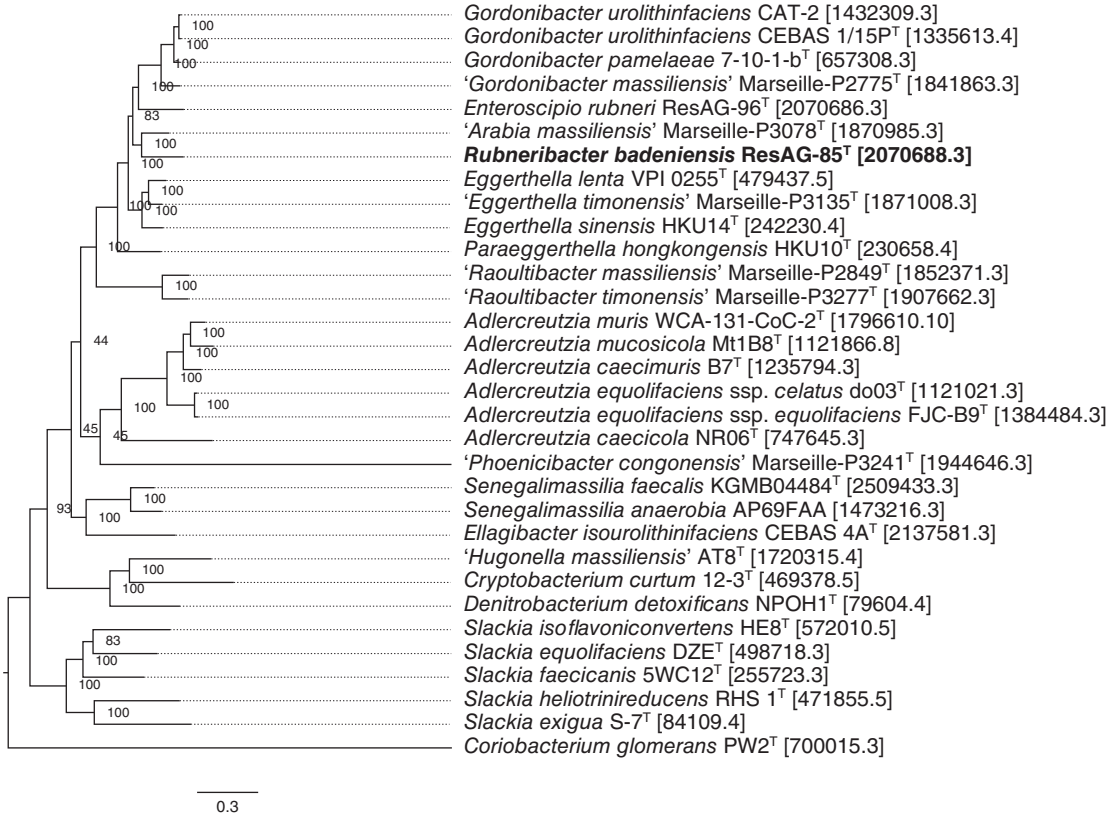
Differentiation from closely related genera

Compared to other strains of the *Eggerthellaceae*, the genus *Rubneribacter* shows some biochemical characteristics using Rapid ID 32A for the identification of anaerobic bacteria. The type strain *R. badeniensis* is positive for the reaction of arginine dihydrolase, proline arylamidase, phenylalanine arylamidase, leucine arylamidase, tyrosine arylamidase, alanine arylamidase, glycine arylamidase, histidine arylamidase, and serine arylamidase. The biochemical pattern of *R. badeniensis* is very similar to the type strain of *Adlercreutzia caecicola* (Clavel et al., 2013; Nouioui et al., 2018) except for the positive reaction of histidine arylamidase. Molecular fingerprinting methods such as ARDRA, BOX-PCR, and rep-PCR can be used to differentiate the genera of the family *Eggerthellaceae* (Danylec et al., 2019; Würdemann et al., 2009). For unambiguous identification of this genus, sequencing of the 16S rRNA gene as well as the whole genome is recommended.

Taxonomic comments

Rubneribacter badeniensis is the only species described within the genus *Rubneribacter*. The phylogenetic relationship of *Rubneribacter* in comparison to other genera within the family *Eggerthellaceae* based on the 16S rRNA gene sequence similarity is shown in Figure 2. A 16S rRNA gene sequence (AY850481) with 99.6% similarity to the sequence of the type strain of *R. badeniensis* was identified in a human fecal metagenomic library of healthy individuals (Manichanh et al., 2008). *Eggerthella* and *Gordonibacter* species are among the nearest validly published neighbors of *R. badeniensis* ResAG-85^T. A phylogenetic tree of strains of the family *Eggerthellaceae* based on all shared proteins is shown in Figure 3. *Rubneribacter badeniensis* ResAG-85^T clusters together with the strain Marseille-P3078^T which represents the effectively but not validly published name '*Arabia massiliensis*' (Traore et al., 2017).

FIGURE 3. Tree based on all shared proteins ($n = 166$ coding sequences) showing the phylogenetic position of *Rubneribacter badeniensis* ResAG-85^T [2070688.3] within the *Eggerthellaceae*. *Coriobacterium glomerans* PW2^T (*Coriobacterium*) was used as an outgroup. The tree was built using the PATRIC server 3.6.3 (Wattam et al., 2017) (https://patricbrc.org/app/PhylogeneticTree) and the RAxML (Randomized Axelerated Maximum Likelihood) algorithm (Stamatakis, 2014). Numbers at nodes indicate support for internal branches within the tree obtained by jackknife analysis (percentages of 100 resamplings). The numbers in square brackets indicate the respective PATRIC Genome IDs.



List of species of the genus *Rubneribacter*

Rubneribacter badeniensis Danylec et al. 2018^{VP}

ba.de.ni.en'sis N.L. masc. adj. *badeniensis* after Badenia, the Roman name of the area Baden, Germany, where the type strain was isolated.

Cells are rod shaped (about 0.98±0.20 µm × 0.30±0.02 µm) and occur as single or in short chains. Cells do not produce endospores. Gram-stain-positive, nonmotile, and strictly anaerobic. Hemolysis does not occur on blood agar plates. Colonies are small (1–2 mm), circular, pale white, and semi-translucent. Catalase-, oxidase-, and urease-negative. Negative for indole production. Positive for production of arginine dihydrolase, proline arylamidase, phenylalanine arylamidase, leucine arylamidase, tyrosine arylamidase, alanine arylamidase, glycine arylamidase, histidine arylamidase, and serine arylamidase. No further biochemical characteristics were detected using API rapid ID 32A. No metabolization of any of the substrates tested with the API 20A. *Trans*-resveratrol is metabolized neither to dihydroresveratrol nor to lunularin. The respiratory quinones are MK-7, MK-6, and MK-5. Polar lipids consist of eight glycolipids, three phospholipids, one phosphatidylglycerol, one diphosphatidylglycerol, and one unidentified lipid. The predominant cellular fatty acids are $C_{14:0}$ iso, $C_{15:0}$ anteiso, $C_{16:0}$ DMA, and one unidentified fatty acid methyl ester. The genome size is 3.36 Mb.

Isolated from a fecal sample of a 30-year-old human, male, moderately obese volunteer in Karlsruhe, Germany.

DNA G + C content (mol%): 65.1% (genome analysis).

Type strain: ResAG-85^T, DSM 105129^T, JCM 32272^T, LMG 30180^T.

EMBL/GenBank accession number (16S rRNA gene): KY595971.

EMBL/GenBank accession number (genome): NZ_PPEL 00000000.

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