

Mitigation of Salt Stress in Rice by the Halotolerant Plant Growth-Promoting Bacterium *Enterobacter asburiae* D2

Zican Ning ^{1,2}, Kexin Lin ¹, Mengya Gao ¹, Xiao Han ¹, Qingjie Guan ^{1,2}, Xiang Ji ^{3,4}, Shuyu Yu ^{3,*} and Lei Lu ^{1,2,*}

¹ College of Life Sciences, Northeast Forestry University, Harbin 150040, China; zicanning@163.com (Z.N.); kx_lin@126.com (K.L.); mengyagao@yeah.net (M.G.); xiaohan093@163.com (X.H.); qingjieguan@126.com (Q.G.)

² Key Laboratory of Saline-Alkali Vegetation Ecology Restoration (Northeast Forestry University), Ministry of Education, Harbin 150040, China

³ College of Water Conservancy and Civil Engineering, Inner Mongolia Agricultural University, Hohhot 010018, China; jixiang@imau.edu.cn

⁴ Hetao College, Bayan Nur 015000, China

* Correspondence: shuyuyu@126.com (S.Y.); llu@nefu.edu.cn (L.L.)

Table S1. Plant growth-promoting abilities of bacteria isolated from the rhizosphere of rice.

Strains	Phosphate solubilization	Siderophore production	ACC deaminase activity
D2	++	+	+
D5	++	+	-
D6	-	+	+
D8	-	-	-
D11	+	++	-
D15	-	-	+

Note: -, not detected; +, low activity; ++, strong activity.

Table S2. Genomic features of *Enterobacter asburiae* D2.

Features	Values
Genome size (bp)	4,659,687
G+C content (%)	55.73
Protein-coding genes (CDS)	4327
Gene total length (bp)	4,158,297
Average gene length (bp)	961.01
Gene/Genome (%)	89.24
rRNAs (5s; 16s; 23s)	8; 1; 0
tRNAs	74
NR annotation	4,316
Swiss-Prot annotation	3,896
Pfam annotation	4,021
COG annotation	3,779
GO annotation	3,160
KEGG annotation	3,082

Table S3. Average nucleotide identity (ANI) (%) of *Enterobacter asburiae* D2 and other closely related *Enterobacter* species based on genome alignments.

	Strain	ANI value (%)
	<i>Enterobacter</i> sp. D2	
	<i>Enterobacter mori</i> LMG 25706(T)	90.05
	<i>Enterobacter asburiae</i> CAV1043	98.29
	<i>Enterobacter cloacae</i> ATCC 23373(T)	88.78
	<i>Enterobacter hormaechei</i> ATCC 49162(T)	87.65
	<i>Enterobacter ludwigii</i> EN-119(T)	88.39
	<i>Enterobacter rogenkampii</i> EN-117(T)	93.11
	<i>Enterobacter cancerogenus</i> ATCC33241(T)	86.79
	<i>Enterobacter sichuanensis</i> WCHECI1597(T)	91.57
	<i>Enterobacter kobei</i> DSM 13645(T)	90.65
	<i>Enterobacter bugandensis</i> EB-247(T)	91.47

Table S4. Genes related to PGP traits and stress response in the genome of *Enterobacter asburiae* D2.

PGP traits	Gene	Gene function
IAA production	<i>trpA</i>	Tryptophan synthase alpha chain [EC:4.2.1.20]
	<i>trpB</i>	Tryptophan synthase beta chain [EC:4.2.1.20]
	<i>trpCF</i>	Indole-3-glycerol phosphate synthase [EC:4.1.1.48]
	<i>trpE</i>	Anthranilate synthase component I [EC:4.1.3.27]
	<i>trpGD</i>	Anthranilate synthase [EC:4.1.3.27]
	<i>trpS</i>	Tryptophanyl-tRNA synthetase [EC:6.1.1.2]
	<i>mtr</i>	Tryptophan permease
	<i>aspC</i>	Aspartate aminotransferase [EC:2.6.1.1]
	<i>ipdC</i>	Indole-3-pyruvate decarboxylase [EC:4.1.1.74]
	<i>aldB</i>	Aldehyde dehydrogenase [EC:1.2.1.-]
Phosphate solubilization	<i>ALDH</i>	Aldehyde dehydrogenase (NAD+) [EC:1.2.1.3]
	<i>pstA/C</i>	Phosphate ABC transporter permease
	<i>pstB</i>	Phosphate ABC transporter ATP-binding protein [EC:7.3.2.1]
	<i>pstS</i>	Phosphate ABC transporter substrate-binding protein
	<i>phoU</i>	Phosphate transport system protein
	<i>phnC</i>	Phosphonate ABC transporter ATP-binding protein [EC:7.3.2.2]
	<i>phnD</i>	Phosphonate ABC transporter substrate-binding protein
	<i>phnE</i>	Phosphonate transport system permease protein
	<i>phnF</i>	Phosphonate metabolism transcriptional regulator
	<i>phnG/H/I/L</i>	Alpha-D-ribose 1-methylphosphonate 5-triphosphate synthase subunit [EC:2.7.8.37]
	<i>phnJ</i>	Alpha-D-ribose 1-methylphosphonate 5-phosphate C-P lyase [EC:4.7.1.1]
	<i>phnK</i>	Putative phosphonate transport system ATP-binding protein
	<i>phnM</i>	Alpha-D-ribose 1-methylphosphonate 5-triphosphate diphosphatase [EC:3.6.1.63]
	<i>phnN</i>	Ribose 1,5-bisphosphokinase [EC:2.7.4.23]
	<i>phnO</i>	Aminoalkylphosphonate N-acetyltransferase [EC:2.3.1.280]
	<i>phnP</i>	Phosphoribosyl 1,2-cyclic phosphate phosphodiesterase [EC:3.1.4.55]
	<i>gcd</i>	Quinoprotein glucose dehydrogenase [EC:1.1.5.2]
	<i>phoA</i>	Alkaline phosphatase [EC:3.1.3.1]
	<i>aphA</i>	Acid phosphatase (class B) [EC:3.1.3.2]

Table S4. Cont.

PGP traits	Gene	Gene function
Nitrogen fixation	<i>nifJ</i>	Pyruvate-ferredoxin oxidoreductase [EC:1.2.7.1]
	<i>iscA</i>	iron-sulfur cluster assembly protein
	<i>iscR</i>	Iron-sulfur cluster assembly transcription factor
	<i>iscS</i>	Cysteine desulfurase [EC:2.8.1.7]
	<i>isclU</i>	Nitrogen fixation protein NifU and related proteins
	<i>sufA/B/D</i>	Fe-S cluster assembly protein
	<i>sufC</i>	Fe-S cluster assembly ATP-binding protein
	<i>sufE</i>	cysteine desulfurization protein
ACC deaminase activity	<i>sufS</i>	cysteine desulfurase
	<i>dcyD</i>	D-cysteine desulphydrase [EC:4.4.1.15]
Siderophore production	<i>fepA</i>	Ferric enterobactin receptor
	<i>fepB</i>	Ferric enterobactin transport system substrate-binding protein
	<i>fepC</i>	Iron-siderophore transport system ATP-binding protein
	<i>fepD/G</i>	Iron-siderophore transport system permease protein
	<i>afuA</i>	Iron (III) transport system substrate-binding protein
	<i>afuB</i>	Iron (III) transport system permease protein
	<i>afuC</i>	Iron (III) transport system ATP-binding protein [EC:7.2.2.7]
	<i>fhuB</i>	Ferric hydroxamate transport system permease protein
	<i>fhuC</i>	Ferric hydroxamate transport system ATP-binding protein [EC:7.2.2.16]
	<i>fhuD</i>	Ferric hydroxamate transport system substrate-binding protein
	<i>efeB</i>	Iron uptake transporter deferochelatase [EC:1.11.1.-]
	<i>efeO</i>	Iron uptake system component
	<i>efeU</i>	High-affinity iron transporter
	<i>entA</i>	2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase [EC:1.3.1.28]
	<i>entB</i>	Bifunctional isochorismate lyase [EC:3.3.2.1]
	<i>entC</i>	Isochorismate synthase [EC:5.4.4.2]
	<i>entD</i>	Enterobactin synthetase component D [EC:6.3.2.14]
	<i>ente</i>	(2,3-dihydroxybenzoyl)adenylate synthase [EC: 6.2.1.71]
	<i>entF</i>	Enterobactin non-ribosomal peptide synthetase [EC: 6.2.1.72]
Cytokinin synthesis	<i>entH</i>	Proofreading thioesterase
	<i>ents</i>	Enterobactin transporter
	<i>iucA</i>	N2-citryl-N6-acetyl-N6-hydroxylysine synthase [EC:6.3.2.38]
	<i>iucB</i>	Acetyl CoA:N6-hydroxylysine acetyl transferase [EC:2.3.1.102]
	<i>iucC</i>	Aerobactin synthase [EC:6.3.2.39]
	<i>iucD</i>	Lysine N6-hydroxylase [EC:1.14.13.59]
	<i>miaA</i>	tRNA dimethylallyltransferase [EC:2.5.1.75]
	<i>miaB</i>	tRNA-2-methylthio-N6-dimethylallyladenosine synthase [EC:2.8.4.3]
Glycine-betaine synthesis	<i>miaE</i>	tRNA-2-(methylsulfanyl)-N6-isopentenyladenosine37 hydroxylase [EC:1.14.99.69])
	<i>betA</i>	Choline dehydrogenase [EC:1.1.99.1]
	<i>betB</i>	Betaine-aldehyde dehydrogenase [EC:1.2.1.8]
	<i>betT</i>	Choline/glycine/proline betaine transport protein

Table S4. Cont.

PGP traits	Gene	Gene function
Proline synthesis	<i>proA</i>	Glutamate-5-semialdehyde dehydrogenase [EC:1.2.1.41]
	<i>proB</i>	Glutamate 5-kinase [EC:2.7.2.11]
	<i>proC</i>	Pyrroline-5-carboxylate reductase [EC:1.5.1.2]
	<i>proP</i>	MFS transporter, MHS family, proline/betaine transporter
	<i>proS</i>	Prolyl-tRNA synthetase [EC:6.1.1.15]
	<i>proV</i>	Glycine betaine/proline transport system ATP-binding protein [EC:7.6.2.9]
	<i>proW</i>	Glycine betaine/proline transport system permease protein
	<i>proX</i>	Glycine betaine/proline transport system substrate-binding protein
	<i>proY</i>	Proline-specific permease ProY
Trehalose synthesis	<i>treB</i>	Trehalose PTS system EIIBC or EIIBCA component [EC:2.7.1.201]
	<i>treS</i>	Maltose alpha-D-glucosyltransferase [EC:5.4.99.16]
	<i>treY</i>	(1->4)-alpha-D-glucan 1-alpha-D-glucosylmutase [EC:5.4.99.15]
	<i>treZ</i>	Maltooligosyltrehalose trehalohydrolase [EC:3.2.1.141]
	<i>otsA</i>	Trehalose 6-phosphate synthase [EC:2.4.1.15 2.4.1.347]
	<i>otsB</i>	Trehalose 6-phosphate phosphatase [EC:3.1.3.12]
EPS synthesis	<i>bcsA</i>	Cellulose synthase (UDP-forming) [EC:2.4.1.12]
	<i>bcsB/C</i>	Cellulose synthase operon protein B/C
	<i>bcsZ</i>	Endoglucanase [EC:3.2.1.4]
	<i>wcaA/B/C/E/F/I/L</i>	Colanic acid biosynthesis glycosyltransferase
	<i>wcad</i>	Colanic acid polymerase
	<i>wcaJ</i>	Undecaprenyl-phosphate glucose phosphotransferase [EC:2.7.8.31]
	<i>wcaK</i>	Colanic acid biosynthesis pyruvyl transferase
	<i>wcam</i>	Colanic acid biosynthesis protein WcaM
	<i>pgaA</i>	Poly-beta-1,6 N-acetyl-D-glucosamine export porin PgaA
	<i>pgaB</i>	Poly-beta-1,6-N-acetyl-D-glucosamine N-deacetylase
	<i>pgaC</i>	Poly-beta-1,6-N-acetyl-D-glucosamine synthase
	<i>pgad</i>	Poly-beta-1,6-N-acetyl-D-glucosamine biosynthesis protein

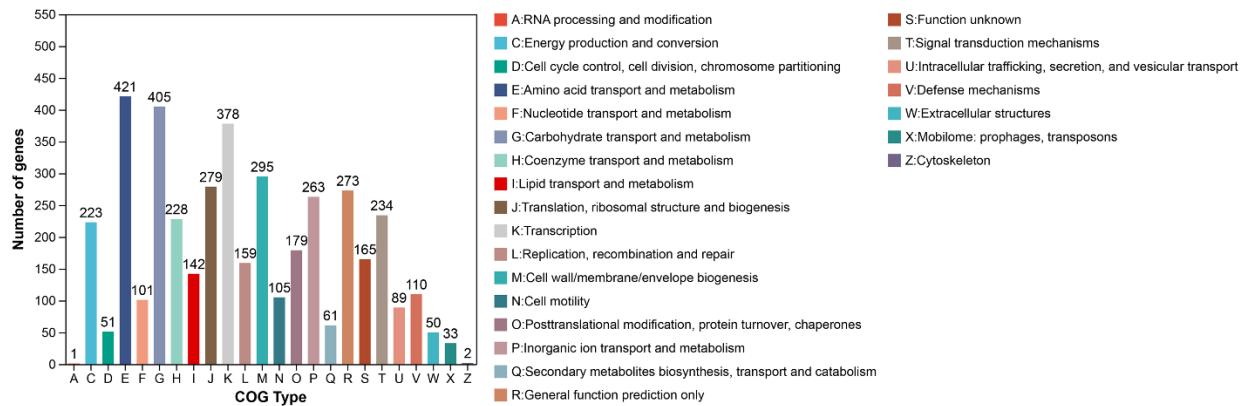


Figure S1. Clusters of Orthologous Groups (COGs) function classification of *Enterobacter asburiae* D2 genome.

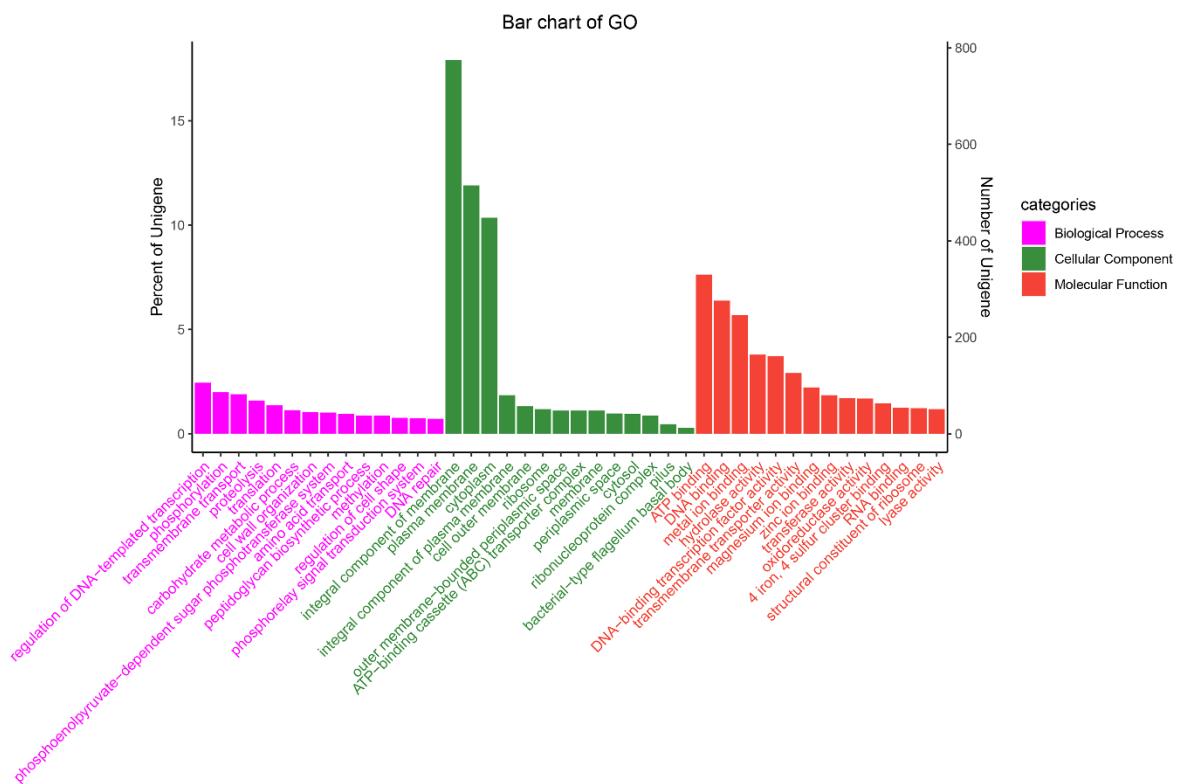


Figure S2. Assignment of Gene Ontology (GO) term for predicted genes of *Enterobacter asburiae* D2 genome.

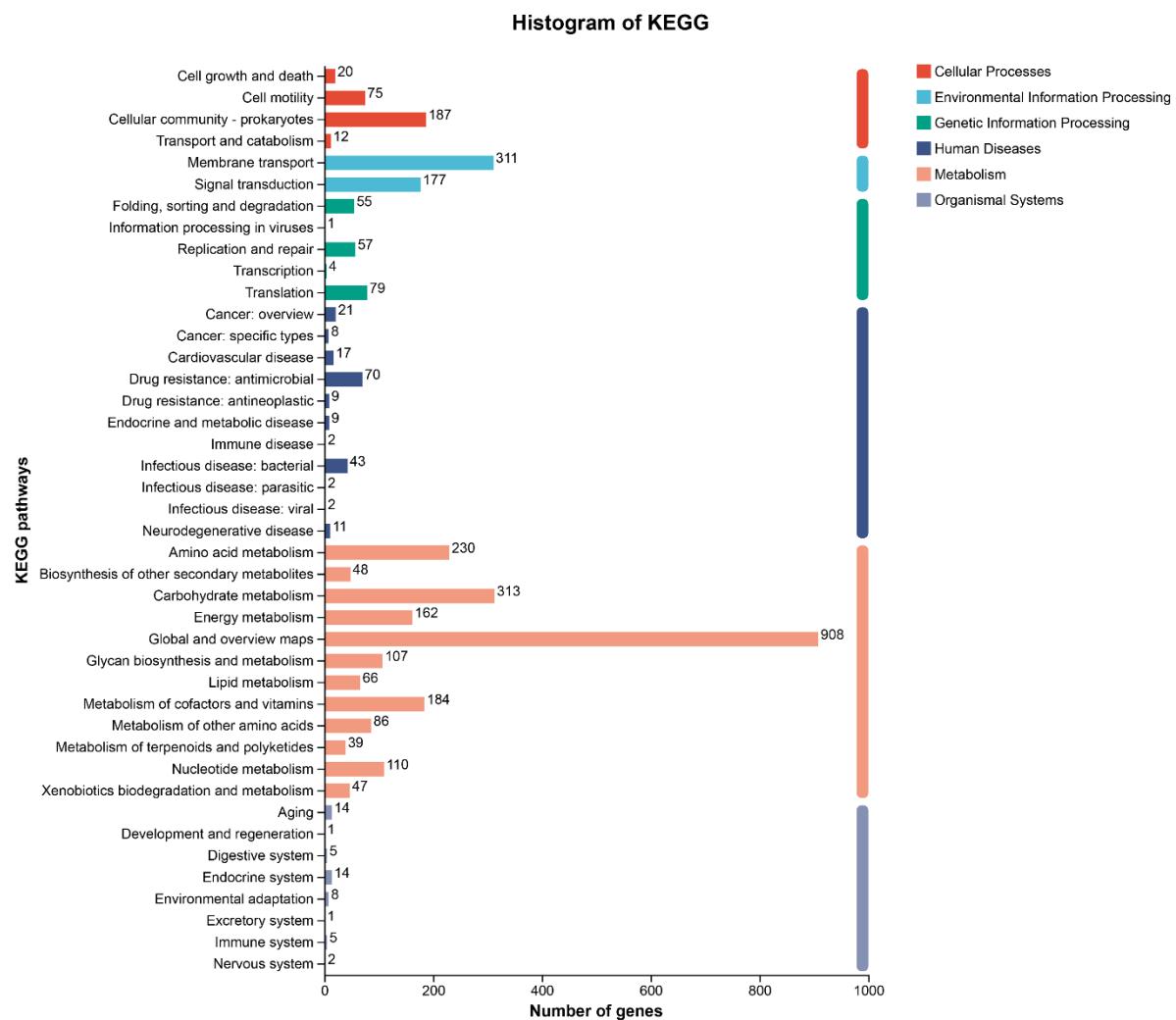


Figure S3. Kyoto Encyclopedia of Genes and Genomes (KEGG) annotation of *Enterobacter asburiae* D2 genome.