Supplemental information for:

Feeding on compatible solutes: a substrate-induced pathway for uptake and catabolism of ectoines and its genetic control by EnuR

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Running title: Ectoines as nutrients

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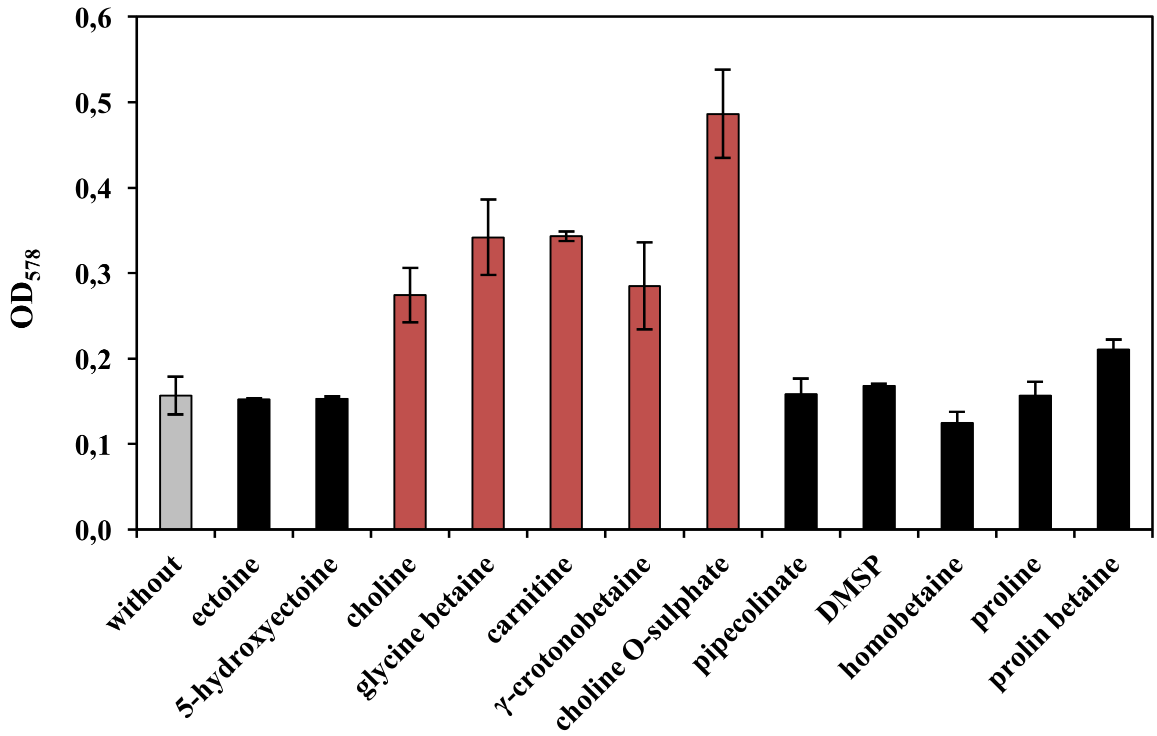


Fig. S1. Identification of osmostress protectants for *R. pomeroyi.* Growth of the *R. pomeroyi* strain J470 in basal minimal medium containing 1 M NaCl in the presence of different compatible solutes (1 mM) is shown. The growth yield of the cultures was recorded after their incubation for 48 h at 30°C. A reference culture of *R. pomeroyi* strain J470 grown without the addition of a compatible solute to the growth medium is marked in grey. Red bars indicate an osmoprotective effect of the added compatible solute. Growth of cultures marked in black bars shows no osmoprotection by the added compatible solute.

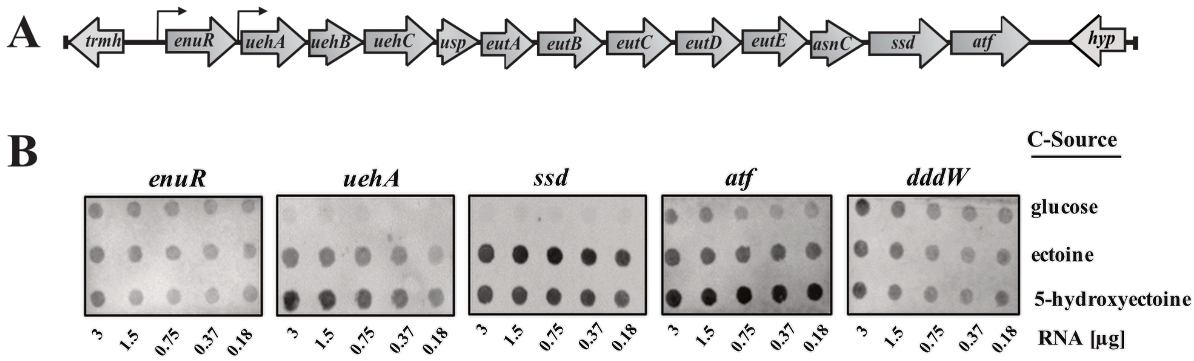


Fig. S2. Transcriptional analysis of the *enuR-uehABC-usp-eutABCDE-asnC-ssd-atf* gene cluster (A) Genetic map of the *enuR-uehABC-usp-eutABCDE-asnC-ssd-atf* genes in the genome of *R. pomeroyi* DSS-3. The approximate position of two promoters mediating the expression of the gene cluster are indicated by bend arrows. (B) Northern Dot Blot analyses of the level of transcription of the *R. pomeroyi enuR*, *uehA*, *ssd*, *atf* and *dddW* genes. Total RNA was prepared from *R. pomeroyi* DSS-3 cells grown in basal medium containing either glucose, ectoine, or 5-hydroxyectoine as the sole carbon source and was spotted at different concentrations (3 µg - 0.18 µg) onto a nylon membrane. The RNA was then hybridized to DIG-labeledanti-sense RNA transcripts prepared *in vitro* for the specific genes under study. The transcript of the DMSP lyase gene (*dddW*) from *R. pomeroyi* DSS-3, a gene involved in DMSP catabolism (Todd et al.,2012a), was used as a control.

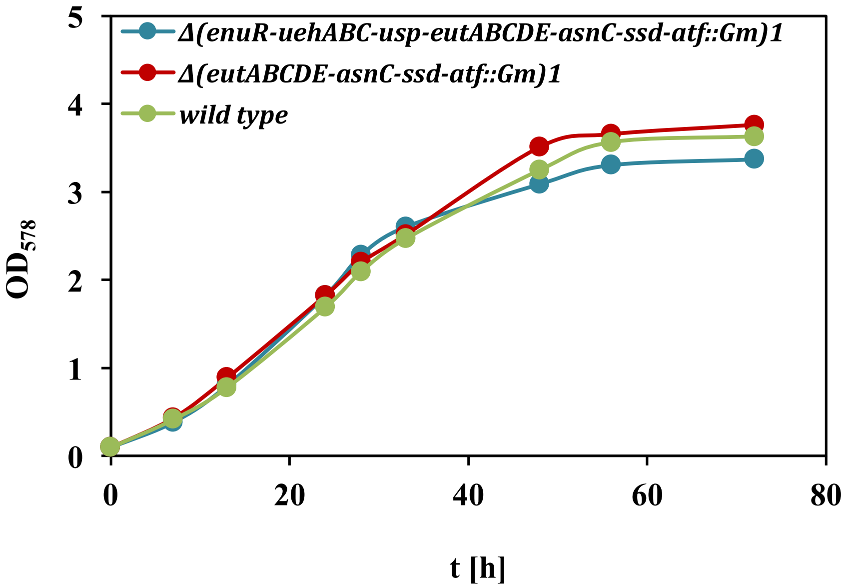
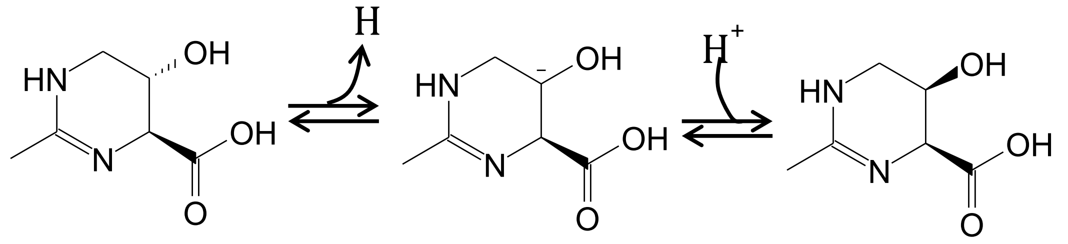


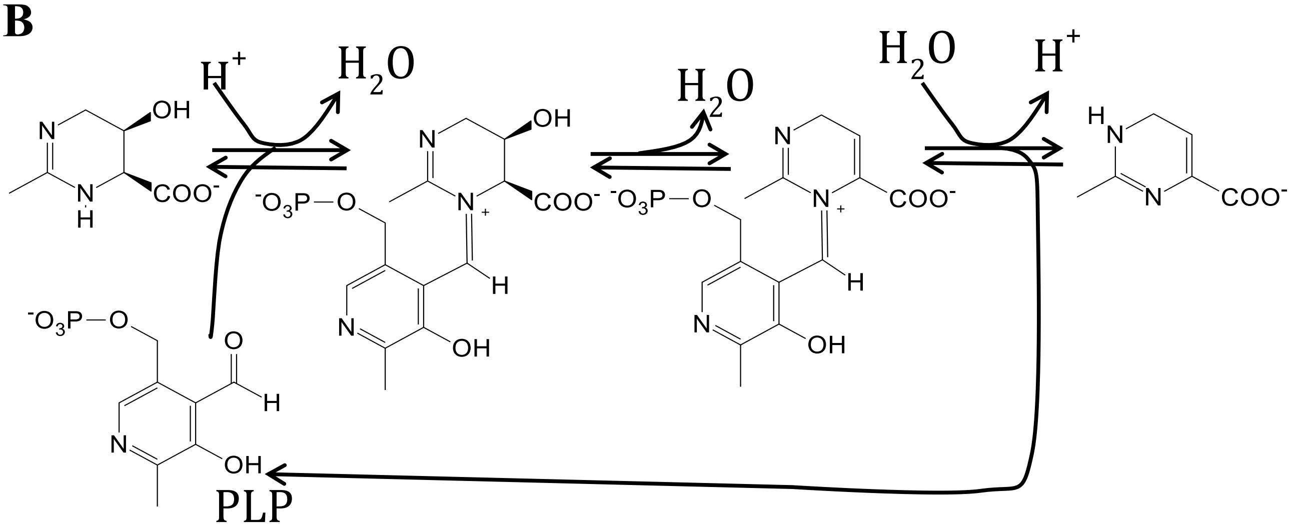
Fig. S3. Growth curves of *R. pomeroyi* cultures in basal media containing glucose (28 mM) as a carbon source and NH_4Cl as a nitrogen source. Shown are *R. pomeroyi* strains J470 (wild type), ASR12 [Δ*(eutABCDE-asnC-ssd-atf::Gm*)1], and ASR6 [Δ(*enuR-uehABC-usp-eutABCDE-asnC-ssd-atf::Gm*)1].

A



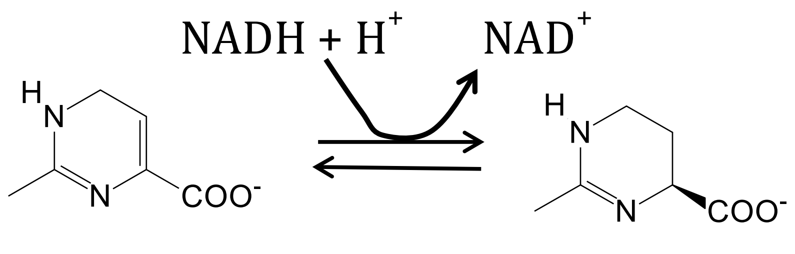
Predicted mechanism of 5-hydroxyectoine racemase EutA

B



Predicted mechanism of 5-(*R*)-hydroxyectoine dehydratase EutB

C



Predicted mechanisms of the ectoine dehydrogenase EutC

Fig. S4. Description of the EutABC-mediated conversion of 5-hydroxyectoine to ectoine. The reaction conducted by each of these enzymes is depicted.

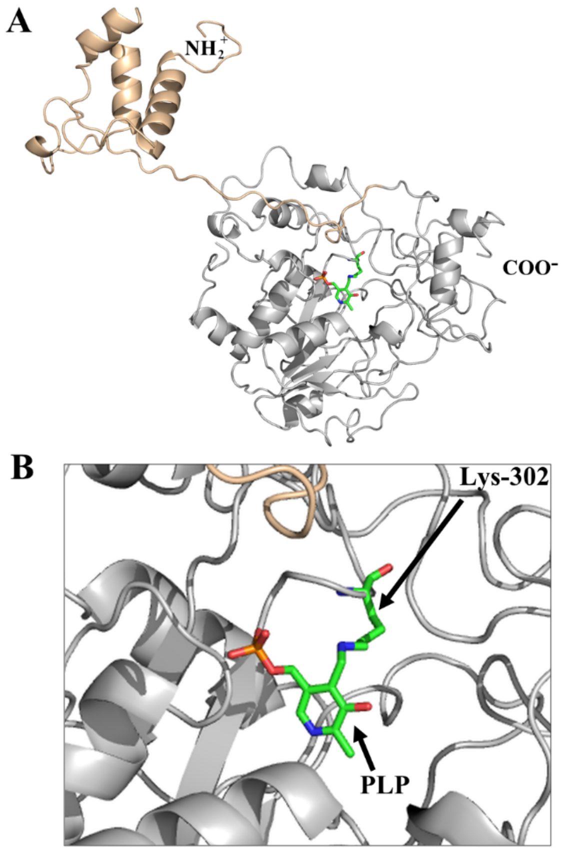
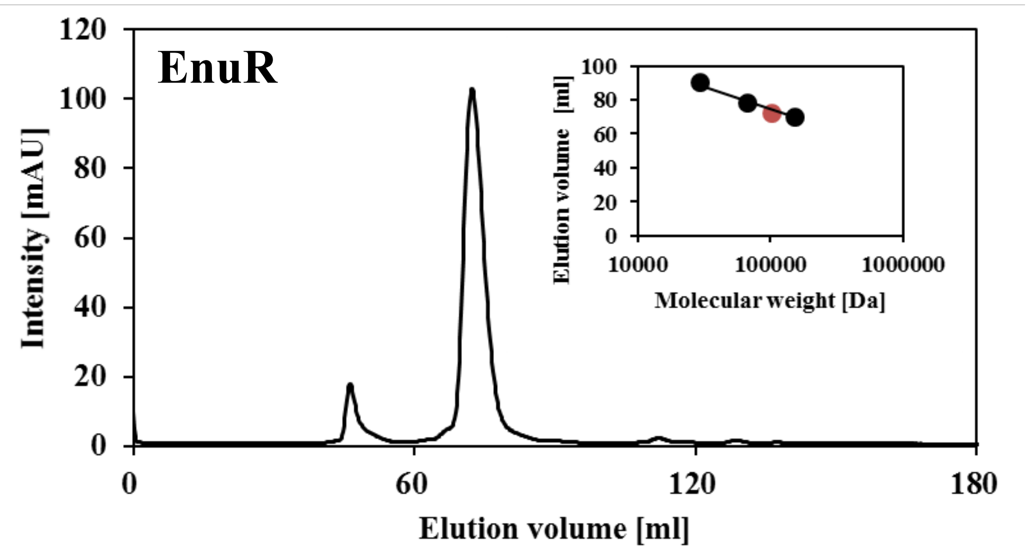


Fig. S5. Model of the EnuR regulatory protein. (A) The crystal structure of the *Bacillus subtilis* GabR protein in complex with PLP (protein database accession code 4N0B) (Edayathumangalam et al., 2013) was chosen as a template to generate an *in silico* model of the transcriptional regulator EnuR. For the visualization of this structure the PyMol suit (https://www.pymol.org/) was used. The helix-turn-helix DNA binding motif of the modelled EnuR protein is depicted in yellow. The C-terminal aminotransferase I-like domain of EnuR is shown in grey. (B) The PLP cofactor (shown as sticks) covalently attached to Lys-302 was modelled into the structure using an overlay of the EnuR model and the GabR crystal structure template.

A



B

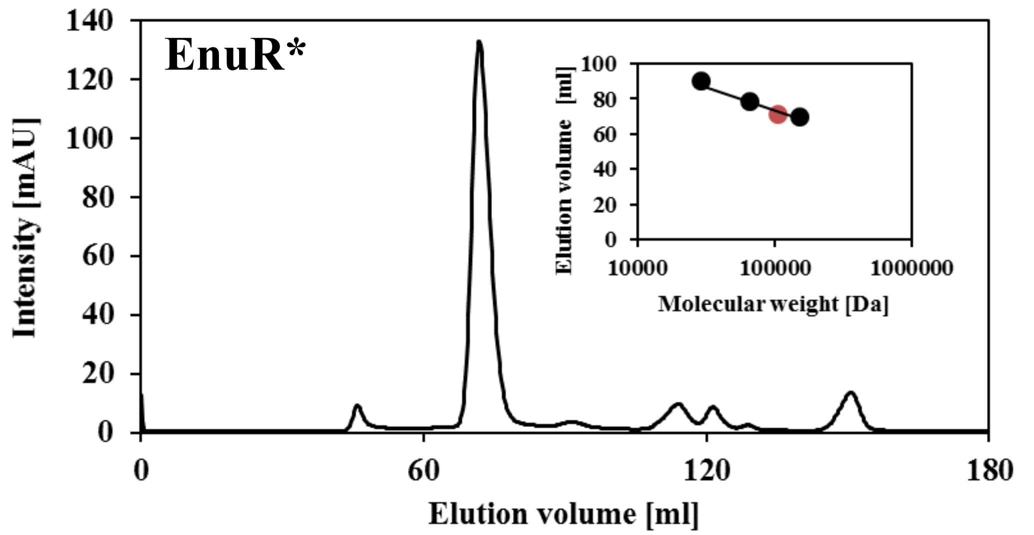


Fig. S6. Assessment of the quaternary assembly of the EnuR-*Strep*-tag II protein and its Lys302/His mutant (EnuR*-*Strep*-tag II). Recombinant EnuR-*Strep*-tag II (A) and EnuR*-*Strep*-tag II (B) were purified by affinity chromatography on a streptactin matrix and analyzed by size exclusion chromatography. In the inserts, the chromatographic behavior of the EnuR-*Strep*-tag II and EnuR*-*Strep*-tag II proteins (approximately 103 kDa) (red dots) is shown relative to that of the marker proteins, carbonic anhydrase from bovine erythrocytes (29 kDa), albumine from bovine serum (66 kDa), and alcohol dehydrogenase from yeast (150 kDa) (black dots).

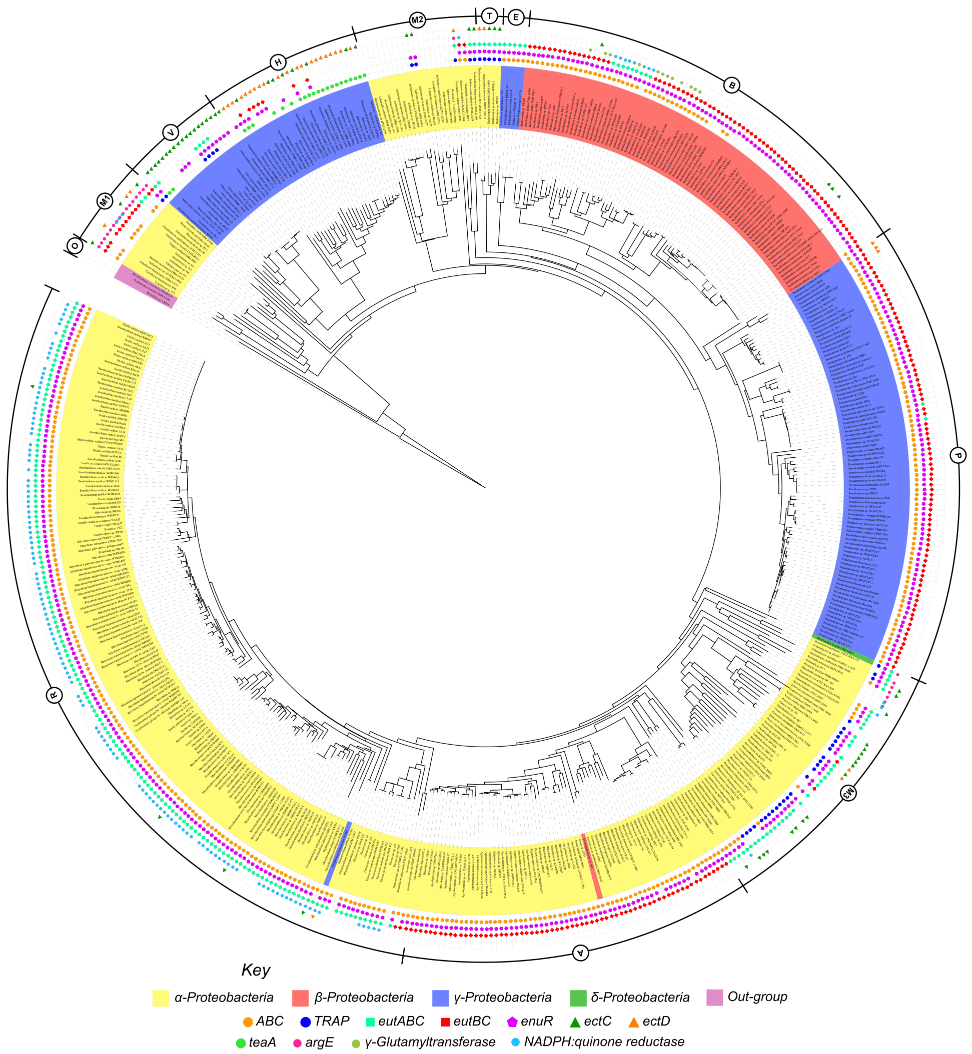


Fig. S7. Taxonomic distribution of 5-hydroxyectoine/ectoine uptake and catabolic genes. An alignment of 539 amino acid sequences homologous to the EutD protein from *Ruegeria pomeroyi* DSS-3 was used to construct a phylogenetic tree. Sequences were identified through a BLAST search on the JGI web server and aligned using ClustalΩ. The phylogenetic tree was constructed using the iTOL web-server (Letunic and Bork, 2011). The color code outlines the distribution of EutD among the classes of the *Proteobacteria*. The presence of a transporter in the direct vicinity of the predicted *eutD* gene cluster is indicated by an orange (ABC-type), or dark blue (TRAP-type), circle. A green circle indicates the presence of a TeaABC-type TRAP transporter. Pink pentagons indicate an association of an *enuR*-type *regulatory* gene with the *eutD* gene cluster. The presence of 5-hydroxyectoine degradation genes is depicted using green (presence of *eutABC* genes) or red (presence of only *eutBC* genes) squares. The presence of genes that were not found in the *eutD* gene clusters derived from *S. meliloti* or *R. pomeroyi* are presented in pink (*argE*), light blue (NADPH:quinone reductase), or green (γ-glutamyltransferase) circles. The presence of an *ectABC* gene cluster (ectoine synthesis genes) is indicated by green triangles; the presence of an *ectD* gene (ectoine hydroxylase) in the respective genome sequences is marked by an orange triangle.

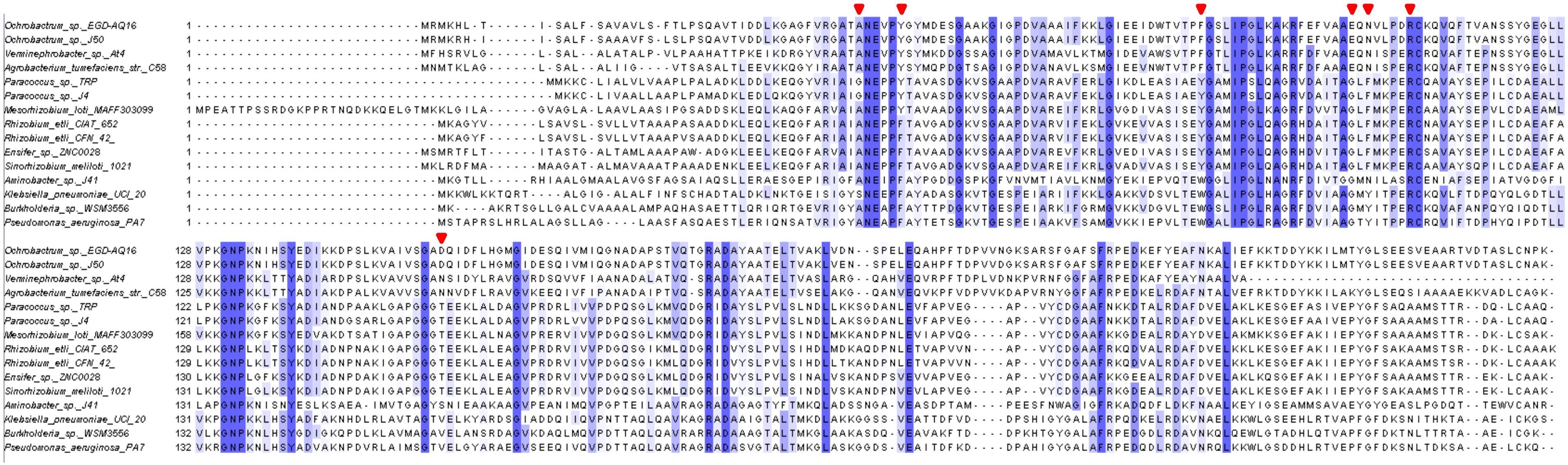


Fig. S8. Amino acid sequence alignment of the 5-hydroxyectoine/ectoine ligand binding protein (EhuB) from the EhuABCD ABC transporter from *S. meliloti* with selected EhuB-type solute receptor proteins from predicted 5-hydroxyectoine/ectoine catabolizing bacteria. The red arrowheads mark those amino acid residues involved in ligand binding (Hanekop et al., 2007).

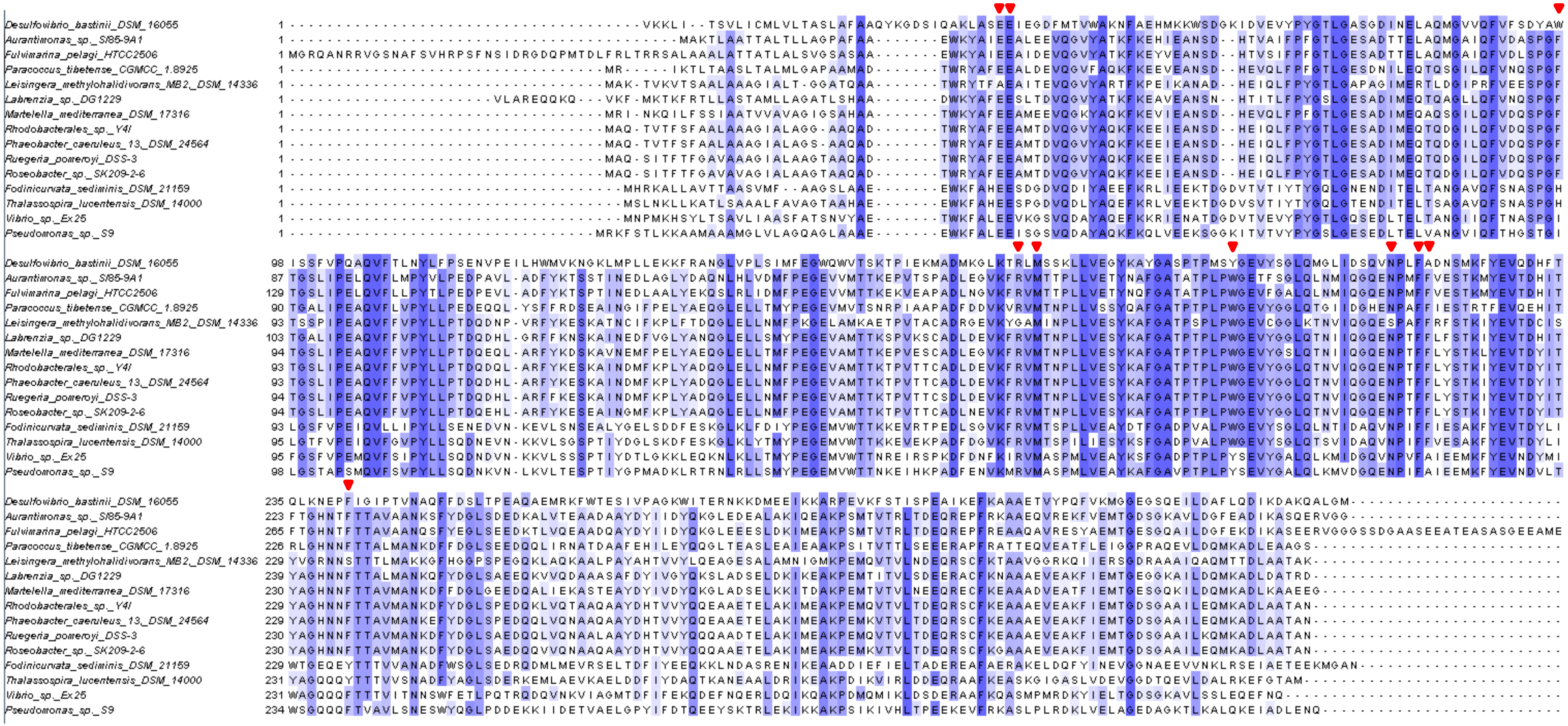


Fig. S9. Amino acid sequence alignment of the 5-hydroxyectoine/ectoine ligand-binding protein (UehA) from the UehABC TRAP transporter from *R. pomeroyi DSS-3* with selected UehA-type solute receptor proteins from predicted 5-hydroxyectoine/ectoine-catabolizing bacteria. The red arrowheads mark those amino acid residues involved in ligand binding (Lecher et al., 2009).

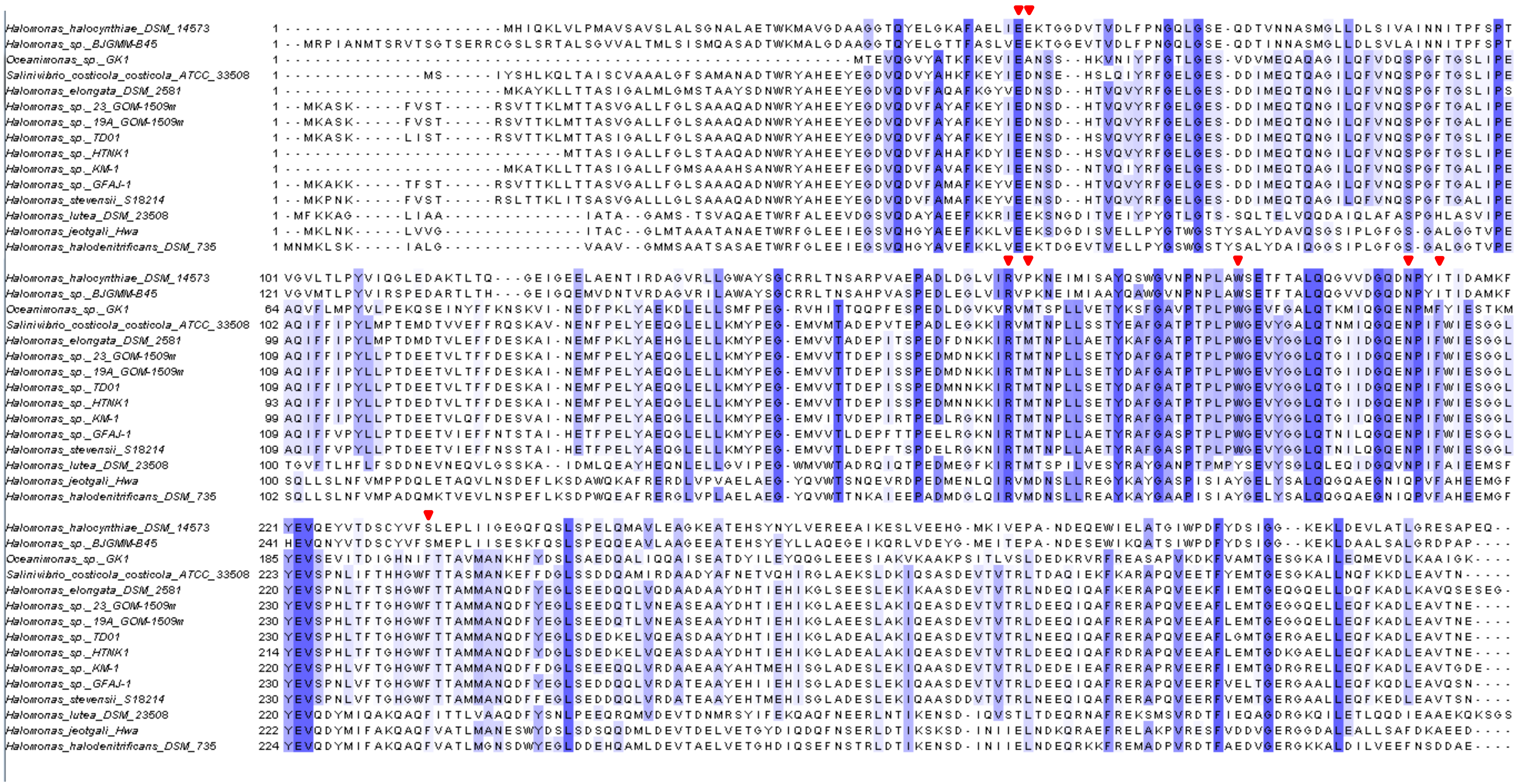


Fig. S10. Amino acid sequence alignment of the 5-hydroxyectoine/ectoine ligand-binding protein (TeaA) from the TeaABC TRAP transporter from *H. elongata* with selected TeaA-type solute receptor proteins from predicted 5-hydroxyectoine/ectoine-catabolizing bacteria. The red arrowheads mark those amino acid residues involved in ligand binding (Kuhlmann et al., 2008).

Table S1. Bacterial strains and plasmids used in this study.

Plasmid	**Genotype/description**	**Reference/source**
pK18mobsacB	Suicide vector for *R. pomeroyi*, Kan^R	(Kvitko and Collmer, 2011)
pBIO1878	Vector with *lacZ* reporter gene	(Todd et al., 2012)
p34S_Gm	Plasmid carry a gentamicin (Gm) resistance cassette	(Dennis and Zylstra, 1998)
pBAS2[1]	Synthetic, codon-optimized *enuR* gene cloned into pENTRY-IBA20	This study
pBAS3	Synthetic, codon-optimized *enuR* gene cloned into pASG-IBA3	This study
pBAS17	pBAS3 with codon exchange mutation (AAA/CAT) in *enuR* leading to the replacement of Lys-302 with a His residue	This study
pBAS19	Upstream region (1.1 kbp) of *enuR* cloned into pBIO1878	This study
pBAS21	Contains 500 bp upstream of *uehA* cloned into pBIO1878	This study
pBAS24	Flanking regions of the *enuR*-operon and a Gm^R cassette cloned into pK18mobsacB	This study
pBAS34	Carries flanking regions of the first 975 bp of the *enuR*-gene and a Gm^R cassette cloned into pK18mobsacB	This study
pBAS35	Carries flanking regions of the *eutD*-gene and a Gm^R cassette cloned into pK18mobsacB	This study
pBAS42	Carries flanking regions upstream of *asnC*and downstream of *atf* and a Gm^R cassette cloned into pK18mobsacB	This study
pBAS44	Carries flanking regions upstream of *eutA*and downstream of *atf* and a Gm^R cassette cloned into pK18mobsacB	This study
pBAS45	Carries flanking regions upstream of *eutA*and downstream of *eutC* and a Gm^R cassette cloned into pK18mobsacB	This study
pBAS49	Carries flanking regions upstream of *ssd*and downstream of *atf* and a Gm^R cassette cloned into pK18mobsacB	This study
Strain		
R. pomeroyi DSS-3	Wild type	(Moran et al., 2004)
J470[2]	Rif^R derivative of *R. pomeroyi* DSS-3	(Todd et al., 2011)
ASR6[2]	Δ(*enuR-uehABC-usp-eutABCDE-asnC-ssd-atf::Gm*)1	This study
ASR7[2]	Δ(*enuR::Gm*)1	This study

ASR8[2]	Δ(*eutD::Gm*)1	This study
ASR10[2]	Δ(*asnC-ssd-atf::Gm*)1	This study
ASR11[2]	Δ(*eutABC::Gm*)1	This study
ASR12[2]	Δ(*eutABCDE-asnC-ssd-atf::Gm*)1	This study
ASR11[2]	Δ(*ssd-atf::Gm*)1	This study

[1]The DNA-sequence of the codon-optimized *enuR* Gene has been deposited in GenBank under accession number KU891821.

[2]These strains are all derivatives of the Rif^R mutant of *R. pomeroyi* J470 (Moran et al., 2004).

Table S2. DNA primers used in this study.

Name	Sequence (5´-3´)	Description
LacZenuR_up_for	GCGAATTCCTTCATGTTCAGCGCCCTC	Forward PCR primer for cloning of pBAS19
LacZenuR_PstI_rev	GCCTGCAGGATCGGGCAGCCAATTTG	Reverse PCR primer for cloning of pBAS19
LacZuehA_PstI_rev	GCCTGCAGCAAAGGTGAAAGTGATGGATTGAG	Forward PCR primer for cloning of pBAS21
LacZuehA_EcoRI_for	GCGAATTCCGGAGCGATACGAAATCG	Reverse PCR primer for cloning of pBAS21
enuR_ehuA_fw	CGTCCGCCAGAGGGAGTTGC	Forward RT_PCR primer for amplification of the intergenic region between *gntR_ehuA*
enuR_ehuA_rev	CGTCCAGATCCGAACAGGTGG	Reverse RT_PCR primer for amplification of the intergenic region between *gntR_ehuA*
ehuA_ehuC_fw	GCTGCGATCCTGAAACAGATGAAGG	Forward RT_PCR primer for amplification of the intergenic region between *ehuA_ehuC*
ehuA_ehuC_rev	CGCCGAACAGCATCAGAAAGCC	Reverse RT_PCR primer for amplification of the intergenic region between *ehuA_ehuC*
ehuC_eutA_fw	GTTCATCCTGATGCTGCTGGGG	Forward RT_PCR primer for amplification of the intergenic region between *ehuC_eutA*

ehuC_eutA_rev	GGGCAATCCGTTTCCTGACG	Reverse RT_PCR primer for amplification of the intergenic region between *ehuC_eutA*
eutA_eutB_fw	CGTCAGGAAACGGATTGCCC	Forward RT_PCR primer for amplification of the intergenic region between *eutA_eutB*
eutA_eutB_rev	CTGCCGGTGATCTGCCTGC	Reverse RT_PCR primer for amplification of the intergenic region between *eutA_eutB*
eutB_eutC_fw	GCTGGTGAGCGGTCAGAACATCG	Forward RT_PCR primer for amplification of the intergenic region between *eutB_eutC*
eutB_eutC_rev	GCACATCGGTCAGATAGCCATTGTCC	Reverse RT_PCR primer for amplification of the intergenic region between *eutB_eutC*
eutC_eutD_fw	GATCACCACAACACCCGCCAGC	Forward RT_PCR primer for amplification of the intergenic region between *eutC_eutD*
eutC_eutD_rev	CGGCTCTCCCTCCAGTGTCAGC	Reverse RT_PCR primer for amplification of the intergenic region between *eutC_eutD*
eutD_eutE_fw	CGAGTGTCTGTCCAATGTGCCG	Forward RT_PCR primer for amplification of the intergenic region between *eutD_eutE*
eutD_eutE_rev	CGCATGGATCAGGACGTTGC	Reverse RT_PCR primer for amplification of the intergenic region between *eutD_eutE*

eutE_ssd_fw	GCAACGTCCTGATCCATGCGG	Forward RT_PCR primer for amplification of the intergenic region between *eutE_ssd*
eutE_ssd_rev	CGCACCACTTGCCGTTTATGTAGG	Reverse RT_PCR primer for amplification of the intergenic region between *eutE_ssd*
ssd_atf_fw	GCGAATGACAGCGAATACGGGC	Forward RT_PCR primer for amplification of the intergenic region between *ssd_atf*
ssd_atf_rev	CCGGCAGATCGAATTTCTTGTGG	Reverse RT_PCR primer for amplification of the intergenic region between *ssd_atf*
NB_gntR_fw	GATTGATCGACGGCCAGGTTGG	Forward primer for amplification of an *enuR* gene fragment that is used for *in-vitro* transcription using T7-RNA Polymerase
NB_gntR_revT7	TAATACGACTCACTATAGGGAGGCAGCAGGAACAGGACCTTGGCG	Reverse primer for amplification of an *enuR* gene fragment that is used for *in-vitro* transcription using T7-RNA Polymerase
NB_uehA_fw	TGGCTCAATCCATCACTTTCACC	Forward primer for amplification of an *uehA* gene fragment that is used for *in-vitro* transcription using T7-RNA Polymerase
NB_uehA_revT7	TAATACGACTCACTATAGGGAGGACCTCGTCCAGATCCGAACAGG	Reverse primer for amplification of an *uehA* gene fragment that is used for *in-vitro transcription* using T7-

		RNA Polymerase
NB_eutD_fw	CTGGATCTTCTGATCGTCACAGACC	Forward primer for amplification of an *eutD* gene fragment that is used for in-vitro transcription using T7-RNA Polymerase
NB_eutD_revT7	TAATACGACTCACTATAGGGAGGCGCATCATAGATATCGGCCACC	Reverse primer for amplification of an *eutD* gene fragment that is used for in-vitro transcription using T7-RNA Polymerase
NB_ssd_fw	AACGCCTGGTGATGGAACTGG	Forward primer for amplification of a *ssd* gene fragment that is used for *in-vitro* transcription using T7-RNA Polymerase
NB_ssd_revT7	TAATACGACTCACTATAGGGAGGGCTGTGCACATAGGCCACCAG	Reverse primer for amplification of a *ssd* gene fragment that is used for *in-vitro* transcription using T7-RNA Polymerase
NB_dddW_for	CATTGACGCCGAAAACGTGCC	Forward primer for amplification of a *dddW* gene fragment that is used for *in-vitro* transcription using T7-RNA Polymerase
NB_dddW_rev	TAATACGACTCACTATAGGGAGGCCGTGCCATGTTCCGCATC	Reverse primer for amplification of a *dddW* gene fragment that is used for *in-vitro* transcription using T7-

		RNA Polymerase
NB_atf_for	GGAAAAGAAAAAGATCATCTCGCGC	Forward primer for amplification of an *atfn*gene fragment that is used for *in-vitro* transcription using T7-RNA Polymerase
deltaGF_up_for	ACAGCTATGACATGATTACGCTTCATGTTCAGCGCCCT	Forward primer for the amplification the fragment upstream of *enuR* for the cloning of plasmid pBAS24
deltaGF_up_rev	GGCTTATGTCAATTCGAGCTGCCGTCCGATACCCTAGC	reverse primer for the amplification the fragment upstream of *enuR* for the cloning of plasmid pBAS24
Gentamycin_GF_for	GCTAGGGTATCGGACGGCAGCTCGAATTGACATAAGCC	Forward primer for the amplification of the Gm-resistance cassette from p34S_Gm for the cloning of plasmid pBAS24
Gentamycin_GF_rev	CTGATGTTGACACGCAGGTTAGGTGGCGGTACTTGGG	reverse primer for the amplification of the Gm-resistance cassette from p34S_Gm for the cloning of plasmid pBAS24
deltaGF_down_for	CCCAAGTACCGCCACCTAACCTGCGTGTCAACATCAG	forward primer for the amplification the fragment downstream of *atf* for the cloning of plasmid pBAS24
deltaGF_down_rev	CCAAGCTTGCATGCCTGCAGGTCGACTCTAGCGCGCTGCTTTATCTGG	reverse primer for the amplification the fragment

		downstream of *atf* for the cloning of plasmid pBAS24
deltaEnuR_ingene_up_for	CACACAGGAAACAGCTATGACATGATTACGCTGAAATGGCTATTGCAGGC	Forward primer for the amplification the fragment upstream of *enuR* for the cloning of plasmid pBAS34
rev_deltaEnuR_ingene_up_rev	CCCAAGTACCGCCACCTAAGCAATACATGTACAATTGAGTGACAC	reverse primer for the amplification the fragment upstream of *enuR* for the cloning of plasmid pBAS34
rev_Gentamycin_ingene_for:	GTGTCACTCAATTGTACATGTATTGCTTAGGTGGCGGTACTTGGG	Forward primer for the amplification of the Gm-resistance cassette from p34S_Gm for the cloning of plasmid pBAS34
rev_Gentamycin_ingene_rev:	CAGCCGAAGACGACAATCCAGCTCGAATTGACATAAGCCTG	reverse primer for the amplification of the Gm-resistance cassette from p34S_Gm for the cloning of plasmid pBAS34
rev_deltaEnuR_ingene_down_for	CAGGCTTATGTCAATTCGAGCTGGATTGTCGTCTTCGGCTG	forward primer for the amplification the fragment in *enuR* for the cloning of plasmid pBAS34
rev_deltaEnuR_ingene_down_rev(XbaI)	CCAAGCTTGCATGCCTGCAGGTCGACTCTAGCTTCGAAAGCATACCGCCAA	reverse primer for the amplification the fragment in *enuR* for the cloning of plasmid pBAS34
deltaeutD_up_for:	CACACAGGAAACAGCTATGACATGATTACGGTCTGCATCATCGGCGCAG	Forward primer for the amplification the fragment upstream of *eutD* for the cloning of plasmid pBAS35
deltaeutD_up_rev	CAGGCTTATGTCAATTCGAGCTGTGCTCGGTTCCTGATTGATCAAC	reverse primer for the amplification the fragment

		upstream of *eutD* for the cloning of plasmid pBAS35
Gentamicin_eutD_for	GTTGATCAATCAGGAACCGAGCACAGCTCGAATTGACATAAGCCTG	Forward primer for the amplification of the Gm-resistance cassette from p34S_Gm for the cloning of plasmid pBAS35
Gentamicin_eutD_rev	GAAATCGGGTTCTTCTGCATCGTTAGGTGGCGGTACTTGGG	reverse primer for the amplification of the Gm-resistance cassette from p34S_Gm for the cloning of plasmid pBAS35
deltaeutD_down_for	CCCAAGTACCGCCACCTAACGATGCAGAAGAACCCGATTTC	forward primer for the amplification the fragment downstream of *eutD* for the cloning of plasmid pBAS35
deltaeutD_down_rev(XbaI)	CCAAGCTTGCATGCCTGCAGGTCGACTCTAGCATTTCCTCGACCGCGGTG	reverse primer for the amplification the fragment downstream of *eutD* for the cloning of plasmid pBAS35
deltaasnC/ssd_up_for:	CACACAGGAAACAGCTATGACATGATTACGCCGAGAAAATCGCCGATTACTTC	Forward primer for the amplification the fragment upstream of *asnC* for the cloning of plasmid pBAS42
deltaasnC/ssd_up_rev:	CAGGCTTATGTCAATTCGAGCTGTCCGCCTCTCAGGCGC	reverse primer for the amplification the fragment upstream of *asnC* for the cloning of plasmid pBAS42
Gentamycin_asnC/ssd_for:	GCGCCTGAGAGGCGGACAGCTCGAATTGACATAAGCCTG	Forward primer for the amplification of the Gm-resistance cassette from p34S_Gm for the cloning of plasmid pBAS42

Gentamycin_atf/ssd_rev:	CCATGCAGGATGCGCCTGTTAGGTGGCGGTACTTGGG	reverse primer for the amplification of the Gm-resistance cassette from p34S_Gm for the cloning of plasmid pBAS42 and 49
deltaatf/ssd_down_for:	CCCAAGTACCGCCACCTAACAGGCGCATCCTGCATGG	forward primer for the amplification the fragment downstream of *atf* for the cloning of plasmid pBAS42 and 49
deltaatf/ssd_down_rev(XbaI)	CCAAGCTTGCATGCCTGCAGGTCGACTCTAGCGGAGGTCATCCGCATCTCG	reverse primer for the amplification the fragment downstream of *atf* for the cloning of plasmid pBAS42 and 49
deltaatf/ssd_up_for	CACACAGGAAACAGCTATGACATGATTACGGAGGCGGACCCATGCAACTG	Forward primer for the amplification the fragment upstream of *ssd* for the cloning of plasmid pBAS49
deltaatf/ssd_up_rev	CAGGCTTATGTCAATTCGAGCTGTTGTCCTCCTCGGGTCTTGCAG	reverse primer for the amplification the fragment upstream of *ssd* for the cloning of plasmid pBAS49
Gentamycin_atf/ssd_for	CTGCAAGACCCGAGGAGGACAACAGCTCGAATTGACATAAGCCTG	Forward primer for the amplification of the Gm-resistance cassette from p34S_Gm for the cloning of plasmid pBAS49
deltaEutABC_up_for	CACACAGGAAACAGCTATGACATGATTACGCGGCACCATCATCACCCTG	Forward primer for the amplification the fragment upstream of *eutA* for the cloning of plasmid pBAS44
deltaEutABC_up_rev	CAGGCTTATGTCAATTCGAGCTTTGGTTTCCTAAACAACAAGTACAGGC	reverse primer for the amplification the fragment

		upstream of *eutA* for the cloning of plasmid pBAS44
Gentamycin_EutABC_for	GAAACGCCTGTACTTGTTGTTTAGGAAACCAAAGCTCGAATTGACATAAGCCTG	Forward primer for the amplification of the Gm-resistance cassette from p34S_Gm for the cloning of plasmid pBAS44
Gentamycin_EutABC_rev	GGGTCTGCCATGTGCTCGGTTCCTGATTGATTAGGTGGCGGTACTTGGG	reverse primer for the amplification of the Gm-resistance cassette from p34S_Gm for the cloning of plasmid pBAS44
deltaEutABC_down_for	CTTTGATATCGACCCAAGTACCGCCACCTAATCAATCAGGAACCGAGCACATG	forward primer for the amplification the fragment downstream of *atf* for the cloning of plasmid pBAS44
deltaEutABC_down_rev	CCAAGCTTGCATGCCTGCAGGTCGACTCTAGGCCCGCATCATAGATATCGGC	reverse primer for the amplification the fragment downstream of *atf* for the cloning of plasmid pBAS44
deltaEutABC_up_for	CACACAGGAAACAGCTATGACATGATTACGCGGCACCATCATCACCCTG	Forward primer for the amplification the fragment upstream of *eutA* for the cloning of plasmid pBAS45
deltaEutABC_up_rev	CAGGCTTATGTCAATTCGAGCTTTGGTTTCCTAAACAACAAGTACAGGC	reverse primer for the amplification the fragment upstream of *eutA* for the cloning of plasmid pBAS45
Gentamycin_EutABC_for	GAAACGCCTGTACTTGTTGTTTAGGAAACCAAAGCTCGAATTGACATAAGCCTG	Forward primer for the amplification of the Gm-resistance cassette from p34S_Gm for the cloning of plasmid pBAS45

Gentamycin_EutABC_rev	GGGTCTGCCATGTGCTCGGTTCCTGATTGATTAGGTGGCGGTACTTGGG	reverse primer for the amplification of the Gm-resistance cassette from p34S_Gm for the cloning of plasmid pBAS45
deltaEutABC_down_for	CTTTGATATCGACCCAAGTACCGCCACCTAATCAATCAGGAACCGAGCACATG	forward primer for the amplification the fragment downstream of *eutC* for the cloning of plasmid pBAS45
deltaEutABC_down_rev	CCAAGCTTGCATGCCTGCAGGTCGACTCTAGGCCCGCATCATAGATATCGGC	reverse primer for the amplification the fragment downstream of *eutC* for the cloning of plasmid pBAS45

References

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