**Supplementary Information to**

**Parallel evolution of high-level aminoglycoside resistance in *Escherichia coli* under low and high mutation supply rates**

**By Claudia Ibacache-Quiroga, Juan Carlos Oliveros, Alejandro Couce and Jesús Blázquez.**

**Supplementary Table S1. Bacterial strains used in this study.**

|  |  |  |
| --- | --- | --- |
| **Strain** | **Description** | **Reference** |
| **MG1655** | F- λ- *ilvG rfb*-50 *rph*-1 | Laboratory collection |
| **BW25113** | F- Δ(*araD*-*araB)*567 Δ*lac*Z4787::rrnB-3. LAM- *rph-1*. Δ(*rhaD*-*rhaB)*568 *hsdR*514 | Laboratory collection |
| **JW0205** | BW25113 Δ*dnaQ*::Kan. KanR | [1] |
| **JW3711** | BW25113 Δ*atpG*::Kan. KanR | [1] |
| **JW0422** | BW25113 ΔcyoA::Kan. KanR | [1] |
| **DH5α** | F-. *endA*1 *glnV*44 *thi*-1 *recA*1 *relA*1 *gyrA*96 *deoR* *nupG* Φ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)U169 *hsdR*17(rK- mK+). λ– | Laboratory collection |
| **CC118** | Δ*(ara-leu) araD* Δ*lacX74 galE galK phoA20 thi-1 rpsE rpoB argE (*Am*) recA1 λpir* phage lysogen | [2] |
| **JM109** | F´ *traD*36 *proA+B*+ *lacI*q Δ[3]M15/ Δ(*lac-proAB*) *glnV*44 e14- *gyrA*96 *recA*1 *relA*1 *endA*1 *thi* hsdR17 | Dr. L.A. Fernández Laboratory |
| **FhuA196** | MG1655-derivatives with and insertion at position 197 | This study |
| **EFG593** | MG1655-derivatives. FusAF593L | This study |
| **PotA208** | MG1655-derivatives. PotAQ208L | This study |

**Supplementary Table S2. Plasmids used in this study.**

|  |  |  |
| --- | --- | --- |
| **Vector** | **Description** | **Reference** |
| **pCA24N** | ColE1-ori *cat* *lacIq* PT5lac. ChlR. | [4] |
| **pCA-*atpG*** | Plasmid derived from pCA24N with *atpG* insertion*.* ChlR. | [4] |
| **pCA-*cyoA*** | Plasmid derived from pCA24N with *cyoA* insertion*.* ChlR. | [4] |
| **pCA-*fhuA*** | Plasmid derived from pCA24N with *fhuA* insertion. ChlR. | [4] |
| **pCA-*fusA*** | Plasmid derived from pCA24N with *fusA* insertion*.* ChlR*.* | [4] |
| **pCA-*potA*** | Plasmid derived from pCA24N with *potA* insertion. ChlR. | [4] |
| **pKD46** | *repA*101(ts) oriR101 *bla* ParaB‐(gam bet exo). AmpR | [5] |
| **pCP20** | λcI857(ts) *repA*101(ts) oriR101 *bla cat* λpR‐FLP. ChlR. AmpR. | [5] |
| **pGE** | R6K-ori. *polylinker* region delimited by two I-*Sce*I restriction sites. ChlR. | [6] |
| **pACBSR** |  p15A-ori. PBAD. I-*Sce*I. λ Red. ChlR. | [7] |

**Supplementary Table S3. Oligonucleotides used in this study**

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene** | **Primer** | **Sequence (5´- 3´)** | **Referece** |
| ***dnaQ*** | dnaQ-F | TAATTGAATCGAACTGTAAAAC | [1]  |
| dnaQ-R | GCTATTTTTAGCGCCTTTCACA |
| ***atpG*** | atpG-F-XhoI | ACACACCTCGAGTTCAAAGCAACCCAATCCTG | This work |
| atpG-R-SpeI | ACACACACTAGTGACAATCTTTCCAGTAGCCATC |
| ***cyoA*** | cyoA-F | CCGAACATCTTTATTCTTCCTCAAC | [1]  |
| cyoA-R | CACACACTTTAAACGCCACCAGA |
| cyoA-F-XhoI | ACACACCTCGAGATGAGACTCAGGAAATACAATAA | This work |
| cyoA-R-SpeI | ACACACACTAGTTCACCTTCTGGCTGGGTCA |
| ***fhuA*** | fhuA-F-XhoI | ACACACCTCGAGATGGCGCGTTCCAAAACTGCTCAG | This work |
| fhuA-R-SpeI | ACACACACTAGTGCGGTTGCAACGACCTGACGTT |
| ***fusA*** | fusA-F-XhoI | ACACACCTCGAGATGGCTCGTACAACACCCATCGC | This work |
| fusA-R-SpeI | ACACACACTAGTTTATTTACCACGGGCTTCAATTAC |
| ***potA*** | potA-F-XhoI | ACACACCTCGAGATGGGACAGAGTAAAAAATTGAATAAAC | This work |
| potA-R-SpeI | ACACACACTAGTTCAGCCAGTACGACCTCCCA |

**Supplementary Table S4. Gentamicin-resistant strains**

|  |  |
| --- | --- |
| **MG1655-derivatives** | ***ΔdnaQ*-derivatives** |
| CIM5A | CIM5T | CIQ1B | CIQ1M | CIQ2G | CIQ4J |
| CIM5C | CIM8B | CIQ1C | CIQ1O | CIQ2H | CIQ4O |
| CIM5F | CIM8C | CIQ1D | CIQ1P | CIQ2I | CIQ4P |
| CIM5G | CIM8D | CIQ1E | CIQ1Q | CIQ2J | CIQ4Q |
| CIM5H | CIM8E | CIQ1F | CIQ1R | CIQ2N | CIQ4S |
| CIM5I | CIM8F | CIQ1G | CIQ1S | CIQ2O |   |
| CIM5K | CIM8G | CIQ1H | CIQ1T | CIQ2P |   |
| CIM5M | CIM8K | CIQ1I | CIQ2B | CIQ2R |   |
| CIM5N | CIM8L | CIQ1J | CIQ2C | CIQ2S |   |
| CIM5P | CIM8M | CIQ1K | CIQ2D | CIQ4B |   |
| CIM5R | CIM8N | CIQ1L | CIQ2E | CIQ4C |   |

**Supplementary Table S5. Susceptibility to different antimicrobials of MG1655- derivatives.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Ancestor** | **Strain** | **Minimal Inhibitory Concentration** (μg/ml) |  |
| **GEN** | **AMP** | **CHL** | **COL** | **CIP** | **FOS** | **TET** | **RIF** | **TRM** | **EtB** |
| MG1655 | MG1655 | 0.25 | 16 | 4 | 1 | 0.008 | 0.25 | 0.25 | 4 | 0.5 | 64 |
| CIM5A | 512 | 16 | 0.25 | 2 | ≤0.0001 | ≥8 | 0.5 | ≥16 | 0.25 | ≤8 |
| CIM5C | 512 | 32 | 2 | 1 | 0.008 | ≥8 | 1 | ≥16 | 0.5 | 128 |
| CIM5F | 512 | 32 | 2 | 1 | 0.008 | ≥8 | 1 | ≥16 | 0.25 | 64 |
| CIM5G | 512 | 32 | 0.5 | 2 | 0.008 | ≥8 | 1 | ≥16 | 0.5 | 32 |
| CIM5H | 512 | 16 | 0.25 | 2 | 0.008 | ≥8 | 0.5 | ≥16 | 0.25 | ≤8 |
| CIM5I | 512 | 8 | 0.5 | 2 | 0.008 | ≥8 | 0.5 | ≥16 | 0.125 | ≤8 |
| CIM5K | 512 | 32 | 1 | 1 | 0.008 | ≥8 | 0.5 | ≥16 | 0.25 | 32 |
| CIM5M | 512 | 32 | 0.5 | 2 | 0.008 | ≥8 | 0.5 | ≥16 | 0.25 | 32 |
| CIM5N | 512 | 32 | 1 | 2 | 0.008 | ≥8 | 1 | ≥16 | 2 | 128 |
| CIM5P | 512 | 16 | ≤0.125 | 2 | ≤0.0001 | ≥8 | 0.5 | ≥16 | 0.125 | 64 |
| CIM5R | 512 | 16 | ≤0.125 | 2 | ≤0.0001 | ≥8 | 0.25 | ≥16 | 0.125 | ≤8 |
| CIM5T | 512 | 32 | 1 | 1 | 0.008 | ≥8 | 1 | ≥16 | 0.25 | 64 |
| CIM8B | 512 | 4 | ≤0.125 | 0.5 | 0.008 | ≥8 | 0.25 | ≥16 | 0.125 | 32 |
| CIM8C | 512 | 16 | 1 | 2 | 0.008 | ≥8 | 0.5 | ≥16 | 0.5 | 32 |
| CIM8D | 512 | 8 | 1 | 1 | 0.008 | ≥8 | 0.063 | ≥16 | 0.25 | ≤8 |
| CIM8E | 512 | 8 | ≤0.125 | 0.5 | 0.008 | ≥8 | 1 | ≥16 | 0.125 | ≤8 |
| CIM8F | 512 | 8 | ≤0.125 | 0.5 | 0.008 | ≥8 | 0.5 | ≥16 | 0.125 | ≤8 |
| CIM8G | 512 | 4 | ≤0.125 | 0.5 | 0.008 | ≥8 | 0.125 | ≥16 | 0.125 | ≤8 |
| CIM8K | 512 | 8 | ≤0.125 | 0.5 | 0.008 | ≥8 | 0.5 | ≥16 | 0.25 | ≤8 |
| CIM8L | 512 | 8 | ≤0.125 | 0.5 | 0.008 | ≥8 | 1 | ≥16 | 0.125 | 32 |
| CIM8M | 512 | 8 | ≤0.125 | 0.5 | 0.008 | ≥8 | 0.5 | ≥16 | 0.125 | 64 |
| CIM8N | 512 | 8 | ≤0.125 | 0.5 | 0.008 | ≥8 | 0.5 | ≥16 | 0.25 | ≤8 |

**Supplementary Table S6. Susceptibility to different antimicrobials of Δ*dnaQ-* derivatives.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Ancestor** | **Strain** | **Minimal Inhibitory Concentration** (μg/ml) |  |
| **GEN** | **AMP** | **CHL** | **COL** | **CIP** | **FOS** | **TET** | **RIF** | **TRM** | **EtB** |
| *ΔdnaQ* | *ΔdnaQ* | 0.25 | 32 | 4 | 1 | 0.016 | 1 | 2 | 4 | 0.5 | 32 |
| CIQ1B | 512 | 64 | 0.25 | 2 | 0.008 | ≥8 | 0.5 | 2 | 0.25 | 64 |
| CIQ1C | 512 | 64 | 1 | 2 | 0.008 | ≥8 | 0.125 | 8 | 0.25 | 64 |
| CIQ1D | 512 | 64 | 1 | 2 | 0.008 | ≥8 | 0.125 | 4 | 0.25 | 16 |
| CIQ1E | 1024 | 64 | 0.25 | 2 | 0.008 | ≥8 | 0.125 | 4 | 0.25 | 32 |
| CIQ1F | 512 | 64 | 1 | 2 | 0.008 | ≥8 | 0.5 | 4 | 0.5 | 64 |
| CIQ1G | 1024 | 16 | 0.25 | 2 | 0.008 | ≥8 | 0.5 | ≤1 | 0.063 | 512 |
| CIQ1H | 1024 | 8 | 1 | 2 | 0.008 | ≥8 | 0.5 | ≤1 | 0.063 | 32 |
| CIQ1I | 512 | 64 | 1 | 2 | 0.008 | ≥8 | 0.5 | 4 | 0.25 | 64 |
| CIQ1J | 1024 | 32 | 0.25 | 2 | 0.008 | ≥8 | 0.5 | 4 | 0.063 | 64 |
| CIQ1K | 512 | 32 | 0.25 | 2 | 0.008 | ≥8 | 1 | 4 | 0.063 | 64 |
| CIQ1L | 512 | 64 | 1 | 16 | 0.008 | ≥8 | 0.125 | 4 | 0.063 | 64 |
| CIQ1M | 256 | 64 | 1 | 2 | 0.008 | ≥8 | 0.125 | 8 | 0.063 | 32 |
| CIQ1O | 512 | 64 | 2 | 2 | 0.008 | ≥8 | 1 | 2 | 0.063 | 64 |
| CIQ1P | 512 | 64 | 2 | 2 | 0.008 | ≥8 | 1 | 2 | 0.5 | 64 |
| CIQ1Q | 1024 | 8 | 1 | 1 | 0.008 | ≥8 | 0.5 | 1 | 0.063 | 32 |
| CIQ1R | 512 | 64 | 1 | 2 | 0.008 | ≥8 | 0.5 | 4 | 0.25 | 128 |
| CIQ1S | 512 | 64 | 0.25 | 2 | 0.008 | ≥8 | 1 | 4 | 0.063 | 8 |
| CIQ1T | 512 | 32 | 0.25 | 2 | 0.008 | ≥8 | 0.125 | ≤1 | 0.25 | 64 |
| CIQ2B | 512 | 64 | 0.25 | 2 | 0.008 | ≥8 | 0.125 | ≤1 | 0.25 | 64 |
| CIQ2C | 512 | 64 | 1 | 1 | 0.008 | ≥8 | 0.5 | ≤1 | 0.5 | 128 |
| CIQ2D | 512 | 64 | 1 | 0.5 | 0.008 | ≥8 | 0.25 | 4 | 0.5 | 64 |
| CIQ2E | 512 | 64 | 1 | 2 | 0.008 | ≥8 | 0.25 | 2 | 0.25 | 64 |
| CIQ2G | 512 | 128 | 1 | 0.5 | 0.008 | ≥8 | 1 | 4 | 0.5 | 128 |
| CIQ2H | 512 | 64 | 1 | 1 | 0.008 | ≥8 | 0.5 | 4 | 0.5 | 64 |
| CIQ2I | 512 | 64 | 1 | 1 | 0.008 | ≥8 | 2 | 4 | 0.5 | 64 |
| CIQ2J | 512 | 128 | 1 | 1 | 0.008 | ≥8 | 0.5 | 2 | 0.5 | 64 |
| CIQ2N | 512 | 64 | 1 | 2 | 0.008 | ≥8 | 0.5 | 8 | 0.5 | 64 |
| CIQ2O | 512 | 128 | 1 | 4 | 0.008 | ≥8 | 0.5 | 2 | 0.5 | 64 |
| CIQ2P | 512 | 128 | 1 | 1 | 0.008 | ≥8 | 1 | 2 | 0.5 | 64 |
| CIQ2R | 512 | 128 | 2 | 1 | 0.008 | ≥8 | 0.25 | 2 | 0.5 | 64 |
| CIQ2S | 512 | 64 | 1 | 2 | 0.008 | ≥8 | 0.25 | 4 | 0.25 | 64 |
| CIQ4B | 512 | 32 | 2 | 2 | 0.008 | ≥8 | 0.063 | 4 | 0.5 | 64 |
| CIQ4C | 512 | 32 | 4 | 2 | 0.008 | ≥8 | 0.25 | 8 | 0.5 | 64 |
| CIQ4J | 512 | 64 | 2 | 2 | 0.008 | ≥8 | 1 | 32 | 1 | 64 |
| CIQ4O | 512 | 32 | 2 | 2 | 0.008 | ≥8 | 0.5 | 8 | 0.5 | 64 |
| CIQ4P | 512 | 64 | 2 | 2 | 0.008 | ≥8 | 1 | 4 | 0.5 | 128 |
| CIQ4Q | 512 | 32 | 2 | 2 | 0.008 | ≥8 | 0.25 | 4 | 0.5 | 64 |
| CIQ4S | 512 | 64 | 2 | 1 | 0.008 | ≥8 | 0.25 | 8 | 0.25 | 64 |

**Supplementary Table S7. Metabolic characterization of the evolved strains.**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Compound** | **MG1655** | **CIM5H** | **CIM5N** | **CIM8C** | **CIM8M** | **Δ*dnaQ*** | **CIQ1E** | **CIQ1G** | **CIQ2J** | **CIQ4J** |
| alpha-Cyclodextrin | - | - | - | - | - | - | - | - | - | - |
| Dextrin | ++ | - | ++ | ++ | - | ++ | + | + | - | ++ |
| Glycogen | - | - | - | - | - | - | - | - | - | - |
| Tween 40 | - | - | - | - | - | - | - | - | - | - |
| Tween 80 | + | - | - | - | - | + | - | - | - | - |
| N- Acetyl-D-Galactosamine | - | - | - | - | - | - | - | - | - | - |
| N- Acetyl-D-Glucosamine | ++ | ++ | ++ | - | ++ | ++ | + | + | ++ | ++ |
| Adonitol | - | - | - | - | - | - | - | - | - | - |
| L-Arabinose | ++ | - | - | - | - | ++ | - | - | - | - |
| D-Arabitol | - | - | - | - | - | - | - | - | - | - |
| D-Cellobiose | - | - | - | - | - | - | + | - | - | - |
| i-Erythritol | - | - | - | - | - | - | - | - | - | - |
| D-Fructose | ++ | - | ++ | - | ++ | ++ | + | - | ++ | ++ |
| D-Fucose | ++ | - | + | - | - | ++ | - | - | - | - |
| D-Galactose | ++ | - | ++ | ++ | ++ | ++ | + | - | + | - |
| Gentobiose | - | - | - | - | - | - | + | - | - | - |
| alpha-D-Glucose | ++ | ++ | ++ | ++ | ++ | ++ | + | + | ++ | ++ |
| m-Inositol | - | - | - | - | - | - | - | - | - | - |
| alpha-D-Lactose | ++ | ++ | ++ | - | - | ++ | + | - | ++ | - |
| Lactulose | ++ | - | ++ | - | - | ++ | - | - | - | - |
| Maltose | ++ | ++ | ++ | - | - | ++ | + | - | - | - |
| D-Mannitol | ++ | ++ | - | - | ++ | ++ | + | + | ++ | ++ |
| D-Mannose | ++ | + | ++ | - | + | ++ | + | + | ++ | ++ |
| D-Melibiose | ++ | ++ | ++ | - | - | ++ | - | - | - | - |
| beta-Methyl-D-Glucoside | + | - | - | - | - | + | + | - | - | - |
| D-Psicose | + | - | - | - | - | + | + | - | - | - |
| D-Raffinose | - | - | - | - | - | - | - | - | - | - |
| L-Rhamnose | ++ | - | - | - | - | ++ | - | - | - | - |
| D-Sorbitol | ++ | - | - | ++ | ++ | ++ | - | + | ++ | ++ |
| Sucrose | - | - | - | - | - | - | + | - | - | - |
| D-Trehalose | ++ | ++ | ++ | - | ++ | ++ | + | + | ++ | ++ |
| Turanose | - | - | - | - | - | - | + | - | - | - |
| Xylitol | - | - | - | - | - | - | - | - | - | - |
| Pyruvic Acid Methyl Ester | ++ | ++ | ++ | - | - | ++ | - | - | ++ | ++ |
| Succinic Acid Mono-Methyl-Ester | + | - | - | - | - | + | - | - | - | - |
| Acetic Acid | + | - | - | - | - | + | - | - | - | - |
| Cis-Aconitic Acid | - | - | - | - | - | - | - | - | - | - |
| Citric Acid | - | - | - | - | - | - | - | - | - | - |
| Formic Acid | - | - | - | - | - | - | - | - | - | - |
| D-Galactonic Acid Lactone | ++ | - | ++ | - | - | ++ | - | - | - | - |

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Compound** | **MG1655** | **CIM5H** | **CIM5N** | **CIM8C** | **CIM8M** | **ΔdnaQ** | **CIQ1E** | **CIQ1G** | **CIQ2J** | **CIQ4J** |
| D-Galacturonic Acid | ++ | - | ++ | ++ | - | ++ | - | - | - | - |
| D-Gluconic Acid | ++ | - | ++ | - | - | ++ | - | + | ++ | ++ |
| D-Glucosaminic Acid | - | - |  | - | - | - | - | - | - | - |
| D-Glucoronic Acid | ++ | - | ++ | - | - | ++ | - | - | - | - |
| alpha-Hydroxybutyric Acid | - | - | - | - | - | - | - | - | - | - |
| beta-Hydroxybutyric Acid | - | - | - | - | - | - | - | - | - | - |
| gamma-Hydroxybutyric Acid | - | - | - | - | - | - | - | - | - | - |
| p-Hydroxy Phenylacetic Acid | - | - | - | - | - | - | - | - | - | - |
| Itaconic Acid | - | - | - | - | - | - | - | - | - | - |
| alpha-Keto Butyric Acid | - | - | - | - | - | - | + | - | - | - |
| alpha-Keto Glutaryc Acid | ++ | - | - | - | - | ++ | - | - | - | - |
| alpha-Keto Valeric Acid | - | - | - | - | - | - | + | - | - | - |
| D,L- Lactic Acid | ++ | - | - | - | - | ++ | - | - | - | - |
| Malonic Acid | - | - | - | - | - | - | - | - | - | - |
| Propionic Acid | ++ | - | - | - | - | ++ | - | - | - | - |
| Quinic Acid | - | - | - | - | - | - | - | - | - | - |
| D-Saccharic Acid | ++ | - | ++ | - | - | ++ | - | - | - | - |
| Sebatic Acid | - | - | - | - | - | - | - | - | - | - |
| Succinic Acid | ++ | - | - | - | - | ++ | - | - | - | - |
| Bromosuccinic Acid | + | - | - | - | - | + | - | - | - | - |
| Succinamic Acid | - | - | - | - | - | - | - | - | - | - |
| Glucuronamide | ++ | - | - | - | - | ++ | - | - | - | - |
| L- Alaninamide | - | - | - | - | - | - | - | - | - | - |
| D-Alanine | ++ | - | - | - | - | ++ | - | - | - | - |
| L-Alanine | ++ | - | - | - | - | ++ | - | - | - | - |
| L-Alanyl-glycine | ++ | - | - | - | - | ++ | - | - | - | - |
| L-Asparagine | ++ | - | - | - | - | ++ | - | - | - | - |
| L-Aspartic Acid | ++ | - | - | - | - | ++ | - | - | - | - |
| L-Glutamic Acid | - | - | - | - | - | - | - | - | - | - |
| Glycyl-L-Aspartic Acid | ++ | - | - | - | - | ++ | - | - | - | - |
| Glycyl-L-Glutamic Acid | + | - | - | - | - | + | - | - | - | - |
| L-Histidine | - | - | - | - | - | - | - | - | - | - |
| Hydroxy-L-Proline | - | - | - | - | - | - | - | - | - | - |
| L-Leucine | - | - | - | - | - | - | - | - | - | - |
| L-Ornithine | - | - | - | - | - | - | - | - | - | - |
| L-Phenylalanine | - | - | - | - | - | - | - | - | - | - |
| L-Proline | + | - | - | - | - | + | - | - | - | - |
| L-Pyroglutamic Acid | - | - | - | - | - | - | - | - | - | - |
| D-Serine | ++ | - | ++ | - | - | ++ | - | - | - | - |
| L-Serine | ++ | - | - | - | - | ++ | - | - | + | + |

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Compound** | **MG1655** | **CIM5H** | **CIM5N** | **CIM8C** | **CIM8M** | **Δ*dnaQ*** | **CIQ1E** | **CIQ1G** | **CIQ2J** | **CIQ4J** |
| L-Threonine | - | - | - | - | - | - | - | - | - | - |
| D, L- Carnitine | - | - | - | - | - | - | - | - | - | - |
| gamma- Amino Butyric Acid | - | - | - | - | - | - | - | - | - | - |
| Urocanic Acid | - | - | - | - | - | - | - | - | - | - |
| Inosine | ++ | ++ | ++ | - | - | ++ | + | - | - | - |
| Uridine | ++ | - | - | - | - | ++ | + | - | - | - |
| Thymidine | ++ | - | - | - | - | ++ | + | - | - | - |
| Phenylethylamine | - | - | - | - | - | - | - | - | - | - |
| Putrescine | - | - | - | - | - | - | - | - | - | - |
| 2-Aminoethanol | - | - | - | - | - | - | - | - | - | - |
| 2,3-Butanediol | - | - | - | - | - | - | - | - | - | - |
| Glycerol | ++ | - | ++ | ++ | ++ | ++ | + | + | ++ | - |
| D,L-alpha-Glycerol Phosphate | + | + | ++ | - | - | + | - | + | - | + |
| alpha-D-Glucosa-1-Phosphate | ++ | ++ | ++ | ++ | ++ | ++ | - | + | ++ | - |
| D-Glucose-6-Phosphate | ++ | - | ++ | - | - | ++ | - | - | ++ | - |

**++** Positive reaction

**+**  Weak positive reaction

 **-** Negative reaction

**Supplementary Table S8. Enzymatic characterization of the evolved strains**.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Enzymatic Activity** | **MG1655** | **CIM5H** | **CIM5N** | **CIM8C** | **CIM8M** | **Δ*dnaQ*** | **CIQ1E** | **CIQ1G** | **CIQ2J** | **CIQ4J** |
| Beta-galactosidase | ++ | ++ | ++ | ++ | ++ | ++ | ++ | + | ++ | ++ |
| Arginine dihydrolase | - | - | - | - | - | - | - | - | - | - |
| Lysine decarboxylase | ++ | ++ | - | ++ | ++ | ++ | + | ++ | ++ | ++ |
| Ornithine decarboxylase | - | - | - | - | - | - | - | - | - | - |
| Citrate utilization | - | - | - | - | - | - | - | - | - | - |
| H2S production | - | - | - | - | - | - | - | - | - | - |
| Urea hydrolysis | - | - | - | - | - | - | - | - | - | - |
| Deaminase | + | - | - | - | - | + | + | + | + | + |
| Indole production | + | ++ | ++ | ++ | ++ | + | ++ | ++ | ++ | ++ |
| Gelatinase | - | - | - | - | - | - | - | - | ++ | - |
| Fermentation/Oxidation of glucose | + | ++ | ++ | + | ++ | + | ++ | ++ | ++ | ++ |
| Fermentation/Oxidation of mannitol | + | ++ | + | + | ++ | + | - | + | + | + |
| Fermentation/Oxidation of inositol | - | - | - | - | - | - | - | - | - | - |
| Fermentation/Oxidation of sorbitol | + | - | - | + | ++ | + | + | + | + | + |
| Fermentation/Oxidation of rhamnose | + | - | - | + | + | + | - | + | + | + |
| Fermentation/Oxidation of sucrose | - | - | - | - | - | - | - | - | - | - |
| Fermentationn/Oxidation of melibiose | + | - | - | + | + | + | - | + | - | + |
| Fermentation/Oxidation of amygdalin | - | - | - | - | + | - | - | - | - | - |
| Fermentation/Oxidation of arabinose | + | ++ | ++ | ++ | ++ | + | + | + | + | + |

**++** Positive reaction

**+**  Weak positive reaction

 **-** Negative reaction

**Supplementary Table S9. CIM8M susceptibility to aminoglycosides**

|  |  |
| --- | --- |
| **Strain** | **MIC** (μg/ml) |
| **GEN** | **AMK** | **KAN** |
| **MG1655** | 0.25 | 2 | 4 |
| **CIM8M** | 512 | ≥ 64 | ≥ 64 |

**Supplementary Table S10. Mutational trajectories**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|

|  |  |
| --- | --- |
| **Genetic element** | **Evolutionary step (μg/ml)a** |
|  | **MG1655b** | **Δ*dnaQ*c** |
| ***fusA*** |  | 0.5 | 0.5 |
| ***cyo* operon** |  | 4 | 0.5 |
| ***potABCD* operon** |  | 4 | 0.5 |
| ***fhuA*** |  | 8 | 2 |
| ***atp* operon** |  | 8 | 8 |

 |

**a** Concentration of gentamicin in chemostats at which mutations on the genetic elements could be identified**.**

**b** MG1655 derivatives

**c** Δ*dnaQ* derivatives

**Supplementary Table S11. Susceptibility to fosfomycin of CIM8M complemented with selected genes.**

|  |  |  |
| --- | --- | --- |
| **Strain** |  | **MIC FOS** (μg/ml) |
| **CIM8M pCA24N** |  | 32 |
| **CIM8M pCA-*atpG*** |  | 32 |
| **CIM8M pCA-*cyoA*** |  | 32 |
| **CIM8M pCA-*fhuA*** |  | 16 |
| **CIM8M pCA-*fusA*** |  | 32 |
| **CIM8M pCA-*potA*** |  | 32 |

|  |
| --- |
|  |

**Supplementary Table S12. Susceptibility to chloramphenicol of single-gene mutants.**

|  |  |  |
| --- | --- | --- |
| **Strain** |  | **MIC CHL** (μg/ml) |
| **MG1655** |  | 4 |
| **FhuA197** |  | 2 |
| **EFG593** |  | 2 |
| **PotA208** |  | 4 |
| **BW25113** |  | 4 |
| **JW3711** |  | 8 |
| **JW0422** |  | 4 |



**A**

**B**



**Supplementary Figure S1. Effect on growth of complementation with wild-type genes in CIM8M.** The effect of complementation of CIM8M was evaluated on M9 minimal media supplemented with glucose without (A) and with gentamicin at a final concentration of 128 μg/ml (B). Error bars correspond to the standard deviation of three independent replicates.

**Supplementary References**

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