Figures and figure supplements

Changes in the genetic requirements for microbial interactions with increasing community complexity

Manon Morin et al
Figure 1. Identification of genes important for growth of *E. coli* alone on cheese curd agar (Figure 1—source data 1). (A) The pooled *E. coli* RB-TnSeq library Keio_ML9 (Wetmore et al., 2015) was grown alone on cheese curd agar (CCA). Gene fitness values were calculated for 3298 genes at days 1, 2, and 3 along with a t-score, which assesses how reliably the fitness value differs from 0. The fitness values obtained at the three timepoints are displayed on a single volcano plot (3 points per gene). A t-score threshold of absolute(t-score) \geq 3 was used to identify genes with strong fitness effects. 97% of the genes fell below this threshold and have no strong and significant fitness effect. Black dots represent genes with strong negative fitness effects. Altogether, they represent 160 different genes that are associated with a significant fitness value for at least one timepoint. (B) These 160 genes were mapped to the KEGG BRITE Database for functional analysis and identification of required functions for *E. coli* growth on CCA. 84 of the 160 genes had hits when mapped to the KEGG BRITE database.

DOI: https://doi.org/10.7554/eLife.37072.003
Figure 1—figure supplement 1. Pipeline of RB-TnSeq experiment using the *E. coli* Keio M9 library: from experimental set-up to normalized gene fitness and t-score calculation. Calculation of gene fitness from the Illumina sequencing files was performed using the Perl script BarSeqTest.pl from (Wetmore et al., 2015). The detailed description of the pipeline can be found in (Wetmore et al., 2015).

DOI: https://doi.org/10.7554/eLife.37072.004
Figure 1—figure supplement 2. Quantification of free amino acids in CCA. Free amino acid characterization and quantification have been carried out by the Proteomics and Mass Spectrometry Facility of the Donald Danforth Plant Science Center and each analysis has been performed in triplicate. Samples were prepared as recommended by the Proteomics and Mass Spectrometry Facility of the Donald Danforth Plant Science Center instructions. For free amino acid analysis, 150 mg CCA was frozen in liquid nitrogen and ground in the presence of 600 μL of water:chloroform:methanol (3:5:12 v/v). Tubes were then centrifuged at full speed for two minutes and supernatant was recovered in a new 2 mL eppendorf tube. A second extraction with 600 μL of water:chloroform:methanol was performed followed by a two minute centrifugation at maximum speed. Supernatant was then recovered and combined with the previous supernatant in a 2 mL eppendorf tube. Then 300 μL of chloroform and 450 μL of water were added before centrifugation at full speed for two minutes. The upper phase was recovered and transferred to a new tube. Samples were dried in a speedvac overnight and stored at −20°C. The total concentration of free amino acids in CCA is 75.3 nmol/mL. Analysis of total amino acids was also performed by the Proteomics and Mass Spectrometry Facility of the Donald Danforth Plant Science Center. It highlights that free aminos are a very small fraction of total amino acid whose concentration is 16.5 ± 2.97 μmol/mL (six times less than the LB concentration measured by Sezonov et al. (2007)). Ala: alanine, Gly: glycine, Ile: isoleucine, Leu: leucine, Pro: proline, Val: valine, Phe: phenylalanine, Trp: tryptophan, Tyr: tyrosine, Asp: aspartate, Glu: glutamate, Arg: arginine, His: histidine, Lys: lysine, Ser: serine, Thr: threonine, Cys: cysteine, Met: methionine, Asn: asparagine, Gln: glutamine.

DOI: https://doi.org/10.7554/eLife.37072.005
Figure 1—figure supplement 3. Comparison of individual growth of enterobactin biosynthesis mutants on LB and CCA. 5% tetrazolium solution (100 μL/L of medium) was added to the media to color colonies and make them visible on CCA. We selected 4 mutants of enterobactin biosynthesis from the Keio collection. 5 μL of each mutant culture was spotted on either LB-tetrazolium or CCA-tetrazolium and grown at room temperature for 3 days. The wild-type strain JW0024 was used as a control.

DOI: https://doi.org/10.7554/eLife.37072.006
Figure 1—figure supplement 4. RB-TnSeq experiments using the P. psychrophila JB418 library. (A) We grew P. psychrophila JB418 alone, in pairwise conditions with H. alvei, G. candidum or P. camemberti and with the full community on CCA. (B) Using the transposon library of P. psychrophila JB418 generated in the laboratory, we identified genes with negative fitness at 1, 2 and 3 days of growth. In each volcano plot, data from the three timepoints have been grouped. We identified 179 genes important for growth alone and the genes with negative fitness for growth in pairwise. We identified three categories: (i) core negative fitness genes with negative fitness at 1, 2 and 3 days of growth alone and all the pairwise conditions, (ii) pairwise-induced negative fitness: genes with negative fitness in at least one pairwise condition but no negative fitness alone and, (iii) pairwise-alleviated: genes with negative fitness in growth alone and no negative fitness in at least one pairwise condition. (E) We further investigated the KEGG functions associated with the core negative fitness, pairwise-induced negative fitness and pairwise alleviated negative fitness. (F) We then compared the community-induced negative fitness and community-alleviated negative fitness for P. psychrophila JB418 with the pairwise-induced and pairwise-alleviated negative fitness.

DOI: https://doi.org/10.7554/eLife.37072.007
Figure 1—figure supplement 5. Competitive assays of 25 mutants of the Keio collection (Baba et al., 2006). Competition assays between single knockouts and the wild-type strain have been carried out for 25 strains associated with genes identified as important for \textit{E. coli} growth using RB-TnSeq (Significant fitness lower than -1 after 1 day of growth). * highlights fitness values different from 0 with a confidence higher than 95%. The competitive assays were performed in at least triplicates. SEM stands for: standard deviation of the mean: 

\[ SEM = \frac{\sigma}{\sqrt{n}} \]

where \( \sigma \) is the standard deviation and \( n \) the number of replicates. Then, \( z = \frac{\mu - \bar{x}}{SEM} \) where \( \mu \) is the experimental fitness value (average of the replicates).

DOI: https://doi.org/10.7554/eLife.37072.009

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<tr>
<th>Keio strain</th>
<th>Gene name</th>
<th>RB-TnSeq fitness</th>
<th>Experimental Fitness value</th>
<th>SEM of Experimental Fitness value</th>
<th>z-score</th>
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<td>JW3140</td>
<td>argG</td>
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<td>-1.38</td>
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<td>JW0391</td>
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<td>JW2974</td>
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<td>JW0890</td>
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<td>JW5576</td>
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<td>JW5566</td>
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Map of the JB418_ECP1 transposon library generated in *P. psychrophila* JB418. We built a barcoded-transposon library in the cheese isolate *P. psychrophila* JB418. 272,329 insertions were mapped to the genome and 143,491 barcodes were located in the central region of a gene. The median of number of insertion mutants per gene is 18, similar to *E. coli*’s library. 4811 genes have at least one insertion in the *P. psychrophila* JB418 chromosome 5.85 Mb.

<table>
<thead>
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<th>Genome size</th>
<th>6.07 Mbp</th>
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<td>Number of protein-coding genes (PCG)</td>
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<td>Number of mapped barcodes</td>
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<tr>
<td>Number of mutants with central insertions in PCG</td>
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<tr>
<td>Median of barcode per gene</td>
<td>18</td>
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<tr>
<td>Number of PCG with central insertion</td>
<td>4811</td>
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</table>
central part of the gene, thus the library covers 83% of *P. psychrophila* JB418 genome. On the chromosome and the plasmids, each bar represents the number of insertions per kb.

DOI: https://doi.org/10.7554/eLife.37072.010
### E. coli RB-TnSeq experiments

<table>
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<th>Sample</th>
<th>gMed</th>
<th>cor12</th>
<th>mad12</th>
<th>adjcor</th>
<th>gcror</th>
<th>quality-test</th>
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<tr>
<td>Alone-Day1</td>
<td>255</td>
<td>0.1723879</td>
<td>0.26568155</td>
<td>0.09779835</td>
<td>0.03594243</td>
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<tr>
<td>with H. alvei -Day1</td>
<td>784.5</td>
<td>0.16093804</td>
<td>0.22950086</td>
<td>0.01344365</td>
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<tr>
<td>with P. camemberti -Day1</td>
<td>694</td>
<td>0.15246077</td>
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<td>0.0781625</td>
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<tr>
<td>with G. candidum -Day 1</td>
<td>615</td>
<td>0.21262254</td>
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<tr>
<td>with Community -Day1</td>
<td>705.5</td>
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<td>Alone-Day2</td>
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<tr>
<td>with G. candidum -Day 2</td>
<td>486.5</td>
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<tr>
<td>with Community -Day2</td>
<td>795</td>
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<tr>
<td>Alone-Day3</td>
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<td>0.16459242</td>
<td>0.26477063</td>
<td>0.04099763</td>
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<tr>
<td>with H. alvei -Day3</td>
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<tr>
<td>with G. candidum -Day 3</td>
<td>1013</td>
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### P. psychrophila JB 418 RB-TnSeq experiments

<table>
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<th>Sample</th>
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<th>mad12</th>
<th>adjcor</th>
<th>gcror</th>
<th>quality-test</th>
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<tr>
<td>with H. alvei -Day1</td>
<td>315.5</td>
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<tr>
<td>with P. camemberti -Day1</td>
<td>375</td>
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<tr>
<td>with G. candidum -Day 1</td>
<td>337</td>
<td>0.28177362</td>
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<td>with Community -Day1</td>
<td>316</td>
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<td>with P. camemberti -Day2</td>
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<td>with G. candidum -Day 2</td>
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**Figure 1—figure supplement 7.** Quality assessment of all RB-TnSeq experiments. For each RB-TnSeq experiment different parameters are calculated to assess the quality of each RB-TnSeq experiment. If all quality parameters are met, then the gene fitness values can be further analyzed. These parameters include: (i) median gene has at least 50 counts (gMed ≥50), (ii) the median of the absolute difference in fitness between the two halves of the gene is less or equal to 0.5 (mad12 ≤0.5), (iii) the Spearman correlation in fitness between the two halves of the gene is at least 0.1 (cor12 ≥0.1), (iv) the correlation between gene GC content and fitness is less or equal to 0.2 (gcror ≤0.2), (v) the Spearman correlation of adjacent genes on different...
insertion mutants is not greater than 0.25 (adjcor ≤ 0.25), and (vi): the sample is not a T0 sample. More details about these metrics can be found in the supplementary material of Wetmore et al. 2015. All of our RB-TnSeq experiments met the required criteria (quality-test = TRUE).

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Figure 2. *E. coli* genes with negative fitness during growth in pairwise conditions (Figure 2—source data 1). (A) We grew *E. coli* in pairwise conditions on CCA with either *H. alvei*, *G. candidum* or *P. camemberti*. Asterisks indicate significant differences in growth of *E. coli* as compared to growth alone at day 3 (Dunnett’s test, adjusted p-value ≤ 0.05). (B) Using the *E. coli* RB-TnSeq library, we identified genes with negative fitness in each pairwise condition at three timepoints (days 1, 2, 3). Each volcano plot shows fitness values of all 3298 genes at all timepoints (three points per gene). We identified 145 genes with a negative fitness during growth alone (black), we obtained a total of 235 unique genes and 131 genes in pairwise culture with *G. candidum* and 142 genes in pairwise culture with *P. camemberti*. Altogether, they constitute 153 genes with negative fitness in pairwise conditions. (C) Comparing these genes (dark blue) to the 160 genes with a negative fitness during *E. coli* growth alone (black), we obtained a total of 235 unique genes and identified 78 genes that have a negative fitness both during growth alone and all pairwise conditions (conserved negative fitness), 75 genes that have a negative fitness in at least one pairwise condition but not alone (pairwise-induced negative fitness) and 82 genes with a negative fitness in growth alone but not in at least one pairwise condition (pairwise-alleviated negative fitness). (D) We selected a gene to illustrate conserved negative fitness (corA, 37 insertion mutants), pairwise-induced negative fitness (lpoB, 31 insertion mutants), and to illustrate pairwise-alleviated negative fitness (argH, 50 insertion mutants). For each gene, we display the number of sequencing reads for associated insertion mutants in the T0 sample, in growth alone day 3 and growth with *G. candidum* day 3. These sequencing reads are the raw data accounting for mutant abundance and used for fitness calculation (f represents each gene’s fitness value). While reads are not rarefied in the fitness calculation pipeline, we used rarefied reads for the purpose of the figure. Asterisks indicate genes with significant fitness values (consistent decrease in the number of reads per insertion mutant in the condition compared to T0). (E) We mapped the genes associated with conserved, pairwise-induced, and pairwise-alleviated negative fitness to the KEGG BRITE database. 41/75, 45/82 and 33/77 genes had hits.

DOI: https://doi.org/10.7554/eLife.37072.013
Figure 2—figure supplement 1. *E. coli* and community member growth curves alone, in pairwise conditions or during community growth. Each graph represents the growth over time of *E. coli*, *H. alvei*, *G. candidum*, or *P. camemberti* alone, in pairwise growth or with the community. Assays have been performed in triplicates. Dunnett’s tests have been performed for each species at day three to compare the number of CFUs of that species in interactive conditions versus growth alone. * adjusted p-value ≤ 5%.

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Figure 2—figure supplement 2. Comparison of the genes important for E. coli growth alone, in each pairwise condition or with the community. (A) Venn diagram of genes with negative fitness during growth alone or in each pairwise condition. The genes are detailed in Figure 2—source data 1. (B) Venn diagram of genes with negative fitness during growth alone, in pairwise conditions and with the community. The genes are detailed in Figure 3—source data 1.

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Figure 3. Comparison of *E. coli* genes with negative fitness within the community and in pairwise conditions (Figure 3—source data 1). (A) Using the *E. coli* RB-TnSeq library, we identified genes required to grow with the community (H. alvei + G. candidum + P. camemberti). During growth with the community, we identified a total of 126 genes with a negative fitness. (B) We compared the pairwise-induced and community-induced genes (Interaction-induced genes) as well as the pairwise-alleviated and community-alleviated genes (Interaction-alleviated genes) to identify conservation of interactions from pairwise to community and emergence of higher-order interactions. (C) Within the alleviated negative fitness, genes associated with numerous amino acid biosynthetic pathways were identified. F6P: fructose-6-phosphate, PRPP: 5-phospho-ribose-1-di-phosphate, G3P: Glyceraldehyde-3-phosphate, PEP: phosphoenol-pyruvate, Pyr: Pyruvate, Oxa: Oxaloacetate, 2-oxo: 2-oxoglutarate.

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Figure 4. Differential expression analysis of E. coli during interactive and non-interactive growth conditions (Figure 4—source data 1). We used RNASeq to investigate E. coli gene expression at three timepoints (1, 2 and 3 days) during growth on CCA alone, in pairwise conditions (with H. alvei, G. candidum or P. camemberti) and with the community. (A) Using DESeq2 (Love et al., 2015), we identified up and downregulated genes during growth in each pairwise condition compared to growth alone as well as up and downregulated genes during growth with the community compared to growth alone. Differential expression analysis has been performed at three timepoints, however, we displayed the results of the three timepoints on a single volcano plot. Only genes associated with an adjusted p-value lower than 1% (Benjamini-Hochberg correction for multiple testing) and an absolute logFC higher than one were considered differentially expressed. (B) We regroup any genes upregulated in at least one pairwise condition as a single set of pairwise-upregulated genes and did the same for pairwise-downregulated genes. Then, we performed functional enrichment analysis on KEGG pathways for pairwise-downregulated genes, community-downregulated genes, pairwise-upregulated genes and community-upregulated genes. Functional enrichment was performed using the R package clusterProfiler (Yu et al., 2012) and only the KEGG pathways enriched with an adjusted p-value lower than 5% (Benjamini-Hochberg correction for multiple testing) were considered. (C) We compared pairwise-upregulated genes with community-upregulated genes and pairwise-downregulated genes with community-downregulated genes to identify if expression regulation from pairwise conditions is conserved in the community context and if we observe specific changes in pairwise or community conditions. (D) Within the genes specifically upregulated during growth with the community, we observed the upregulation of multiple genes associated with the nitrogen starvation response. Most of these genes were also downregulated in pairwise conditions.

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Figure 4—figure supplement 1. RNASeq analysis of *E. coli* gene expression during growth alone and in pairwise conditions. We used RNASeq to investigate *E. coli* gene expression at three timepoints (1, 2 and 3 days) during growth on CCA alone, in pairwise conditions (with *H. alvei*, *G. candidum* or *P. camemberti*) and with the community. (A) We carried out a principal component analysis on the rlog transformed expression data (Love et al., 2015). (B) For each pairwise condition, we identified up and downregulated genes compared to alone at each timepoint. Then, for each pairwise condition, we pooled together upregulated genes at any timepoint and did the same for downregulated genes. Comparing the up or downregulated genes in the different pairwise conditions, we observe overlapping as well as specific responses depending on the partner.

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