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Supplementary Information for

Type IV Pili Trigger Episymbiotic Association of Saccharibacteria with Its Bacterial Host

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Validation of symbiont detection using epicPCR.

To validate the robustness of symbiont detection using epicPCR, we constructed an artificial TM7–host symbiont association. *Escherichia coli* transformed with a plasmid containing a TM7 16S rRNA gene sequence was used to simulate a TM7–*E. coli* symbiotic association and mimic the close contact between the host and TM7 (Fig. S1). Three representative mock samples were prepared, including a mock TM7-positive community sample that combined transformed *E. coli* and nontransformed *Staphylococcus aureus*, a mock TM7-free community composed of wild-type *E. coli* mixed with *S. aureus*, and a pure "TM7–host symbiont" containing only transformed *E. coli* (Fig. S1). After epicPCR was conducted with the above three sample types, fusion PCR successfully detected the artificial TM7–host symbiont in the positive community (84.9% of total reads), while the pure TM7–host symbiont culture comprised 98.5% of the total epicPCR reads. In contrast, the TM7-free community exhibited a lack of nested PCR products and fused amplicons, indicating the high level of specificity and robustness of the epicPCR method (Fig. S1).

Table S1. Transcription of fimbriae-related genes of *Leucobacter aridicollis* J1 from 48-h cultures.

Gene	KO number	Definition	Gene copies	RPKM of pure J1	RPKM of coculture
spaA	-	SpaA-type pili spaA putative type 1 fimbrial protein	1	32	77
srtD	K07284	Class C sortase	2	62	139

Notes: RPKM indicates reads per kilobase per million mapped reads. Transcriptomic data of both pure culture of J1 and coculture of TM7i–J1 are shown. KO number indicates KEGG Orthology accession.

Table S2. Transcription of TM7i's type IV pili genes from a 48-h coculture with *Leucobacter aridicollis* J1.

Gene	KO number	Definition	Gene copies	RPKM
-	-	possible prepilin-type protein 1	1	158
-	-	possible prepilin-type protein 2	1	29
-	-	possible prepilin-type protein 3	1	28
pilC	K02653	membrane core protein	2	181
pilM	K02662	membrane accessory protein	2	139
pilD	K02654	prepilin peptidase	1	81
pilT	K02669	type IV pilus retraction ATPase	1	56
pilB	K02652	type IV pilus assembly ATPase	2	115

Notes: RPKM indicates reads per kilobase per million mapped reads. KO number indicates KEGG Orthology accession.

Table S3. PCR Primers used for TM7 detection and epicPCR.

Primer	Sequence (5' – 3')	Description
R1-F2-27F-910R	CTGAGCCAKGATCAAACTCTG TCCCCGTCAATTCCTTTATG	Combination of the TM7-specific primer 910R (1) and a reverse complement of universal primer 27F (2); used in fusion PCR.
F1-TM7-580F	AYTGGGCGTAAAGAGTTGC	A TM7-specific primer (3) used in PCR testing for the existence of TM7 and fusion PCR
R2-519R	GWATTACCGCGGCKGCTG	A universal primer (4) used in fusion PCR and epicPCR
nest_806F	ATTAGAWACCCBNGTAGTCC	Reverse complement of universal primer 806R (5), used in nested PCR and epicPCR
nest_341R	CTGSTGCVYCCCRTAGG	Reverse complement of universal primer 341F (5), used in nested PCR and epicPCR
910R	GTCCCCGTCAATTCCTTTATG	A TM7-specific primer (1) used in PCR to detect TM7.

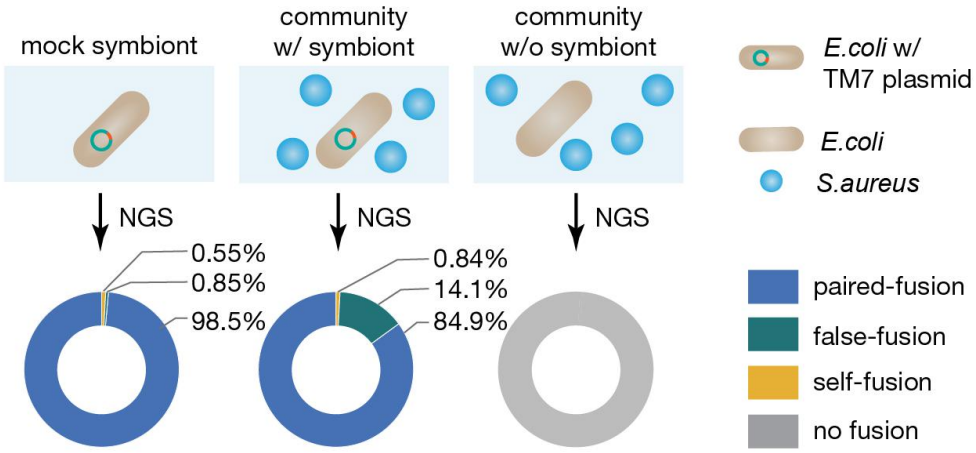

Fig. S1. The Validation of TM7-specific epicPCR system using mock samples. (**A**) Detection of bacteria carrying a TM7-specific plasmid in mock community samples using epicPCR. We built the mock symbiotic host of TM7 using *E. coli* by introducing a plasmid containing the TM7 16S rRNA gene sequence. EpicPCR detected paired fusion of the 16S rRNA genes of TM7 and *E. coli* in samples containing the plasmid-transfected *E. coli* but not in the negative control. Self-fusion indicates that the 16S rRNA sequence of TM7 fused with itself. False-fusion indicates that the 16S rRNA gene sequence of TM7 fused with those of *Staphylococcus aureus*.

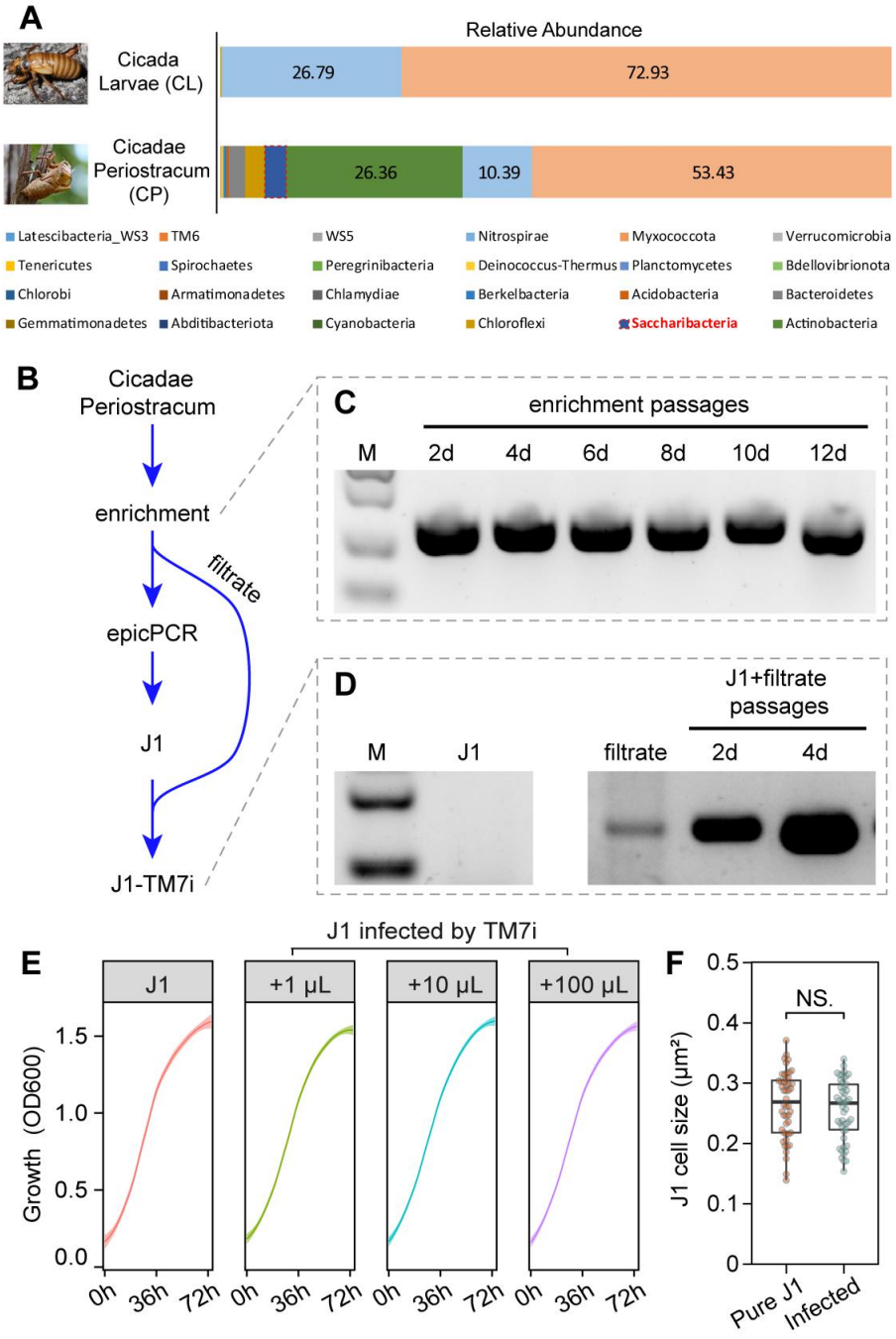

Fig. S2. The epicPCR-directed isolation and confirmation of TM7i–J1 symbiont from Cicadae Periostracum (CP). (**A**) 16 rRNA gene sequencing of the original microbial community from Cicada Larvae (CL) and CP. Phylum-level profiles are shown. (**B**) Workflow of TM7 episymbiont isolation from the Cicadae Periostracum enrichment. (**C**) The existence of the TM7–host symbiont was confirmed by continuous passages and PCR detection of TM7. (**D**) The obtained TM7i–J1 symbiont was also validated by PCR detection of TM7 in multiple passages. (**E**) Growth curve of pure J1 and infected J1 treated with different volumes of TM7i-containing filtrate (mean ± s.e.m., n = 3 biologically independent experiments). (**F**) SEM-measured J1 cell sizes with or without TM7i infection. (n=98, $^{NS.}$ P = 0.58).

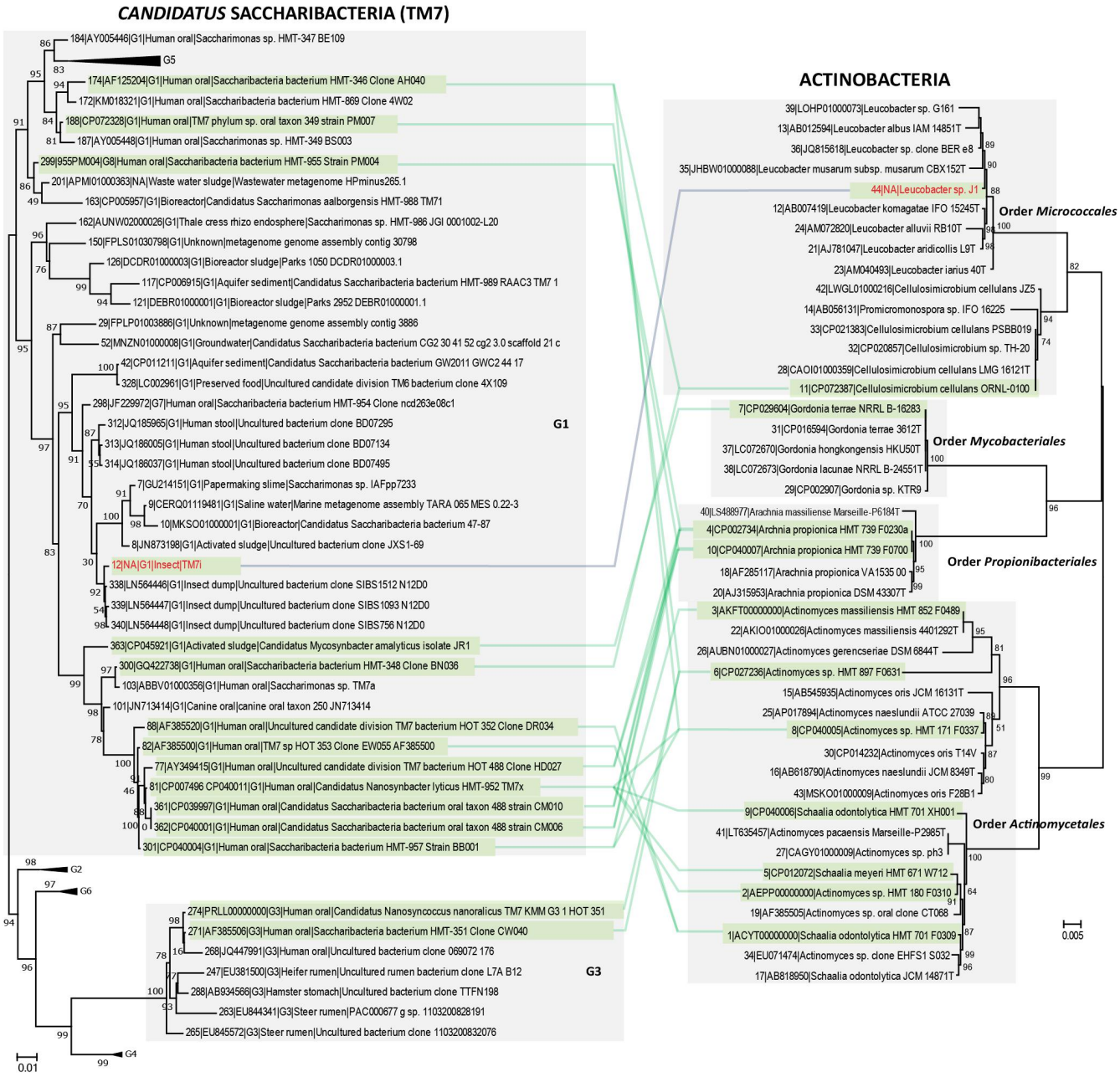

Fig. S3. Phylogenetic analysis by maximum-likelihood of 16S rRNA gene sequences from the ARB/SILVA release 138.1 database shows strain TM7i within the G1 group of *Candidatus* Saccharibacteria. Sequences for TM7 bacteria included in this tree were detected in human oral and open environments, identified by sequence accession numbers from GenBank/EMBL/DDBJ, along with six operational groupings (G1–6). Lines connecting TM7 and actinobacterial species indicate TM7-host symbionts previously identified (green) and in this study (gray).

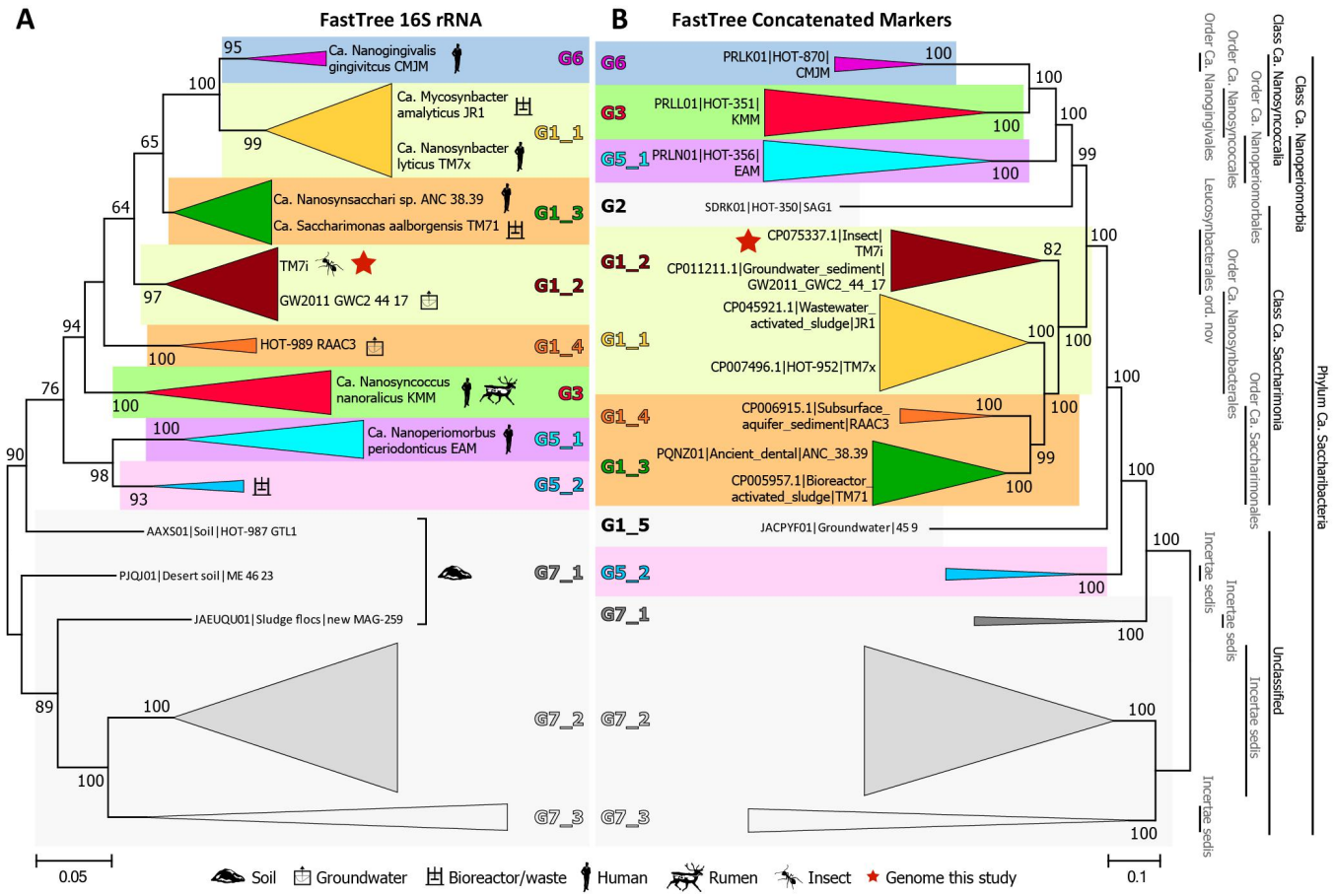

Fig. S4. Phylogenetic relationships within the expanded phylum *Candidatus Saccharibacteria* using maximum-likelihood inferences of 16S rRNA genes (A) and concatenated housekeeping proteins (B). The concatenated protein tree was built by the UBCG marker proteins annotated from the new genome of strain TM7i and 183 previously published genome assemblies containing 16S rRNA genes. The 16S rRNA gene tree was built by sequences extracted from the same dataset of the 184 genome sequences. Comparisons between both phylogenies of 16S rRNA genes and concatenated marker proteins supported the formerly established taxonomy of classes *Ca*. *Saccharimonia*, *Ca*. *Nanosyncoccalia*, and *Ca*. *Nanoperiomorbia*. Class *Ca*. *Saccharimonia*, also known as group G1, included orders of *Ca*. *Saccharimonales* (subgroup G1_3 and G1_4), *Ca*. *Nanosynbacterales* (G1_1), and *Ca*. *Leucosynbacterales* ord. nov. (G1_2), which was proposed by this study based on the evolutionary depth and paraphyletic topology observed within this class. Strain TM7i from this study represented the first laboratory isolate of the new order, with the suggested nomenclature of *Ca*. *Leucosynbacter cicadicola* gen. nov., sp. nov. Class *Ca*. *Nanosyncoccalia* included established orders of *Ca*. *Nanosyncoccales* (G3) and *Ca*. *Nanogingivales* (G6). Class *Ca*. *Nanoperiomorbia* was composed of the single order *Nanoperiomorbales* (G5_1). We also observed several lineages (G2, G1_5, G5_2, G7_1, G7_2, and G7_3) falling out of a previously published classification system, which expanded the diversity of phylum *Ca*. *Saccharibacteria* to a great extent. The red filled star marks the genome of strain TM7i. Graphic icons indicate source materials of the genome data.

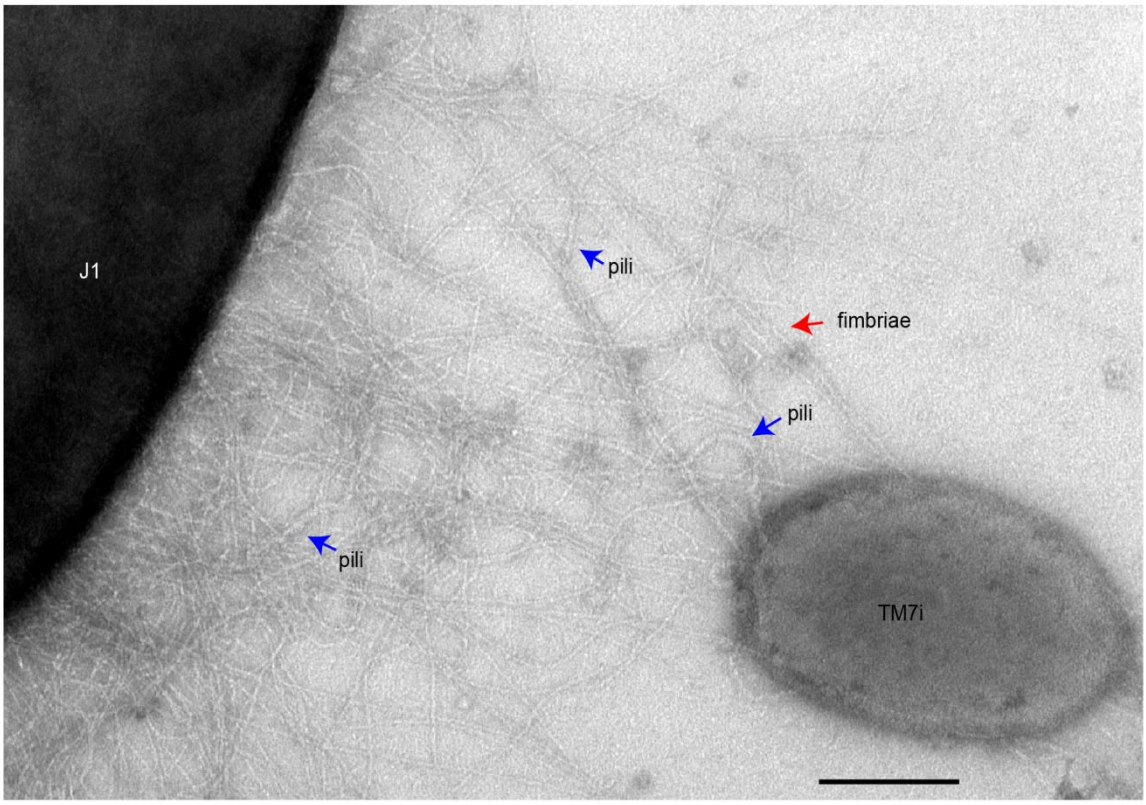

Fig. S5. Transmission electron microscopy showing T4P of the TM7i intermediate stage between free and sessile. The intertwinement of T4P from TM7i and type 2 fimbriae of J1 can be seen. The black arrow indicates the T4P of TM7i. Scale bar=100 nm.

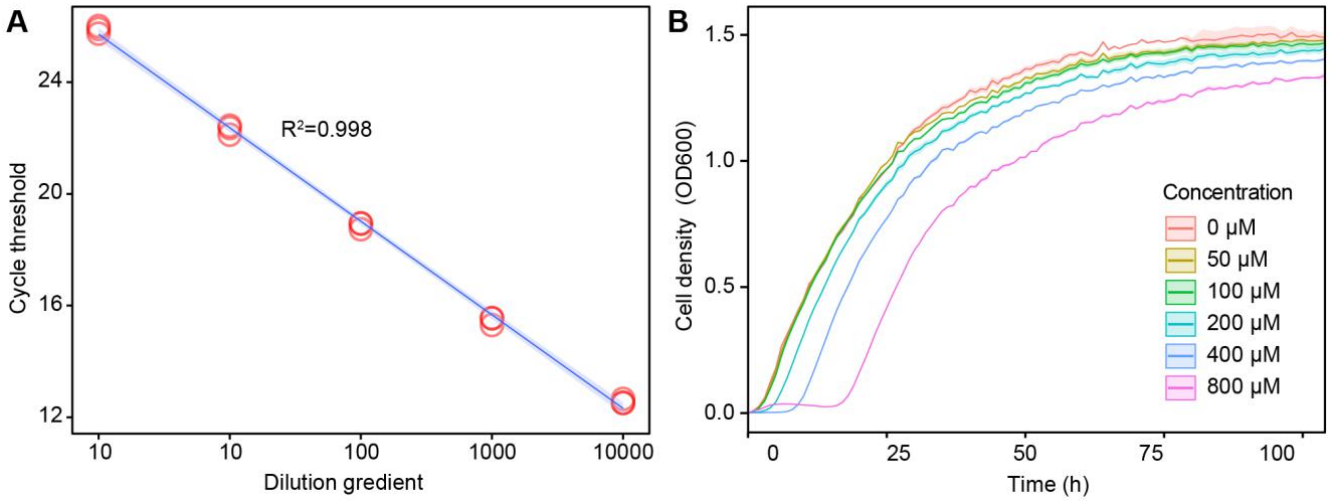

Fig. S6. Supplementary data for the influence of quercetin on the growth of the TM7i–J1 symbiont. (A) Calibration curve for quantitative PCR measurement of TM7i density. **(B)** Addition of quercetin does not significantly inhibit the growth of *L. aridicollis* J1 at a concentration of 200 μM.

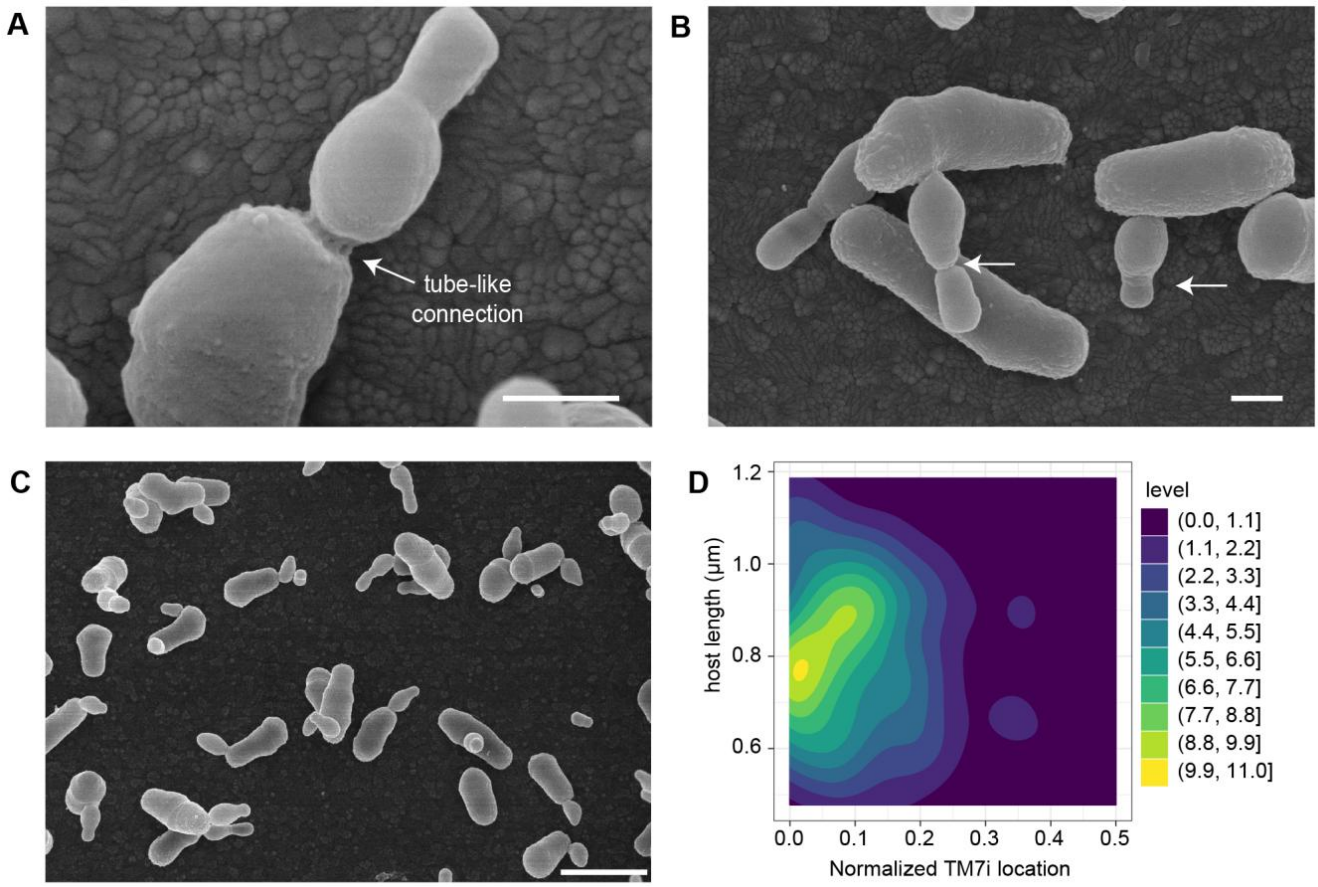

Fig. S7. SEM imaging of the TM7i–J1 symbiont. (**A**) An SEM image showing a tube-like connection between J1 and epibiotic TM7i. (**B**) An SEM image showing TM7i proliferation. The daughter TM7i is usually smaller than its parent. Scale bar=200 nm. (**C**) An SEM image showing the TM7i cell prefers to be located on the polar sides of the host cell; scale bar=1 μm. (**D**) Polar and near-polar locations of TM7i cells on its host J1 cells. The 0.0 value on the x-axis indicates that TM7i is located at the polar end of the host cell, while 0.5 indicates that TM7i is located in the middle of the host cell.

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Movie S1. Inhibition of TM7i T4P affects cell motility. The bacterial suspension containing pure J1 or J1 mixed with TM7i was sandwiched within a thin layer between a gel pad and a cover glass. TM7i cells were spotted and tracked to show motility. TM7i cells exhibited motility in the presence of J1 cells. The T4P inhibitor reversibly inhibited the motility of TM7i.

Movie S2. T4P trigger TM7i motility and interaction with its host. TM7i and J1 cells were separately labeled with red and green fluorescent NHS ester probes and sandwiched within a thin layer between a gel pad and a cover glass for dynamic structured illumination microscopy (SIM) imaging. 1) A TM7i cell dynamically extruded and retracted its pili, which is typical for T4P; 2) T4P triggered the continuous translocation of the TM7i cell on the surface; 3) A TM7i cell moved toward a J1 cell with T4P-mediated motility and became sessile on the J1 cell.

Movie S3. Inhibition of TM7i T4P affects host infection. A 3-h long-term microscopic imaging of the TM7i–J1 symbiont was carried out. The bacterial suspension containing pure J1 or mixed with TM7i was sandwiched within a thin layer between a gel pad and a cover glass. TM7i cells exhibited active motility after division. The T4P inhibitor immobilized free-floating and progeny TM7i cells, preventing them from adhering to potential host cells.