

Supplementary Information

Enhanced Galactose Utilization in *Bacillus subtilis* via Adaptive Laboratory Evolution

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13 **Table S1 Mutations of *Bacillus subtilis* BSGA14 strain**

Ref. Position	Ref. Base	Qry. Base	Ref. FeatureName	Mutation Type	SNP Type	Gene
46223	T	C	BS_00063	Synonymous	Substitution	DNA-directed DNA polymerase
87567	C	T	BS_00103	R12K	Substitution	Aspartate 1-decarboxylase
89432	.	T	.	Intergenic	Insertion	
242186	.	T	.	Intergenic	Insertion	
294025	A	.	BS_00345	Non-Synonymous	Deletion	uncharacterized protein
452700	G	A	BS_00533	A106V	Substitution	Cytoplasm; Kinase; Phosphotransferase system; Sugar transport; Transferase; Transport.
453765	T	C	BS_00535	D66G	Substitution	Cytoplasm; Kinase; Phosphotransferase system; Sugar transport; Transferase; Transport.
465022	C	T	BS_00544	P329S	Substitution	Putative peptidoglycan O-acetyltransferase YrhL
606392	C	T	.	Intergenic	Substitution	
626536	G	A	BS_00705	R120Q	Substitution	FAD; Flavoprotein; Oxidoreductase.
668251	G	A	BS_00746	Synonymous	Substitution	DNA damage; DNA repair; DNA replication; DNA-binding; DNA-directed DNA polymerase; Exonuclease; Hydrolase; Nuclease; Nucleotidyltransferase; Transferase.
675565	A	.	.	Intergenic	Deletion	
681172	C	T	BS_00757	E175K	Substitution	Antibiotic resistance; ATP-binding; Cytoplasm; Fatty acid biosynthesis; Fatty acid metabolism; Ligase; Lipid biosynthesis; Lipid metabolism; Nucleotide-binding.
752525	T	.	BS_00827	Non-Synonymous	Deletion	Cell membrane; Membrane; Transmembrane; Transmembrane helix.

Ref. Position	Ref. Base	Qry. Base	Ref. FeatureName	Mutation Type	SNP Type	Gene
752942	.	A	BS_00827	Non-Synonymous	Insertion	Cell membrane; Membrane; Transmembrane; Transmembrane helix.
765263	T	G	.	Intergenic	Substitution	
831129	G	A	BS_00905	Synonymous	Substitution	1,4-dihydroxy-2-naphthoyl-CoA synthase
857276	.	C	.	Intergenic	Insertion	
858417	C	T	.	Intergenic	Substitution	
859874	C	T	.	Intergenic	Substitution	
859908	A	G	.	Intergenic	Substitution	
859990	G	A	.	Intergenic	Substitution	
882853	C	T	BS_00972	A448T	Substitution	ATP-binding; Disulfide bond; Kinase; Magnesium; Nucleotide-binding; Rhamnose metabolism; Transferase.
889303	A	.	.	Intergenic	Deletion	
920669	A	G	BS_01009	Y143C	Substitution	Histidine kinase
929196	A	G	BS_01015	T447A	Substitution	Na(+)-malate symporter
1141683	T	.	BS_01225	Non-Synonymous	Deletion	Cu(+) exporting ATPase
1168180	A	.	.	Intergenic	Deletion	
1172514	C	T	BS_01261	Synonymous	Substitution	hypothetical protein
1220431	A	G	BS_01325	H226R	Substitution	Arabinose metabolism transcriptional repressor
1270572	C	T	BS_01373	A397V	Substitution	Para-nitrobenzyl esterase
1303639	.	A	BS_01406	Non-Synonymous	Insertion	Sensory transduction protein BceR
1307971	T	C	BS_01412	E30G	Substitution	Nucleotide-binding protein YvcJ
1474174	.	T	BS_01576	Non-Synonymous	Insertion	HTH-type transcriptional repressor GlcR
1538617	A	.	.	Intergenic	Deletion	
1542967	C	T	BS_01656	C224Y	Substitution	UDP-N-acetylglucosamine 1-carboxyvinyltransferase
1650934	C	T	BS_01760	D44N	Substitution	Rod shape-determining protein RodA
1809288	T	C	BS_01920	K2E	Substitution	Uncharacterized N-acetyltransferase YxeL
1890202	G	A	BS_02005	H119Y	Substitution	Formaldehyde dehydrogenase

Ref. Position	Ref. Base	Qry. Base	Ref. FeatureName	Mutation Type	SNP Type	Gene
1907448	C	T	BS_02024	G22D	Substitution	Two-component system YycFG regulatory protein
1947921	T	C	BS_02075	K665E	Substitution	Formate dehydrogenase
1972167	T	C	.	Intergenic	Substitution	
2036440	.	T	BS_02167	Non-Synonymous	Insertion	Non-specific serine/threonine protein kinase
2216241	T	C		Intergenic	Substitution	
2306909	A	G	BS_02455	Y214C	Substitution	Proline-responsive transcriptional activator PutR
2392663	.	G	BS_02522	Non-Synonymous	Insertion	Uncharacterized ABC transporter permease protein YclO
2410605	A	G	BS_02540	E172G	Substitution	Protein-N(pi)-phosphohistidine--D-mannitol phosphotransferase
2486868	A	G	BS_02625	D125G	Substitution	Non-specific serine/threonine protein kinase
2504043	A	.	BS_02656	Non-Synonymous	Deletion	uncharacterized protein
2545634	A	.	.	Intergenic	Deletion	
2587112	T	.	.	Intergenic	Deletion	
2616306	A	G	BS_02778	T49A	Substitution	Redox-sensing transcriptional repressor Rex
2706301	.	T	BS_02859	Non-Synonymous	Insertion	hypothetical protein
2832606	C	T	BS_02980	Synonymous	Substitution	Probable tRNA-dihydrouridine synthase
2882228	A	G	BS_03026	H205R	Substitution	Probable siderophore transport system permease protein YfhA
2906994	C	T	BS_03058	Synonymous	Substitution	uncharacterized protein
2983659	T	C	BS_03154	W88R	Substitution	Glycerol kinase
3413566	T	C	BS_03622	I259V	Substitution	S-methyl-5-thioribose kinase
3488491	.	T	.	Intergenic	Insertion	
3776519	C	.	BS_03984	Non-Synonymous	Deletion	DNA mismatch repair protein MutL
3817238	T	.	.	Intergenic	Deletion	
3926950	A	.	.	Intergenic	Deletion	
4001235	G	A	BS_04230	A130T	Substitution	RsbT co-antagonist protein RsbRC

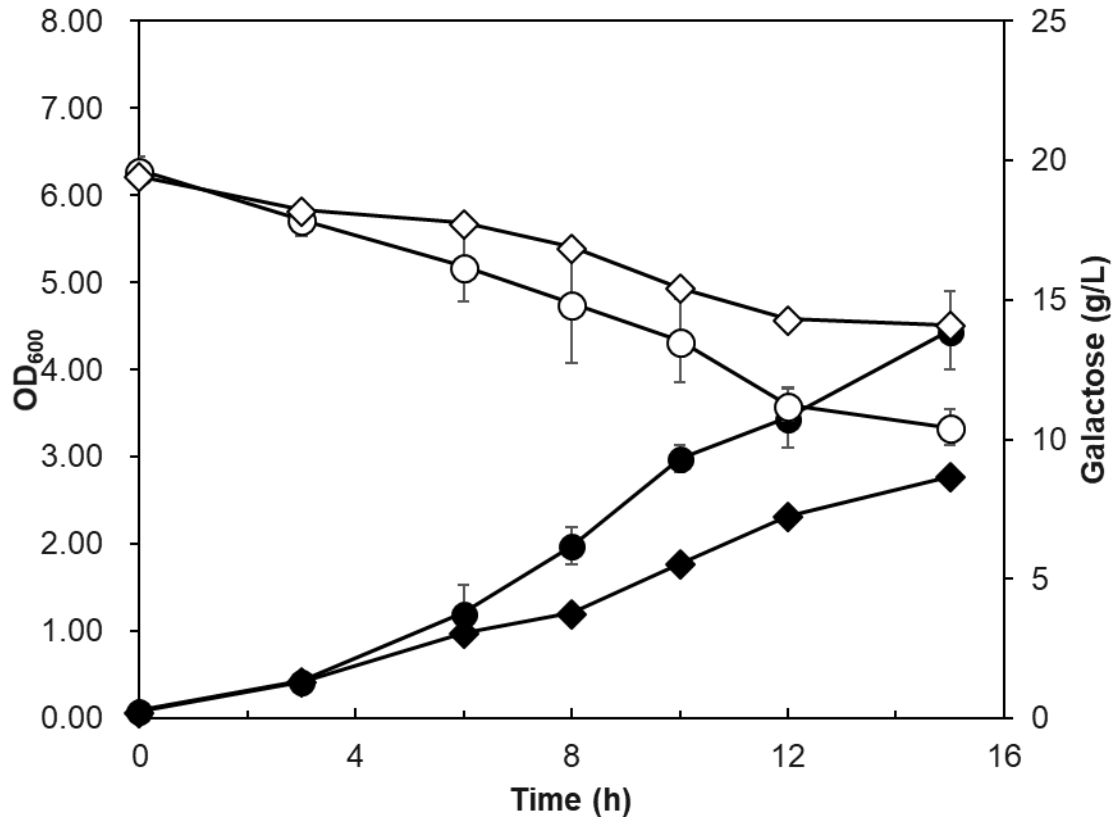


Fig. S1 Reverse engineering of *B. subtilis* J46 introduced mutation of *araR*^{H226R} and Δ *araR* (a) The growth profile and galactose consumption of BSGALE1 and BSGALE2 in M9 minimal medium containing 2 % (w/v) galactose. Black and white symbols mean optical density at 600nm and galactose concentration (g/L), respectively. Triangles and diamonds represent samples from BSGALE1 and BSGALE2 respectively. The standard deviation estimated from triplets is represented by the error bars.