Supplementary information:

Rapid genetic adaptation during the first four months of survival under resource exhaustion

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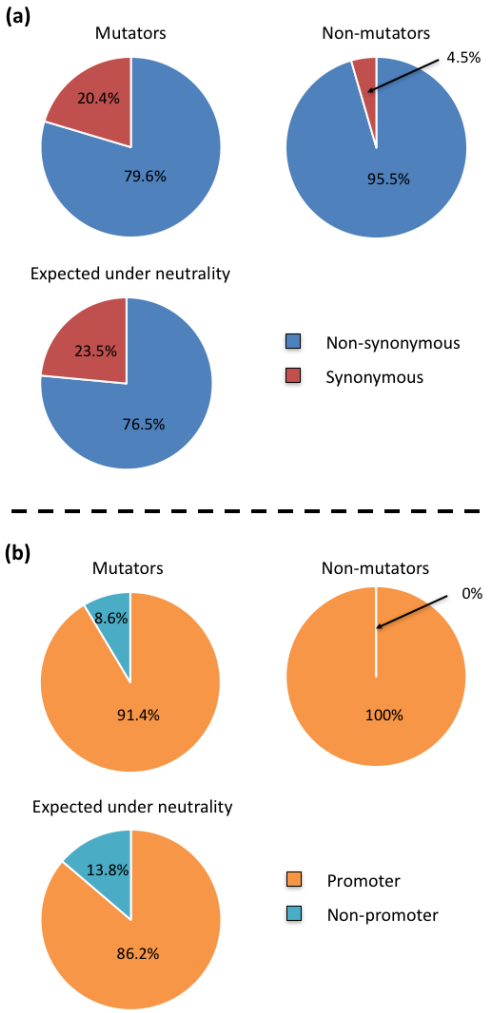
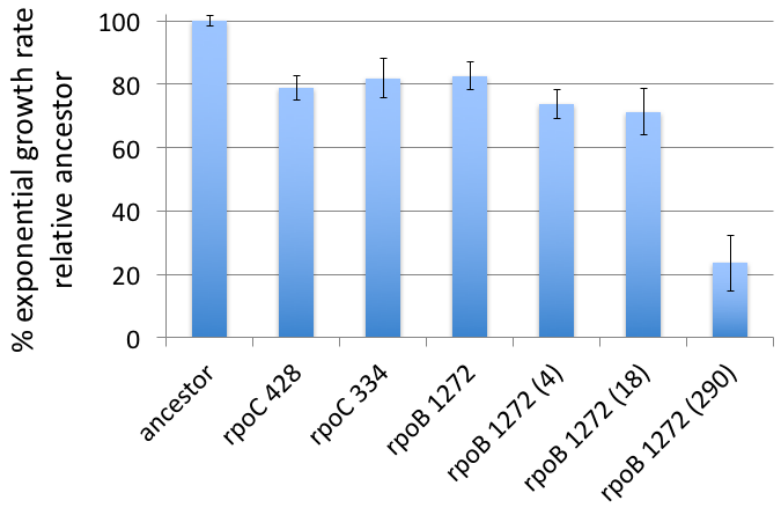
Supplementary Table S3. List of fully sequenced bacteria carrying the RpoC 428S LTSP allele

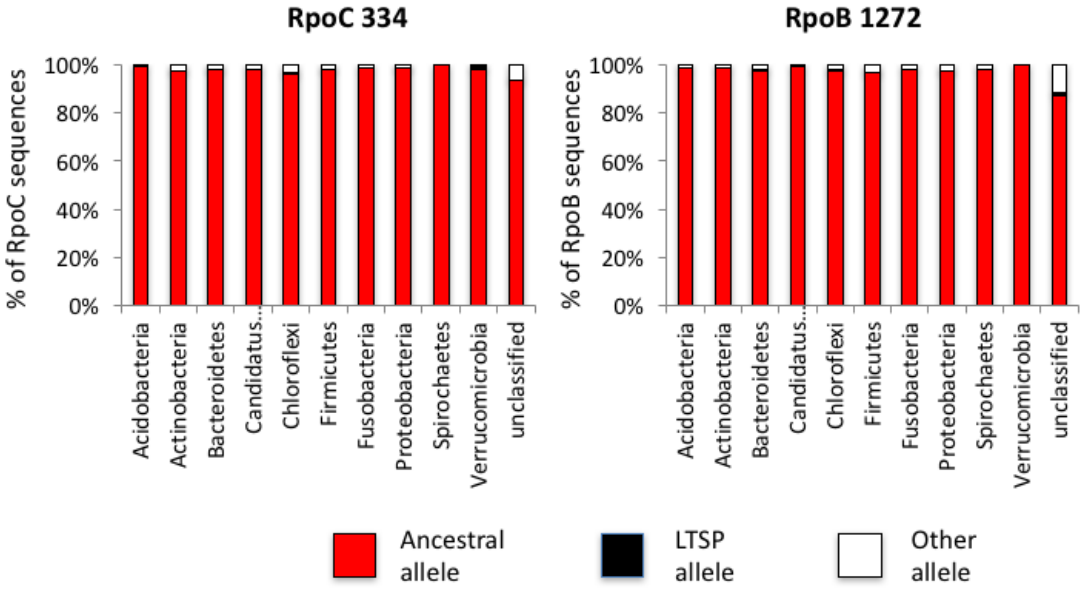
Accession	Name	Phyla
WP_023794300.1|GCF_000503915.1	*Candidatus Saccharibacteria* bacterium RAAC3_TM7_1	Candidatus Saccharibacteria phyla
YP_001634495.1|GCF_000018865.1	*Chloroflexus aurantiacus*	Chloroflexales
WP_012660547.1|GCF_000022185.1	*Chloroflexus* sp. Y-400-fl	Chloroflexales
WP_015941431.1|GCF_000021945.1	*Chloroflexus aggregans*	Chloroflexales
WP_011956021.1|GCF_000016665.1	*Roseiflexus* sp. RS-1	Chloroflexales
WP_012120118.1|GCF_000017805.1	*Roseiflexus castenholzii*	Chloroflexales
WP_047264431.1|GCF_001021085.1	*Candidatus Liberibacter africanus*	Rhizobiales
WP_012778362.1|GCF_000023765.2	*Candidatus Liberibacter asiaticus*	Rhizobiales
WP_012778362.1|GCF_000590865.2	*Candidatus Liberibacter asiaticus*	Rhizobiales
WP_045490085.1|GCF_000829355.1	*Candidatus Liberibacter asiaticus*	Rhizobiales
WP_012778362.1|GCF_000346595.1	*Candidatus Liberibacter asiaticus*	Rhizobiales
WP_007557346.1|GCF_000496595.1	*Candidatus Liberibacter americanus*	Rhizobiales
WP_013462401.1|GCF_000183665.1	*Candidatus Liberibacter solanacearum*	Rhizobiales



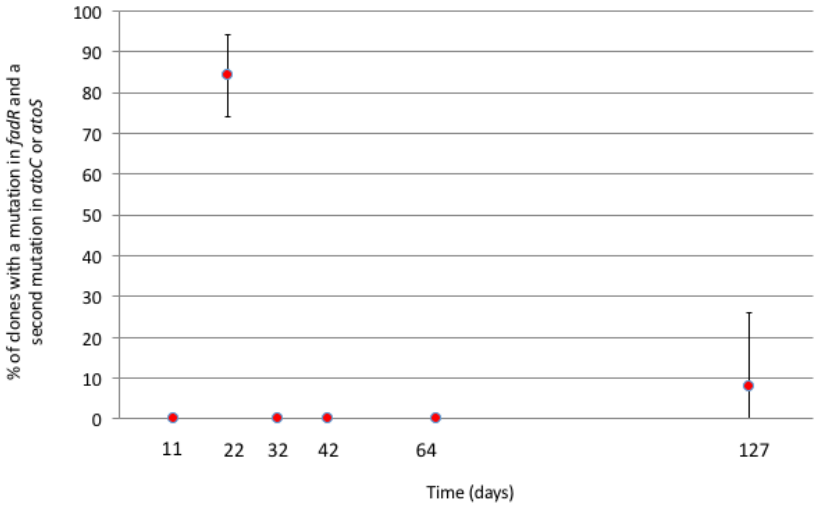
Supplementary fig. S1. Significant enrichment in categories of mutations that are more likely to carry a functional effect within LTSP clones. (a) Significant enrichment in non-synonymous (i.e. amino-acid altering) vs. synonymous (i.e. non-amino-acid altering) mutations, relative neutral expectations. (b) Significant enrichment in mutations within intergenic regions likely to contain promoters, vs. intergenic regions less likely to contain promoters, relative neutral expectations. In both cases neutral expectations were estimated based on the distribution of sites within the *E. coli* K12 MG1655 genome (Materials and Methods).



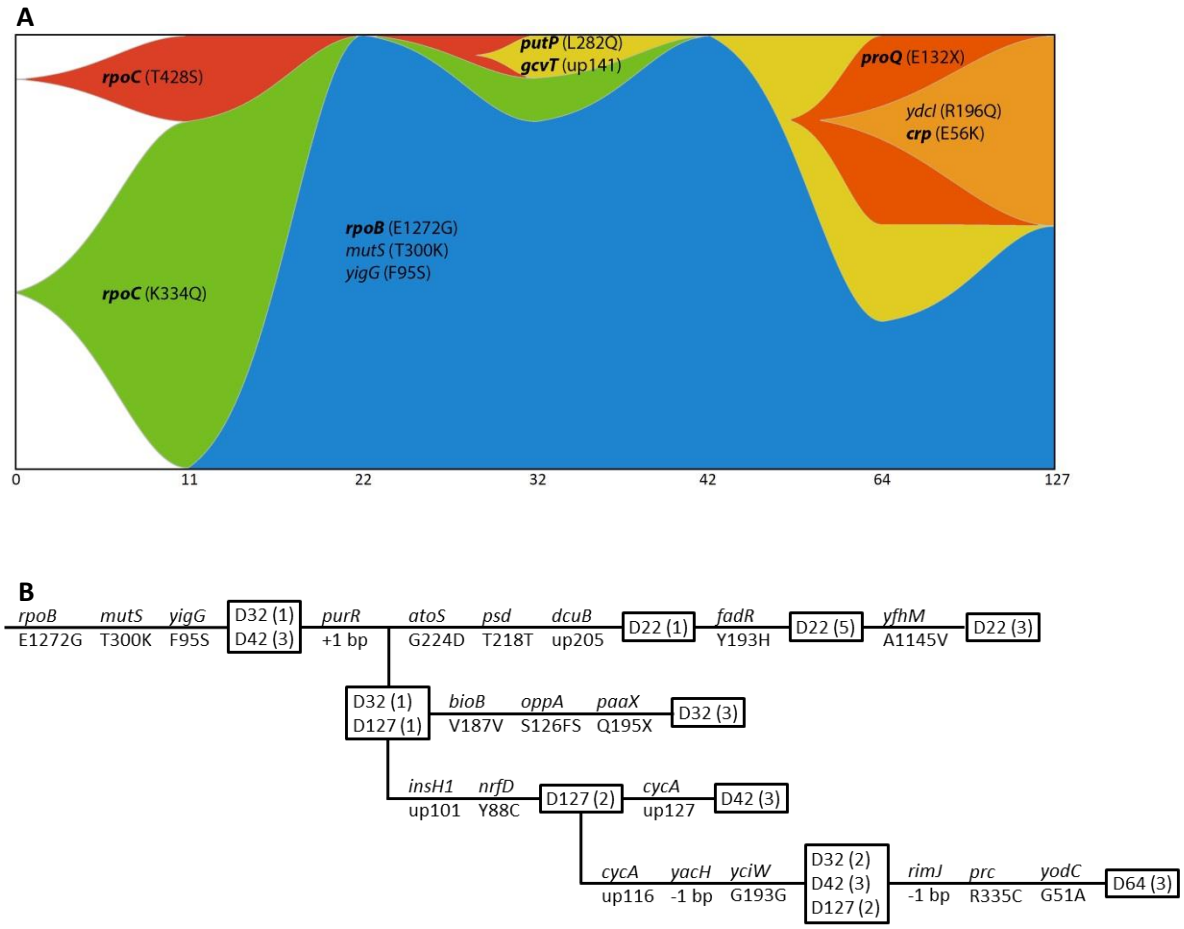
Supplementary fig. S2. LTSP clones suffer significant reductions in exponential growth-rates in fresh LB. Depicted are the mean exponential growth rates of each clone tested, relative the mean exponential growth rate of the ancestor *E. coli* strain. For each clone five independent growth-experiments were carried out using a plate reader. Error bars represent standard deviations across the five growth experiments.



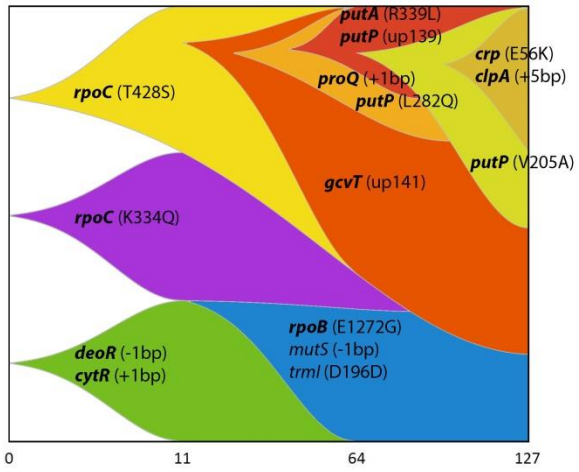
Supplementary fig. S3. Extremely high conservation of RpoC position 334 K and RpoB position 1272 E alleles within natural microbiomes. Depicted is the relative frequency of the *E. coli* ancestral allele (Red), the LTSP adaptive allele (black) and all other alleles (white) within RpoC and RpoB sequences extracted from a large collection of metagenomic samples (Materials and Methods). Only phyla for which at least 100 RpoB or RpoC sequences could be analyzed are depicted.



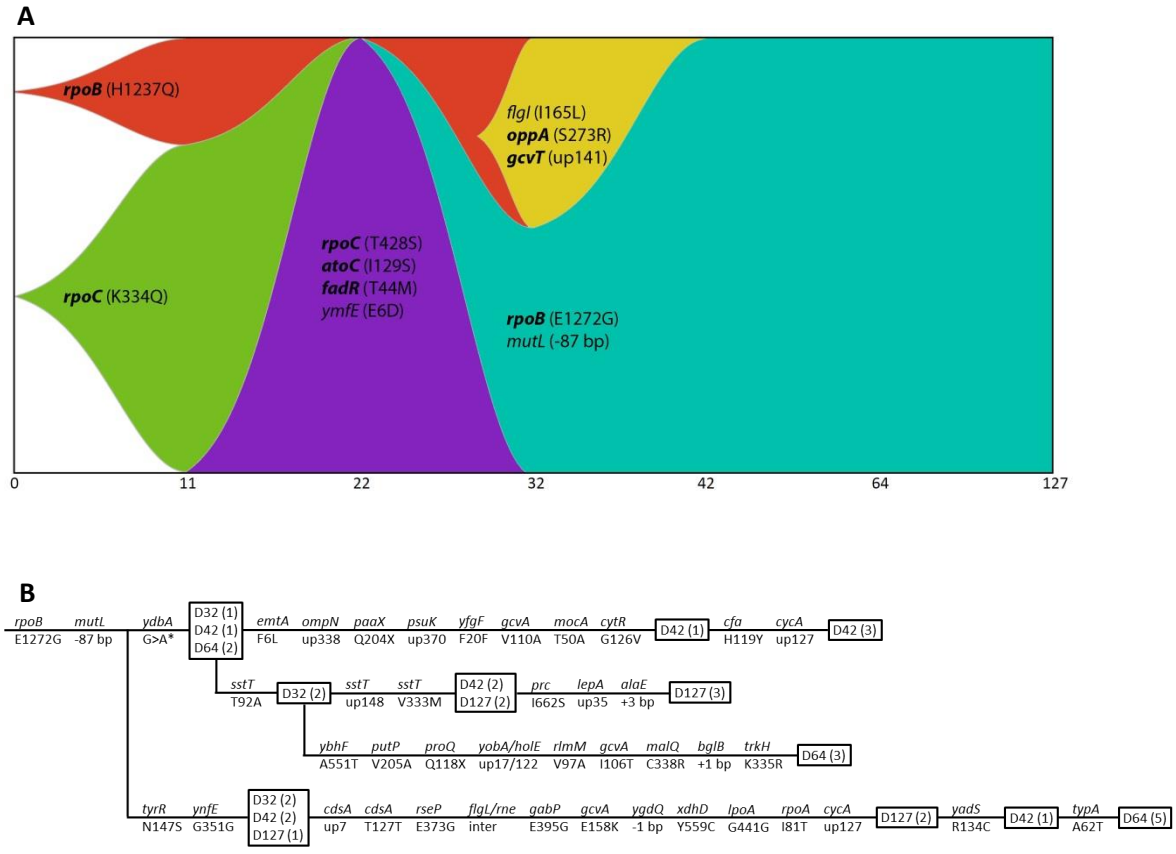
Supplementary fig. S4. Temporal adaptation via modifications to the regulation of fatty acid metabolism. Depicted is the mean percentage of sequenced clones carrying a mutation in *fadR* and a second mutation in *atoC* or *atoS* across all examined populations. Error bars represent the standard deviation across the three or five populations (depending on how many populations were sampled at that time point, Figure 2).



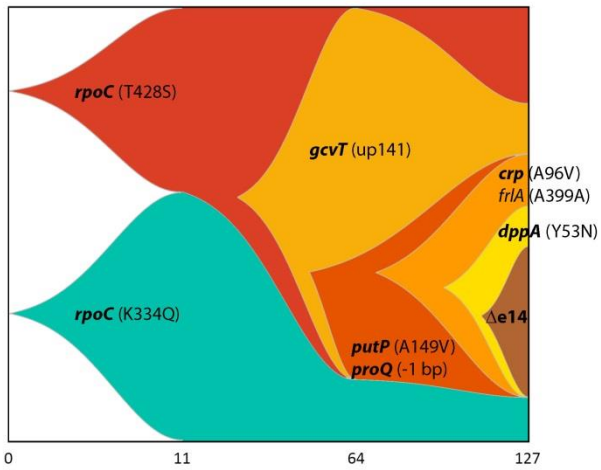
Supplementary fig. S5. Clear pattern of clonal interference observed within LTSP population number 2. (A) Presented is a Muller diagram depicting the relative frequencies of different haplotypes segregating within LTSP population 2. The X-axis indicates the sampling times. Only mutations appearing in 30% or more of population 2 clones, in at least one time point, were used to generate the plot. Gene names appearing in bold represent genes that are mutated in at least three of the five LTSP populations. The pattern of haplotypes derived from the mutator linage colored in blue is too complex to draw in a Muller diagram, for this reason we include: (B) A diagram depicting the relative frequencies of different haplotypes derived of the mutator lineage colored in blue in panel A. Only mutations appearing in 30% or more of population 2 clones, in at least one time point, were used to generate the plot. D# (#) = day of clone isolation (# of clones carrying these mutations); up = upstream.



Supplementary fig. S6. Clear pattern of clonal interference observed within LTSP population number 3. Presented is a Muller diagram depicting the relative frequencies of different haplotypes segregating within LTSP population 3. The X-axis indicates the sampling times. Only mutations appearing in 30% or more of population 3 clones, in at least one time point, were used to generate the plot. Gene names appearing in bold represent genes that are mutated in at least three of the five LTSP populations. up = upstream.



Supplementary fig. S7. Clear pattern of clonal interference observed within LTSP population number 4. (A) Presented is a Muller diagram depicting the relative frequencies of different haplotypes segregating within LTSP population 4. The X-axis indicates the sampling times. Only mutations appearing in 30% or more of population 4 clones, in at least one time point, were used to generate the plot. Gene names appearing in bold represent genes that are mutated in at least three of the five LTSP populations. The pattern of haplotypes derived from the mutator linage colored in turquoise is too complex to draw in a Muller diagram, for this reason we include: (B) A diagram depicting the relative frequencies of different haplotypes derived of the mutator lineage colored in turquoise in panel A. Only mutations appearing in 30% or more of population 4 clones, in at least one time point, were used to generate the plot. D# (#) = day of clone isolation (# of clones carrying these mutations); up = upstream.



Supplementary fig. S8. Clear pattern of clonal interference observed within LTSP population number 5. Presented is a Muller diagram depicting the relative frequencies of different haplotypes segregating within LTSP population 5. The X-axis indicates the sampling times. Only mutations appearing in 30% or more of population 5 clones, in at least one time point, were used to generate the plot. Gene names appearing in bold represent genes that are mutated in at least three of the five LTSP populations. up = upstream; Δe14 = deletion of prophage e14.