

Bullifex porci DSM 105750^T (VUNN0000000) Victivallis lenta DSM 107290^T (VUNS0000000) Victivallis vadensis DSM 107450 (JABAEW00000000) Hallerella succinigenes DSM 104698^T (ASM279767v1) Fibrobacter intestinalis DSM 104696 (ASM230044v1) Hallerella porci DSM 104699^T (ASM314888v1) Alistipes shahii DSM 107272 (JABAGP000000000) Sodaliphilus pleomorphus DSM 108610^T (CP045696) Parabacteroides distansonis DSM 108610^T (CP045696) Prevotella mizrahii DSM 108495^T (VUNG00000000) Prevotella copri DSM 108494 (VUNF00000000) Bacteroides vulgatus DSM 107446 (VULU00000000) Bacteroides fragilis DSM 103087 (JABAGK000000000 Bacteroides eggerthii DSM 107245 (JABAGL00000000) Escherichia coli DSM 106279 (VUMJ00000000) Escherichia dai Domi Todz'i (10M30000000) Enterobacter asburiae DSM 10527 (10M400000000) Desuffovibrio porci DSM 105247 (10MH0000000) Desuffovibrio piger DSM 106036 (JABAFY00000000) Berryella intestinalis DSM 10837 (10MF0000000) Collinsella aerdaciens DSM 10837 (10MF0000000) Collinsella aerofaciens DSM 108492 (VUME00000000) Olsenella porci DSM 105246^T (VUNC00000000) Olsenella umbonata DSM 105184 (JABAGR000000000) Olsenella umbonata DSM 105334 (VUND00000000) Cutibacterium porci DSM 101006⁷ (VUMG00000000) Rhodococcus sp. DSM 105337 (JABAGQ000000000) Rhodococcus sp. DSM 106145 (JABAFB000000000) Corynebacterium xerosis DSM 107249 (JABAGA000000000) Corynebacterium stationis DSM 107248 (JABAFZ00000000) Corynebacterium ammoniagenes DSM 104746 (JABAGB000000000) Micrococcus luteus DSM 105846 (JABAFF000000000) Arthrobacter sp. DSM 105845 (VULS000000) Actinobacter sp. DSM 105845 (VULS000000) Cellulosimicrobium aquatile DSM 105328 (JABAGH00000000) Mobiluncus porci DSM 108840^T (VUMY00000000) Scrofimicrobium canadense DSM 105338^T (VULO0 Schaalia hyovaginalis DSM 106277 (VUNK0000000) Bifidobacterium tsurumiense DSM 104387 (VULW00000000) Bifidobacterium infantis DSM 107246 (VULV00000000) Bifidobacterium boum DSM 102857 (JABAGJ00000000) Bifidobacterium thermophilum DSM 102827 (JABAGI000000000 Pyramidobacter porci DSM 105193^T (VUNH00000000) Cloacibacillus porcorum DSM 25858 (NZ CP016757.1) Cloacibacillus porcorum DSM 105753 (JABAGT000000000) Fusobacterium perfoetens DSM 105865 (JABAFQ000000000) Fusobacterium mortiferum DSM 108838 (VUMQ00000000) Acidaminococcus fermentans DSM 105754 (VULN00000000) Anaerovibrio slackiae DSM 108025^T (VUNR00000000) Selenomonas montiformis DSM 106892⁷ (VUNL0000000) Selenomonas bovis DSM 100960 (JABAFA00000000) Megasphaera hexanoica DSM 106893 (JABAFG000000000) Megasphaera elsdenii DSM 10095 (JABAFH00000000) Megasphaera elsdenii DSM 10961 (JABAFH00000000) Sharpea porci DSM 108165^T (VUNM00000000) Stecheria intestinalis DSM 109718^T (VUMN00 Clostridium innocuum DSM 100998 (VUMA00000000) Faecalicoccus pleomorphus DSM 103368 (JABAFR000000000) Holdemanella porci DSM 105256^T (VUMR00000000) Floccifex porci DSM 104670^T (VUMM00000000) Aneurinibacillus aneurinilyticus DSM 105329 (JABAGO00000000) Bacillus velezensis DSM 100955 (JABAGN000000000) Bacillus altitudinis DSM 108493 (JABAGM00000000) Psychrobacillus sp. DSM 106035 (JABAFC00000000) Staphylococcus cohnii DSM 107449 (VUNO00000000) Staphylococcus hominis DSM 104142 (JABAEZ00000000) Staphylococcus epidermidis DSM 106280 (JABAEY00000000) Streptococcus sp. DSM 105354 (JABAEX000000000) Streptococcus alactolyticus DSM 100950 (VUNP0000000) Enterococcus cecorum DSM 100908 (JABAFV000000000) Enterococcus gallinarum DSM 104116 (JABAFS00000000) Enterococcus faecalis DSM 100906 (JABAFU00000000) Enterococcus hirae DSM 100949 (JABAFT000000000) Enterococcus faecium DSM 105332 (JABAGS000000000) Enterococcus faecium DSM 105332 (VUMK00000000) Enterococcus faecium DSM 100905 (VUML00000000) Lactobacillus salivarius DSM 103789 (JABAFL0000000) Lactobacillus agilis DSM 102821 (JABAFP00000000) Lactobacillus ruminis DSM 107447 (JABAFM000000000) Lactobacillus sp. DSM 102820 (JABAFO000000000) Lactobacillus reuteri DSM 108836 (JABAFN000000000) Lactobacillus equicursoris DSM 104994 (VUMW00000000) Lactobacillus porci DSM 105804 (VUMX00000000) Lactobacillus iohnsonii DSM 106897 (JABAFI00000000) Lactobacillus amylovorus DSM 107288 (JABAFJ000000000) Lactobacillus crispatus DSM 105842 (JABAFK000000000) Clostridium cochlearium DSM 107247 (JABAGF000000000) Clostridium cadaveris DSM 100963 (JABAGG000000000) Clostridium perfringens DSM 106278 (JABAGE00000000) Inconstantimicrobium porci DSM 108839⁷ (VULX00000 Clostridium sp. DSM 107452 (JABAGC000000000) Clostridium beijerinckii DSM 105335 (JABAGD00000000) Peptostreptococcus porci DSM 106284^T (VUNE000000 Paraclostridium sp. DSM 107287 (JABAFD00000000) Paraciostridium sp. 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Spirochaetes Lentisphaerae Fibrobacteres

Bacteroidetes

Proteobacteria

Actinobacteria

Synergistetes

Fusobacteria Firmicutes

Supplementary Fig. 1 / Phlyogenomic tree of isolates within PiBAC.

The tree was constructed based on 400 marker genes in PhyloPhlan (v0.99). The accession numbers of genomes used for the analysis are indicated in brackets next to species names. Novel taxa are written in bold letters. Colors indicate phyla.



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(a) Occurrence of genes involved in cell morphology and division, peptidoglycan synthesis, and cell wall formation within the genome of Bullifex porci gen. nov. sp. nov. and related species within the family Spirochaetaceae.

(b) List of most frequent antimicrobial resistance (AMR) genes (top; bluish bars) and antimicrobial substances (middle; brown) within the entire collection along with PiBAC species with highest numbers of AMR genes (bottom; gray).

(c) Global functional annotation of the 117 strains within PiBAC according to EggNOG categories. The bar chart shows average % per functional category by EggNOG-Mapper; data is presented as mean values ± SD.





Supplementary Fig. 3 / Short-chain fatty acid (SCFA) profile of new taxa provided in PiBAC. Triplicate cultures of each strain were incubated in modified YCFA broth (DSMZ medium 1611). Unless otherwise stated in brackets, the incubation time was 48 h at 37 °C under anaerobic conditions. The concentrations of SCFA were determined by HPLC-RI as detailed in the Methods. Results are represented as mean with SD whereby delta-values below 0.5 mM (threshold) were not plotted.

Bacteria were: (a) Victivalis lenta DSM 107290; (b) Suipraeoptans intestinalis DSM 104945; (c) Peptostreptococcus porci DSM 106284; (d) Peptoniphilus porci DSM 104947; (e) Velocimicrobium porci DSM 107250; (f) Clostridium porci DSM 100959; (g) Pyramidobacter porci DSM 105193 (96 h); (h) Tissierella pigra DSM 105185; (i) Hornefia butyriciproducens DSM 104962; (j) Hornefia porci DSM 104948; (k) Eisenbergiella porci DSM 101007; (l) Anaerococcus porci DSM 101005; (m) Roseburia porci DSM 107448; (n) Selenomonas montiformis DSM 106892; (o) Olsenella porci DSM 105246 (96 h); (p) Cutibacterium porci DSM 101006; (q) Anaerovibrio slackiae DSM 108025; (r) Scrofimicrobium canadense DSM 105338; (s) Hallerella succinigenes DSM 104698; (t) Prevotella mizrahii DSM 108495. Suppl. Fig. 4a 165 rRNA gene-based phylogenetic tree of Berryella intestinalis gen. nov., sp. nov. and Olsenella porci sp. nov.



Taxonomy: (P) Actinobacteria, (F) Atopobiaceae

Suppl. Fig. 4b 16S rRNA gene-based phylogenetic tree of Cutibacterium porci sp. nov.



Suppl. Fig. 4c 165 rRNA gene-based phylogenetic tree of Mobiluncus porci sp. nov. and Scrofimicrobium canadense gen. nov., sp. nov.



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Actinomyces meyeri ATCC 35568 (X82451)

Suppl. Fig. 4e 16S rRNA gene-based phylogenetic tree of Prevotella mizrahii sp. nov.





Taxonomic note:

16S rRNA gene-based phylogeny of the genus Prevotella is unstable and phylogenomic analysis clearly shows that this genus is heterogenous and requires extensive taxonomic amendments. Although somewhat contradictory, the various genome-based parameters determined to delineate the status of our isolate suggest that the creation of a novel genus is required to accomodate it. However, the strain clearly falls within the Prevotella genus cluster (according to both 16S rRNA gene-based and genomic trees) as per the current status of valid names, and GTFB-Tk also places the isolate within this genus. Furthermore, whilst the species name Hallella serengens is valid, the taxonomy of this and related species is not congruent. Altogether, to avoid generating further instability within the genus Prevotella, the creation of a novel species, Prevotella mizrahii, is proposed to accomodate strains DSM 108495. Taxonomic reclassifcations within the genus Prevotella requires extensive work. It is out of scope of the present manuscript and will be performed in future studies.

Suppl. Fig. 4f 16S rRNA gene-based phylogenetic tree of Inconstantimicrobium porci gen nov., sp. nov.





Taxonomy: (P) Firmicutes, (F) *Clostridiaceae* Highest log likelihood: -5,525.46 No. of nucleotide positions: 1,370

Suppl. Fig. 4g 16S rRNA gene-based phylogenetic tree of *Pseudoramibacter porci* sp. nov.









Suppl. Fig. 4i 16S rRNA-based phylogenetic tree of *Floccifex porci* gen. nov., sp. nov., *Holdemanella porci* sp. nov., Stecheria intestinalis gen. nov., sp. nov., and *Sharpea porci* sp. nov.



2 µm

Suppl. Fig. 4j 16S rRNA gene-based phylogenetic tree of Roseburia porci sp. nov.



* The proposition to accomodate Eubacterium rectale into the genus Agathobacter' has been thereafter refuted by genome analysis², which indicated that the species is located deeply inside the Roseburia cluster. There is no genome available for Agathobacter ruminis, preventing further analysis. The taxonomic status of Agathobacter spp. is thus ambiguous and requires amendment.

Suppl. Fig. 4k 16S rRNA gene-based phylogenetic tree of *Bilifractor porci* gen. nov., sp. nov., *Porcincola intestinalis* gen. nov., sp. nov. and *Oliverpabstia intestinalis* gen. nov., sp. nov.



Suppl. Fig. 41 16S rRNA gene-based phylogenetic tree of *Eisenbergiella porci* sp. nov., *Velocimicrobium porci* gen. nov., sp. nov. and *Waltera intestinalis* gen. nov., sp. nov.



Suppl. Fig. 4m 165 rRNA gene-based phylogenetic tree of Suipraeoptans intestinalis gen nov., sp. nov.





Taxonomy: (P) Firmicutes, (F) *Lachnospiraceae* Highest log likelihood: -4,461.46 No. of nucleotide positions: 1,268

Suppl. Fig. 4n 16S rRNA gene-based phylogenetic tree of Clostridium porci sp. nov.



Suppl. Fig. 40 165 rRNA gene-based phylogenetic tree of *Mogibacterium kristiansenii* sp. nov., *Baileyella intestinalis* gen. nov., sp. nov., *Hornefia butyriciproducens* gen. nov., sp. nov., and *Hornefia porci* sp. nov.



Suppl. Fig. 4p 165 rRNA gene-based phylogenetic tree of Anaerovibrio slackiae sp. nov. and Selenomonas montiformis sp. nov.



Suppl. Fig. 4q 165 rRNA gene-based phylogenetic tree of *Peptoniphilus porci* sp. nov., *Peptostreptococcus porci* sp. nov., and *Anaerococcus porci* sp. nov.



Suppl. Fig. 4r 16S rRNA gene-based phylogenetic tree of Victivalis lenta sp. nov.



Suppl. Fig. 4s 16S rRNA-based phylogenetic tree of Desulfovibrio porci sp. nov.





Taxonomy: (P) Proteobacteria, (F) *Desulfovibrionaceae* Highest log likelihood: -4,147.33 No. of nucleotide positions: 1,392

Suppl. Fig. 4t 16S rRNA gene-based phylogenetic tree of Bullifex porci gen. nov., sp. nov.



Suppl. Fig. 4u 16S rRNA gene-based phylogenetic tree of *Pyramidobacter porci* gen nov., sp. nov.



Suppl. Fig. 4v 16S rRNA gene-based phylogenetic tree of Hallerella porci gen nov., sp. nov. and Hallerella succinigenes sp. nov.



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Supplementary Fig. 5a / Scanning electron micrographs of *Pseudoramibacter porci* DSM 106894^T.

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Supplementary Fig. 5b / Transmission electron micrographs of *Pseudoramibacter porci* DSM 106894^T
(1) Cells fixed with 2% (v/v) formaldehyde and 2.5% (v/v) glutaraldehyde (protocol 1 with ruthenium red).
(2) Cells were processed by high pressure freezing (protocol 2; see methods section).

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Supplementary Fig. 6a / Electron micrographs of Stecheria intestinalis DSM 109718^T.
(1) Negative staining of cells observed by TEM indicating the presence of an extra-cellular matrix surrounding cells.
(2) Cells imaged by means of scanning electron microscopy after fixation with 3% (v/v) glutaraldehyde.

Electron Microscopy Center at Wageningen University



Supplementary Fig. 6b / Transmission electron micrographs of *Stecheria intestinalis* DSM 109718^T.
(1) Cells fixed with 2% (v/v) formaldehyde and 2.5% (v/v) glutaraldehyde (protocol 1 with ruthenium red).
(2) Cells were processed by high pressure freezing (protocol 2; see methods section).









Supplementary Fig. 7a / Electron micrographs of *Tissierella pigra* DSM 109718^T performed at RWTH university.

(1) Negative staining of cells observed by TEM indicating the presence of an extracellular matrix surrounding cells.

(2) Cells imaged by means of scanning electron microscopy after fixation with 3% (v/v) glutaraldehyde.

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Supplementary Fig. 7b / Transmission electron micrographs of *Tissierella pigra* DSM 109718^T.
 (1) Cells fixed with 2% (v/v) formaldehyde and 2.5% (v/v) glutaraldehyde (protocol 1 with ruthenium red).
 (2) Cells were processed by high pressure freezing (protocol 2; see methods section).





2a



Supplementary Fig. 8a / Electron micrographs of *Bullifex porci* DSM 105750^T performed at RWTH university. (1) Scanning electron micrographs. (2a) Transmission electron micrographs (1.5%-glutaraldehyde fixation, embedding in 1% agarose, high-pressure freezing, ethanol-dehydration).

Electron Microscopy Facility of RWTH Aachen University



Supplementary Fig. 8a / Electron micrographs of *Bullifex porci* DSM 105750^T performed at RWTH university. Transmission electron micrographs: (2b) 1.5%-glutaraldehyde fixation, embedding in 1% agarose, high-pressure freezing, ethanol-dehydration; (2c) Same with 0.2%-glutaraldehyde fixation.

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Supplementary Fig. 8b / Electron micrographs of Bullifex porci DSM 105750^T performed at Wageningen university.
 (2d) Transmission electron micrographs of cells fixed with 2% (v/v) formaldehyde and 2.5% (v/v) glutaraldehyde (protocol 1).
 (2e) Transmission electron micrographs of cells processed by high pressure freezing followed by freeze substitution (protocol 2).



Supplementary Fig. 8b / Electron micrographs of Bullifex porci DSM 105750^T performed at Wageningen university.
(2f) Transmission electron micrographs of cells fixed with 2% (v/v) formaldehyde and 2.5% (v/v) glutaraldehyde (protocol 1).
(2g) Transmission electron micrographs of cells processed by high pressure freezing followed by freeze substitution (protocol 2; see methods section). White arrows point at intact membranes, indicating the presence of viable cells



a

Supplementary Fig. 9a / Impact of medium dilution on the growth and cell morphology of *Bullifex porci* **DSM 105750^T.** The strain was grown for 7 days (168 h) at 37 °C with constant shaking (220 rpm) under anaerobic conditions in Hungate tubes containing 10 ml of media. A gradient of medium dilutions (100, 90, 75, 50, 25, and 10%, as indicated in the images) was obtained by mixing the basal medium (BHI broth supplemented with 10% rumen fluid) with autoclaved and gassed distilled water in appropriate volume ratios. Triplicate cultures were tested for each condition. Data is represented as mean ± SD. The osmolarity of all media was measured using an OSMO Station OM-6050 (Arkray, Kyoto, Japan). Growth was followed overtime by measuring the OD_{600 nm} directly within the Hungate tubes using a CO8000 Cell Density meter. After one week of growth, cells were centrifuged (3,500 x g, 10 min) and re-suspended in a lower volume of the corresponding growth medium to increase cell density. Cell morphology was observed by phase contrast microscopy as in Fig. 2.



b

Supplementary Fig. 9b / Impact of NaCI supplementation on the growth and cell morphology of Bullifex porci DSM 105750^T. The strain was grown for 7 days (168 h) at 37 °C with constant shaking (220 rpm) under anaerobic conditions in Hungate tubes containing 10 ml of media. For increasing osmolarity, the basal medium (BHI broth with 10% rumen fluid) was supplemented with the appropriate amount of NaCI (0, 0.25, 0.50, 0.75, 1.00, and 2.50% (w/v) as indicated in the microscopy images) prior to gassing and autoclaving. Triplicate cultures were tested for each condition. Data is represented as mean ± SD. The osmolarity of all media was measured using an OSMO Station OM-6050 (Arkray, Kyoto, Japan). Growth was followed overtime by measuring the OD_{600 nm} directly within the Hungate tubes using a CO8000 Cell Density meter. After one week of growth, cells were centrifuged (3,500 x g, 10 min) and re-suspended in a lower volume of the corresponding growth medium to increase cell density. Cell morphology was observed by phase contrast microscopy as in Fig. 2.













Supplementary Fig. 10 / Fluorescence microscopy images of *Bullifex porci* DSM 105750^T.

The strain was grown for 4 days in BHI medium supplemented rumen fluid, cysteine and DTT under anaerobic conditions. Cells were stained with FM4-64 (1 μ g/ml; red; membrane) and DAPI (2 μ g/ml; blue; DNA) after a wash in PBS (2 min, 5,000 rpm). They were then visualized using an Axio Imager.Z2 microscope (Zeiss, Jena, Germany) equipped with a Plan-Apochromat x 63 phase contrast objective lens, appropriate filter sets, and an ORCA-Flash 4.0 LT digital CMOS camera (Hamamatsu Photonics, Shizuoka, Japan) using the Zeiss Zen Blue software. Brightness and contrast level of the images were adjusted using ImageJ. The bar in the images represents 5 μ m.



Supplementary Fig. 11 / Detected peptidoglycan in Bullifex porci.

Visible and total ion chromatograms obtained from peptidoglycan preparations of *B. porci* did not show any obvious muropeptide profile, pointing at low amounts of peptidoglycan isolated from liters of culture. Use of a UNIFI compound library allowed the detection of muropeptides with m/z value and fragmentation pattern corresponding to a murotetrapeptide with L-Orn linked to a single glycine, as reported previously for phylogenetically related bacteria³⁻⁴

a, Chemical structure proposed for the muropeptides detected in the isolated peptidoglycan. NAG, *N*-acetylglucosamine; NAM, *N*-acetylmuramic acid. **b**, Extracted ion chromatogram of the detected M4 (L-Orn) + Gly ions. **c**, Theoretical and observed m/z values for the different ion adducts of the M4 (L-Orn) + Gly muropeptide detected. **d**, Low- (top panel) high- (bottom) energy mass spectrum obtained during the fragmentation of the molecular ion of M4 (L-Orn) + Gly molecule (m/z value 941.42 Da) indicating the different adduct ions detected (top) and the fragmentation pattern of the molecule (bottom)



Supplemental Figure 12 / KEGG pathway coverage by the PiBAC collection. KEGG orthologs (KO) were searched for within each of the 117 isolate genomes and the prevalence of each KO visualised over the Metabolic pathways map (map01100). Prevalence is represented via KO colour from dark red to light red.



Supplementary Fig. 13 / Cultured fractions as determined using study-specific 16S rRNA gene amplicon data.

To assess the coverage of sequence-based diversity by all PiBAC isolates in relation to parameters such as diet, age, and gut locations, we processed three datasets:

Dataset 1: Published data⁵ investigating the impact of dietary protein content on the fecal microbiota of castrated male Duroc pigs at the age of 165 days;

Datasets 2: Stool samples from ten German landrace pigs from the animal facility of Thalhausen (TU Munich, Germany) at the age of 8, 24, and 52 weeks;

Dataset 3: Samples from six wildtype pigs from the MIDY biobank,⁶ including five locations within the gastro-intestinal tract.

For study 2 and 3, samples were processed as previously described (see also method section).⁷ All sequencing data were processed using IMNGS⁸ and Rhea.⁹

Cultured fractions were determined by searching for matches between the amplicon sequences of all study-specific OTUs (those occurring at a relative abundance of ≥0.25% in at least one sample within the respective dataset) and 16S rRNA gene sequences of the isolates using blastn (E-value <1e-25, 80% query coverage) at two different sequence identity thresholds: 97% (as proxy for species level) and 95% (genus level). Data are shown in a box plots, center line: median, bounds of box: quartile, whiskers: Tukey.



Open the lid of the flask and rapidly transfer ca. 5g of feces using sterile instruments (*e.g.* single-use plastic loops). Thereby, a dilution of ca. 1:10 (w/v) is obtained.
 Close the lid and hand-shake vigourusly until fecal material is re-suspended. Remember, the environment now contains oxygen, so be quick (<1 min).
 Let to stand for 1 min to sediment debris. Collect 5 ml of fecal slurry using a syringe with sterile needle (>1 mm ø) via the rubber stopper (avoid particles).

3-- Let to stand for 1 min to sediment debits. Collect 5 min of letal study using a symile with steme needle (31 min 6) via the rubber stopper (avoid particular study of the stopper (avoid particular study of th

4-- Transfer to new flask through rubber stopper. Thereby, a cumulative dilution of ca. 1:100 is obtained. Mix gently to homogenize.

5-- After transport to the lab, transfer ca. 200 µl of fecal slurry to Hungate tubes using a syringe. Thereby, a final dilution of ca. 1:5,000 is obtained. Alternatively, dilution series can be plated directly onto agar plates.

In all steps, be mindful of contaminations. For instance, when opening flasks or transferring slurries with syringes through the rubber stoppers. Flasks and Hungates were autoclaved with aluminium foil over theirs lids for the sake of transportation. These were removed just prior to handling.

Supplementary Fig. 14 / Schematic representation of the fecal sample collection procedure for anaerobic cultivation.

Supplementary References

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