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Supplementary Information for

Acquisition of Arginine Deiminase System benefits epiparasitic Saccharibacteria and its host bacteria in mammalian niche environment

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Supplemental Material and Methods

Monitor the expression of genes in the ADS operon under different conditions. Primers for ADS related genes are as follows: *arcA*, forward 5'- GTAATGGCGATCGGTGTGTC -3', reverse 5'- GTCGCGAATTTCTGGGTGAA -3'; *arcB*, forward 5'- CGGTATTGAGTTTCGCGGTT -3', reverse 5'- TATTACGGCCATCACCCACA -3'; *arcC*, forward 5'- CACATTGATGGCGGTCAGTT -3', reverse 5'- TGAGCCGTTGATTGCTTCAG -3'; and *arcE*, forward 5'- ATGACGCTGTTTGCTATCGG -3', reverse 5'- AATGGTTGAACCGAGCACAC -3'. TM7x housekeeping gene primer sequences are: forward 5'- CCGAAGTCCGATTCGTCGG -3', and reverse 5'- CCTAGAAAGGAGGTAATCCATCC -3'.

Specifically, XH001/TM7x coculture were first incubated in saline for 3 hr, ADS gene expression of XH001/TM7x were tested under four conditions: saline, saline supplemented with 10 mM arginine, saline supplemented 10 mM glucose and saline supplemented with 10 mM arginine and 10 mM glucose. After 0.5 hr of incubation at 37°C, mRNAs were isolated using RNA extraction kit (RNeasy Mini Kit, Qiagen, REF 74104), then reverse transcribed into cDNA (PrimeScript™ 1st strand cDNA Synthesis Kit, TaKaRa, Cat. #6110A) to perform quantitative PCR.

Figures S1 to S4

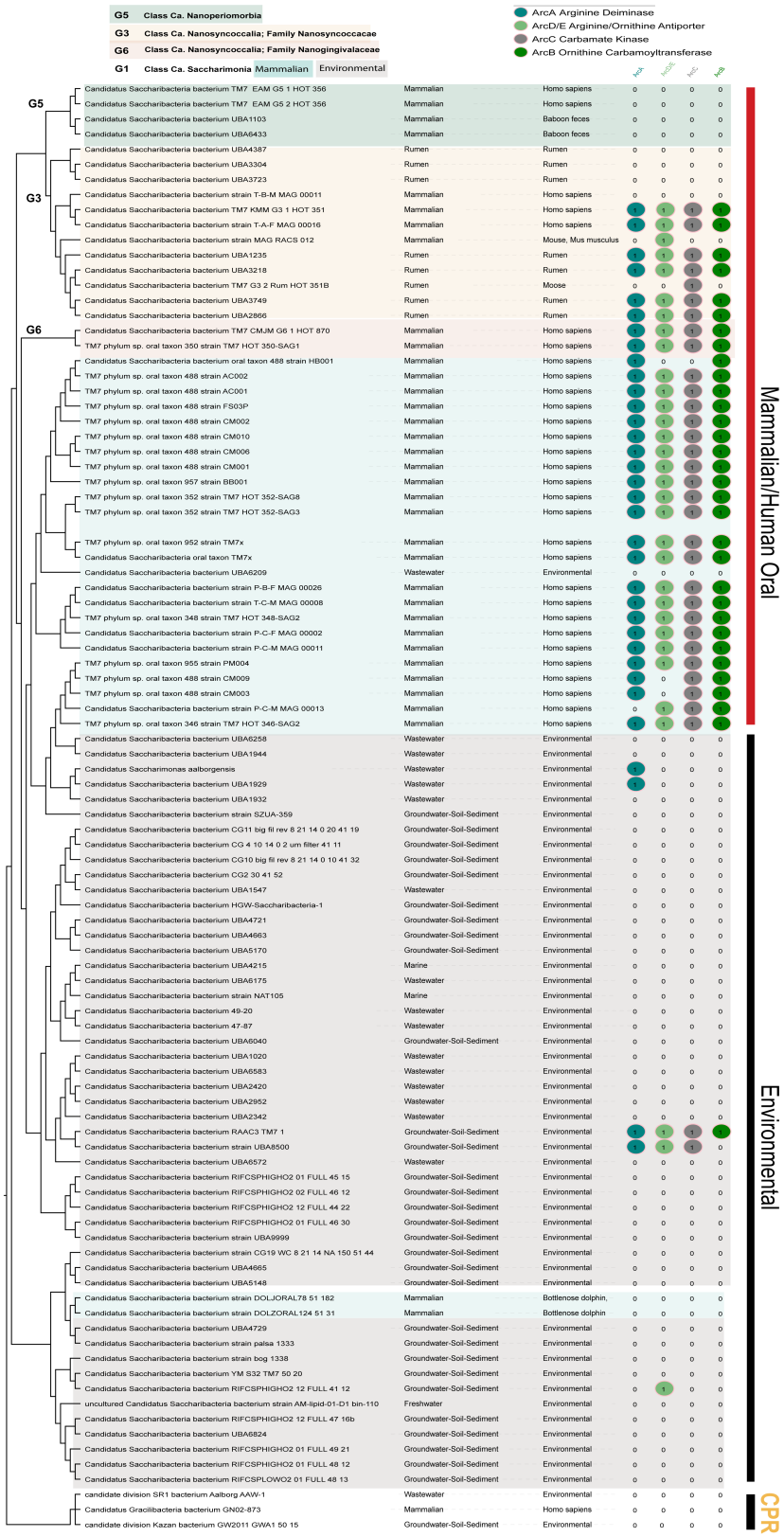

Fig. S1. Arginine deiminase system (ADS) within TM7 phylum.

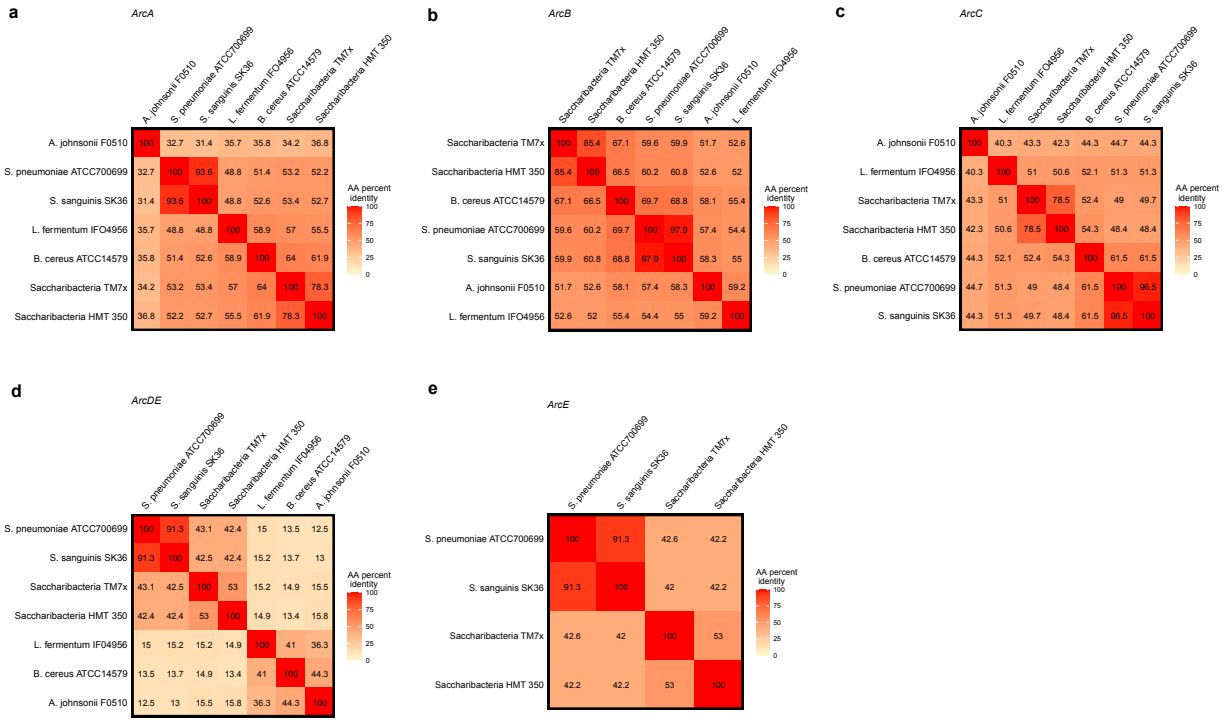

Fig. S2. Heatmap of proteins in ADS pathway showing amino acid sequence percent identity from pairwise alignments for ADS genes in TM7x, Saccharibacteria HMT 350, *B. cereus* ATCC 14579 and four oral bacteria. Amino acid sequence percentage identity of ArcA (a), ArcB (b), ArcC (c), ArcD/E (d) and ArcE (e) genes are showed respectively.

Fig. S3. Phylogeny of 304 representative amino acid sequences for ArcA spanning known bacteria. Tips and branches corresponding to Saccharibacterial ArcA sequences are colored in red, while known hosts for oral Saccharibacteria are shown in blue.

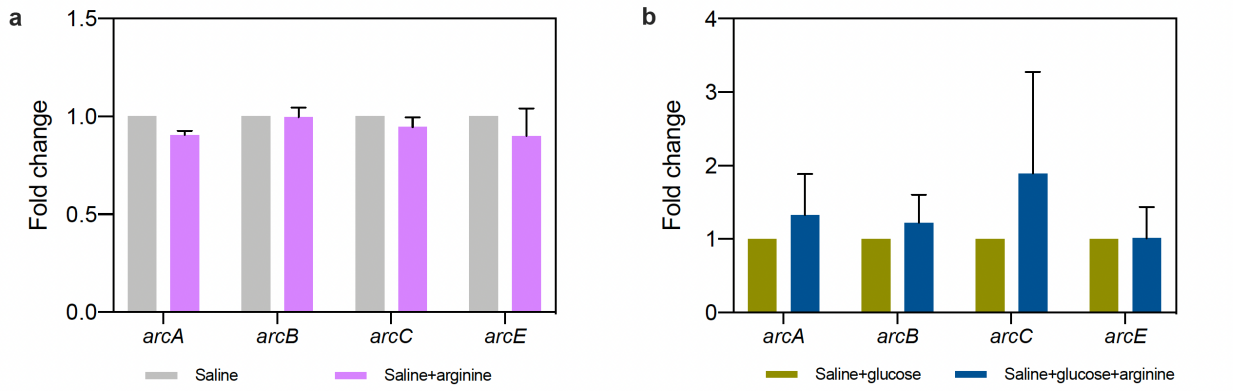

Fig. S4. Expression of the TM7x ADS genes in TM7x/XH001 coculture under different conditions. The impact of the presence of arginine (10 mM) on the expression of ADS genes was tested when XH001/TM7x coculture was incubated in saline (pH ≈ 7) **(a)**, as well as in saline + glucose (10 mM) **(b).** After 0.5 hr of incubation at 37°C anaerobically, mRNAs were isolated to perform quantitative reverse transcription PCR. **Note:** As shown in the main Figure 4b, when XH001/TM7x cells were incubated in saline with glucose, there is a fast drop in pH (close to 5, mimicking acid stress) within 0.5 hr, while the presence of arginine will maintain the pH above 5.5.