Phyllobacterium calauticae sp. nov. isolated from a microaerophilic veil transversed by cable bacteria in freshwater sediment

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Received: 19 July 2021 / Accepted: 23 August 2021 / Published online: 7 September 2021
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Abstract Microaerophilic veils of swimming microorganisms form at oxic-anoxic interfaces, mostly described in sediments where sulfide from below meets oxygen diffusing in from the water phase. However, microaerophilic veils form even when these gradients do not overlap, for example when cable bacteria activity leads to a suboxic zone. This suggests that veil microorganisms can use electron donors other than sulfide. Here we describe the extraction of microorganisms from a microaerophilic veil that formed in cable-bacteria-enriched freshwater sediment using a glass capillary, and the subsequent isolation of a motile, microaerophilic, organoheterotrophic bacterium, strain R2-JL T, unable to oxidize sulfide. Based on phenotypic, phylogenetic, and genomic comparison, we propose strain R2-JL T as a novel Phyllobacterium species, P. calauticae sp. nov.. The type strain is R2-JL T (= LMG 32286 T = DSM 112555 T). This novel isolate confirms that a wider variety of electron donors, including organic compounds, can fuel the activity of microorganisms in microaerophilic veils.

Keywords Chemotaxis · Gradient · Microaerophile · Motility · Oxic-anoxic interface · Sulfide

Introduction

Oxic-anoxic interfaces (OAIs) are present in all environments where oxygen is consumed faster than replenished, such as aquatic sediments, and are home to microbial communities that are well adapted to micro-oxic conditions (Brune et al. 2000; Thar and Kühl 2002; Thar and Fenchel 2005). Motile members of these communities seek out low oxygen concentrations by chemotaxis and form dense accumulations of swimming microorganisms right at the OAI, termed the microaerophilic veil (Barbara and Mitchell 1996; Brune et al. 2000; Brune et al. 2000; Thar and Fenchel 2005; Scilipoti et al. 2021). These microaerophilic veils typically form where sulfide diffusing up from below meets oxygen. Thus the characterization of veil microorganisms has been focused on sulfide-oxidizing bacteria (Thar and Kühl 2002; Muyzer et al. 2005).
However, microaerophilic veils form even when sulfide and oxygen are separated by a layer without detectable oxygen and sulfide, a suboxic zone (Froelich et al. 1979; Berner 1981). Such suboxic layers are found, for example, in sediments with active cable bacteria (Nielsen et al. 2010; Pfeffer et al. 2012; Schauer et al. 2014). Cable bacteria are up to centimetres-long, multi-cellular, filamentous, sulfide-oxidizing bacteria (Pfeffer et al. 2012). They spatially separate their metabolic redox half reactions by transporting electrons from sulfide oxidation to the oxic surface sediment, where oxygen is reduced (Pfeffer et al. 2012), and thus create a suboxic zone up to several centimetres wide (Schauer et al. 2014). While sulfide and oxygen never directly meet in these zones, microaerophilic veils form along the OAI of cable bacteria-induced suboxic zones (Bjerg et al. 2016; Scilipoti et al. 2021). This suggests that organisms in these microaerophilic veils use electron donors other than sulfide. The aim of this study was to isolate and characterize bacteria from such a veil, with a focus on chemoorganotrophs. To extract bacterial cells from the veil, we adapted a glass capillary technique, used for molecular identification of veil bacteria (Muyzer et al. 2005), and proceed here to describe a novel species of the genus Phyllobacterium.

Materials and methods

Isolation and cultivation of bacteria

For the visualization of the microaerophilic veil, an in-house designed “trench slide” was used (Bjerg et al. 2016, 2018; Thorup et al. 2021). The glass slide chamber system was adapted by raising the coverslip, which was done by adding 2 broken parts of a glass coverslip on each side of the sediment chamber (Fig. 1). The glass fragments were anchored in place with Vaseline. The trench slide allows space for sediment in the middle, a layer of water that is increased slightly by the smalladaptation, and has a glass surface around the central sediment space to facilitate light passing through. Oxygen diffuses in from the side, while sulfide and other compounds diffuse from the sediment into the water layer, creating geochemical gradients.

Sediment was collected from a pond at Aarhus University campus (Vennelyst Park), Denmark (56.164672, 10.207908), at a water depth of 0.5–1 m and stored with overlying water at 15 °C for two weeks. After storage, the sediment was homogenized, sieved (pore size: 0.5 mm), and autoclaved at 121 °C for 20 min in 2 L bottles, cooled down to 15 °C, homogenized and distributed into 50 ml Falcon tubes. The sediment was then inoculated with a pre-grown sediment enrichment culture (at 15 °C) containing a single cable bacterium species along with diverse sediment bacteria (Thorup et al. 2021). After 2 weeks a clump of sediment (~ 5 mm³) was transferred into the modified trench slide. The trench slide was incubated in a humid environment at 15 °C over night.

With a phase-contrast microscope at 50 × magnification (Leica 020–507.010, Germany) the microaerophilic veil that formed in the glass trench slide was localized. Cells were extracted from the microaerophilic veil with a handmade glass capillary (diameter: 80–150 μm, sterilised by 1.5 h of UV-radiation). Through use of the microscope and a micromanipulator (Unisense A/S, Denmark), we kept control over the location of extraction. The extracted volume was regulated by connecting the capillary to a flowrate-controlled (100–200 μl min⁻¹), programmable syringe pump (TSE Systems, Germany) that held a 10 ml syringe filled with milliQ water to prevent introduction of bubbles. The capillary was inserted by wedging the capillary in between the glass slide and its coverslip (Fig. 1). When the veil re-established itself after forced movement from water flow pressure, cells were visibly sucked into the capillary. The flow rate and location was adapted during extraction to exclude cable bacteria and sediment particles. Extracted cells were transferred into a PCR tube and diluted 1:1 with 1:10 diluted R2A (18 g l⁻¹, Sigma-Aldrich, Germany) and kept at 4 °C for approximately 5–15 min until inoculation.

Nutrient broth (8 g NB l⁻¹, Scharlau, Spain), R2A, and Tryptic Soy Broth (30 g TSB l⁻¹, Scharlau) plates (15 g agar l⁻¹, Scharlau) were inoculated with 2–10 μl extract, as well as with 1:10, 1:100, and 1:1000 diluted extract (in R2A). All plates were incubated at 15 °C in the dark. After 2 weeks, distinct colonies were selected and transferred. The novel isolate originated from a 1:1000 diluted inoculum grown for 7 days on NB agar plates. Liquid cultures of the novel isolate were grown in NB without stirring with a headspace of approx. 50% atmospheric air.

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Taxonomy screen based on 16S rRNA

After distinct colonies were visible on the second generation plates a colony PCR was performed to obtain an initial taxonomic classification. Samples were PCR amplified for the 16S rRNA gene with primers 27F/1492R (Lane 1991), DNA concentrations were determined with a Nanodrop (Thermo Fisher), and sequencing of the 16S rRNA gene was performed by Macrogen Europe B.V. (Sanger sequencing). Taxonomic classification of the isolates was determined after trimming off the primer and poor quality bases determined with Chromas (Technelysium). Sequences were used as query sequences with nucleotide BLAST (Camacho et al. 2009) against the Nucleotide collection (nr/nt) database, excluding uncultured/environmental sample sequences.

Genome sequencing, assembly and annotation

After two transfers, genomic DNA was extracted from colonies of five *Phyllobacterium* isolates with a DNeasy PowerLyzer PowerSoil DNA Isolation Kit (Qiagen). Concentrations and length distribution were scanned using the Agilent 4200 TapeStation with a Genomic DNA ScreenTape in the size range of 200 to > 60,000 bp (Agilent Technologies). A Nextera XT DNA Library Preparation kit (Illumina) was used and sequencing was performed in-house using 2 × 300 bp paired-end reads on the Illumina MiSeq platform, following the standard Illumina protocol for MiSeq reagent kit v3. Raw sequence quality was assessed using FastQC v0.11.5 (Wingett and Andrews 2018) before and after trimming with Trimmomatic v.0.39 (Bolger et al. 2014): headcrop 20; sliding window 4:20; minimum length 100 bp; adapter trimming with parameters 2:40:15. Trimmed paired-end and non-paired reads were de novo assembled with SPAdes v3.14.1 (Bankevich et al. 2012) with: default k-mers 21, 33, 55, 77, 99, 127; the option careful; automatic coverage cutoff. Completeness, contamination, and quality were estimated with CheckM v1.1.3 (Parks et al. 2015) and Quast v4.3 to determine the quality of the final assembly. Using Prokka v1.14.5 (Seemann 2014) with a minimal contig length of 300, first a genus database was generated based on *Phyllobacterium* genomes (*P. myrsinacaerum* DSM 5892^T^, *P. sp* 628, *P. bourgognense* 21–35^T^). This curated database was used as primary annotation source against which the genome of R2-JLT^T^ was searched using protein–protein BLAST within the Prokka pipeline. Additionally, protein-encoding genes were also annotated through BlastKOALA and KEGG Mapper (Kanehisa and Goto 2000; Kanehisa et al. 2016Kanehisha 2019). The web services were also used to determine absence or presence of metabolic pathways.

Taxonomic and additional genomic analyses

To determine the taxonomy of the strain, all genomes designated to the genus *Phyllobacterium* were
downloaded from NCBI and compared by calculating average nucleotide identities using FastANI v1.32 (Jain et al. 2018) with minimal fragment size set to 50. Estimates from FastANI were checked by running ANI calculations using the web service (http://enve-omics.ce.gatech.edu/ani/; Goris et al. 2007) between the sample and P. myrsinacearum DSM 5892 T. Phylogenetic marker genes were identified in all the genomes by GTDB-Tk v1.4.0 (Chaumeil et al. 2020; Parks et al. 2018; Matsen et al. 2010; Jain et al. 2018; Eddy 2011), which uses Prodigal (Hyatt et al. 2010) for this purpose. Using GTDB-Tk align, the identified 120 marker genes were translated to protein sequences and a concatenated multiple sequence alignment was made. A maximum likelihood tree with 1000 bootstrap replicates was calculated from this data with FastTree v2.1.11 SSE3 (Price et al. 2010, 2009). The tree was rooted with Mesorhizobium loti LMG 6125 T.

MEGA X (Kumar et al. 2018) alignment with Clustal ω (Sievers et al. 2011) and BLAST were used to determine similarities between the cytochrome bd complexes of Escherichia coli and strain R2-JL.

Physiological testing

Cells were harvested from plates to perform three API (20E, 20NE, ZYM; BioMérieux) tests and to inoculate a Biolog Gen III microplate (Agilent). The API tests were performed as described in the manual, with the exception of the incubation that was executed in the dark at room temperature for 24–72 h depending on the test. The Biolog Gen III plate was measured with a FLUOstar Omega microplate reader which makes use of their Omega 3.00 R2 software (BMG Labtech, France). Gram-staining was performed on liquid cultures. An OF Hugh-Leifson test for glucose fermentation was performed with additional 0.5 g L⁻¹ yeast extract and 10 g L⁻¹ glucose added before autoclaving. Growth at different temperatures was tested in duplicate or triplicate where a 3–7 day old single colony of R2-JL was aseptically streaked on fresh NB plates. Plates were incubated under dark conditions at 0.5, 4, 15, 22, 30, 35, 37, 40 and 42 °C, and checked regularly for growth or dehydration up to 2 weeks after inoculation. To test for catalase presence one or two successive drops of cold hydrogen peroxide were dripped onto a three- and a five-day-old colony.

Light microscopy

A 0.5 ml sample of shaken liquid culture of R2-JL was added to a normal glass microscopy slide and covered with a coverslip, this was performed in duplicate at 22 °C. Microaerophilic veil formation was observed for 5–10 min before capturing pictures. The microaerophilic veil formation was captured using phase contrast on a ZEISSL Observer Z1 (Zeiss, Göttingen, Germany) inverted microscope with PALM automated stage. Images were taken at 100 × magnification, with Zen Black edition (Zeiss).

Scanning Electron Microscopy (SEM)

Liquid cultures of 3–5 days old were diluted (1:2 in milliQ), air dried onto a silicon wafer and sputtered with 2 nm of platinum with a Leica EM SCD 500 platinum coater (Leica Microsystems, Wetzlar, Germany) at 35 mA under argon atmosphere. Wafers were subsequently analysed with a high vacuum SEM: Versa 3D, dual beam system (FEI, Oregon, USA). Scanning parameters: 13 pA and 5 kV.

Results and Discussion

We isolated 30 bacterial strains from freshwater sediment containing a single species of cable bacteria; Ca. Electronema aureum. The bacteria were extracted from a microaerophilic veil that formed in the modified trench slide, filled with freshwater sediment where cable bacteria thrive (Thorup et al. 2021). The 30 isolates belonged to 12 different genera, and seven of them belonged to the genus Phyllobacterium (Table S1). Five of these isolates were whole genome sequenced and appeared identical with a FastANI score greater than 99.99%. We therefore focused our efforts on one of these isolates: R2-JL.

Morphological and Physiological analyses

Phyllobacterium sp. R2-JL, the isolated strain, was found to be a Gram-negative, catalase-positive bacterium that is motile with at least one (sub)polar flagellum. Cells were slightly curved, rod-shaped, and approx. 2 × 0.6 μm in size (Fig. 2a). The cells formed a microaerophilic veil even in pure culture (Fig. 2b), suggesting chemotactic behaviour specific to oxygen.
The isolate did not reduce nitrate, and was OF-test negative suggesting that it cannot ferment glucose. Strain R2-JL can physiologically be distinguished from all other phyllobacteria based on its ability to grow on D-raffinose, arabinose and the absence of growth on quinic acid (Table 1). It can be distinguished from its currently closest relative P. myrsinacearum by growth of R2-JL on α-D-lactose, D-melibiose, D-raffinose, glucuronamide, N-acetyl-D-galactosamine, and by absence of growth of R2-JL on a-keto-glutaric acid, D-gluconic acid, D-glucose-6-phosphate, D-lactic acid methyl ester, γ-amino-butyric acid, glycerol, inosine, L-lactic acid, L-serine, N-acetyl-b-D-mannosamine and the absence of urease (Table 1).

R2-JL appears to grow exclusively on organic compounds, specifically on hexoses in mono-, di- or polysaccharide form, like glucose, sucrose, and pectine, L-rhamnose, and esculine (Table S2), which are found commonly in plants. The freshwater sediment where strain R2-JL originates from has an input of dead or partially degraded plant material, explaining the access to these carbon compounds. The novel strain is currently the only Phyllobacterium that has been isolated from sediment, where all other species have been isolated from plant rhizospheres, root or leaf nodules of tropical ornamental plants (Knösel 1962) (Table 1). Until now, only 2 species have been identified that were not isolated from nodules: P. phragmitis (Liang et al. 2019) and P. catacumbae (Jurado et al. 2005).

Phylogenetic analyses and genomic characteristics

The 16S rRNA gene sequence of R2-JL is identical to that of P. myrsinacearum, and highly identical (> 98%) to 5 other phyllobacteria (Table 1). The genomes of R2-JL and P. myrsinacearum have an ANI of 92.64% and all other Phyllobacterium species are less than 81% identical to R2-JL (Table 1, Table S3). These results were suggestive of a novel species of Phyllobacterium for our strain (Richter and Rosselló-Móra 2009; Jain et al. 2018), which clustered within the Phyllobacterium genus (Fig. 3). Figure 3, like Table 1, shows that P. myrsinacearum DSM 5892T is the closest known relative to the novel strain. It also clustered all P. myrsinacearum in a different cluster than R2-JL (bootstrap value [99% out of 1000 iterations), except for P. myrsinacearum AN3 which is in yet a third cluster.

Evidence from phylogenetic, genomic and phenotypic analyses, strengthened by its distinct isolation source that is free of living plants, and its apparent free-living lifestyle, leads to the conclusion that strain R2-JL belongs to a novel species within the genus Phyllobacterium. As the novel bacterium was isolated from a microaerophilic veil (in freshwater sediment), we propose the name Phyllobacterium calauticae sp. nov., with the type-strain R2-JL.

The genome of P. calauticae R2-JLT consists of 31 contigs, is 5,288,098 bp in size with a G+C content of 59.12%, and completeness was estimated to be 100% with 0.00% contamination. Gene prediction and
Table 1 Differential characteristics of strain R2-JL<sup>T</sup> (1, this study) compared to 8 closely related *Phyllobacterium* species.

| Similarity to R2-JL (%) | 1   | 2    | 3    | 4    | 5    | 6    | 7    | 8    |
|-------------------------|-----|------|------|------|------|------|------|------|
| Genome (FastANI)<sup>a</sup> | NA  | 92.64| 80.89| 80.22| 80.07| 79.86| 79.80| 78.42|
| 16S rRNA gene (BLAST)<sup>a</sup> | NA  | 100  | 98.79| 98.38| 98.65| 98.65| 98.38| 97.72|

**Characteristic**

| Isolation source<sup>a</sup> | Sed | Nod | Nod | Nod | Nod | Nod | Nod | Nod |
|-------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|

**Growth at/on**

|               | pH 6 | pH 5 | 1% NaCl | 2% NaCl | 3% NaCl | 4% NaCl | Acetic acid | A-D-lactose | A-keto-glutaric acid | B-hydroxy-D,L-butyric acid | B-methyl-D-glucoside | Citric acid | Dextrin | D-galactose | D-galacturonic acid | D-glucic acid | D-glucose-6-PO4 | D-glucaric acid | D-glucaric acid methyl ester | D-melibiose | D-raffinose | D-saccharic acid | D-saccharic acid methyl ester | Glucuronamide | Glycerol | Inosine | L*alanine | L-aspartic acid | L-fucose | L-glutamic acid | L-histidine | L-lactic acid | L-serine | Maltose asm | Methyl pyruvate | N-acetyl-β-D-mannosamine | N-acetyl-D-galactosamine | N-acetyl-D-glucosamine | (Potassium) gluconate asm | Propionic acid |
|---------------|------|------|---------|---------|---------|---------|-------|-------------|-------------|----------------------|-----------------------|-------------------|------------|--------|-------------|-------------------|---------------|----------------|---------------|--------------------------|-------------|--------------|----------------|--------------------------|----------------|----------------|-----------|---------------|----------|------------|----------------|------------------------|----------------|----------------|---------------|-----------------|----------|--------------|----------------|-----------------|-----------------|----------------|
Table 1 continued

| Similarity to R2-JL (%) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|------------------------|---|---|---|---|---|---|---|---|
| Quinic acid            | v | v | v | v | v | v | v | v |
| Sucrose                | + | + | + | + | + | + | + | v |
| Tween 40               | w | + | + | + | + | + | + | + |
| Urease                 | - | + | + | nr | + | + | w | - |
| Acid produced from     |   |   |   |   |   |   |   |   |
| Arabinose              | w | v | nr | +c | +c | +c | +c | + |
| Melibiose              | - | - | - | - | - | - | - | + |

Species: (2) *P. myrsinacearum*, 14 strains; (3) *P. zundukense*, 4 strains; (4) *P. sophorae* CCBAU 03422 T; (5) *P. brassicacearum*, 2 strains; (6) *P. bourgognense*, 2 strains; (7) *P. trifolii*, 2 strains; (8) *P. endophyticum*, 2 strains. Data from Liang et al. 2019, León-Barrios et al. 2018, Jiao et al. 2015, Mantelin et al. 2006, Mergaert et al. 2002, Safronova et al. 2018, Sanchez et al. 2014, Valverde et al. 2005, Flores-Félix et al. 2013.

NA, not applicable; Sed, microaerophilic veil in freshwater sediment; Nod, plant nodule; +, growth; −, no growth; w, weak growth; v, (type) strain results are variable (v/#, # indicates the result of the type strain); nd, not determined; nr, no data reported.

aValues and source are based on the type strain of the species.
bD/L not specified.
cMeasured as L-arabinose.

Fig. 3 Phylogenetic tree highlighting the placement of Phyllobacterium calauticae R2-JL T (in bold) within the genus Phyllobacterium. Phylogenetic marker genes translated to protein sequences were identified in genomes available from NCBI with GTDB-Tk identify and alignment was subsequently performed with GTDB-Tk align. The maximum likelihood tree was inferred using FastTree2. Bootstrap support (1000 replicates) is shown as closed circles > 99%, half open circles > 85%, and open circles > 60% likelihood. *Mesorhizobium loti* LMG 6125 T was used as outgroup to root the tree. Scale bar 0.04 substitutions per amino acid position.
annotation identified 5111 putative protein-coding genes, whereof 1119 (21.69%) are hypothetical proteins. Of these, 2768 (54.2%) could be annotated with BlastKOALA (Kanehisha et al. 2016).

Genomic and phenotypic insights into R2-JL\textsuperscript{T}\textsuperscript{7}’s ecophysiology and lifestyle

In agreement with phenotypic data, we find a flagellar assembly pathway (02035), several genes related to chemotaxis, and aerobic respiratory pathways in the genome of \textit{P. calauticae} R2-JL\textsuperscript{T}. Chemotaxis related genes that were found are \textit{cheY} that is a flagellar switch (Szurmant et al. 2003; Miller et al. 2009), PA1976 which is used for swarming and biofilm formation (Hsu et al., 2008), and \textit{regAB} that is used to sense redox signals (Emmerich et al. 2000). Presence of sensitivity towards redox signals, the veil formation, a flagellar and swarming switch, all indicate that R2-JL\textsuperscript{T} has a chemotaxis towards oxygen.

For energy conservation, R2-JL\textsuperscript{T} has an F-type ATPase (M00157), and a respiratory electron transport chain, including NADH quinone oxidoreductase (M00144), succinate dehydrogenase (M00149), ubiquinone-cytochrome \textit{c} reductase and oxidase (M00151, M00155), and likely both a low affinity terminal oxidase (cytochrome \textit{o} ubiquinol oxidase, M00417), and a high affinity oxidase (cytochrome \textit{bd} ubiquinol oxidase (cydAB, M00153)). R2-JL\textsuperscript{T}’s CydA resembles that of \textit{Escherichia coli} K-12 (protein id: QHB69574.1) based on alignments (data not shown) and BLASTp comparisons (59.4% identity). \textit{E. coli}’s cytochrome \textit{bd} complex is described as a very high affinity terminal oxidase ($K_m$ 3–8 nM O\textsubscript{2}) and it likely facilitates growth under low- or micro-oxic conditions (Rice and Hempfling 1978; D’mello et al. 1996; Borisov et al. 2011). Thus, the cytochrome \textit{bd} complex of \textit{P. calauticae} R2-JL\textsuperscript{T} potentially has a high affinity for oxygen and may enable micro-aerobic respiration of R2-JL\textsuperscript{T} under the micro-oxic conditions that are found in the veil. Neither genomic nor physiological data showed indications of an anaerobic metabolism such as nitrate reduction or fermentation to support life on the anoxic side of the veil.

On the electron donor side, there is good phenotypic evidence that R2-JL\textsuperscript{T} utilizes a broad spectrum of different organic compounds (Table 1, Table S2). On the other hand, there are no phenotypic or genomic indications for the use of sulfide as electron donor, and both Sox and rDSR pathways are absent. Thus, \textit{P. calauticae} R2-JL\textsuperscript{T} was not only isolated from a microaerophilic veil, but seems to be a microaerophile that uses organic compounds (for example plant-derived hexoses) for its metabolism, in contrast to most other bacteria described from microaerophilic veils, which make use of the sulfide-oxygen gradient (Thar and Kühl 2002; Muyzer et al. 2005). Species of \textit{Phyllobacterium} are usually associated with plants (Knösel 1962), or have at least been isolated from root or leaf nodules (Table 1). Two \textit{Phyllobacterium} species, \textit{P. trifolii} (Valverde et al. 2005) and \textit{P. sophorae} (Jiao et al. 2015), possess the genes necessary for nodule formation (\textit{nodACD}) or nitrogen fixation (\textit{nif}DKH). In contrast, the genome of \textit{P. calauticae} R2-JL\textsuperscript{T} did not contain any of the plant signalling pathways (MAPK), \textit{nodACD} genes, \textit{nif}DKH genes, or plant-pathogen interaction pathways. This strongly indicates that R2-JL\textsuperscript{T} lacks the ability to form symbiotic, commensal or pathogenic interactions with plants and belongs to the free-living \textit{Phyllobacterium} (Safronova et al. 2018).

Conclusion

\textit{P. calauticae} R2-JL\textsuperscript{T} (= LMG 32286\textsuperscript{T} = DSM 112555\textsuperscript{T}), the first \textit{Phyllobacterium} from sediment, is an organoheterotrophic, aerobic bacterium, isolated from a microaerophilic veil that formed in cable-bacteria-enriched freshwater sediment. With its motility, chemotaxis, and presumably high affinity terminal oxidase, R2-JL\textsuperscript{T} appears adapted to the moving counter-gradients typical of a microaerophilic veil, where oxygen overlaps with reduced organic compounds diffusing from the organic-rich, anoxic sediment. Ultimately, these results expand our view of the bacteria in microaerophilic veils and the compounds they utilise other than sulfide.

Description of \textit{P. calauticae} R2-JL\textsuperscript{T} (type strain)

\textit{Phyllobacterium calauticae} (ca’ lau. ti. cae N.L. gen. n. \textit{calauticae} of \textit{calautica}; a headdress or turban reaching to the shoulders i.e. veil, from which the type strain was isolated).

Cells are Gram-stain-negative, aerobic, slightly curved rods (2 \textmu m \times 0.6 \textmu m) that are motile by at least one (sub)polar flagellum. They form mucoid...
milky-white undulate-shaped colonies (2–5 mm diameter) and often present with transparent edges and radial whiteness emerging from the centre, after 48 h of incubation at 15 °C. P. calauticae. R2-JL T is catalase positive, and OF-test negative for glucose, but arabinose and glucose showed weak growth. Salinity is tolerated to at least 4.0% (w/v) NaCl, it can grow at a pH of at least 5.0–7.2, and at a temperature of 0.5–37 °C, with weak growth at 0.5, 4, and 37 °C. The G + C content of the genomic DNA is 59.12%.

Type strain R2-JL T (= LMG 32286 T = DSM 112555 T) was isolated from a microaerophilic veil forming in a microscopy chamber with freshwater sediment that contained a single strain enrichment of cable bacteria (Thorup et al. 2021). The extracted sample was collected in March 2019 and cultivated until a pure culture was reached in June 2019. The accession number for P. calauticae R2-JLT’s draft genome sequence is JAGENB000000000.

Acknowledgements We thank Susanne Nielsen and Lars B. Pedersen for their work and support in the molecular and microbiological laboratories; Pia Bomholt Jensen for excellent SEM images; and Ronny Mario Baaske for care of the cable bacteria enrichments. This research was supported by the Danish National Research Foundation (DNRF136) and the Carlsberg Foundation (CF19-0666).

Authors’ contributions JL, JB, AS, and IM conceived and designed the study; JL and AS performed research; JL analysed data; JL, JB, and IM contributed new methods or models; JL, AS, and IM wrote the paper; all authors have read and approved the final manuscript.

Funding This research was supported by the Danish National Research Foundation (DNRF136) and the Carlsberg Foundation (CF19-0666).

Availability of data and material P. calauticae R2-JL T is available from BCCM/LMG Gent, Belgium (LMG 32286 T) and DSMZ, Braunschweig, Germany (DSM 112555 T). The draft genome sequence is available from NCBI under the accession number JAGENB000000000.

Declarations

Conflicts of interest The authors declare that they have no conflicts of interest.

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