pathogens

MDPI

Supplementary Material

Adaptive Evolution Compensated the plasmid Fitness Costs brought by specific Genetic Conflicts

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Table S4. Detailed function of non-synonymous SNP-genes in E. coli C600 carrying the pHN330 plasmid.

Gene	Product and function	Supplementary description
ptrA	Protease III; hydrolyzes oligopeptides and peptides; involved in the protein catabolic process of bacteria.	Swamy et al. found that lack of PtrA has no effect on bacterial growth [1].
lpdA	Lipoamide dehydrogenase; component of the pyruvate dehydrogenase complex, which catalyzes the conversion of pyruvate to acetyl-CoA.	Murarka et al. found that pyruvate dehydrogenase deficient *E. coli* had decreased growth and glucose metabolism rates [2].
bamA	Outer membrane protein assembly factor BamA; component of the BAM complex, which is essential to constructing and maintaining the outer membrane.	Lack of BamA causes defects in assembly of β-barrel protein complex, influencing the construction of the outer membrane [3].
melA	α-Galactosidase; hydrolyzes melibiose to glucose and galactose.	Melibiose is a common nutrient. Belyaeva et al. found that mutants in the *melA* gene affect the growth of bacteria with melibiose as the carbon source [4].
gatA	Galactitol-specific PTS enzyme IIA component; involved in galactinol phosphorylation and transport.	Galactinol is transported into the cytosol by the PTS system and is a carbon source that provides energy for bacterial growth. Lengeler et al. found that bacteria with a mutated *gatA* gene cannot transport galactinol [5].
mdoB	Phosphoglycerol transferase I; catalyzes phosphoglycerol residues from phosphatidylglycerol into membrane-derived oligosaccharide.	Membrane-derived oligosaccharide plays an important role in maintaining the stability of the bacterial membrane [6].
yihV	6-Deoxy-6-sulfofructose kinase; involved in the sulphoglycolysis pathway.	A mutation in the *yihV* gene influences the utilization of sulfoquinovose in bacteria [7].
mntH	Divalent metal cation transporter MntH; transports most metal ions, with high affinity for Mn (II).	Makui et al. found that inactivation of MntH did not affect bacterial growth in nutrient rich medium [8].

trkA	NAD-binding component of Trk potassium transporters; K^+ uptake mediated by Trk transport system with hypotonic conditions.	TrkA plays an important role in maintaining potassium ion homeostasis under hypotonic conditions [9].
ydfK	Cold shock protein; unknown function.	It has been reported that after a cold shock, the expression of YdfK is upregulated [10].

Table S5. Detailed functions of non-synonymous SNP-genes in *Escherichia coli* C600N500.

Gene	Product and function	Supplementary description
bamA	Outer membrane protein assembly factor BamA; BamA is a component of the BAM complex, which is essential to constructing and maintaining the outer membrane.	Lack of BamA causes defects in the assembly of the β-barrel protein complex, influencing the construction of the outer membrane [3].
bcsG	Cellulose phosphoethanolamine transferase; the BcsE-BcsF-BcsG pathway controls the modification of cellulose by direct protein–protein interactions.	Exopolysaccharide cellulose is the major component of the self-produced extracellular matrix in biofilms, which enhances antibiotic resistance. The *bcsG* gene is essential for the formation of cellulose; lack of the *bcsG* gene results in the inability to form a biofilm [11].
lptF	Lipopolysaccharide transport system protein LptF; involved in lipopolysaccharide transport.	Sherman et al. found that LptF is essential for the transport of LPS. LPS plays a vital role in cell mobility, biofilm formation, antibiotic resistance, intestinal colonization, and is responsible for protecting the bacterial cell [12].
mreC	Cell shape determining protein MreC; *mreC* gene is a component of the *mreBCD* operon; encodes for cell cytoskeletal proteins.	The function of the bacterial cytoskeleton is highly conserved. Kruse T et al. found that the bacterial cell will undergo morphological change then lysis when the MreBCD protein is depleted [13].
sfmD	Fimbrial usher protein SfmD	Involved in the functioning of pili—macromolecular structures on the surface of gram-negative bacteria; defects in SfmD will not affect the biofilm formation of *E. coli* [14].

rfaF	ADP-heptose–LPS heptosyltransferase 2; involved in the synthesis of the inner core of LPS.	The inner core of LPS is highly conserved and responsible for the function of the outer membrane barrier [15].
gudD	D-glucarate dehydratase; involved in the D-glucarate catabolic pathway.	D-glucarate is not a common product in glucose metabolism [16].
araC	DNA-binding transcriptional dual regulator AraC; in the presence of arabinose, AraC stimulates the transcription of arabinose utilization genes.	Generally, bacteria utilize glucose as a carbon source due to carbon catabolite repression [17].
pepQ	Xaa-Pro dipeptidase; PepQ is capable of X-proline dipeptide hydrolysis.	Mutants in the *pepQ* gene of *E. coli* have no observable change in phenotype [18].
paaJ	Beta-ketoadipyl-CoA thiolase; the *paa* gene cluster is involved in phenylacetic acid metabolism.	Phenylacetic acid is not a common energy source.
dgt	Deoxyguanosinetriphosphate triphosphohydrolase; catalytic hydrolysis of dGTP to deoxyguanosine and triphosphate.	dGTPase is involved in maintaining proper dNTP pools and plays a vital role in cell survival and DNA replication. A mutation in the *dgt* gene reduces the fidelity of DNA replication [19].
mshA	D-inositol 3-phosphate glycosyltransferase; involved in the synthesis of extracellular colanic acid.	Colanic acid, an extracellular polysaccharide secreted by *E. coli*, is thought to promote biofilm formation and is important for survival. Ren et al. knocked out four consecutive genes of the CA operon (*wza*, *wzb*, *wzc*, and *wcaA*) and found that the strain was unable to synthesize colanic acid [20].

Table S6. Detailed functions of non-synonymous SNP-genes in *E. coli* C600Y500.

Gene	Product and Function	Supplementary description
fliY	Cystine ABC transporter periplasmic binding protein; FliY together with the L-cysteine exporter EAMA constitute the L-cysteine/L-cystine shuttle system involved in oxidative stress resistance	Ohtsu et al. found that deletion of the *fliY* gene results in decreased *E. coli* growth in LB broth [21].
bamA	Outer membrane protein assembly factor BamA; BamA is a component of the BAM complex, which is essential to constructing and maintaining the outer membrane.	Lack of BamA causes defects in the assembly of the β-barrel protein complex, influencing the construction of the outer membrane [3].
yghQ	Putative transport protein YghQ	No study of the *yghQ* gene has yet been conducted.

caiA	Crotonobetainyl-CoA reductase; Convert crotonobetainyl to γ-butyrobetaine under anaerobic conditions.	The expression of *caiA* suppressed is suppressed under aerobic conditions [22].
gloA	Glyoxalase I; involved in the glutathione-dependent glyoxalase I pathway that metabolizes methylglyoxal.	Endogenous synthesis of methylglyoxal occurs in the glycolytic pathway; 300 μM methylglyoxal causes growth arrest and higher concentrations cause cell death. The glutathione-dependent glyoxalase I pathway is the major pathway for methylglyoxal detoxification [23].
acnA	Aconitate hydratase A; catalyzes citrate and isocitrate via cisaconitate in the citric acid and glyoxylate cycles.	Mutations in the *acnA* gene has no effect on the growth of *E. coli* [24].
rlmB	23S rRNA methyltransferase RlmB; responsible for the methylation of Gm2251 in 23S rRNA	RlmB has no important role in ribosome assembly or function in *E. coli* [25].
ydiF	Acetate-CoA transferase; displays enzymatic activity with short-chain acyl-CoAs.	Rangarajan et al. found that YdiF is involved in metabolism of short-chain fatty acid [26].
sbp	Sulfate/thiosulfate ABC transporter periplasmic binding protein Sbp; regulates the composition of sulfate–thiosulfate transporters.	Mutants in the *sbp* gene do not affect thiosulfate and sulfate binding or uptake [27].
hycF	Formate hydrogenlyase subunit HycF; enables utilization of formic acid as a source of carbon in *E. coli*.	HycF functions only under anaerobic conditions [28].
tfaE	Putative tail fiber assembly protein TfaE.	No study of the *tfaE* gene has yet been conducted.
ycjX	Putative protein YcjX.	No study of the *ycjX* gene has yet been conducted.

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