**S2 Table. Non-synonymous mutations identified in the genomes of derived genotypes and their predicted impact on protein functioning.** The effect of mutations in coding regions on the functioning of the corresponding protein was predicted using the PROVEAN algorithm [1]. Gene names and their functional assignment are based on Ecocyc [2]. *Strain identity* code: X-Y-Z where X refers to a particular population (1-8), Y refers to the regime, i.e. AA = amino acid, or NA = no amino acid, and Z refers to the phenotype, i.e. either AT = auxotrophic or PT = prototrophic. *Type of change* indicates whether the mutation was identified in a structural (**S**, encoding functions directly involved in enzymatic reactions of biosynthesis pathways) or a regulatory gene (**R**, encoding functions, which can directly or indirectly control expression or activity of enzymes involved in amino acid biosynthesis). Genes and proteins with unknown functional annotations are designated as 'NA'. Mutations in structural genes: AT: 18, PT: 5, mutations in regulatory genes: AT: 19, PT: 6.

| Gene, Blattner ID, accession number | Strain identity | Protein function | Mutation | Predicted effect | Type of change |
|---|---|---|---|---|---|
| *ptsP,* b2829, ECK2825 | 2-AA-AT | Phosphoenol-pyruvate-protein phosphor-transferase | M604R | Neutral | S |
| *yoaA,* b1808, ECK1806 | 2-AA-AT | Conserved protein | D121Y | Neutral | NA |
| *hemF,* b2436, ECK2431 | 3-AA-AT | Coproporphyrinogen III oxidase | G127V | Deleterious | S |
| *dhaM,* b1198, ECK1186 | 3-AA-AT | Dihydroxyacetone kinase subunit M | V196A | Neutral | S |
| *sspA,* b3229, ECK3218 | 5-AA-AT | Stringent starvation protein A | Q24* | Deleterious | R |
| *stpA,* b2669, ECK2663 | 6-AA-AT | H-NS-like DNA-binding protein with RNA chaperone activity | R49S | Neutral | R |
| *yhdW,* b3268, ECK3255 | 8-AA-AT | Putative transport protein | C→A, pseudogene | NA | NA |
| *rpoB,* b3987, ECK3978 | 7-NA-AT,<br>8-NA-AT | RNA polymerase, β subunit | T135P | Deleterious | R |
| *yqiB,* b3033, ECK3024 | 7-NA-AT,<br>8-NA-AT | Predicted dehydrogenase | Q117K | Deleterious | S |
| *metG,* b2114, ECK2107 | 7-NA-AT | Methionyl-trna synthetase | R600P | Deleterious | R |
| *cra /ilvH* | 4-AA-AT | Intergenic | C→T, intergenic | Deleterious | R |
| *dxr/frr* | 8-NA-AT | Intergenic | T→A, intergenic | Deleterious | R |
| *ynaE/pinR* | 7-NA-AT,<br>8-NA-AT | Intergenic | A→G, intergenic | Deleterious | R |
| *yfjM /yfjL* | 4-AA-AT | Intergenic | (CATAGTGC) 6→7,inter- | Deleterious | R |

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| | | | genic | | |
| *uspC/flhD* | 6-AA-AT | Intergenic | +4 bp, intergenic | Deleterious | R |
| *ΔykfC,* b0258, ECK0260 | 6-AA-AT | CP4-6 prophage; conserved protein | Deletion | Deleterious | S |
| *ΔinsN,* b0255, ECK0257 | 6-AA-AT | CP4-6 prophage; partial regulator of insertion element IS911A | Deletion | Deleterious | S |
| *ΔinsI1,* b0256, ECK0258 | 6-AA-AT | Transposase of IS30 | Deletion | Deleterious | S |
| *ΔperR* b0254 ECK0256 | 6-AA-AT | Perr transcriptional regulator | Deletion | Deleterious | R |
| *ΔykfA,* b0253, ECK0255 | 6-AA-AT | Predicted GTP-binding protein | Deletion | Deleterious | R |
| *ΔyafZ,* b0252, ECK0254 | 6-AA-AT | CP4-6 prophage; conserved protein | Deletion | Deleterious | NA |
| *ΔykfK,* b4628, ECK4444 | 6-AA-AT | Pseudogene | Deletion | Deleterious | NA |
| *ΔykfL,* b4627, ECK4445 | 6-AA-AT | Pseudogene | Deletion | Deleterious | NA |
| *ΔyafY,* b0251, ECK0253 | 6-AA-AT | CP4-6 prophage; inner membrane lipoprotein | Deletion | Deleterious | NA |
| *ΔykfB,* b0250, ECK0252 | 6-AA-AT | Predicted protein | Deletion | Deleterious | NA |
| *ΔykfF,* b0249, ECK0251 | 6-AA-AT | Predicted protein | Deletion | Deleterious | NA |
| *ΔyafX,* b0248, ECK0250 | 6-AA-AT | Predicted protein | Deletion | Deleterious | NA |
| *ΔykfG,* b0247, ECK0249 | 6-AA-AT | Predicted DNA repair protein | Deletion | Deleterious | NA |
| *ΔykfH,* b4504, ECK0248 | 6-AA-AT | Predicted protein | Deletion | Deleterious | NA |
| *ΔyafW,* b0246, ECK0247 | 6-AA-AT | Antitoxin of the ykfi-yafw toxin-antitoxin pair | Deletion | Deleterious | R |
| *ΔykfI,* b0245, ECK0246 | 6-AA-AT | Toxin of the ykfi-yafw toxin-antitoxin system | Deletion | Deleterious | R |
| *ΔykfN, NA* | 6-AA-AT | Pseudogene | Deletion | Deleterious | S |
| *ΔthrW,* b0244, ECK0245 | 6-AA-AT | Trna: trna$^{thrw}$ | Deletion | Deleterious | R |
| *ΔproA,* b0243, ECK0244 | 6-AA-AT | Component of glutamate-5-semialdehyde | Deletion | Deleterious | S |

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|---|---|---|---|---|---|
| | | dehydrogenase | | | |
| *ΔproB,* b0242, ECK0243 | 6-AA-AT | Component of γ-glutamyl kinase | Deletion | Deleterious | S |
| *ΔinsF1,* b0299, ECK0299 | 2-AA-AT | IS3 element protein insf | Deletion | Deleterious | NA |
| *ΔinsE1,* b0298, ECK0298 | 2-AA-AT | IS3 element protein inse | Deletion | Deleterious | NA |
| *ΔgatR,* b2087, ECK2083 | 2-AA-AT | Negative DNA-binding transcriptional regulator of galactitol metabolism | Deletion | Deleterious | R |
| *ΔyegS,* b2086, ECK2082 | 2-AA-AT | Lipid kinase | Deletion | Deleterious | S |
| *ΔyegR,* b2085, ECK2081 | 2-AA-AT | Predicted protein | Deletion | Deleterious | NA |
| *ΔyegZ,* b2083, ECK2080 | 2-AA-AT | Predicted protein fragment | Deletion | Deleterious | NA |
| *ΔogrK,* b2082, ECK2079 | 2-AA-AT | DNA-binding transcriptional regulator, prophage P2 remnant | Deletion | Deleterious | R |
| *ΔyegQ,* b2081, ECK2077 | 2-AA-AT | Predicted peptidase | Deletion | Deleterious | NA |
| *ΔyegP,* b2080, ECK2076 | 2-AA-AT | Predicted protein | Deletion | Deleterious | NA |
| *ΔbaeR,* b2079, ECK2075 | 2-AA-AT | Baer transcriptional regulator | Deletion | Deleterious | R |
| *ΔbaeS,* b2078, ECK2074 | 2-AA-AT | Baes sensory histidine kinase | Deletion | Deleterious | R |
| *ΔmdtD,* b2077, ECK2073 | 2-AA-AT | Putative transport protein, major facilitator superfamily (MFS) | Deletion | Deleterious | S |
| *ΔmdtC,* b2076, ECK2072 | 2-AA-AT | Mdtabc-tolc multidrug efflux system - membrane subunit | Deletion | Deleterious | S |
| *ΔmdtB,* b2075, ECK2071 | 2-AA-AT | Mdtabc-tolc multidrug efflux system - membrane subunit | Deletion | Deleterious | S |
| *wcaN,* b2042, ECK2036 | 6-AA-AT | Predicted uridylyltransferase subunit with galu | -1 bp, | Deleterious | S |
| *gltD,* b3213, ECK3203 | 5-AA-AT,<br>6-AA-AT.<br>8-AA-AT,<br>5-AA-PT<br>6-AA-PT | Glutamate synthase, small subunit | N354D | Neutral | S |
| *crr/ptsI* | 5-AA-PT | Intergenic | A→C | NA | R |
| *rcnR/thiM* | 5-AA-PT,<br>6-AA-PT | Intergenic | A→G | NA | R |
| *rph,* b3643, ECK3633 | 3-AA-AT,<br>4-AA-AT,<br>6-AA-AT, , | Coding | - 82 bp | Deleterious | S |

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|---|---|---|---|---|---|
|  | 6-AA-PT |  |  |  |  |
| *rph/pyrE* | 5-AA-AT,<br>3-AA-PT,<br>4-AA-PT,<br>7-NA-AT,<br>8-NA-AT,<br>8-NA-PT | Intergenic and coding | -1 bp | Deleterious | S |
| *wzxC/wcaK* | 8-AA-AT,<br>7-NA-PT,<br>8-NA-PT | Intergenic | +85 bp | NA | S |
| *rpsK,*<br>b3297,<br>ECK3284 | 5-AA-PT | 30S ribosomal subunit protein S11 | I116N | Neutral | S |
| *cra,*<br>b0080,<br>ECK0081 | 3-AA-PT,<br>4-AA-PT | DNA-binding transcriptional dual regulator | L191F | Neutral | R |
| *fecR,*<br>b4292<br>ECK4282 | 6-AA-PT | Regulator for fec operon | A12G | Neutral | R |
| *hflC,*<br>b4175,<br>ECK4171 | 6-AA-PT | Regulator of ftsh protease | G268D | Neutral | R |
| *mppA/pgrR* | 8-NA-PT | Intergenic | IS5 (–) +4 bp | NA | R |

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