Polyphasic characterization of four soil-derived phenanthrene-degrading *Acidovorax* strains and proposal of *Acidovorax carolinensis* sp. nov.

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Supplementary Information

Table A.1. Genome blast distance phylogeny values for all publicly available *Acidovorax* genomes to strains described in this work.

		NA2			**NA3^{T}**			**P3**			**P4**		
Species	**Strain**	**DDH**	**Model C.I.**	**Prob DDH ≥70%**	**DDH**	**Model C.I.**	**Prob. DDH ≥70%**	**DDH**	**Model C.I.**	**Prob. DDH ≥70%**	**DDH**	**Model C.I.**	**Prob. DDH ≥70%**
sp.	NA2	100	[100 - 100%]	98.3	72.4	[69.4 - 75.2%]	82.24	75.5	[72.5 - 78.3%]	86.38	75.7	[72.7 - 78.5%]	86.56
sp.	NA3^{T}	72.4	[69.4 - 75.2%]	82.2	100	[100 - 100%]	98.30	73.2	[70.2 - 76%]	83.46	73.3	[70.3 - 76.2%]	83.63
sp.	P3	75.5	[72.5 - 78.3%]	86.4	73.2	[70.2 - 76.0%]	83.46	100	[100 - 100%]	98.30	98.3	[97.5 - 98.8%]	97.91
sp.	P4	75.7	[72.7 - 78.5%]	86.6	73.3	[70.3 - 76.2%]	83.63	98.3	[97.5 - 98.8%]	97.91	100	[100 - 100%]	98.30
acenae	T10_61	22.4	[20.1 - 24.8%]	0.00	22.4	[20.1 - 24.8%]	0.00	22.4	[20.1 - 24.9%]	0.00	22.4	[20.1 - 24.9%]	0.00
avenae	ATCC 19860^{T}	22.4	[20.1 - 24.8%]	0.00	22.3	[20.0 - 24.7%]	0.00	22.3	[20.0 - 24.8%]	0.00	22.3	[20.1 - 24.8%]	0.00
acenae	RS-1	22.4	[20.1 - 24.9%]	0.00	22.4	[20.1 - 24.8%]	0.00	22.6	[20.3 - 25.0%]	0.00	22.6	[20.3 - 25.0%]	0.00
caeni	R-24608^{T}	22.2	[19.9 - 24.6%]	0.00	22.3	[20.0 - 24.7%]	0.00	22.4	[20.1 - 24.8%]	0.00	22.4	[20.1 - 24.8%]	0.00
cattleyae	DSM 17101	22.1	[19.8 - 24.5%]	0.00	22.0	[19.7 - 24.4%]	0.00	22.1	[19.9 - 24.6%]	0.00	22.1	[19.9 - 24.6%]	0.00
citrulli	AAC00-1	22.3	[20.0 - 24.7%]	0.00	22.3	[20.1 - 24.8%]	0.00	22.4	[20.1 - 24.8%]	0.00	22.4	[20.1 - 24.8%]	0.00
citrulli	DSM 17060	22.2	[20.0 - 24.7%]	0.00	22.2	[19.9 - 24.6%]	0.00	22.3	[20.0 - 24.7%]	0.00	22.3	[20.0 - 24.7%]	0.00
citrulli	M6	22.2	[19.9 - 24.7%]	0.00	22.2	[19.9 - 24.6%]	0.00	22.3	[20.0 - 24.7%]	0.00	22.3	[20.0 - 24.7%]	0.00
citrulli	pslb65	22.2	[20.0 - 24.7%]	0.00	22.2	[19.9 - 24.6%]	0.00	22.3	[20.0 - 24.7%]	0.00	22.3	[20.0 - 24.7%]	0.00
citrulli	tw6	22.3	[20.0 - 24.7%]	0.00	22.3	[20.0 - 24.7%]	0.00	22.3	[20.1 - 24.8%]	0.00	22.3	[20.1 - 24.8%]	0.00
citrulli	ZJU1106	24.0	[21.7 - 26.5%]	0.00	24.0	[21.6 - 26.4%]	0.00	24.0	[21.7 - 26.4%]	0.00	24.0	[21.7 - 26.4%]	0.00
delafieldii	2AN	30.0	[27.6 - 32.5%]	0.11	29.9	[27.6 - 32.4%]	0.10	30.0	[27.7 - 32.6%]	0.11	30.0	[27.7 - 32.5%]	0.11
delafieldii	CCH3-G3	26.1	[23.8 - 28.6%]	0.02	26.0	[23.7 - 28.5%]	0.02	26.6	[24.3 - 29.1%]	0.02	26.6	[24.3 - 29.1%]	0.02
delafieldii	CCH4-A2	18.0	[15.9 - 20.4%]	0.00	16.8	[14.7 - 19.1%]	0.00	17.3	[15.2 - 19.6%]	0.00	17.3	[15.2 - 19.6%]	0.00
ebreus	TPSY	22.4	[20.1 - 24.8%]	0.00	22.6	[20.3 - 25.0%]	0.00	22.4	[20.2 - 24.9%]	0.00	22.4	[20.2 - 24.9%]	0.00
konjaci	DSM 7481^{T}	22.1	[19.8 - 24.5%]	0.00	22.2	[20.0 - 24.7%]	0.00	22.2	[19.9 - 24.6%]	0.00	22.2	[19.9 - 24.6%]	0.00
oryzae	ATCC 19882^{T}	22.2	[19.9 - 24.7%]	0.00	22.3	[20.0 - 24.7%]	0.00	22.3	[20.0 - 24.7%]	0.00	22.3	[20.0 - 24.7%]	0.00
radicis	N35^{T}	25.9	[23.5 - 28.3%]	0.01	26.0	[23.6 - 28.5%]	0.02	26.0	[23.7 - 28.5%]	0.02	26.0	[23.7 - 28.5%]	0.02
radicis	N35v	25.9	[23.5 - 28.3%]	0.01	26.0	[23.6 - 28.5%]	0.02	26.0	[23.7 - 28.5%]	0.02	26.0	[23.7 - 28.5%]	0.02
soli	DSM 25157	34.0	[31.6 - 36.5%]	0.47	33.8	[31.4 - 36.3%]	0.44	34.2	[31.8 - 36.7%]	0.51	34.2	[31.8 - 36.7%]	0.51
temperans	KY4	24.0	[21.7 - 26.5%]	0.01	24.2	[21.9 - 26.6%]	0.01	24.3	[22.0 - 26.7%]	0.01	24.3	[22.0 - 26.7%]	0.01
valerianellae	DSM 16619	22.6	[20.3 - 25.1%]	0.00	22.7	[20.5 - 25.2%]	0.00	22.7	[20.4 - 25.1%]	0.00	22.7	[20.4 - 25.1%]	0.00

		NA2			NA3^{T}			P3			P4		
Species	**Strain**	**DDH**	**Model C.I.**	**Prob DDH ≥70%**	**DDH**	**Model C.I.**	**Prob. DDH ≥70%**	**DDH**	**Model C.I.**	**Prob. DDH ≥70%**	**DDH**	**Model C.I.**	**Prob. DDH ≥70%**
wautersii	DSM 27981	23.3	[21.0 - 25.7%]	0.00	23.1	[20.8 - 25.6%]	0.00	23.4	[21.1 - 25.8%]	0.00	23.3	[21.0 - 25.8%]	0.00
sp.	65-7	25.7	[23.4 - 28.2%]	0.01	25.9	[23.6 - 28.4%]	0.01	25.9	[23.6 - 28.4%]	0.01	25.9	[23.6 - 28.4%]	0.01
sp.	12322-1	20.3	[18.1 - 22.7%]	0.00	20.6	[18.4 - 23.1%]	0.00	20.6	[18.3 - 23.0%]	0.00	20.6	[18.3 - 23.0%]	0.00
sp.	121606	20.1	[17.9 - 22.5%]	0.00	20.4	[18.2 - 22.8%]	0.00	20.4	[18.2 - 22.8%]	0.00	20.4	[18.1 - 22.8%]	0.00
sp.	202149	20.1	[17.9 - 22.6%]	0.00	20.4	[18.2 - 22.8%]	0.00	20.4	[18.2 - 22.8%]	0.00	20.4	[18.2 - 22.8%]	0.00
sp.	CCH12-A4	24.8	[22.4 - 27.2%]	0.01	24.7	[22.4 - 27.2%]	0.01	26.2	[23.9 - 28.7%]	0.02	26.3	[23.9 - 28.8%]	0.02
sp.	CF316	25.1	[22.8 - 27.6%]	0.01	25.3	[22.9 - 27.7%]	0.01	25.3	[23.0 - 27.8%]	0.01	25.3	[23.0 - 27.8%]	0.01
sp.	GW101-3H11	26.1	[23.7 - 28.6%]	0.02	26.0	[23.7 - 28.5%]	0.02	26.1	[23.7 - 28.6%]	0.02	26.1	[23.7 - 28.6%]	0.02
sp.	JHL-3	35.1	[32.6 - 37.6%]	0.67	34.9	[32.5 - 37.4%]	0.64	35.3	[32.9 - 37.8%]	0.73	35.3	[32.9 - 37.8%]	0.73
sp.	JHL-9	34.5	[32.1 - 37.1%]	0.57	34.5	[32.1 - 37.0%]	0.56	34.7	[32.3 - 37.2%]	0.60	34.7	[32.3 - 37.2%]	0.60
sp.	JS42	23.1	[20.8 - 25.6%]	0.00	23.4	[21.1 - 25.8%]	0.00	24.1	[21.8 - 26.6%]	0.01	24.1	[21.8 - 26.6%]	0.01
sp.	KKS102	25.9	[23.5 - 28.4%]	0.01	26.1	[23.8 - 28.6%]	0.02	25.9	[23.6 - 28.4%]	0.01	25.9	[23.6 - 28.4%]	0.01
sp.	Leaf76	26.0	[23.7 - 28.5%]	0.02	25.9	[23.5 - 28.4%]	0.01	25.9	[23.6 - 28.4%]	0.02	25.9	[23.6 - 28.4%]	0.02
sp.	Leaf78	25.8	[23.5 - 28.3%]	0.01	25.7	[23.4 - 28.2%]	0.01	25.8	[23.4 - 28.3%]	0.01	25.8	[23.4 - 28.2%]	0.01
sp.	Leaf84	26.0	[23.7 - 28.5%]	0.02	25.9	[23.5 - 28.4%]	0.01	25.9	[23.6 - 28.4%]	0.01	25.9	[23.6 - 28.4%]	0.01
sp.	Leaf160	22.8	[20.5 - 25.3%]	0.00	22.9	[20.6 - 25.3%]	0.00	22.8	[20.5 - 25.2%]	0.00	22.8	[20.5 - 25.2%]	0.00
sp.	Leaf191	26.0	[23.7 - 28.5%]	0.02	25.9	[23.5 - 28.3%]	0.01	25.9	[23.6 - 28.4%]	0.02	25.9	[23.6 - 28.4%]	0.02
sp.	MR-S7	22.8	[20.5 - 25.2%]	0.00	22.7	[20.4 - 25.2%]	0.00	22.9	[20.6 - 25.3%]	0.00	22.9	[20.6 - 25.3%]	0.00
sp.	NO-1	25.9	[23.6 - 28.4%]	0.02	26.0	[23.7 - 28.5%]	0.02	26.1	[23.8 - 28.6%]	0.02	26.2	[23.8 - 28.6%]	0.02
sp.	RAC01	25.5	[23.2 - 28.0%]	0.01	25.4	[23.1 - 27.9%]	0.01	25.7	[23.4 - 28.2%]	0.01	25.7	[23.3 - 28.2%]	0.01
sp.	Root70	25.8	[23.5 - 28.3%]	0.01	25.7	[23.4 - 28.2%]	0.01	25.8	[23.5 - 28.3%]	0.01	25.8	[23.5 - 28.3%]	0.01
sp.	Root217	25.2	[22.8 - 27.6%]	0.01	25.2	[22.8 - 27.7%]	0.01	25.3	[23.0 - 27.8%]	0.01	25.3	[23.0 - 27.8%]	0.01
sp.	Root219	25.2	[22.9 - 27.7%]	0.01	25.3	[22.9 - 27.8%]	0.01	25.4	[23.0 - 27.8%]	0.01	25.4	[23.0 - 27.8%]	0.01
sp.	Root267	25.8	[23.5 - 28.3%]	0.01	25.8	[23.5 - 28.3%]	0.01	25.9	[23.6 - 28.4%]	0.01	25.9	[23.6 - 28.4%]	0.01
sp.	Root275	25.9	[23.6 - 28.4%]	0.01	26.0	[23.7 - 28.5%]	0.02	26.1	[23.7 - 28.6%]	0.02	26.1	[23.7 - 28.6%]	0.02
sp.	Root402	25.7	[23.4 - 28.2%]	0.01	25.8	[23.4 - 28.2%]	0.01	25.8	[23.5 - 28.3%]	0.01	25.8	[23.5 - 28.3%]	0.01
sp.	Root568	25.9	[23.6 - 28.4%]	0.01	25.9	[23.6 - 28.4%]	0.01	25.8	[23.5 - 28.3%]	0.01	25.8	[23.5 - 28.3%]	0.01
sp.	SCN_65-28	25.2	[22.8 - 27.6%]	0.01	25.2	[22.9 - 27.7%]	0.01	25.4	[23.1 - 27.9%]	0.01	25.4	[23.1 - 27.9%]	0.01

		NA2			NA3^T			P3			P4		
Species	**Strain**	**DDH**	**Model C.I.**	**Prob DDH ≥70%**	**DDH**	**Model C.I.**	**Prob. DDH ≥70%**	**DDH**	**Model C.I.**	**Prob. DDH ≥70%**	**DDH**	**Model C.I.**	**Prob. DDH ≥70%**
sp.	SCN_65-108	25.3	[23.0 - 27.8%]	0.01	25.1	[22.8 - 27.6%]	0.01	25.3	[23.0 - 27.8%]	0.01	25.3	[23.0 - 27.8%]	0.01
sp.	SCN_68-22	21.7	[19.4 - 24.1%]	0.00	22.0	[19.7 - 24.4%]	0.00	21.6	[19.3 - 24.0%]	0.00	21.6	[19.4 - 24.0%]	0.00
sp.	SD340	26.1	[23.7 - 28.6%]	0.02	25.9	[23.6 - 28.4%]	0.02	26.4	[24.1 - 28.9%]	0.02	26.4	[24.1 - 28.9%]	0.02

Table A.2. Average nucleotide identity (ANI) between *Acidovorax* strains from this study and characterized *Acidovorax* strains with publicly available genomic data, including strains without species designation.

Species	**Strain**	**NA2**	**NA3^{T}**	**P3**	**P4**
sp.	NA2	100	96.9	97.1	97.1
sp.	NA3^{T}	96.9	100	97.0	97.1
sp.	P3	97.1	97.0	100	100
sp.	P4	97.1	97.1	100	100
acenae	T10_61	80.6	80.8	80.7	80.7
avenae	ATCC 19860^{T}	80.8	80.7	80.8	80.8
acenae	RS-1	80.7	80.7	80.7	80.8
caeni	R-24608^{T}	80.5	80.4	80.5	80.4
cattleyae	DSM 17101	80.7	80.5	80.7	80.7
citrulli	AAC00-1	80.9	80.9	80.9	80.9
citrulli	DSM 17060	80.7	80.6	80.8	80.7
citrulli	M6	80.8	80.8	80.9	80.8
citrulli	pslb65	80.8	80.7	80.8	80.9
citrulli	tw6	80.8	80.7	80.9	80.8
citrulli	ZJU1106	81.7	81.7	81.8	81.8
delafieldii	2AN	85.6	85.6	85.7	85.6
delafieldii	CCH3-G3	83.9	83.9	84.2	84.2
delafieldii	CCH4-A2	n.d.	n.d.	n.d.	n.d.
ebreus	TPSY	81.1	81.2	81.0	81.1
konjaci	DSM 7481^{T}	80.3	80.5	80.4	80.4
oryzae	ATCC 19882^{T}	80.6	80.8	80.7	80.8
radicis	N35^{T}	84.0	83.8	83.9	83.8
radicis	N35v	83.9	83.8	83.8	83.8
soli	DSM 25157	87.4	87.3	87.6	87.6
temperans	KY4	82.7	82.7	82.8	82.7
valerianellae	DSM 16619	81.3	81.4	81.2	81.4
wautersii	DSM 27981	81.6	81.7	81.7	81.8
sp.	65-7	83.5	83.6	83.6	83.6
sp.	12322-1	78.1	78.1	78.2	78.1
sp.	121606	78.1	78.1	78.1	78.2
sp.	202149	78.2	78.2	78.2	78.2
sp.	CCH12-A4	83.0	83.0	84.4	84.5
sp.	CF316	83.0	82.9	83.1	83.1
sp.	GW101-3H11	83.7	83.7	83.8	83.8
sp.	JHL-3	88.0	88.0	88.1	88.1
sp.	JHL-9	88.0	87.9	87.9	87.9
sp.	JS42	82.0	82.0	83.2	83.2
sp.	KKS102	83.7	83.8	83.8	83.8
sp.	Leaf76	83.6	83.5	83.4	83.5
sp.	Leaf78	83.4	83.3	83.4	83.3
sp.	Leaf84	83.5	83.4	83.4	83.5
sp.	Leaf160	81.3	81.3	81.4	81.3
sp.	Leaf191	83.4	83.4	83.5	83.5
sp.	MR-S7	80.8	81.0	81.0	81.0
sp.	NO-1	84.1	84.0	84.2	84.2
sp.	RAC01	83.7	83.4	83.7	83.6
sp.	Root70	83.6	83.6	83.7	83.7
sp.	Root217	83.0	83.1	83.1	83.1
sp.	Root219	82.9	82.9	83.0	83.1
sp.	Root267	83.9	83.9	83.9	83.9
sp.	Root275	83.9	83.9	84.0	84.0
sp.	Root402	83.8	83.7	83.8	83.8
sp.	Root568	83.9	83.7	83.7	83.7
sp.	SCN_65-28	83.0	83.0	83.2	83.2
sp.	SCN_65-108	83.4	83.2	83.3	83.3
sp.	SCN_68-22	80.2	80.8	80.3	80.3
sp.	SD340	83.8	83.8	84.0	84.0

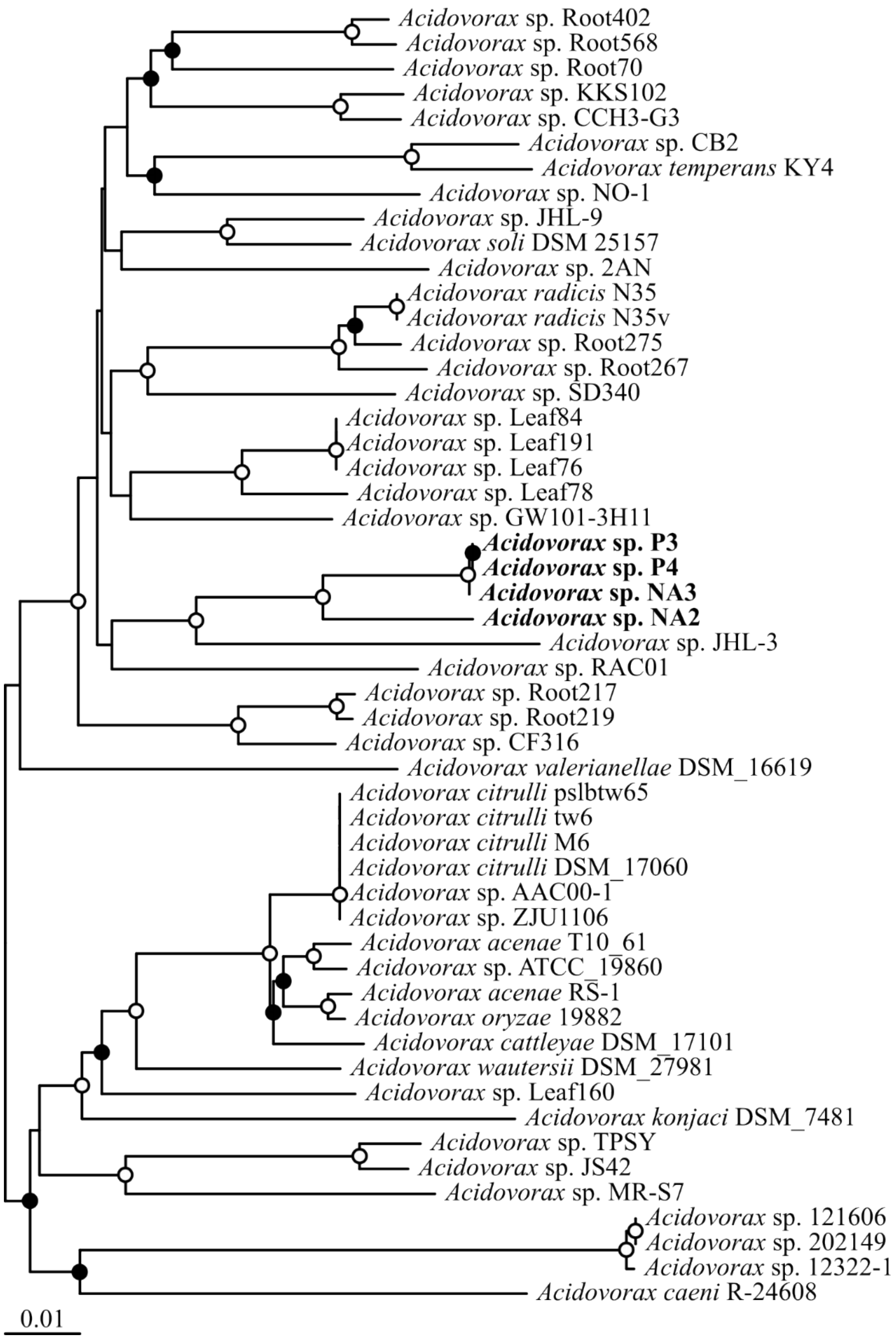


Figure A.1. Neighbor-joining phylogenetic tree of *Acidovorax* strains based on the *rpoB* gene. Sequences were identified based on blastn searches of genomes using the NA3^T annotated-*rpoB* gene nucleotide sequence. Open and closed circles at nodes indicate >95 and >50% bootstrap support, respectively (1000 iterations). *Variovorax paradoxus* strain EPS was used as an outgroup (not shown).

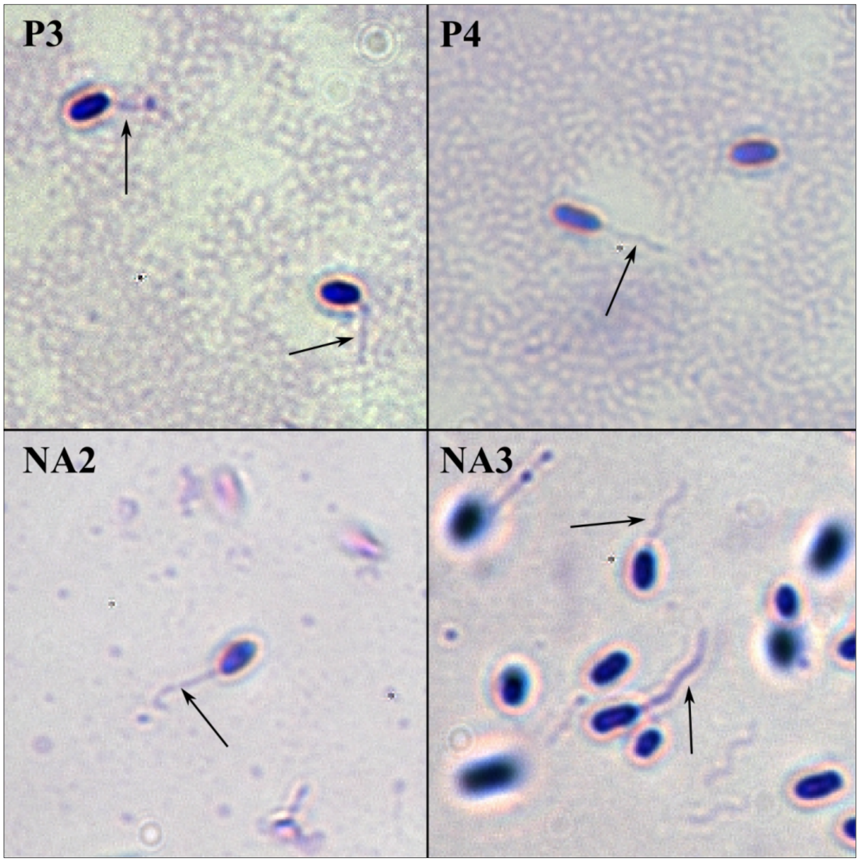
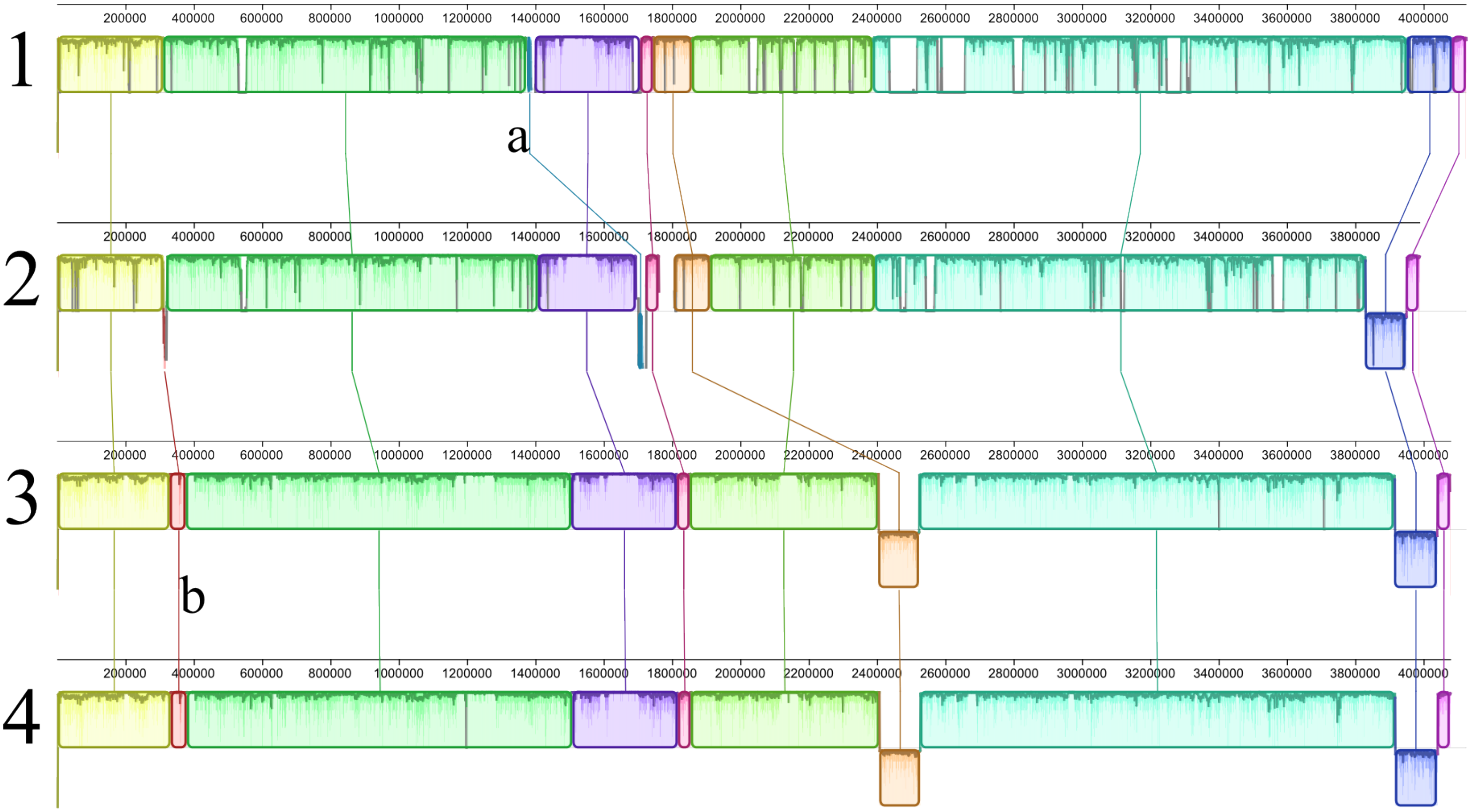


Figure A.2. Flagella-stained cells of described *Acidovorax* strains acquired using light microscopy. Cell-attached flagella are indicated in each panel with arrows.

Fig. A.3. Mauve alignment of *Acidovorax* chromosomes from this study – (1) *Acidovorax* sp. NA3^{T}, (2) *Acidovorax* sp. NA2, (3) *Acidovorax* sp. P3, reversed, and (4) *Acidovorax* sp. P4. Colored blocks indicate regions of high similarity, arrangement, and homology. Letters (a) and (b) indicate blocks specific to strains NA2/NA3^{T} and P3/P4, respectively. Numbers above alignments indicate position on the chromosome. Blocks below the center line indicate inversions with respect to homologous regions of other chromosomes.



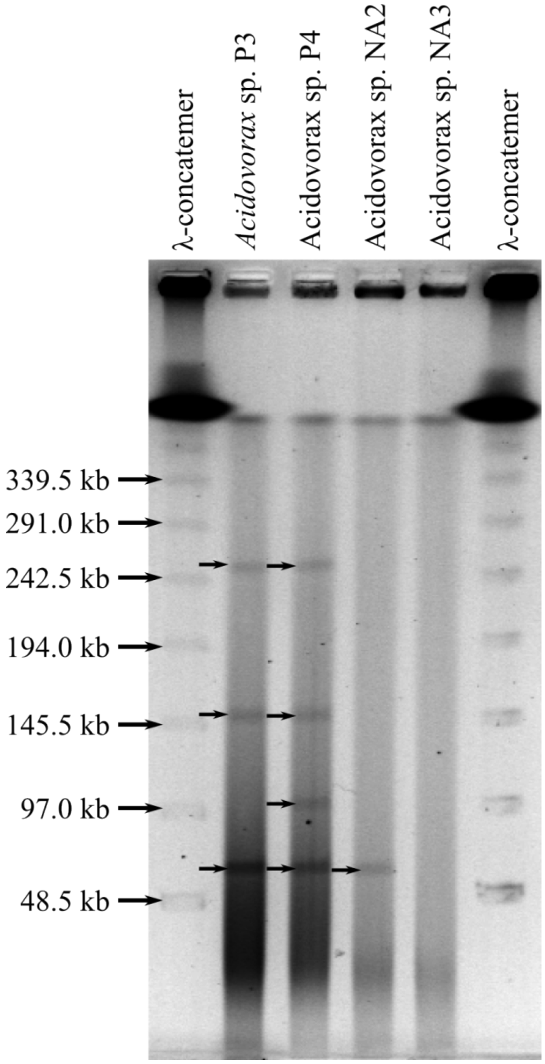


Figure A.4. PFGE image showing presence of extrachromosomal elements in described *Acidovorax* strains. Lambda concatemer marker sizes are shown on the left side of the gel and putative plasmids within each strain are indicated with arrows. The gel image was inverted in color and levels adjusted for best display quality using the GNU Image Manipulation Program ver. 2.8.