

Role of EmaSR in Ethanol Metabolism by *Acinetobacter baumannii*

Supplementary Materials

Fig. S1A

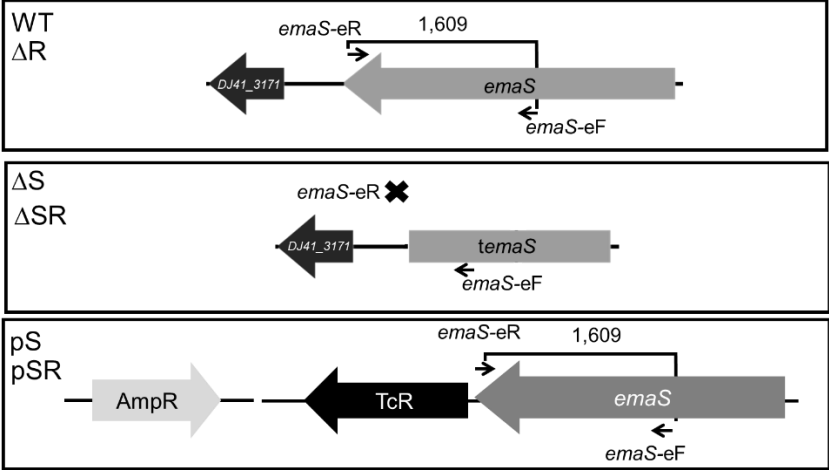


Fig. S1B

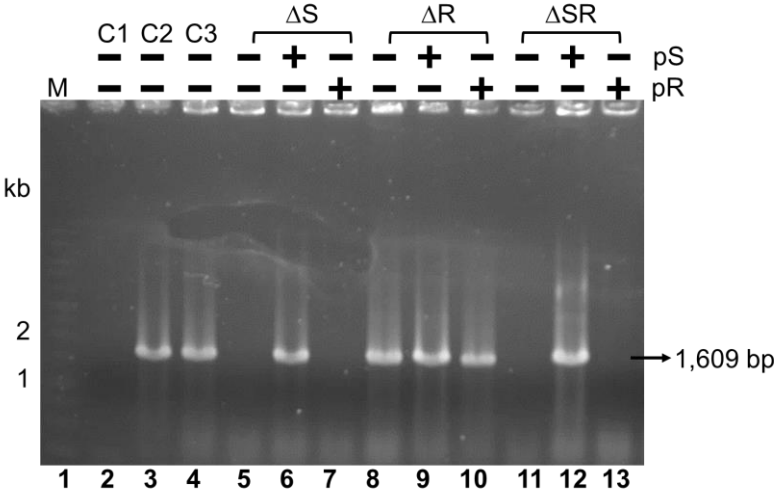


Figure S1. *EmaS* gene analysis of mutants and complementary strains. (A) Diagram of gene localization and primers for mutant and complementary strains. WT represents the wild-type strain. ΔR , ΔS , and ΔSR respectively represent the *emaR*, *emaS*, and *emaSR* mutants, while the pS and pSR strains respectively contain the complementation plasmids for *emaS* and *emaSR*. (B) PCR products analyzed by electrophoresis on a 0.8% agarose gel. The *emaS* gene was amplified by the *emaS*-eR and *emaS*-eF primers, with PCR fragment size of 1,069 bp. *EmaS* mutant strains have no PCR product. C1 is a negative control that used ddH₂O as a template, while C2 used the wild-type chromosomal DNA as a template, and C3 used pS in *E. coli* DH5 α as a template. M represents the DNA size marker.

Fig. S2A

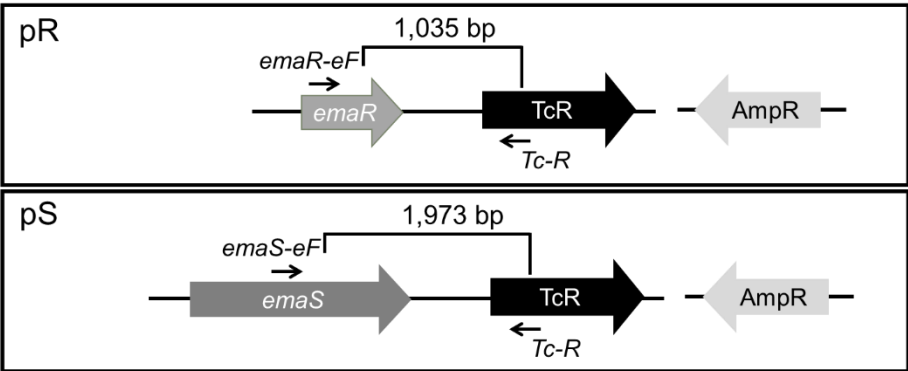


Fig. S2B

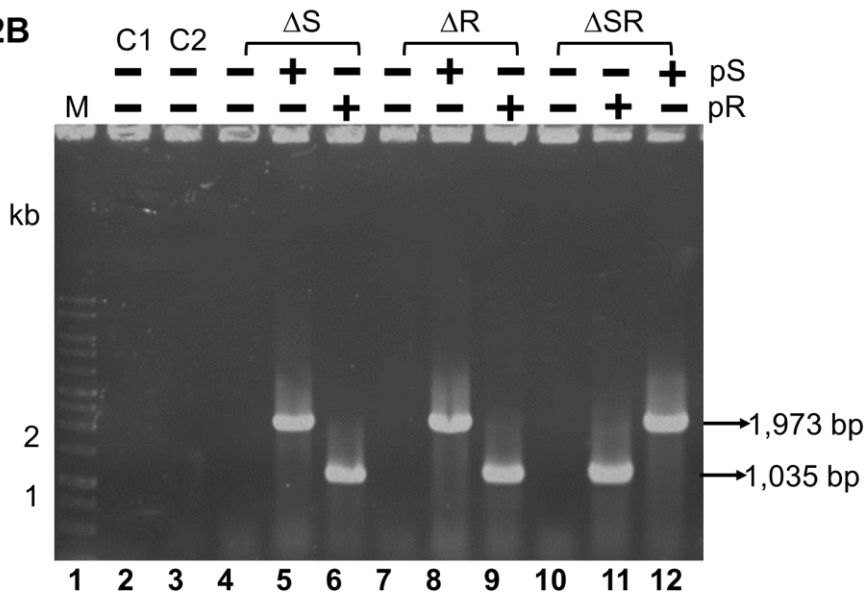


Figure S2. Complementary strains as confirmed by PCR. (A) Diagram of gene localization and primers for pR and pS, with pR confirmed using *emaR-eF* and *Tc-R* as primers, with expected amplification product of 1,035 bp, and pS confirmed using *emaS-eF* and *Tc-R* to amplify a DNA fragment of 1,973 bp in size. (B) PCR products analyzed by electrophoresis on a 0.8% agarose gel. C1 is a negative control that used ddH₂O as a template, while C2 used the wild-type strain without complementary plasmid as a template.

Table S1. Differentially regulated genes in the *ΔemaSR* mutant strain versus the ATCC 19606 wild-type strain in key functional groups.

Category and locus tag	Gene	Log ₂ fold change ^a	p-value	Predicted function
Carbon metabolism				
DJ41_2796	-	6.21	3.53×10^{-9}	succinate CoA transferase family protein
DJ41_569	-	5.78	2.12×10^{-8}	E3 binding domain protein
DJ41_568	<i>lpdA</i>	4.01	1.82×10^{-5}	dihydrolipoyl dehydrogenase
DJ41_2528	-	3.33	0.00019	isocitrate lyase
DJ41_1153	<i>pta</i>	2.34	0.00574	phosphate acetyltransferase
DJ41_1152	<i>ackA</i>	2.23	0.00789	acetate kinase
DJ41_3568	<i>gltA</i>	1.66	0.03356	citrate (Si)-synthase
DJ41_227	<i>fumC</i>	1.61	0.03873	fumarate hydratase, class II
Phenylalanine metabolism				
DJ41_3060	<i>hppD</i>	3.51	0.00012	4-hydroxyphenylpyruvate dioxygenase
DJ41_2050	<i>paaJ</i>	2.43	0.00461	phenylacetate-CoA oxygenase, PaaJ subunit
DJ41_2049	-	2.08	0.01162	2Fe-2S iron-sulfur cluster binding domain protein
DJ41_2052	-	1.96	0.02361	phenylacetic acid degradation B family protein
DJ41_3262	-	1.94	0.01876	pyridine nucleotide-disulfide oxidoreductase family protein
DJ41_2053	<i>paaA</i>	1.90	0.01821	phenylacetic acid degradation protein paaA
DJ41_2051	<i>paaC</i>	1.84	0.02349	phenylacetic acid degradation protein paaC
DJ41_2054	<i>paaN</i>	1.73	0.02970	phenylacetic acid degradation protein paaN
DJ41_2048	-	1.61	0.04592	enoyl-CoA hydratase/isomerase family protein
Biosynthesis of cofactors				
DJ41_568	<i>lpdA</i>	4.01	1.82×10^{-5}	dihydrolipoyl dehydrogenase
DJ41_3060	<i>hppD</i>	3.51	0.00012	4-hydroxyphenylpyruvate dioxygenase
Biosynthesis of siderophore group nonribosomal peptides				
DJ41_1728	-	3.75	8.51×10^{-5}	isochorismate synthase family protein
DJ41_1721	<i>entB</i>	2.59	0.00266	isochorismatase
DJ41_512	-	2.55	0.005	short chain dehydrogenase family protein
DJ41_1720	-	2.25	0.00716	(2,3-dihydroxybenzoyl)adenylate synthase
Purine metabolism				
DJ41_2077	-	-4.36	8.52×10^{-5}	cytidine and deoxycytidylate deaminase zinc-binding region family protein
Tyrosine metabolism				
DJ41_3063	<i>maiA</i>	3.85	8.99×10^{-5}	maleylacetoacetate isomerase
DJ41_3064	<i>fahA</i>	3.53	0.00013	fumarylacetoacetase
DJ41_3060	<i>hppD</i>	3.51	0.00012	4-hydroxyphenylpyruvate dioxygenase
Two-component system				

DJ41_2555	-	2.35	0.00479	TonB-dependent siderophore receptor family protein
Sulfur metabolism				
DJ41_1587	-	-2.11	0.03619	sulfate ABC transporter, sulfate-binding family protein
DJ41_3169	<i>msuE</i>	-2.31	0.02509	FMN reductase

^a Log₂ fold change = Log₂(WT/ Δ *emaSR*)

Table S2. Differentially regulated genes in the $\Delta emaR$ mutant strain versus the ATCC 19606 wild-type strain in key functional groups.

Category and locus tag	Gene	Log ₂ fold change ^a	p-value	Predicted function
Carbon metabolism				
DJ41_2796	-	7.03	2.93×10^{-10}	succinate CoA transferase family protein
DJ41_569	-	4.88	1.37×10^{-6}	e3 binding domain protein
DJ41_568	<i>lpdA</i>	4.64	3.77×10^{-6}	dihydrolipoyl dehydrogenase
DJ41_2528	-	2.82	0.00199	isocitrate lyase
DJ41_1153	<i>pta</i>	2.75	0.00304	phosphate acetyltransferase
DJ41_1152	<i>ackA</i>	2.68	0.00371	acetate kinase
Phenylalanine metabolism				
DJ41_3262	-	1.97	0.0293	pyridine nucleotide-disulfide oxidoreductase family protein
Biosynthesis of cofactors				
DJ41_3276	-	4.72	0.00084	hypothetical protein
DJ41_568	<i>lpdA</i>	4.64	3.77×10^{-6}	dihydrolipoyl dehydrogenase
DJ41_3509	<i>nqO2</i>	3.86	0.01529	ribosyldihydronicotinamide dehydrogenase (quinone)
Biosynthesis of siderophore group nonribosomal peptides				
DJ41_1728	-	2.17	0.01904	isochorismate synthase family protein
Purine metabolism				
DJ41_3122	<i>uraD</i>	3.95	0.03722	OHCU decarboxylase
DJ41_3118	-	2.96	0.01487	FAD binding domain protein
DJ41_3123	-	2.66	0.04244	polysaccharide deacetylase family protein
DJ41_2077	-	-3.83	0.00025	cytidine and deoxycytidylate deaminase zinc-binding region family protein
Two-component system				
DJ41_3249	-	16.78	0.00028	autoinducer synthetase family protein
DJ41_3468	-	2.29	0.01665	cheW-like domain protein
DJ41_1407	-	2.17	0.04247	PAS fold family protein
Bacterial secretion system				
DJ41_1170	<i>tatC</i>	4.47	0.00751	twin arginine-targeting protein translocase TatC

^a Log₂ fold change = Log₂(WT/ $\Delta emaR$)

Table S3. Differentially regulated genes in the *ΔemaS* mutant strain versus the ATCC 19606 wild-type strain in key functional groups.

Category and locus tag	Gene	Log ₂ fold change ^a	p-value	Predicted function
Carbon metabolism				
DJ41_2796	-	6.66	6.88×10^{-10}	succinate CoA transferase family protein
DJ41_2528	-	2.65	0.00208	isocitrate lyase
DJ41_1152	<i>ackA</i>	2.47	0.00443	acetate kinase
DJ41_1153	<i>pta</i>	2.47	0.00433	phosphate acetyltransferase
DJ41_227	<i>fumC</i>	1.73	0.03169	fumarate hydratase, class II
DJ41_1959	-	-2.15	0.03472	catalase family protein
DJ41_2119	-	-2.67	0.00812	D-ala D-ala ligase family protein
DJ41_568	<i>lpdA</i>	-2.91	0.00352	dihydrolipoyl dehydrogenase
DJ41_569	-	-3.05	0.00219	e3 binding domain protein
Phenylalanine metabolism				
DJ41_3060	<i>hppD</i>	3.21	0.00037	4-hydroxyphenylpyruvate dioxygenase
DJ41_3262	-	2.51	0.00415	pyridine nucleotide-disulfide oxidoreductase family protein
DJ41_2051	<i>paaC</i>	2.03	0.01538	phenylacetic acid degradation protein paaC
DJ41_2054	<i>paaN</i>	2.00	0.01565	phenylacetic acid degradation protein paaN
DJ41_2050	<i>paaJ</i>	1.97	0.01879	phenylacetate-CoA oxygenase, PaaJ subunit
DJ41_2052	-	1.73	0.04316	phenylacetic acid degradation B family protein
DJ41_2053	<i>paaA</i>	1.57	0.04764	phenylacetic acid degradation protein paaA
Biosynthesis of cofactors				
DJ41_3509	<i>nqO2</i>	3.28	0.02682	ribosyldihydronicotinamide dehydrogenase quinone
DJ41_3060	<i>hppD</i>	3.21	0.00037	4-hydroxyphenylpyruvate dioxygenase
DJ41_3276	-	3.06	0.01392	hypothetical protein
DJ41_572	<i>lipA</i>	-2.65	0.01033	lipoyl synthase
DJ41_568	<i>lpdA</i>	-2.91	0.00352	dihydrolipoyl dehydrogenase
Biosynthesis of siderophore group nonribosomal peptides				
DJ41_1728	-	4.34	1.29×10^{-5}	isochorismate synthase family protein
DJ41_1721	<i>entB</i>	3.80	5.65×10^{-5}	isochorismatase
DJ41_1720	-	3.51	0.00014	(2,3-dihydroxybenzoyl)adenylate synthase
DJ41_512	-	3.44	0.00041	short chain dehydrogenase family protein
Purine metabolism				
DJ41_3122	<i>uraD</i>	3.52	0.04837	OHCU decarboxylase
DJ41_3123	-	2.84	0.02402	polysaccharide deacetylase family protein
DJ41_2077	-	-4.56	4.28×10^{-5}	cytidine and deoxycytidylate deaminase zinc-binding region family protein

Tyrosine metabolism				
DJ41_3063	<i>maiA</i>	3.95	7.4×10^{-5}	maleylacetoacetate isomerase
DJ41_3064	<i>fahA</i>	3.58	0.00012	fumarylacetoacetase
Two-component system				
DJ41_3249	-	3.85	0.03103	autoinducer synthetase family protein
DJ41_2555	-	3.54	0.00010	TonB-dependent siderophore receptor family protein
DJ41_3060	<i>hppD</i>	3.21	0.00037	4-hydroxyphenylpyruvate dioxygenase
DJ41_3468	-	1.84	0.03365	cheW-like domain protein
Bacterial secretion system				
DJ41_1170	<i>tatC</i>	4.13	0.00921	twin arginine-targeting protein translocase TatC
DJ41_1169	<i>tatB</i>	3.81	0.04078	twin arginine-targeting protein translocase TatB

Table S4. List of plasmids used in this study.

Name	Sequence (5'-3')	Function
<i>emaSupF</i>	ACATGACCCAAGGGGTAAGCTAATCATTTATCGCTTCCC	Construction of <i>emaS</i> mutant
pK18_emaSupR	GAGCTCGGTACCCGGGAATCAACAAAGAGAGCTGCG	Construction of <i>emaS</i> mutant
pK18_emaSdownF	ACGACGGCCAGTGCCATGCATCAACAGGTATGTTAC	Construction of <i>emaS</i> mutant
<i>emaSdownR</i>	TGGGAAGCGATAAATGATTAGCTTACCCCTTGGGTCATGT	Construction of <i>emaS</i> mutant
<i>emaRupF</i>	TTCATGAAACAGCTTCGGTTATGTTCCACTTAGGCAAGGC	Construction of <i>emaR</i> mutant
pK18_emaRupR	GAGCTCGGTACCCGGGTCACCTTATACGACTTTAGT	Construction of <i>emaR</i> mutant
pK18_emaRdownF	ACGACGGCCAGTGCCATAACTGTTCACTTTTCACCG	Construction of <i>emaR</i> mutant
<i>emaRdownR</i>	GCCTTGCCTAAGTGGAACATAACCGAAGCTGTTTCATGAA	Construction of <i>emaR</i> mutant
<i>emaS-eF</i>	<i>Bam</i> HI GGATCCGCTTTAGGACAAGTGGCTAA	Expression of <i>emaS</i>
<i>emaS-eR</i>	<i>Hind</i> III AAGCTTTTAGTCTGTCTGCTGGCAA	Expression of <i>emaS</i>
<i>emaR-eF</i>	<i>Bam</i> HI TTGGATCCATGGAAAATGGGGCAGAACCTGA	Expression of <i>emaR</i>
<i>emaR-eR</i>	<i>Sma</i> I TTCCCGGGTTAGATTTTTTTTATCTTCAA	Expression of <i>emaR</i>
pWH-emaScF	GACAGCTTATCATCGATAAACCAAACCTTACATAGTG	Complement of <i>emaS</i>
pWH-emaScR	GATAAACTACCCCATTAATTAGTCTGTCTGCTGGCAA	Complement of <i>emaS</i>
pWH-emaRcF	CCCTTTCGTCTTCAAGGATTTGGTTCCTAAGCCTAA	Complement of <i>emaR</i>
pWH-emaRcR	AGCTGTCAAACATGAGTTAGATTTTTTTTATCTTCAA	Complement of <i>emaR</i>
DJ41_571-rF	TGCAATTAACGGAAGAGCAA	RT-PCR
DJ41_571-rR	TGTCCACGGTGTGTGAAGT	RT-PCR
DJ41_2796-rF	TGGAATGACCGTTGGTATGA	RT-PCR
DJ41_2796-rR	TAAACATGACTTCGCCGTTG	RT-PCR
DJ41_3170-rF	AATCGTTGATGATCATCCTT	RT-PCR
DJ41_3170-rR	TCTTCGTGTGCAGAAACCAC	RT-PCR
DJ41_3172-rF	CCTCACCCCTCAACCCAAATA	RT-PCR
DJ41_3172-rR	CTTACCCCTTGGGTCATGTG	RT-PCR
DJ41_3174-rF	TATTACCAAATGGGCGGCTA	RT-PCR
DJ41_3174-rR	CCCGAGTGAATAGAGCAAGC	RT-PCR
DJ41_3688-rF	GGCGGTTTATCTGAGTTTGT	RT-PCR
DJ41_3688-rR	TTTGTGGAATGTTGTTTGTG	RT-PCR
DJ41_3771-rF	AAGGGCAACTCTCGTCTCAA	RT-PCR
DJ41_3771-rR	CAGGCGCAAATACATCAAAA	RT-PCR
DJ41_571-rF	TGCAATTAACGGAAGAGCAA	RT-PCR

* Start codon (Met) as 0.