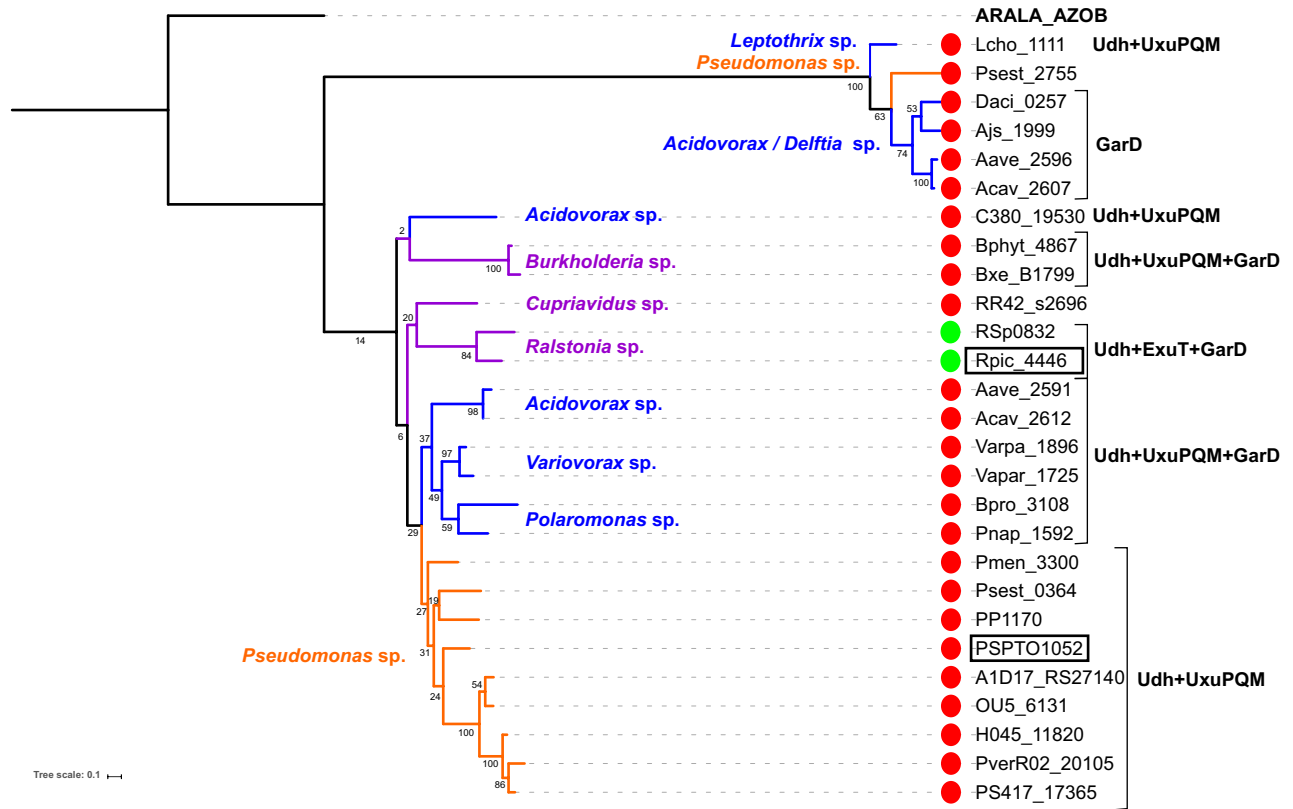
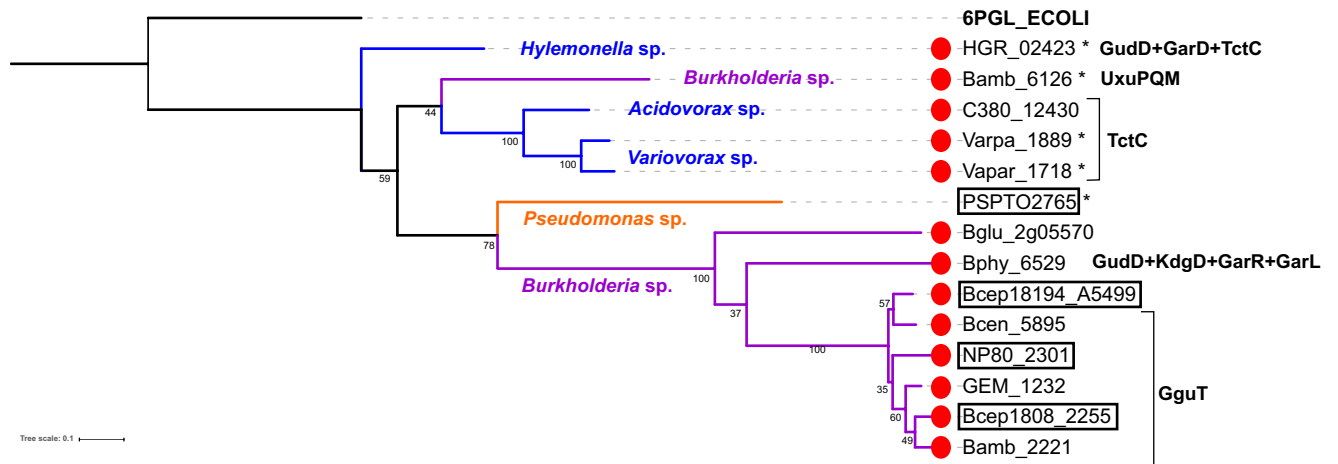


### (A) UxuL proteins from the PF08450 family



### (B) UxuF proteins from the PF10282 family



**Taxonomic groups:** Comamonadaceae, Burkholderiaceae, Pseudomonadaceae

### FIG S1. Maximum likelihood phylogenetic trees of novel UxuF and UxuL lactonases.

The tree was constructed using the PhyML software; the bootstrap values (100 replicates) are shown for each node.

Representative previously characterized lactonases from both families included as outgroups are in bold:

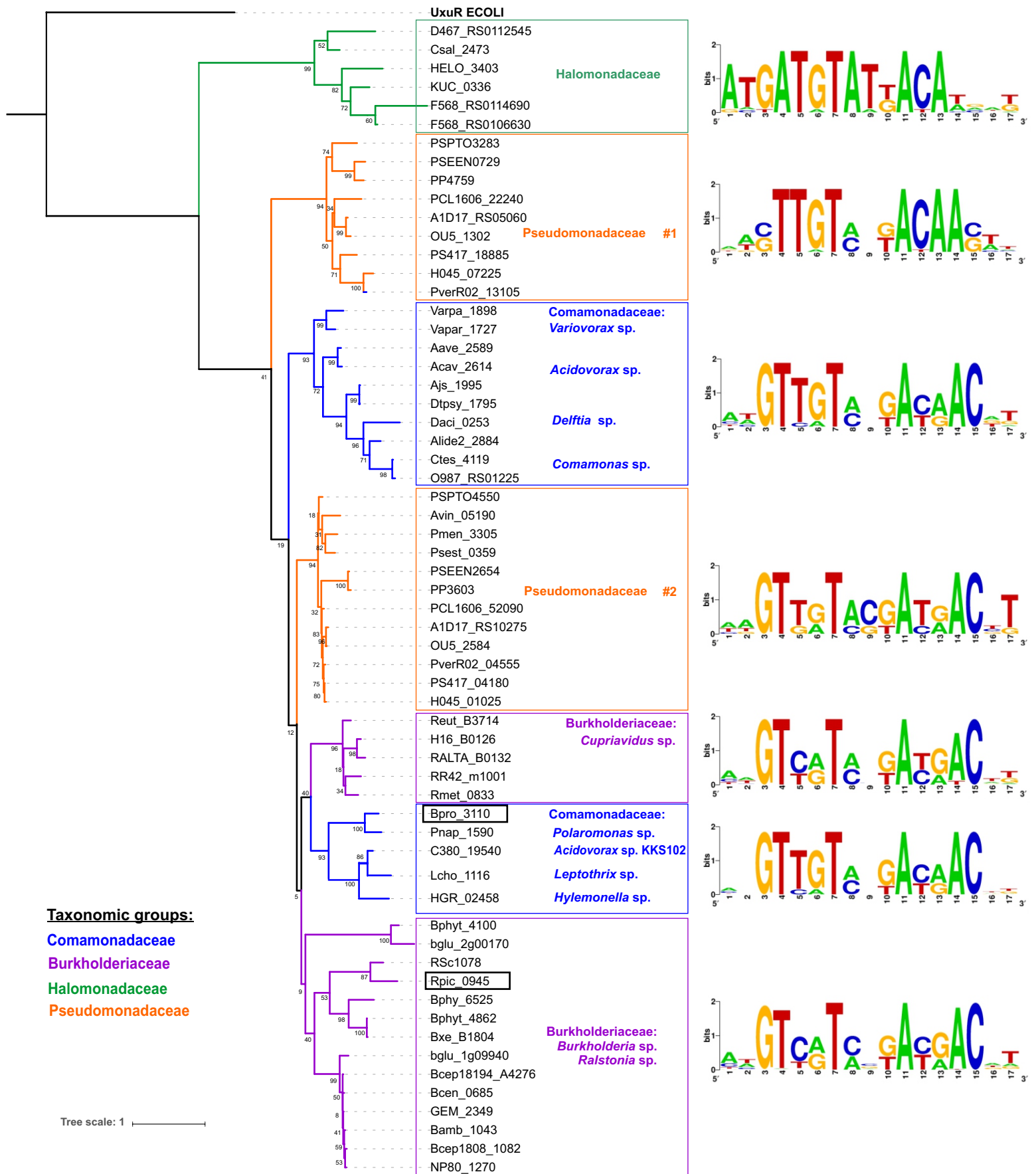
6-phosphogluconolactonase from *Escherichia coli* (6PGL\_ECOLI), L-arabinolactonase from *Azospirillum brasilense* (ARALA\_AZOB).

Lactonase genes from the reconstructed GguR and GulR regulons are marked with red ● and green ● circles.

Enzymes biochemically characterized in this work are boxed (   ).

Secreted enzymes as predicted using the SignalP software are marked with asterisk (\*).

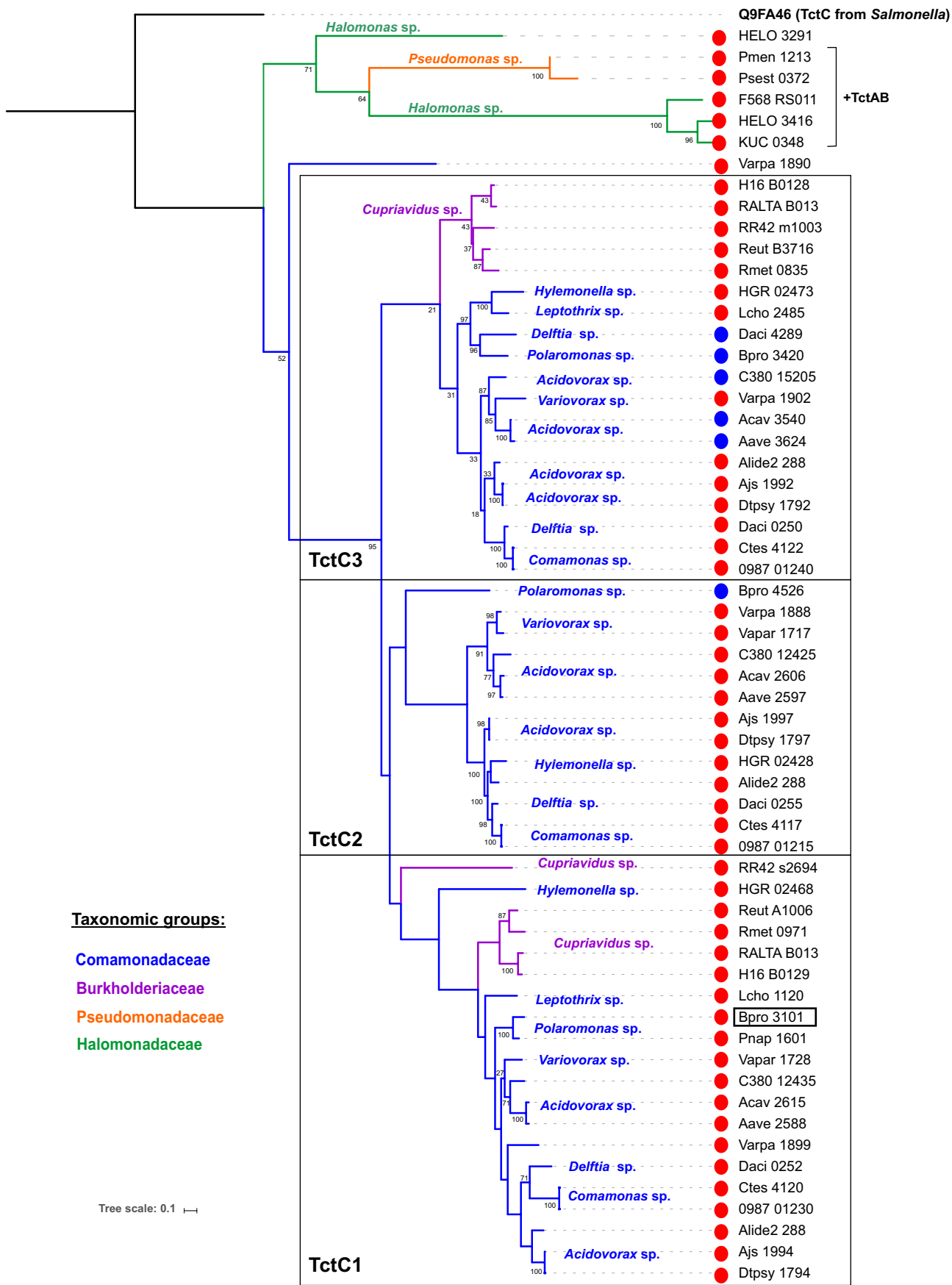
Genomic clustering of *uxuL* and *uxuF* with other hexuronate utilization genes is shown on the right.



**FIG S2. Maximum likelihood phylogenetic tree of the GguR regulators and their DNA motifs.**

The tree was constructed using the PhyML software; the bootstrap values (100 replicates) are shown for each node.

Regulators characterized in this work are boxed ( Rpic\_0945 ). DNA motifs constructed using the WebLogo tool.



**FIG S3. Maximum likelihood phylogenetic tree of novel aldarate transporters TctC.**

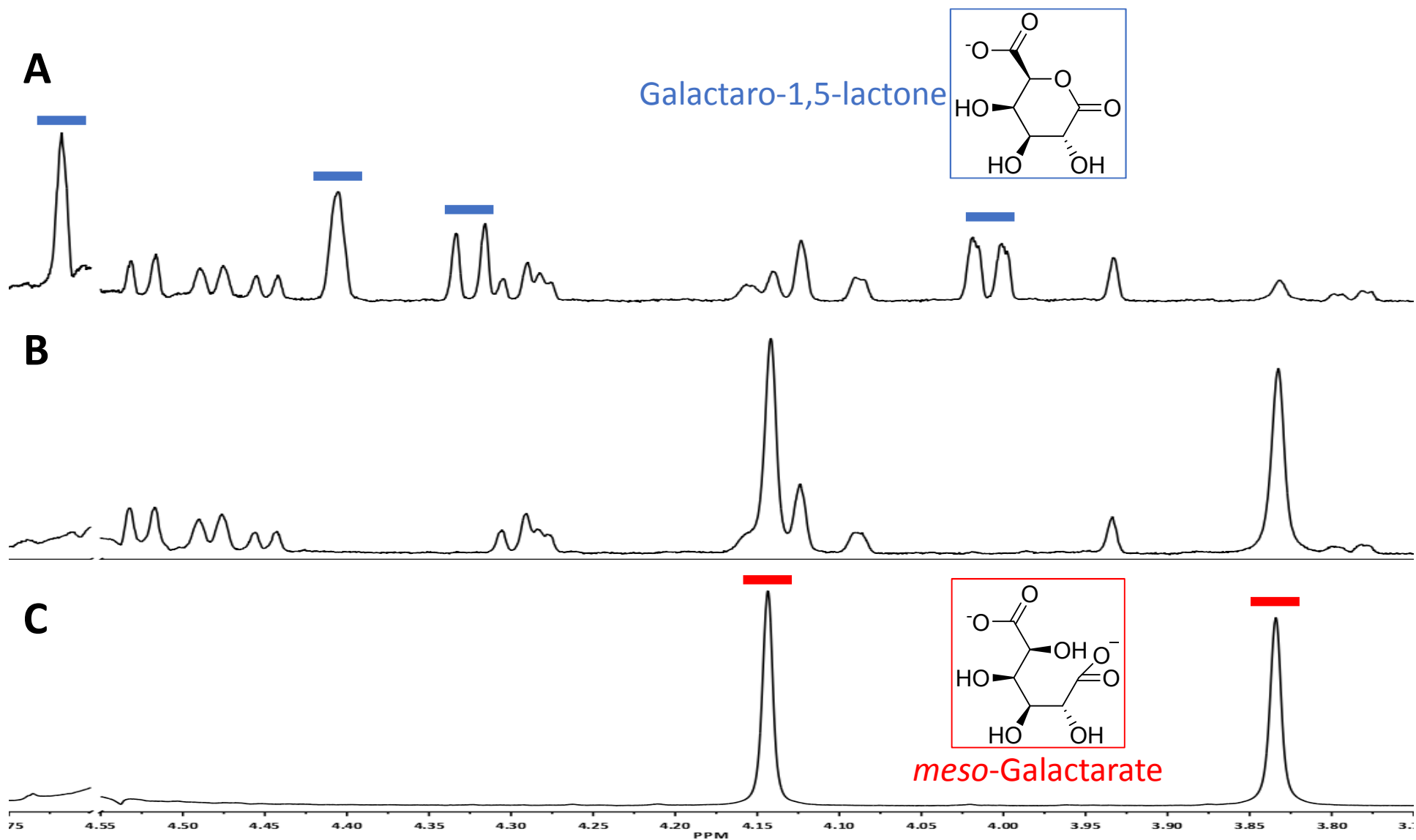
The tree was constructed using the PhyML software; the bootstrap values (100 replicates) are shown for each node.

Previously characterized TctC protein from *Salmonella* was included as outgroup.

*tctC* genes from the reconstructed GguR and GudR regulons are marked with red ● and blue ● circles.

Experimentally characterized in this work D-glucarate binding TctC protein is boxed.

Genomic clustering of *tctC* with other aldarate utilization genes is shown on the right.



**FIG S4 Proton NMR spectra for activity screening of the lactone hydrolase Rpic\_4446 from *Ralstonia pickettii*.** (A)  $^1\text{H}$  NMR spectrum of a mixture of galactaro-1,5 & 1,4-lactones. The 1,5-lactone is unstable and converts to the 1,4-lactone spontaneously. (B) Spectrum 2 minutes after the addition of Rpic\_4446. The 1,4-lactone peaks remain, while two new peaks emerge. (C) Spectrum of pure *meso*-galactarate. The peaks in (B) match those of *meso*-galactarate, suggesting it to be the product of the lactone hydrolase reaction.

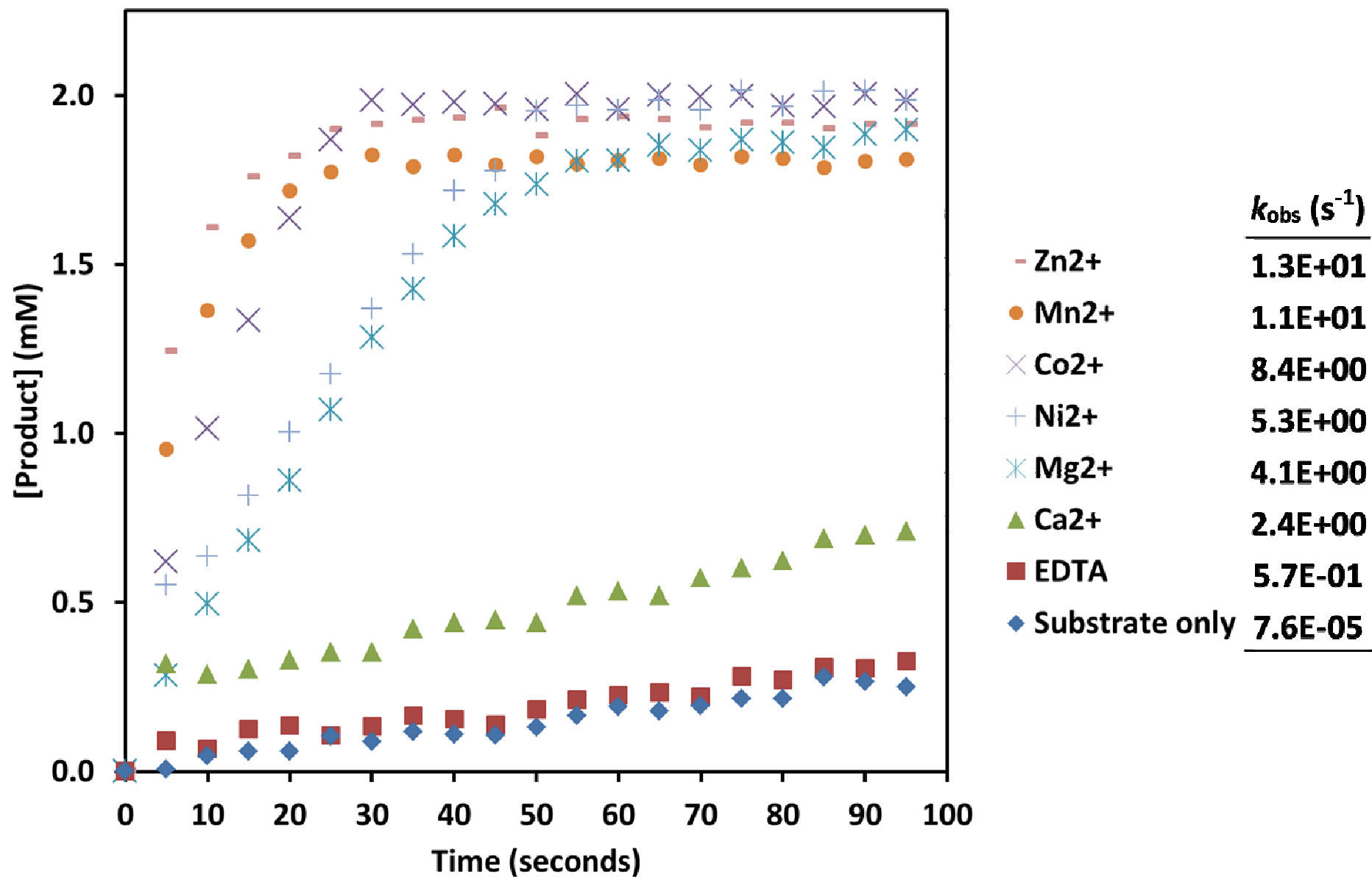


FIG S5 Metal screening of lactone hydrolase from *Ralstonia pickettii* 12J. The reaction was monitored in the presence of Zn<sup>2+</sup>, Mn<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, and EDTA. Blue diamonds represent the progress curve for substrate only. Zn<sup>2+</sup> is the best activator.

Table S1. Reconstructed catabolic pathways and regulons for hexuronate and aldarate utilization in Proteobacteria.

(A) Distribution of transcriptional regulators, enzymes, and transporters involved in hexuronate/aldarate utilization in the analyzed genomes.

Table with columns: Proteobacterial Genomes, Regulators (GguR, GudR, GguR, UdhR), Upstream enzymes (Udh, UxuL, UxuF, Gci, Gli), Downstream enzymes (GarD, Guld, KgdD, KgsD/KgsD2, GarL, GarR), Transporters (UxuPQM, TctCAB, GarP (GudP), GguT, ExuT), Additional GguR-regulated genes (Pgl, AIdE, SerA, Agl, Omp, Exu-II).

Color abbreviations: Tested in this work: enzymes (UxuL, UxuF), transporters (TctC), and regulators (GguR, GudR)

genes in GguR regulons

genes in GudR regulons

genes in GguR regulons

genes in UdhR regulons

genes in chromosomal loci

genes in chromosomal loci

genes in chromosomal loci

genes in chromosomal loci

\* has Signal peptide (secreted enzyme)



Table S1. Reconstructed catabolic pathways and regulons for hexuronate and aldarate utilization in Proteobacteria.

(B) Reconstructed GguR regulons and predicted GguR-binding sites in the analyzed genomes.

Genome	TF locus tag	Target locus tag	Site position	Site score	Site sequence	Target putative operon
<b>Halomonadaceae</b>						
Chromohalobacter salexigens DSM 3043	Csal_2473	Csal_2479	-339	5.53	ATGATGTATgACAaCAT	uxuPQM-gci-gli-udh--gguR
		Csal_2487	-57	5.51	ATGATGTATgACATgAT	kdgD-gudD-TRAP-gudD
Halomonas elongata DSM 2581	HELO_3403	HELO_3410	-102	5.01	ATGATGTATTAtAagAg	uxuPQM-gci-gli-udh-X-gguR
		HELO_3417	-64	5.54	ATGATGTATTACATgAg	kdgD-tctCAB-garD-gudD-kgsD
		HELO_3291	-207	5.44	ATGATGTATTACATgAa	tctC2
Halomonas boliviensis LC1	KUC_0336	KUC_0343	-76	5.19	ATGATGTATTACATagg	kdgD-uxuPQM-gci-gli-udh-gguR-kgsD
		KUC_0348	-203	5.39	ATGATGTATTACATagT	tctCAB-garD-gudD
		KUC_0336	-124	<u>4.76</u>	AcGATaTAATACAaCcT	gguR
Halomonas lutea DSM 23508	F568_RS0106630, F568_RS0114690	F568_RS0106595	-90	5.21	ATGATGTATgACATaAg	kdgD2-uxuPQM-gci-gli-udh-gguR
		F568_RS0114660	-87	4.81	ATGATGTATgACAcCtT	kdgD1-tctCAB-garD-gudD-gguR2-kgsD
Halomonas zhanjiangensis DSM 21076	D467_RS0112545	D467_RS0112560	-38	5.18	gTGATGTATTACAaCAT	kdgD1-gli-udh-gguR-kgsD
		D467_RS0112665	-85	4.92	ATGATGTATgACAaatT	kdgD2-uxuPQM-gci
		D467_RS0112585	-314	<u>4.39</u>	AatATGTATgACATgtg	porin-kdgm-rhgT- pmeA-pelY
<b>Comamonadaceae</b>						
Acidovorax avenae subsp. citrulli AAC00-1	Aave_2589	Aave_2589	-37	5.22	ATGTTGTCGTACAACGC	gguR
		Aave_2590	-18	5.22	CGTTGTACGACAACAT	udh-uxuL1-uxuPQM-garD-uxuL2-tctC2
		Aave_2588	-37	<u>4.73</u>	CAGTTGTATGATGACGT	tctC1-kdgD
Acidovorax avenae subsp. avenae ATCC 19860	Acav_2614	Acav_2613	-43	5.22	gcGTTGTaCGACAACAT	udh-uxuL1-uxuPQM-garD-uxuL2-tctC2
		Acav_2614	-39	5.22	ATGTTGTCGtACAACgc	gguR
		Acav_2615	-43	4.87	AaGTTGTatGAtgAcgT	tctC-kdgD
Acidovorax sp. KKS102	C380_19540	C380_19535	-68	4.84	tcGTTGTatGACAACtg	udh-uxuL1-uxuPQM-garD-X-aldE
		C380_19540	-71	4.84	caGTTGTatGACAACgA	gguR
		C380_19545	-168	5.06	ATGTTGTaCGAtgACAT	kgsD
		C380_12430	-348	<u>4.54</u>	taGTcaTCGtACAACtg	uxuF-tctC2
		C380_12435	-146	<u>4.77</u>	tGTTGTatGACgACAT	tctC-kdgD-DUF4440-serA
Acidovorax sp. JS42	Ajs_1995	Ajs_1994	-50	5.02	ATGTTGTACGATGACGT	tctC1-kdgD-tctC3-serA-gudD
		Ajs_1995	-38	5.22	ATGTTGTCTGATAACGT	gguR
		Ajs_1996	-75	5.22	ACGTTATCAGACAACAT	gudD2-tctC2-garD-uxuL2
Acidovorax ebreus TPSY	Dtpsy_1795	Dtpsy_1795	-38	5.14	ATGTTGTcTtAtAACgT	gguR
		Dtpsy_1796	-78	5.14	AcGTTaTaaGACAACAT	gudD2-tctC2
		Dtpsy_1794	-50	5.02	ATGTTGTaCGAtgAcgT	tctC1-kdgD-tctC3-serA-gudD
Alicyclophilus denitrificans BC	Alide2_2884	Alide2_2883	-21	4.73	AaGTcaTcGACgACAT	gudD2-tctC2-garD
		Alide2_2884	-103	4.73	ATGTcGTCtGAtgAcTt	gguR
		Alide2_2885	-80	4.92	ATGTTGTatGAtgACAT	tctC1-kdgD-tctC3-serA-gudD-kgsD
Comamonas testosteroni KF-1 (old)	CtesDRAFT_4119	CtesDRAFT_4118	-79	5.38	caGTTGTaCGACAACAT	gudD2-tctC2-garD
		CtesDRAFT_4119	-81	5.38	ATGTTGTCGtACAACtg	gguR
		CtesDRAFT_4120	-42	<u>4.40</u>	taGTTGTatGAtgAcTg	tctC1-kdgD-tctC3-X-serA-gudD-kgsD
Comamonas testosteroni TK102	O987_RS01225	O987_01220	-79	5.38	caGTTGTaCGACAACAT	gudD2-tctC2-garD
		O987_01225	-81	5.38	ATGTTGTCGtACAACtg	gguR
		O987_01230	-42	<u>4.40</u>	taGTTGTatGAtgAcTg	tctC1-kdgD-tctC3-tctC4-serA-gudD-kgsD
Delftia acidovorans SPH-1	Daci_0253	Daci_0252	-42	5.23	CAGTTGTACGACAACGT	tctC1-kdgD-tctC3-serA-gudD-kgsD
		Daci_0253	-25	5.48	ACGTTGTCGTACAACCT	gguR
		Daci_0254	-74	5.48	AAGTTGTACGACAACGT	gudD2-tctC2-garD-uxuL2
Hylemonella gracilis ATCC 19624	HGR_02458	HGR_02453	-95	5.02	tGTTGTaCGACAACtg	udh-uxuPQM-gudD2-tctC3-uxuF-garD
		HGR_02458	-105	5.02	caGTTGTCGtACAACa	gguR
		HGR_02463	-112	4.91	ATGTTGTaCGAtgAcTg	kdgD-tctC1-tctC2-gudD
Polaromonas sp JS666	Bpro_3110	Bpro_3110	-60	<u>4.58</u>	AGTTGTCGTGATGACTG	gguR
		Bpro_3109	-96	<u>4.58</u>	CAGTCATCAGACAACCT	udh-uxuL1-uxuPQM-garD-garL-garR-tctC1-aldE
Polaromonas naphthalenivorans CJ2	Pnap_1590	Pnap_1590	-57	<u>4.68</u>	ACGTTGTCGTGATGACTC	gguR
		Pnap_1591	-99	<u>4.68</u>	GAGTCATCAGACAACGT	udh-uxuL1-uxuPQM-kdgD-garD-kgsD-garL-garR-tctC1
Variovorax paradoxus S110	Vapar_1727	Vapar_1726	-39	5.02	taGTTGTaCGACAACg	udh-uxuL1-uxuPQM-garD-X-tctC3-uxuF-tctC2
		Vapar_1727	-39	5.02	cGTTGTCGtACAACtA	gguR
		Vapar_1728	-44	<u>4.54</u>	taGTTGTaCGAtgAcTg	tctC1-kdgD-DUF4440-gudD
Variovorax paradoxus EPS	Varpa_1898	Varpa_1897	-148	5.02	taGTTGTaCGACAACg	udh-uxuL1-uxuPQM-garD-X-tctC4-uxuF-tctC2



Leptothrix cholodnii SP-6	Lcho_1116	Varpa_1898	-40	5.02	cTGTTCGtACAACta	gguR
		Varpa_1899	-78	<u>4.54</u>	taGTTGTaCGAtgACTg	tctC1-kdgD-DUF4440-tctC3-gudD-X-kgsD
		Lcho_2484	-66	5.70	ATGTTGTACGACAACAT	garD
		Lcho_1117	-65	5.10	ATGTTGTACGATGACAT	kdgD-gudD-kgsD-tctC1-serA-garL-garR
		Lcho_1115	-95	5.40	ACGTTGTACGACAACCTG	udh-uxuPQM-uxuL2
		Lcho_1116	-73	5.40	CAGTTTCGtACAACGT	gguR
<b>Burkholderiaceae</b> Burkholderia phytotfirmans PsJN	Bphyt_4862; Bphyt_4100	Bphyt_4100	-332	4.85	AcGtTtGtCtGACGACag	gguR2
			-202	4.06	AgGatGTCCGAtGACaa	
		Bphyt_4101	-238	4.06	ttGTCaTCGGACatCcT	gguT-gudD-kdgD2
			-108	4.85	ctGTCGTCaGACaACgT	
		Bphyt_4856	-103	4.56	AcGtTtaTCGtACaACTa	udh2-exuT2
		Bphyt_4860	-191	4.67	AgGTCaTCCTaCaACaT	garL-garR
			-79	4.34	AAGaCaTaCGACGACcT	
		Bphyt_4861	-160	4.34	AgGTCGTCGtAtGtCTT	kgsD
			-48	4.67	AtGtTGTaGGAtGACcT	
		Bphyt_4862	-69	4.86	cAGTCGTCaGACGACgg	gguR
		Bphyt_4864	-176	4.89	AcGTCaTCCTaCaACaT	kdgD-garP
			-81	4.65	ttGtTGTaCGAtGACgT	
		Bphyt_4865	-160	4.65	AcGTCaTCGtACaACaa	garD-udh-uxuL
			-65	4.89	AtGtTGTaGGAtGACgT	
		Bphyt_4868	-302	4.96	AtGTCGTCGtACaACgT	uxuPQM-omp
Burkholderia phymatum STM815	Bphy_6525	Bphy_6382	-67	4.58	ATGTTGTACGATGACGC	garD
		Bphy_6525	-51	4.51	CGTTGTCGGACAACGT	gguR
		Bphy_6526	-48	4.31	TTGTTGTACGATGACCG	kgsD
			-171	4.49	ATGTCATAGGATGTCTG	
		Bphy_6527	-77	4.49	CAGACATCCTATGACAT	garL-garR-uxuF-gudD-kdgD
			-200	4.31	CGGTCATCGTACAACAA	
		Bphy_6596	-92	4.75	ATGTCGTCAGACAACCT	garP
			-223	4.86	ACGTCGTCGTACAACCTG	
Burkholderia ambifaria AMMD	Bamb_1043	Bamb_1041	-270	5.13	AAGTCATCCGACGACTG	gudD
		Bamb_1043	-107	5.13	CAGTCGTCGGATGACTT	gguR
		Bamb_2220	-86	5.12	ATGTCATACGACGACAT	gguT-uxuF
		Bamb_3566	-106	4.80	CAGTCGTCAGATAACGT	exuT-agl-omp
		Bamb_4675	-48	4.82	ATGTCGtATGATGACTA	garD
			-153	4.65	ATGTCATCGTACAACGA	
		Bamb_4676	-207	4.82	TAGTCATCATAACGACAT	kdgD
			-102	4.65	TCGTTGTACGATGACAT	
		Bamb_4679	-239	5.00	AcGTCGTCGtAtGACaT	kgsD
		Bamb_4680	28	5.00	AtGTCaTaCGACGACgT	garP
		Bamb_4904	-73	4.43	ATGTAGTACGACGACGT	pgl
		Bamb_6123	-341	4.59	CAGTCATCCTATGACGA	uxuPQM-uxuF2
		Burkholderia vietnamiensis G4	Bcep1808_1082	Bcep1808_1081	-333	5.13
Bcep1808_1082	-107			5.13	CAGTCGTCGGATGACTT	gguR
Bcep1808_2255	-239			5.12	ATGTCATACGACGACAT	uxuF
Bcep1808_3560	-49			4.82	ATGTCGtATGATGACTA	garD
	-155			4.58	ATGTCATCGTACAACGC	
Bcep1808_3561	-198			4.82	TAGTCATCATAACGACAT	kdgD
	-92			4.58	CGGTTGTACGATGACAT	
Bcep1808_3562	-241			5.00	ACGTCGTCGtATGACAT	kgsD
Bcep1808_3563	-26			5.00	ATGTCATACGACGACGT	garP
Bcep1808_3851	-70			4.51	ATGTGATACGACGACGT	pgl
Bcep1808_6572	-329			4.78	CAGTCATCCGACAACta	uxuPQM
Burkholderia glumae BGR1	bglu_1g09940; bglu_2g00170			bglu_1g09940	-99	5.06
		bglu_2g00170	-276	<u>4.05</u>	AgGAcGTCTGACaACaa	gguR2
			-67	<u>4.03</u>	ggGACaTCGGAtGACaT	
		bglu_2g00180	-207	4.90	ATGTCATCAGACAACGT	gudD-kdgD
		bglu_2g03700	-69	5.10	AAGTTGTACGACGACAT	garP
		bglu_2g03710	-212	5.10	ATGTCGTCGTACAACCT	kgsD
		bglu_2g03730	-186	4.83	CAGTCATCATAACACAT	kdgD
			-75	5.10	ATGTTGTACGATGACAT	
		bglu_2g03740	-164	5.10	ATGTCATCGTACAACAT	garD
			-53	4.83	ATGTTGTATGATGACTG	
		bglu_2g04250	-90	4.50	AAGTGGTACGATGACTG	exuT-agl-omp
		bglu_2g05570	-114	4.76	AAGTTATATGATGACGT	uxuF
		bglu_2g07210	-77	4.93	ATGTCATCGTATGACGT	pgl
		bglu_2g13770	-497	4.83	CAGTCGTCtTATAACAT	uxuPQM
Burkholderia xenovorans LB400	Bxe_B1804	Bxe_B0539	-106	4.89	CTGTCATCGTACAACAT	gudD
		Bxe_B1798	-322	5.08	ATGTCGTCGTACAACAT	uxuPQM-omp
		Bxe_B1801	-159	4.65	ACGTCATCGTACAACAA	garD-udh-uxuL
			-64	4.89	ATGTTGTAGGATGACGT	
		Bxe_B1802	-83	4.65	TTGTTGTACGATGACGT	kdgD-garP
			-178	4.89	ACGTCATCCTACAACAT	
		Bxe_B1804	-70	4.79	CAGTCATCAGACGACGG	gguR
Bxe_B1805	-48	4.67	ATGTTGTAGGATGACCT	kgsD		

		Bxe_B1807	-103	4.56	AcGTTaTCGtACaACTa	udh2-exuT2		
Burkholderia lata sp. 383	Bcep18194_A4276	Bcep18194_A4276	-107	5.13	CAGTCGTCGGATGACTT	gguR		
		Bcep18194_A4275	-268	5.13	AAGTCATCCGACGACTG	gudD		
		Bcep18194_A5498	-89	5.12	ATGTCATACGACGACAT	gguT-uxuF		
		Bcep18194_B0327	-25	4.88	ACGTCATACGACGACGT	garP		
		Bcep18194_B0328	-239	4.88	ACGTCGTCGTATGACGT	kgsD		
		Bcep18194_B0332	-198	4.82	TAGTCATCATAACGACAT	kdgD		
		Bcep18194_B0333	-92	4.65	TCGTTGTACGATGACAT	garD		
			-154	4.65	ATGTCATCGTACAACGA			
			-48	4.82	ATGTCGTATGATGACTA			
				Bcep18194_B1871	-196	4.92	CAGTCGTCAGATAACAT	exuT-agl-omp
		Bcep18194_C7436	-347	4.71	CAGTCATCCTATGACAA	uxuPQM		
Burkholderia multivorans ATCC BAA-247	NP80_1270	NP80_1269	-194	5.06	AAGTCaTCCGAtGACTg	gudD		
		NP80_1270	-108	5.06	cAGTCaTCGGAtGACTT	gguR		
		NP80_2300	-98	5.12	AtGTCaTaCGACGACaT	gguT-uxuF		
		NP80_3332	-77	4.50	AAGTaGtACGAtGACaT	pgl		
		NP80_4291	-272	4.55	AAGgCGTcTGACGACTT	omp		
		NP80_5140	-153	4.58	AtGTCaTCGtACaACgC	garD		
			-47	4.82	AtGTCGTatGAtGACTa	kdgD		
		NP80_5141	-199	4.82	tAGTCaTCatACGACaT			
			-93	4.58	gcGTtGTaCGAtGACaT			
		NP80_5150	-237	4.92	AcGTCGTcAtAtGACaT	kgsD		
		NP80_5151	-27	4.92	AtGTCaTatGACGACgT	garP		
		NP80_5675	-367	4.72	cAGTCaTCCTAtaACgT	uxuPQM		
		NP80_5676	-177	4.72	AcGTTaTaGGAtGACTg	pgl*		
		Burkholderia cepacia GG4	GEM_2349	GEM_1233	-86	5.12	AtGTCaTaCGACGACaT	gguT-uxuF
				GEM_2349	-106	5.13	cAGTCGTCGGAtGACTT	gguR
GEM_2350	-335			5.13	AAGTCaTCCGACGACTg	gudD		
GEM_5878	30			5.00	AtGTCaTaCGACGACgT	garP		
GEM_5879	-239			5.00	AcGTCGTCGTAtGACaT	kgsD		
GEM_5882	-197			4.82	tAGTCaTCatACGACaT	kdgD		
GEM_5883	-50			4.82	AtGTCGTatGAtGACTa	garD		
Burkholderia cenocepacia AU 1054	Bcen_0685			Bcen_0684	-266	5.13	AAGTCaTCCGACGACTg	gudD
		Bcen_0685	-108	5.13	cAGTCGTCGGAtGACTT	gguR		
		Bcen_3044	-25	5.00	AtGTCaTaCGACGACgT	garP		
		Bcen_3045	-239	5.00	AcGTCGTCGTAtGACaT	kgsD		
		Bcen_3049	-197	4.82	tAGTCaTCatACGACaT	kdgD		
		Bcen_3050	-49	4.82	GAtTCGTatGAtGACTa	garD		
		Bcen_4207	-196	4.92	cAGTCGTCaGAtaACaT	exuT-agl-omp		
		Bcen_5663	-336	4.48	cAGTCaTCCTAtaACgA	uxuPQM		
		Bcen_5896	-89	5.00	AtGTCaTaCGACGACgT	gguT-uxuF		
		Cupriavidus taiwanensis LMG 19424	RALTA_B0132	RALTA_B0132	-91	4.83	CTGTCATCGTATGACTG	gguR
RALTA_B0133	-109			4.83	CAGTCATACGATGACAG	gudD-tctC3-tctC1		
RALTA_B0137	-301			4.86	AcGTTtGTcAGACaACaT	kdgD-kgsD		
	-93			4.78	ttGTCGTaCGAtGACTg			
RALTA_B0782	-155			4.66	ATGTTGTCTGTAACAACAC	garD		
	-54			4.86	ATGTTGTCTGACACAACGT			
Cupriavidus eutropha H16	H16_B0126	H16_B0965	-57	5.00	AAGTTGTCTGACACAACGT	garD		
			-157	4.60	GTGTTATCGTACAACATA			
		H16_B0131	-94	4.71	TTGTCATACGATGACTG	kdgD-kgsD		
			-289	4.85	ACGTTGTCCTACAACAT			
		H16_B0126	-81	4.83	CTGTCATCGTATGACTG	gguR		
H16_B0127	-90	4.83	CAGTCATACGATGACAG	gudD-tctC3-tctC1				
Cupriavidus basilensis 4G11	RR42_m1001	RR42_m1000	-148	4.57	AtGTTtGtAGGAtGtCTT	kgsD		
		RR42_m1001	-81	4.49	cgGTCGTcAtAtaACTT	gguR		
		RR42_m1002	-35	4.49	AAGTtaTatGACGACcg	gudD-tctC3		
		RR42_s1730	-97	4.67	tAGTtGTaCGAtGACgT	kdgD		
		RR42_s1731	-107	5.07	AAGTCaTaCGAtGACaT	garP-garD		
		RR42_s2695	-75	4.71	tAGTCaTaGGAtGACgT	serA-tctC1		
		RR42_s2696	-214	4.71	AcGTCaTCCTAtGACTa	uxuL		
		Cupriavidus eutropha JMP134	Reut_B3714	Reut_A1006	-45	5.00	ATGTTGTATGACGACTG	tctC1
-180	5.00				ATGTCATCAGACAACAG			
Reut_B3688	-317			5.02	AtGTCGTCGTAtGACTg	kdgD		
	-101			4.67	tAGTtGTaCGAtGACgT			
Reut_B3689	-284			4.67	AcGTCaTCGtACaACTa	garP-garD		
	-68			5.02	cAGTCaTaCGACGACaT			
Reut_B3713	-96			4.17	AtGTCaTatGAtGtCcT	kgsD		
Reut_B3714	-80			5.04	CAGTCGTCGTATGACTT	gguR		
Reut_B3715	-87	5.04	AAGTCATACGACGACTG	gudD-tctC3				
Cupriavidus metallidurans CH34	Rmet_0833	Rmet_0832	-98	4.53	ATGTCATATGATGTCTT	kgsD		
		Rmet_0833	-30	4.68	CGGTCGTCGTATGACTT	gguR		
		Rmet_0834	-80	4.68	AAGTCATACGACGACCG	gudD-tctC3		

		Rmet_4735	-326	4.45	AAGTCaTCGTACaACca	garP-garD
			-77	5.13	AAGTCGTatGACGACaT	
		Rmet_4736	-359	5.13	AtGTCGTcAtACGACTT	kdgD
			-110	4.45	tgGtTGTaCGAtGACTT	
		Rmet_0971	-161	5.13	AAGTCATCCGACGACTG	tctC1
<i>Ralstonia pickettii</i> 12J	Rpic_0945	Rpic_0946	-17	4.67	GTGTTGTACGACGACTG	gudD-exuT-agl-omp-aldE
		Rpic_0945	-85	4.67	CAGTCGTCGTACAACAC	gguR
<i>Ralstonia solanacearum</i> GMI1000	RSc1078	RSc1078	-90	4.98	CAGTCGTCGTACAACAT	gguR
		RSc1079	-26	4.98	ATGTTGTACGACGACTG	gudD-exuT-agl-omp-aldE
<b><i>Pseudomonadaceae</i></b>						
<i>Pseudomonas entomophila</i> L48	PSEEN0729, PSEEN2654	PSEEN2654	-63	4.80	ATGTGATCGTACAACCTG	gguR2
		PSEEN2658	-71	5.50	ATGTTGTACGATAACTT	kdgD-garP-garD-kgsD
		PSEEN0730	-105	5.30	GTGTTGTGTACAACCTT	gguT-gudD
		PSEEN0729	-201	5.30	AAGTTGTACGACAACAC	gguR1
<i>Pseudomonas mendocina</i> ymp	Pmen_3305	Pmen_1214	-450	4.70	ACGTTATCGTACAACAA	gudD2-tctCBA
			-251	5.10	AGGTCATACGACAACAT	
		Pmen_1215	-275	5.10	ATGTTGTGTATGACCT	kdgD-gudD-garP-garD-serA
			-76	4.70	TTGTTGTACGATAACGT	
		Pmen_3299	-316	4.80	ACGTTGTACAACCTT	udh-uxuL-uxuPQM-X
			-60	5.00	AAGTCATACGACAACGT	
		Pmen_3306	-54	4.80	CAGTTGTACGATGACAC	aldE
		Pmen_3305	-63	4.80	GTGTCATCGTACAACCTG	gguR
<i>Pseudomonas putida</i> KT2440	PP4759, PP3603	PP1173	-251	4.82	tAGTTGTACGACAACc	omp
		PP1171	-43	5.10	AAGTCATACGACAACCT	udh-uxuL-uxuPQM-X-aldE
		PP3599	-71	5.30	AGGTTGTACGATAACTT	kdgD-garP-garD-kgsD
		PP3603	-47	4.60	ATGTGATCGTACAACCG	gguR2
		PP4758	-79	5.10	ATCTTGTGTACAACCTT	gguT-gudD
		PP4759	-104	5.10	AAGTTGTACGACAAGAT	gguR1
<i>Pseudomonas syringae</i> pv. tomato str. DC3000	PSPTO3283, PSPTO4550	PSPTO4549	-77	5.10	CAGTTGTACGATGACTT	kdgD-garP-garD
		PSPTO3323	-163	5.10	AAGTTGTACGATGACTG	kgsD
		PSPTO1054	-297	4.85	tgGTTGTACGACAACcT	omp
		PSPTO1053	-48	5.10	AAGTCATACGACAACAG	udh-uxuL-uxuPQM-X-aldE
		PSPTO5219	-385	4.20	CAGTCATCGTATAACGA	exuT
			-265	5.60	ATGTTGTACGACAACAT	
		PSPTO3283	-128	3.81	AgcTTGTAAcTACAgtTc	gguR1
		PSPTO3284	-358	3.81	gAcTTGTATAcAAgtTc	gguT-gudD
<i>Pseudomonas simiae</i> WCS417	PS417_18885, PS417_04180	PS417_04195	-79	4.78	tAGTTaTACGAtgACgT	kdgD-kgsD2-garP-garD
		PS417_17355	-113	4.86	gAGTcaTCGTaTAACTT	omp
			-55	5.20	AtGTTGTACGACgACgT	
		PS417_17360	-36	4.79	tAGTcaTACGACAACcT	udh-uxuL-uxuPQM-aldE
		PS417_14775	-172	4.62	ttGTcaTCCtACAACtG	exuT-II
			-63	5.01	cAGTTGTACGACgACaT	
		PS417_18885	-99	4.26	tgGTTGTcATACAgtTT	gguR2
		PS417_18890	-257	4.26	AAcTTGTATgACAACca	gguT-gudD
<i>Pseudomonas fluorescens</i> FW300-N1B4	A1D17_RS05060, A1D17_RS10275	A1D17_RS27150	-117	5.23	AAGTcaTCGTaTAACTT	omp
			-58	5.20	AtGTTGTACGACgACgT	
		A1D17_RS27145	-59	5.11	AAGTcaTACGACAACcT	udh-uxuL-aldE
		A1D17_RS10260	-80	5.11	AAGTTGTACGAtgACcT	kdgD-kgsD2-garP-garD
		A1D17_RS05065	-283	4.36	AAcTTGTATgACAACc	gguT-gudD
		A1D17_RS05060	-101	4.36	gtGTTGTcATACAgtTT	gguR1
		A1D17_RS30165	-322	4.69	cAGTTGTACGACgACg	exuT-II
<i>Pseudomonas stutzeri</i> RCH2	Psest_0359	Psest_0373	-394	5.03	AcGTTaTCGTACAACtA	gudD2-tctCBA
			-179	4.68	tAGTcGTACGACgACcT	
		Psest_0374	-288	4.68	AgGtGTCGTACgACTa	kdgD-kgsD2-garP-garD-serA-aldE
			-73	5.03	tAGTTGTACGAtAACgT	
		Psest_0359	-64	4.84	tAGTTaTCGTACAACtG	gguR
		Psest_0365	-49	4.52	AtGTcaTATgACAACg	udh-uxuL-uxuPQM
		Psest_2755	-169	4.84	AtGTcaTATgACAACaT	uxuL2
<i>Pseudomonas poae</i> RE_1-1-14	H045_07225, H045_01025	H045_01030	-81	4.85	tAGTTGTACGAtgACgT	kdgD-kgsD2-garP-garD
		H045_07220	-210	4.41	AAcTTGTATgACAACaa	gguT-gudD
		H045_07225	-69	4.41	ttGTTGTcATACAgtTT	gguR1
		H045_11810	-119	4.91	tAGTcaTCGTaTAACTT	omp
			-61	5.38	AtGTTGTACGACAACgT	
		H045_11815	-39	5.11	AAGTcaTACGACAACcT	udh-uxuL-uxuPQM-aldE
		H045_09475	-170	4.75	ctGTcaTCCtACAACcT	exuT-II
			-61	5.01	cAGTTGTACGACgACaT	

Pseudomonas veronii R02	PverR02_13105,	PverR02_04575	-81	4.85	tAGTTGTACGAtgACgT	kdgD-kgsD2-garP-garD
	PverR02_04555	PverR02_13100	-265	4.09	tAcTTGTATGACAACaa	gguT-gudD
		PverR02_13105	-70	4.09	tTGTGTCaTACAAGTa	gguR1
		PverR02_20095	-116	4.91	tAGTcaTCGTatAACTT	omp
			-59	5.20	AtGTTGTACGACgACgT	
		PverR02_20100	-39	4.79	tAGTcaTACGACAACcT	udh-uxuL-uxuPQM-aldE
		PverR02_17090	-171	4.57	gtGTcaTCCtACAACtA	exuT-II
		-61	5.01	cAGTTGTACGACgACaT		
Pseudomonas chlororaphis PCL1606	PCL1606_22240,	PCL1606_22240	-96	4.76	cAcTtaTCCGACAACtT	gguR1
	PCL1606_52090	PCL1606_22260	-275	4.76	AAGTTGTCGGatAAgTg	gguT-gudD
		PCL1606_52060	-78	4.79	tAGTTGTACGAtgACcT	kdgD-kgsD2-garP-garD
		PCL1606_52090	-62	4.36	cgGTgaTCGTACAACtT	gguR2
Pseudomonas mandelii JR-1	OU5_1302, OU5_2584	OU5_1302	-80	4.36	gtGTTGTCaTACAAGtT	gguR1
		OU5_1304	-230	4.36	AAcTTGTATGACAACac	gguT-gudD
		OU5_2579	-79	5.10	AAGTTGTACGAtgACcT	kdgD-kgsD2-garP-garD
		OU5_6133	-74	5.23	AAGTcaTCGTatAACTT	omp
			-16	5.20	AtGTTGTACGACgACgT	
		OU5_6132	-46	5.11	AAGTcaTACGACAACcT	udh-uxuL-uxuPQM-X-aldE
Azotobacter vinelandii AvOP	Avin_05190	Avin_05220	-73	5.10	AAGTcaTACGAtAACgT	kdgD-garP-garD-serA
		Avin_05180	-120	4.63	AAGTcaTAtGAtgACaT	gguR
		Avin_05190	-214	4.63	AtGTcaTCaTatgACTT	kgsD
Binding sites experimentally tested in this work		genes in divergons		4.10	weak sites	
		genes in divergons				

Table S1. Reconstructed catabolic pathways and regulons for hexuronate and aldarate utilization in Proteobacteria.

(C) Reconstructed GudR regulons and predicted GudR-binding sites in the analyzed genomes.

Genome	TF locus tag	Target locus tag	Site position	Site		Target putative operon
				score	Site sequence	
<b>Burkholderiales</b>						
Acidovorax avenae subsp. citrulli AAC00-1	Aave_3625	Aave_3625	-110	5.9	GGATGTTAACGTTAACATCC	gudR
		Aave_3624	-40	5.9	GGATGTTAACGTTAACATCC	tctC3-gudD
Acidovorax avenae subsp. avenae ATCC 19860	Acav_3542	Acav_3542	-111	6.12	GAATGTTAaCGtTAACATTC	gudR
		Acav_3540	(-1286)	6.12	GAATGTTAaCGtTAACATTC	tctC3-gudD
Acidovorax sp. KKS102	C380_15215	C380_15215	-130	5.61	tAATGTTAaCGtTAACATca	gudR
		C380_15210	-110	5.61	tgATGTTAaCGtTAACATTa	kdgD-tctC3-gudD
Polaromonas sp. JS666	Bpro_4527, Bpro_3418	Bpro_4527	-24	4.9	GCAAGTTAGCGTTAACATCA	gudR2
		Bpro_4526	-79	5.9	AATTGTTAGCGCTAACATTC	tctC2
		Bpro_3418	-33	5.2	GACTGGTAGCGCTATCGTCG	gudR1
		Bpro_3419	-29	6	AAAAGGTAGCGCTACCATTC	kdgD-tctC3-gudD-kgsD
	-2	5.5	CAATGGTAGCGCTACCTTA			
Polaromonas naphthalenivorans CJ2	Pnap_1131	Pnap_1130	-93	5.7	AAATGTTACCGATAACATTG	gudD
		Pnap_1131	-67	4.67	tgccGgTAtCGgTAtCATTC	gudR
Delftia acidovorans SPH-1	Daci_4288	Daci_4288	-131	4.95	aAATGaTAtCGtTgtCATTt	gudR
		Daci_4289	-118	4.95	aAATGacAaCGaTAtCATTt	tctC3

Binding sites experimentally tested in this work

	(transposase)	4.1	weak sites
genes in divergons			
genes in divergons			
			tandem sites
			tandem sites

Table S1. Reconstructed catabolic pathways and regulons for hexuronate and aldarate utilization in Proteobacteria.

(D) Reconstructed GulR regulons and predicted GulR-binding sites in the analyzed genomes.

Genome	TF locus tag	Target locus tag	Site position	Site score	Site sequence	Target putative operon	
<u>GulR regulon in Ralstonia spp.</u> Ralstonia pickettii 12J	Rpic_4453	Rpic_4452	-339	5.37	TCGATTCAAAAacgGTATgGA	kdgD-kgsD-garP-gudD2-garD-udh-uxuL-exuT2	
			-104	5.69	TCaATACAAGTTcTGTATtaA		
		Rpic_4453	-256	5.69	TtaATACAgAAcTTGTATtGA		gulR
			-21	5.37	TCcATACcgtTTTTGAATCGA		
Ralstonia solanacearum GMI1000	RSp0823	RSp0823	92	5.06	TCGATAAcgTTTTGAATCGA	gulR	
		RSp0824	-111	5.06	TCGATTCAAAAcgTaTATCGA	uxuL2	
		RSp0826	-109	6.05	TCGATTCAAAAaaTGAATtGA	kdgD-kgsD-garP-gudD2-garD-udh-uxuL-pgl-exuT2	

**Table S1. Reconstructed catabolic pathways and regulons for hexuronate and aldarate utilization in Proteobacteria.**

**(D) Reconstructed UdhR regulons and predicted UdhR-binding sites in the analyzed genomes.**

Genome	TF locus tag	Target locus tag	Site position	Site score	Site sequence	Target putative operon
<b><u>UdhR regulon in Burkholderia spp.</u></b>						
Burkholderia phymatum STM815	Bphy_5674	Bphy_5673	-40	6.5	TcGGAAAACGTTTTCCtA	udh
		Bphy_5674	-107	6.5	TaGGAAAACGTTTTCCgA	udhR
		Bphy_5675	-136	6.5	TaGaAAAACGTTTTCCAA	exuT
Burkholderia ambifaria AMMD	Bamb_5235	Bamb_5235	-156	6.6	TcGGAAAACGTTTTCCAA	udhR
		Bamb_5236	-87	6.6	TTGGAAAACGTTTTCCgA	udh
		Bamb_5234	-145	6.2	TTGaAAAACGTTTTCCAt	exuT
Burkholderia lata sp. 383	Bcep18194_B2717	Bcep18194_B2716	-52	6.6	TTGGAAAACGTTTTCCgA	udh
		Bcep18194_B2717	-158	6.6	TcGGAAAACGTTTTCCAA	udhR
		Bcep18194_B2718	-133	5.8	cTGA AAAACGTTTTCCAg	exuT
Burkholderia multivorans ATCC BAA-247	NP80_3553	NP80_3553	-156	6.33	gcGGAAAACGTTTTCCAA	udhR
		NP80_3554	-61	6.33	TTGGAAAACGTTTTCCgc	udh
		NP80_3552	-130	6.2	TTGaAAAACGTTTTCCAt	exuT
Burkholderia cepacia GG4	GEM_5231	GEM_5230	-64	6.2	TTGGAAAACGTTTTCCgg	udh
		GEM_5231	-47	6.2	ccGGAAAACGTTTTCCAA	udhR
		GEM_5232	-142	6.2	TTGaAAAACGTTTTCCAg	exuT
Burkholderia vietnamiensis G4	Bcep1808_3988	Bcep1808_3988	-156	6.2	ccGGAAAACGTTTTCCAA	udhR
		Bcep1808_3989	-88	6.2	TTGGAAAACGTTTTCCgg	udh
		Bcep1808_3987	-143	6.08	TgGaAAAACGTTTTCCAc	exuT

**Table S2. (A) Primers used for cloning of analyzed transcriptional regulators.**

Gene locus tag	Uniprot ID	Direction / Restriction site	Gene name	Primer sequence
Rpic_0945	B2U998	forward / NdeI reverse / BamHI	<i>gguR</i>	GAGACCATATGCCACCCGCGAGTTC CGTACAACATGGATCCGTATTGGCCGG
Bpro_3110	Q128L8	forward / NdeI reverse / BamHI	<i>gguR</i>	CATTTGCTTGTCAACGGTTTTCATATGACCCTCAGGAAAACC GCAACAGAGGTGTACGGATCCAGTCAGGCCTGGTTC
Bpro_3418	Q127G5	forward / NdeI reverse / BamHI	<i>gudR</i>	CGTCGATAGAATCGGCATATGAACCACGAGGAC GTGGTGGGATCCAAATGCGGGGTCACTC

**(B) Primers used for cloning of analyzed lactonases and TctC transporters.**

Gene locus tag	Uniprot ID	Direction / Restriction site	Gene name	Primer sequence
Rpic_4446	B2UIY8	forward / reverse /	<i>uxuL</i>	TACTTCCAATCCATGGATGGCGTTTGAATGGTGCTTG TATCCACCTTTACTGTTACTACTCCCCTACCTGGCGC
PSPTO_1052	Q888H2	forward / reverse /	<i>uxuL</i>	TACTTCCAATCCATGGATGCCGAAGTATAGTCGATGC TATCCACCTTTACTGTTAGCCCTGAAAACGGGTTCCTC
Bcep1808_2255	A4JG52	forward / reverse /	<i>uxuF</i>	TACTTCCAATCCATGCCGAACACTGATCGCCTGAC TATCCACCTTTACTGTTAAATCATCGCGATCCAGTTCGCAC
BMULJ_02167	A9AG49	forward / reverse /	<i>uxuF</i>	TACTTCCAATCCATGCCGAACACTGACCTTCCGAC TATCCACCTTTACTGTTAGACGATCGCGATCCAGTTCG
Bcep18194_A5499	Q39EM3	forward / reverse /	<i>uxuF</i>	TACTTCCAATCCATGCCGAACACTGACCCGCGC TATCCACCTTTACTGTTAGACGATCGCGACCCAGTTC
PSPTO_2765	Q881W7	forward / reverse /	<i>uxuF</i>	TACTTCCAATCCATGGTGCAGGCAGTACCTTCG TATCCACCTTTACTGTTATTTGTCCGGTGGCTGAGC
Bpro_3101	Q128M7	forward / reverse /	<i>tctC</i>	TACTTCCAATCCATGCCAAACGCCAAACTGGCCGC TATCCACCTTTACTGTTACTCCAGCTTGATACCCGCG

**(C) Candidate regulator-binding sites tested by DNA-binding assays.**

Regulator	Organism <sup>1</sup>	First gene locus tag <sup>2</sup>	Regulated operon	DNA fragments used in EMSA and FPA DNA-binding assays <sup>3</sup>
<b>FPA</b>				
Bpro_3110 (GguR)	POL	Bpro_3109	<i>udh-uxuL1-uxuPQM-garD-garL-garR-tctC1-aldE</i>	a c c c c C A G T C A T C A G A C A C C T c c c c c
Rpic_0945 (GguR)	RP	Bpro_3110 (NC)	-	a c c c c a t a a g a g t a c a c t c c c c c
		Rpic_0946	<i>gudD-exuT-agl-omp-aldE</i>	a c c c c G T T G T A C G A C G A C T G c c c c c
		Rpic_0946 (NC)	-	a c c c c a t g t t a c a c g c a c c g t a c c c c
<b>EMSA</b>				
Rpic_0945 (GguR)	RP	Rpic_0946	<i>gudD-exuT-agl-omp-aldE</i>	g g g g g a t a g t c g c a t c c g t g t g t a c g a c g a c t g a t g a c a t a c g t g g g g g
Bpro_3418 (GudR)	POL	Bpro_3419 (sites 1&2)	<i>kdgD-tctC4-gudD-kgsD</i>	g g g g g t t c A A A A G G T A G C G C T A C C A T T C a a g g e t t c A A T G G T A G C G C T A C C T T T A c c t g g g g g

<sup>1</sup> RP, *Ralstonia pickettii* 12J; POL, *Polaromonas* sp. JS666.

<sup>2</sup> NC, a negative control DNA fragments contain scrambled sequence of the original target DNA fragment.

<sup>3</sup> Candidate binding sites are capitalized. For each DNA fragment, two complimentary single-stranded oligonucleotides have been synthesized by IDT.

The fragments were 5' labeled by either 6-carboxyfluorescein (for FP) or biotin (for EMSA) as marked by red letters.

**(D) Primers used for RT-qPCR in *Ralstonia pickettii* 12J.**

Gene locus tag	Gene name	Direction	Primer sequence
<b>(I) GguR regulon</b>			
Rpic_4453	<i>gulR</i>	forward	ATTGACGCTGAAGGAGATGGTG
		reverse	CACGGCACGGCAATCAG
Rpic_4452	<i>kdgD</i>	forward	GTCTACAACCGCCCAACC
		reverse	GGCCCGGCATAGGTAAG
Rpic_4451	<i>kgsD</i>	forward	TCCGCATATTCAGGCAGTGG
		reverse	AGTGCCTCGGGCAACAGG
Rpic_4450	<i>garP</i>	forward	TGGCGGTCGTGGAGAG
		reverse	GTTTCGGACCTCAGCCTTG
Rpic_4449	<i>gudD2</i>	forward	TTGCCGATTTCAAGCTCAAGG
		reverse	TGAGCGACCATGCACCATTC
Rpic_4448	<i>garD</i>	forward	GCCGGTCTGCGTCTG
		reverse	CTGGCGCTTTCAGATGTT
Rpic_4447	<i>udh</i>	forward	GTTCCGACGCTCGAACCC
		reverse	GCTGTTCCGCAACACCTTG
Rpic_4446	<i>uxuL</i>	forward	CCGCTCGGCAAGCTGTATC
		reverse	ATGTCGTAATCGAAGGCCAGAC
Rpic_4445	<i>exuT2</i>	forward	TGTGGAGCGTGTGGTTC
		reverse	CGACAGAAAGCGCGGAATG
<b>(II) GguR regulon</b>			
Rpic_0945	<i>gguR</i>	forward	GTCGCCATTGAGACGGAGTG
		reverse	GAGGTGGCGCTGTCGTTG
Rpic_0946	<i>gudD</i>	forward	CGTGCGCCACGAAGAAG
		reverse	ATCGCGGCTCAGCACAC
Rpic_0947	<i>exuT2</i>	forward	TCTACCGTTCGCCGAAAGACC
		reverse	AGCGCAATCGCCAGAAAC
Rpic_0948	<i>agl</i>	forward	GGGTGCTGATTTGCGATG
		reverse	GCTCTGACGTTGGCCTTCC
Rpic_0949	<i>omp</i>	forward	CCCACGTTTGGCGTCTC
		reverse	AACGCCTGGTAGTCATAACGCATC
Rpic_0950	<i>aldE</i>	forward	AACTCGGCTTGCCTATGTG
		reverse	TGCGGGCGAAATATGGATG



**Table S3. Database identifiers for proteins characterized in this work.**

<b>Locus tag</b>	<b>UniProt Accession</b>	<b>Name</b>	<b>Genbank Accession</b>	<b>Current Genbank annotation</b>	<b>Newly proposed annotation</b>
Bcep1808_2255	A4JG52	UxuF	ABO55255	3-carboxymuconate cyclase-like protein	Uronate lactonase, PF10282 family
Bcep18194_A5499	Q39EM3	UxuF	ABB09093	3-carboxymuconate cyclase-like protein	Uronate lactonase, PF10282 family
BMULJ_02167	A9AG49	UxuF	BAG44069	6-phosphogluconolactonase	Uronate lactonase, PF10282 family
Bpro_3101	Q128M7	TctC	ABE45015	Uncharacterized protein UPF0065	Substrate binding protein of aldarate transporter, TTT family
Bpro_3110	Q128L8	GguR	ABE45024	transcriptional regulator, GntR family	Transcriptional regulator for hexuronate/aldarate utilization, GntR family
Bpro_3418	Q127G5	GudR	ABE45327	transcriptional regulator, LacI family	Transcriptional regulator for glucarate utilization, LacI family
PSPTO_1052	Q888H2	UxuL	AAO54583	senescence marker protein-30 family protein	Uronate lactonase, PF08450 family
PSPTO_2765	Q881W7	UxuF	AAO56265	conserved hypothetical protein	Uronate lactonase, PF10282 family
Rpic_0945	B2U998	GguR	ACD26095	GntR domain protein	Transcriptional regulator for hexuronate/aldarate utilization, GntR family
Rpic_4446	B2UIY8	UxuL	ACD29539	SMP-30/Gluconolactonase/LRE domain protein	Uronate lactonase, PF08450 family