**S1 Table. Amino acid substitutions identified in high virulence mutants**

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| --- | --- | --- | --- | --- |
| Strain | Reference | SNP | Amino acid substitution | Gene and product |
| HV1 | 55372 | C>T | G580S | lptD, exported protein required for envelope biosynthesis and integrity |
| 304209 | C>T | R74H | yagV, hypothetical protein |
| 488205 | G>A | V816M | kefA, fused mechanosensitive channel proteins |
| 1916014 | C>T | D173N | prc, carboxy-terminal protease for penicillin-binding protein 3 |
| HV10 | 628162 | C>T | A130V | entA, 2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase |
| 672325 | G>A | T95I | lptE, minor lipoprotein |
| 1705201 | C>T | M274I | ydgJ, predicted oxidoreductase |
| 1784384 | G>A | A426T | ydiS, predicted oxidoreductase, FAD/NAD(P)-binding domain |
| 1880237 | C>T | S270L | yeaP, predicted diguanylate cyclase |
| 1938991 | C>T | T209I | yebK, predicted DNA-binding transcriptional regulator |
| 1976099 | C>T | A314T | cheA, fused chemotactic sensory histidine kinase |
| 2017518 | C>T | Q168Stop | fliG, flagellar motor switching and energizing component |
| 2846216 | G>A | A561V | hycC, hydrogenase 3, membrane subunit |
| 3647237 | G>A | A129T | hemD, uroporphyrinogen III synthase |
| 4234763 | G>A | A274T | yjbC, 23S rRNA pseudouridine synthase |
| 4243042 | G>A | D607N | yjbH, predicted porin |
| 4361245 | G>A | A684V | cadA, lysine decarboxylase 1 |
| 4485435 | C>T | G342G | yjgN, conserved inner membrane protein |
| 4519790 | G>A | A523V | fecA, ferric citrate outer membrane transporter |
| HV11 | 134958 | G>A | Q209Stop | speD, S-adenosylmethionine decarboxylase |
| 237849 | C>T | S172F | yafT, predicted aminopeptidase |
| 672325 | G>A | T95I | lptE, minor lipoprotein |
| 886635 | G>A | A376V | ybjJ, predicted transporter |
| 1087662 | C>T | G203S | ycdQ, predicted glycosyl transferase |
| 1579155 | C>T | G60E | yddB, predicted porin protein |
| 2748857 | C>T | V170I | grpE, heat shock protein |
| 2785512 | G>A | A37T | ygaR, hypothetical protein |
| 2832389 | G>A | A420T | norV, flavorubredoxin oxidoreductase |
| 3121936 | C>T | E176K | glcB, malate synthase G |
| 3526504 | C>T | R83H | cdh, CDP-diacylglycerol phosphotidylhydrolase |
| 3591713 | C>T | R257H | yihF, hypothetical protein |
| 3704036 | G>A | R444C | trkD, potassium transporter |
| 3711196 | C>T | A60V | gidA, glucose-inhibited cell-division protein |
| 4588434 | G>A | T1021M | hsdR, endonuclease R |

‘Reference’ means a position in *E. coli* W3110 genome. ‘SNP’ is single nucleotide polymorphism.