Adaptive Evolution of *Escherichia coli* K-12 MG1655 during Growth on a Nonnative Carbon Source, L-1,2-Propanediol^{▽†}

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Laboratory adaptive evolution studies can provide key information to address a wide range of issues in evolutionary biology. Such studies have been limited thus far by the inability of workers to readily detect mutations in evolved microbial strains on a genome scale. This limitation has now been overcome by recently developed genome sequencing technology that allows workers to identify all accumulated mutations that appear during laboratory adaptive evolution. In this study, we evolved *Escherichia coli* K-12 MG1655 with a nonnative carbon source, L-1,2-propanediol (L-1,2-PDO), for ~700 generations. We found that (i) experimental evolution of *E. coli* for ~700 generations in 1,2-PDO-supplemented minimal medium resulted in acquisition of the ability to use L-1,2-PDO as a sole carbon and energy source so that the organism changed from an organism that did not grow at all initially to an organism that had a growth rate of 0.35 h⁻¹; (ii) six mutations detected by whole-genome resequencing accumulated in the evolved *E. coli* mutant over the course of adaptive evolution on L-1,2-PDO; (iii) five of the six mutations were within coding regions, and IS5 was inserted between two *fuc* regulons; (iv) two major mutations (mutations in *fucO* and its promoter) involved in L-1,2-PDO catabolism appeared early during adaptive evolution; and (v) multiple defined knock-in mutant strains with all of the mutations had growth rates essentially matching that of the evolved strain. These results provide insight into the genetic basis underlying microbial evolution for growth on a nonnative substrate.

Evolution of microorganisms in the laboratory offers the possibility of relating acquired mutations to increased fitness of the organism under the conditions used. Complete identification of mutations over defined evolutionary periods is necessary to fully understand the evolutionary change because spontaneous mutation is the foundational biological source of phenotypic variation (52). Since microbes grow rapidly and have large population sizes and since ancestors can be preserved by freezing them for later direct comparison of evolved types, laboratory evolution using microorganisms provides a powerful context for studying the genetics of evolutionary adaptation (5, 12, 14, 19, 43) due to the advent of new technologies for genome-wide detection of mutations (30, 33). A large number of studies of experimental evolution with various microbes have been carried out using natural carbon sources, especially glucose (12, 19, 47, 55), since glucose is the preferred carbon and energy source for most bacteria and eukaryotic cells (4, 50). Recently, a few studies have investigated the adaptive evolution of *Escherichia coli* at the genetic and metabolic levels with gluconeogenic carbon sources, including lactate (34) and glycerol (20). Compared to experimental evolution with native carbon sources, microorganisms might be more capable of adapting to various nonnative carbon compounds because microorganisms are able to adapt to environmental changes by using a number of strategies to meet their growth requirements and to achieve optimal overall perfor-

mance in the new conditions (20, 21, 34). However, a comprehensive analysis of the genetic basis of adaptation to nonnative carbon sources has not been performed.

The K-12 MG1655 strain of *E. coli* is not able to utilize L-1,2-propanediol (L-1,2-PDO) as a sole carbon and energy source. However, *E. coli* has an enzyme, L-1,2-PDO oxidoreductase (POR), which is involved in fermentative L-fucose metabolism and catalyzes the oxidation of L-1,2-PDO to L-lactaldehyde (Fig. 1A). The *E. coli* POR is encoded by the *fucO* gene of the fucose regulon (11, 23), which consists of two divergent operons (*fucAO* and *fucPIKUR*) under positive control of FucR (Fig. 1B) (9). FucR is activated by fucose-1-phosphate, which is the inducer of the *fuc* regulon (3). In *E. coli*, fucose metabolism is initiated by the sequential actions of a permease (encoded by *fucP*), an isomerase (encoded by *fucI*), a kinase (encoded by *fucK*), and an aldolase (encoded by *fucA*). The aldolase catalyzes the cleavage of fucose-1-phosphate to dihydroxyacetone phosphate and L-lactaldehyde. Under aerobic respiratory conditions, L-lactaldehyde is oxidized to L-lactate by an NAD-linked aldehyde dehydrogenase with broad functions (encoded by *aldA*). L-Lactate is then oxidized to pyruvate by a flavin adenine dinucleotide (FAD)-dependent L-lactate dehydrogenase (encoded by the *lldD* gene of the *lldPRD* operon [formerly the *lctPRD* operon]). Under anaerobic fermentative conditions, however, redox balance requires sacrifice of the L-lactaldehyde as a hydrogen acceptor at the expense of NADH (Fig. 1A). This reaction is catalyzed by the POR. The terminal fermentation product, L-1,2-PDO, is then released by a permease (57). Although the POR catalyzes the oxidation of L-1,2-PDO to L-lactaldehyde, L-1,2-PDO cannot be utilized by wild-type (WT) *E. coli* as a sole carbon source under aerobic conditions because this compound cannot induce expression of the *fuc* regulon (11). Indeed, the *fuc* regu-

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FIG. 1. Metabolic pathway and *fuc* regulon for L-fucose and L-1,2-PDO. (A) Metabolic pathway for L-fucose and L-1,2-PDO. In *E. coli*, fucose metabolism is initiated by the sequential actions of a permease (encoded by *fucP*), an isomerase (encoded by *fucI*), a kinase (encoded by *fucK*), and an aldolase (encoded by *fucA*). The aldolase catalyzes cleavage of fucose-1-phosphate to dihydroxyacetone phosphate and L-lactaldehyde. Under aerobic respiratory conditions, the L-lactaldehyde is further oxidized by a series of enzymes to pyruvate, which subsequently enters central metabolism. Under anaerobic fermentative conditions, the L-lactaldehyde is reduced to L-1,2-PDO by oxidoreductase (encoded by *fucO*). (B) Genetic organization of the *fuc* regulon. The *fuc* regulon for L-fucose uptake and metabolism consists of two divergent operons, *fucAO* and *fucPIKUR*.

lon was not expressed under any conditions when a database of 213 expression profiles produced in our laboratory was examined (38). Furthermore, even if the POR is expressed, it is oxidatively inactivated by a metal-catalyzed oxidation (MCO) mechanism (7).

Sridhara et al. (48) previously described *E. coli* mutants that were isolated from an *E. coli* K-12 derivative treated with the mutagen ethyl methanesulfonate and were able to grow aerobically on L-1,2-PDO as a sole carbon source. Previous studies showed that an IS5 insertion between the *fucAO* and

fucPIKUR operons caused constitutive expression of the *fucAO* operon (9, 41) at a level that enabled the *E. coli* mutant to grow on L-1,2-PDO. In addition, mutations resulting in increased resistance to MCO under aerobic conditions were found in the N-terminal domain of POR (39). However, at present, little is known about the accumulated genome-wide mutations and their effects on the fitness in *E. coli* that has acquired the ability to use L-1,2-PDO because previous studies have focused on mutations in POR and its regulatory region.

In an attempt to investigate the genetic basis of adaptive

evolution of *E. coli* during growth on L-1,2-PDO, we first isolated an *E. coli* mutant able to use L-1,2-PDO using experimental evolution without a mutagen, and we then characterized this evolved *E. coli* mutant. Using whole-genome sequencing, we identified all accumulated mutations of the evolved *E. coli* mutant related to the known ancestor and also determined the fitness benefits and phenotypic behaviors of the mutations discovered. Our results offer a systematic view of the genetic basis underlying microbial adaptation to a nonnative substrate.

MATERIALS AND METHODS

Strains and media. All strains and plasmids used in this study are listed in Table S1 in the supplemental material. A glycerol-evolved *E. coli* K-12 MG1655 strain [GC strain; $F^- \lambda^-$ *ilvG rfb-50 rph-1 glpK(G184T)*] (30) was used as a parent strain for adaptive evolution on L-1,2-PDO. Evolution of the GC strain was carried out at 37°C using 200 ml of M9 minimal medium supplemented with 2 g/liter of L-1,2-PDO (catalogue number 398039; Sigma Aldrich) in 500-ml Erlenmeyer flasks containing magnetic stir bars for aeration. M9 minimal medium contained (per liter of deionized water) 0.8 g of NH_4Cl , 0.5 g of NaCl , 7.5 g of $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, and 3.0 g of KH_2PO_4 . The following components were sterilized separately and then added (per liter [final volume] of medium): 2 ml of 1 M MgSO_4 , 0.1 ml of 1 M CaCl_2 , and 0.5 ml of a trace element solution containing (per liter) 1 g of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 0.18 g of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.12 g of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, 0.12 g of $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, and 0.18 g of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$. During the early stage of adaptive evolution, the minimal medium was also supplemented with 2 g/liter of glycerol, and the concentration of this compound was gradually decreased while the L-1,2-PDO concentration was increased to keep the total carbon source concentration in the minimal medium 2 g/liter. *E. coli* K-12 MG1655 (= ATCC 700926) obtained from the American Type Culture Collection (Manassas, VA) and the GC strain were used as parent strains for construction of all knock-in mutants in this study. During the knock-in process, the strains were cultured on Luria-Bertani (LB) medium supplemented with 50 $\mu\text{g}/\text{ml}$ of kanamycin or 100 $\mu\text{g}/\text{ml}$ of ampicillin when necessary.

Generation of mutant strains. The knock-in *E. coli* mutants were generated by homologous recombination using the gene gorging method or the lambda Red recombinase system (15, 29). The gene gorging method introduces a desired mutation without direct selection and therefore leaves no antibiotic resistance gene or other trace of having been used. Plasmids pKD46, pKD13, and pACBSR were used for introduction of the recombinase gene, for homologous recombination with the target gene, and for removal of antibiotic resistance markers, respectively. In order to confirm the genotypes of all mutants, colonies were isolated from solid medium and tested using PCR. Wild-type *E. coli* colonies were tested in parallel as a negative control.

Adaptive evolution. At the start of adaptive evolution, the GC strain was cultured on solid M9 minimal medium containing 2 g/liter of glycerol and incubated overnight at 37°C. A single colony was selected from the plate that was incubated, resuspended in 10 μl of sterile water, and inoculated into three 500-ml Erlenmeyer flasks containing 200 ml of M9 minimal medium supplemented with 2 g/liter of glycerol. The flasks were incubated at 37°C using a stir bar for mixing and aeration (~1,000 rpm). For adaptive evolution cultures, the optical density at 600 nm (OD_{600}) was determined, and cells were transferred into fresh medium. The dilution used for each passage was adjusted to account for changes in the growth rate (typically between 5×10^5 and 5×10^8 cells were transferred during each inoculation) and to ensure that cultures did not enter the stationary phase before the next passage. The OD_{600} was typically $\leq 2.4 \times 10^{-6}$ when it was calculated. This batch growth and serial passage process was carried out for 60 days until a stable growth rate was observed for more than 5 days. The number of generations was estimated daily by calculating the starting optical density of each batch culture and determining how many doublings occurred during batch growth until the culture was transferred into fresh medium. The total number of cell divisions was calculated daily by determining how many doublings occurred during batch growth and how many cells were in 1 ml of medium at a normalized OD_{600} of 1. Triplicate cultures were evolved concurrently under identical conditions. This process resulted in three evolved mutants with end points that were designated eBOP12, eBOP13, and eBOP14. The amount of glycerol added to the medium was reduced exponentially during the first 11 days of evolution. Cultures were screened every other day for contamination by performing PCR with primers for the V2 region of 16S rRNA genes and Sanger sequencing. Samples

were stored at -80°C every day over the course of evolution. Primers used in this study are described in the supplemental material.

Phenotype assessment. The OD_{600} was measured to monitor batch cell growth. To measure the growth rate, the substrate uptake rate (SUR), and the by-product secretion rate, each population was grown in batch culture at 37°C under aerobic conditions. Aerobic cultivation was conducted in 500-ml Erlenmeyer flasks containing 200 ml of M9 minimal medium with trace elements and 2 g/liter of L-1,2-PDO as the sole carbon source. The temperature was controlled at 37°C by using a circulating water bath, and aeration was controlled with a stir bar (~1,000 rpm). Samples were taken from the batch cultures periodically throughout exponential growth, filtered through a 0.22- μm filter, and stored at -20°C for substrate uptake and by-product secretion analyses. The depletion of L-1,2-PDO and the secretion of acetate and other metabolites in the medium were determined using filtered supernatants by performing high-performance liquid chromatography (HPLC) (Waters). Samples were injected into an Aminex HPLC-87H column (Bio-Rad) connected to a cation-H guard column (Bio-Rad) at 65°C. Metabolites were eluted with 5 mM sulfuric acid at a flow rate of 0.5 ml/min for 30 min. Detection was performed using a differential refractive index detector at 35°C, and the compounds were compared to standards. The identities of metabolites and organic acids in the fermentation broth were further verified with enzymatic kits (R-Biopharm).

Solexa resequencing. Five micrograms of genomic DNA isolated from a single colony (eBOP12-6) of the end point eBOP12 population was used to generate a genomic DNA library using an Illumina genomic DNA library generation kit by following the manufacturer's protocol (Illumina Inc., San Diego, CA). Briefly, bacterial genomic DNA was fragmented by nebulization. The ends of fragmented DNA were repaired by T4 DNA polymerase, Klenow DNA polymerase, and T4 phosphonucleotide kinase. The exonuclease-negative Klenow DNA polymerase was then used to add an A base to the 3' end of the DNA fragments. After ligation of the adapters to the ends of the DNA fragments, the ligated DNA fragments were subjected to electrophoresis on a 6% 1 $\times$ Tris-borate-EDTA (TBE) gel. DNA fragments ranging from 190 bp to 220 bp long were recovered from the gel and purified using a Qiagen minigel purification kit. Finally, the adapter-modified DNA fragments were enriched by PCR. The final concentration of the genomic DNA library was determined by using a NanoDrop instrument (Thermo Scientific), and the results were validated by using a 6% 1 $\times$ TBE gel. The genomic DNA library was used to generate a cluster on a Flowcell by following the manufacturer's protocol. The V2 genomic sequencing primer was used for all DNA sequencing. A 36-cycle sequencing program was used with an Illumina genome analyzer II by following the manufacturer's protocol.

Genome sequence assembly and identification of polymorphism. The Solexa output for the resequencing run was first curated to remove any sequences containing a period. We then used MosaikAligner, developed by M. P. Stromberg and G. T. Marth (unpublished data), to iteratively align reads with the *E. coli* reference sequence (gi 48994873); in each iteration a limit was placed on the number of alignment mismatches allowed. This iterative limit increased from 0 to 5, and unaligned reads were used as input for the next iteration, which had a more lenient mismatch limit. An in-house script (available upon request) was then used to compile the read alignments into a nucleotide resolution alignment profile. The consistency and coverage were then assessed to identify likely polymorphic locations. Locations at which coverage was greater than 10 $\times$ and for which indels were observed or the count for a single-nucleotide polymorphism (SNP) was greater than twice the count for the nucleotide matching the reference sequence were considered to be likely polymorphic locations. False-negative rates were determined by this sequencing method by carrying out polymorphism identification analysis using an *E. coli* reference sequence which had 1,000 SNPs, deletions, and insertions added at random and known locations. Insertion sizes were randomly and uniformly distributed between 1 and 4 bp, and deletions were between 1 and 99 bp long. Mutations were not permitted to overlap. The rates of detection of SNPs, deletions, and insertions were determined separately by determining the fraction of each type of mutation that was marked as polymorphic by the script described above when sequence data from an end point were mapped on the mutated reference genome.

qPCR. RNA samples were taken from exponentially growing cells and added to 2 volumes of RNA Protect (Qiagen). Total RNA was isolated using an RNeasy kit (Qiagen). Reverse transcription was performed with 10 μg of total RNA. The reverse transcription mixture (60 μl) contained 10 μg total RNA, 75 μg random primers, 1 $\times$ 1st Strand buffer, 10 mM dithiothreitol, 0.5 mM deoxyribonucleotide triphosphates, 30 U of SUPERase, and 1,500 U of Superscript II. The mixture was incubated in an iCycler (Bio-Rad) at 25°C for 10 min, at 37°C for 1 h, and then at 42°C for 1 h. Then the reaction mixture was incubated at 70°C for 10 min to inactivate the Superscript II. The RNA was then degraded by adding 20 μl of 1 N NaOH and incubation at 65°C for 30 min. After the incubation, 20 μl

of 1 N HCl was added to neutralize the solution. QIAquick PCR purification kits were used to clean up the cDNA synthesis product. Following purification, the cDNA was quantified and then directly used in real-time quantitative PCR (qPCR). The 50- μ l qPCR mixtures contained 25 μ l of SYBR green PCR master mixture (Qiagen), 0.2 μ M forward primer, 0.2 μ M reverse primer, and cDNA as a template. Each qPCR was performed in triplicate in the iCycler using the following conditions: 95°C for 15 min and then 40 cycles of denaturation 94°C for 15 s, annealing at 52°C for 30 s, and extension at 72°C for 30 s. The gene expression of evolved strains was analyzed using minimal medium containing glycerol and was compared to that of the wild-type strain under same growth conditions. In order to determine the binding affinity of each primer set, a standard curve was constructed for each primer, and the reaction efficiency was obtained by using it. Using the standard curve, the relative quantity of cDNA was determined for each gene by normalizing the quantity to the quantity of *acpP* (acyl carrier protein) cDNA in the same sample. *acpP* was chosen as the internal control gene since it is constitutively expressed in wild-type *E. coli* K-12 (13).

POR expression, purification, and assay. The WT and mutant alleles of the *fucO* gene encoding POR were amplified by PCR using genomic DNA of the GC strain and the L-1,2-PDO-evolved mutant as templates, respectively, and were cloned into pGEX-6P-1 (GE Healthcare Life Sciences). Potential clones were checked for the presence of the correct insert in the correct orientation by restriction enzyme analysis, followed by Sanger sequencing. *E. coli* BL21 (New England Biolabs) was used for expression of WT and mutant alleles of the *fucO* gene and was cultured in LB medium supplemented with 100 μ g/ml ampicillin at 30°C. Protein overexpression was induced with isopropyl- β -D-thiogalactoside (IPTG), and cell extracts were prepared by sonication and centrifugation. WT and mutant POR encoded by *fucO* were purified using a GSTrap Fast Flow column (GE Healthcare Life Sciences), and the glutathione S-transferase (GST) moiety was removed by on-column cleavage with PreScission protease (GE Healthcare Life Sciences), resulting in intact POR. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was conducted to confirm the purity of the purified samples. POR was routinely assayed using a method described previously (39). Glycoaldehyde was used as a substrate because it has been used as an alternative substrate for the forward reaction (L-lactaldehyde $\rightarrow$ L-1,2-PDO) of the enzyme (6, 39) and is commercially available. The activity of POR was measured in the forward direction and in the reverse direction (L-1,2-PDO $\rightarrow$ L-lactaldehyde) by monitoring NAD(H) release at 340 nm. The L isomer of 1,2-PDO (catalogue number 540250; Sigma) was used for the POR assay. A calibration curve was constructed using enzyme standards and chemicals purchased from Sigma Chemicals. The protein content was determined with the Quant-It assay system (Invitrogen) by following the manufacturer's instructions.

Growth rate measurement. Growth rate experiments with knock-in *E. coli* mutants were performed by measuring the OD₆₀₀ of triplicate cultures at several time points at which the OD₆₀₀ was >0.05 but <0.30 . The growth conditions used were identical to the conditions used for adaptive evolution, except that flasks were placed in a 37°C water bath instead of the 37°C air incubator used for adaptive evolution. The growth rate was defined as the slope of the linear best-fit line in a plot of $\ln(\text{OD}_{600})$ versus time (hours).

Allele frequency estimation. For allele frequency estimation, 15 clones were randomly selected from M9 minimal agar plates containing L-1,2-PDO inoculated with frozen stocks of the day 60 adaptive evolution culture. A 200- to 300-bp region surrounding each mutation was amplified from extracted DNA by PCR and Sanger sequenced to determine its presence in each clone.

Allele appearance estimation. In order to estimate the temporal appearance of acquired mutations, the approximate time point that each mutation was fixed in the relevant population was estimated by screening the frozen stocks of cultures saved at intermediate time points during each evolution with L-1,2-PDO. The predominant presence or absence of each mutation at a time point was determined by performing PCR for the 200- to 300-bp regions surrounding the mutation, followed by Sanger sequencing. If the mutation was not fixed, we considered the larger peak the dominant signal for two peaks at one position in a Sanger sequencing chromatogram.

RESULTS

Adaptive evolution. Initially, WT strain MG1655 and the GC strain were each examined to determine their abilities to utilize 1,2-PDO as a sole source of carbon and energy. Both strains showed no growth in M9 minimal medium supplemented with 4 g/liter of a racemic 1,2-PDO mixture. Therefore, another carbon source was needed to support the growth of these

strains in minimal medium containing 1,2-PDO. We assumed that *E. coli* could grow on a natural carbon source and then utilize the 1,2-PDO during adaptation. Although glucose is often the preferred carbon for *E. coli* cell growth, it inhibits utilization of other carbon sources by a mechanism known as catabolite repression (16). Unlike the non-phosphotransferase system (PTS) sugars (e.g., lactose, melibiose, maltose, gluconate, and glucose-6-phosphate) that cause catabolite repression in *E. coli* (31, 32), glycerol is a non-PTS carbon source for which there is not catabolite repression (18). However, it is a poor carbon source for WT strain MG1655 cell growth in M9 minimal medium. Our lab has generated several evolved *E. coli* mutants that grow well on minimal medium containing glycerol (20). One of these glycerol-evolved strains, the GC strain, has only one mutation in the *glpK* gene (encoding glycerol kinase), which was revealed by genome sequencing (30), and it showed the smallest change in the global gene expression profile (20) when its profile was compared to the WT strain MG1655 profile. Thus, the initial evolution experiment was conducted to generate a 1,2-PDO-evolved mutant by further experimental evolution of the GC strain with 2 g/liter of glycerol. Then the amount of glycerol in the medium was decreased gradually while the concentration of 1,2-PDO was increased to keep the total concentration of the two carbon sources 2 g/liter. A rapid decrease in the growth rate was observed when the amount of glycerol was reduced and the concentration of 1,2-PDO was increased during serial passage in M9 minimal medium (Fig. 2A). After the glycerol was completely removed from the medium, the average growth rate was $0.0035 \pm 0.0005 \text{ h}^{-1}$ in 1,2-PDO-supplemented minimal medium. However, the growth rate then steadily increased during laboratory evolution over the following ~ 450 generations. Laboratory evolution of the GC strain on 1,2-PDO-supplemented minimal medium stopped once a stable growth rate was achieved and resulted in the evolved eBOP12, eBOP13, and eBOP14 populations. The number of cell divisions that occurred during evolution was estimated based on the amount of cells transferred each day and the doubling time. The total number of cell divisions was $3.85 \times 10^{11} \pm 0.3 \times 10^{11}$ (Fig. 2A).

Phenotypic characterization. Phenotypic characterization of the evolved populations revealed that the populations at the three evolutionary end points had similar metabolic phenotypes. Three evolved populations, eBOP12, eBOP13, and eBOP14, had acquired the ability to grow on 1,2-PDO-supplemented minimal medium (Fig. 2A). The specific growth rate continued to increase over the course of experimental evolution, and the maximal average growth rate was $0.35 \pm 0.04 \text{ h}^{-1}$ at the end point of adaptive evolution, which corresponded to a ~ 100 -fold increase in the growth rate after the three evolved populations started to utilize only 1,2-PDO. To determine whether both optical isomers of 1,2-PDO were utilized, three populations evolved on a racemic mixture of 1,2-PDO were cultivated using M9 minimal medium containing 2 g/liter of the D isomer, the L isomer, or a racemic mixture. No growth was detected with D-1,2-PDO. The maximal growth yield on L-1,2-PDO was 18.6 g (dry weight) cells mol^{-1} L-1,2-PDO degraded. It was also found that with the racemic mixture, one-half of the diol content remained after growth had stopped. Thus, only the L isomer could be utilized by the evolved populations. In order to further probe the metabolic phenotypes after adaptive

FIG. 2. Evolutionary trajectory and phenotype characterization of L-1,2-PDO-evolved *E. coli* populations. (A) Evolutionary trajectory. The growth rates of the evolved population and cell division are expressed as a function of time of evolution. The final average growth rate was $0.35 \pm 0.04 \text{ h}^{-1}$. The amounts of glycerol added with 1,2-PDO are indicated by gray bars. The total number of cell divisions for the entire period of adaptation is also shown. (B) Phenotype characterization. Three populations at the end point of adaptive evolution were used to determine the L-1,2-PDO uptake rate, the acetate secretion rate, and the biomass yield. Each population stored at -80°C was grown in batch culture under the same adaptive evolution conditions. Metabolites were detected by HPLC. ●, L-1,2-PDO uptake rate; ■, acetate secretion rate; ▲, biomass yield. DW, dry weight.

evolution, the biomass yield, SUR, and product secretion rate were monitored for the three evolved populations (Fig. 2B). The results showed that there was a 9.6-fold increase in the average dry weight yield compared to the yield of the ancestral population after utilization of L-1,2-PDO was started. On average, a 5.8-fold increase in the SUR, from $1.24 \pm 0.6 \text{ mmol g (dry weight)}^{-1} \text{ h}^{-1}$ to $7.17 \pm 0.51 \text{ mmol g (dry weight)}^{-1} \text{ h}^{-1}$, was observed after the evolved populations started to utilize L-1,2-PDO. The major carbon flow in the evolved populations grown aerobically on L-1,2-PDO was acetate carbon flow,

which decreased from $4.3 \pm 0.1 \text{ mmol g (dry weight)}^{-1} \text{ h}^{-1}$ to $3.4 \pm 0.2 \text{ mmol g (dry weight)}^{-1} \text{ h}^{-1}$ at the end point of evolution (Fig. 2B). Ethanol, another metabolite derived from intracellular acetyl coenzyme A (acetyl-CoA), was not secreted. Lactate and succinate were also produced, but at very low levels.

Whole-genome sequencing. To determine the genetic basis of adaptation, the evolved eBOP12 population was selected because it had the highest SUR and growth rate of the three evolutionary end point populations (see Table S2 in the sup-

TABLE 1. Mutations discovered in sequenced L-1,2-PDO-adapted *E. coli*^a

Gene	Product or function	Mutation	Gene position	Region	Genome position	Effect	Frequency
<i>fucO</i>	L-1,2-Propanediol oxidoreductase	C → A	22	Coding	1791921	Leu → Met	1.00
<i>ilvG1</i>	Acetohydroxy acid synthase II	1-bp deletion (A)	977	Coding	3949559	Frameshift	1.00
<i>rrlD</i>	23S rRNA	GAT → ATG	2547–2549	NA	3422257–3422259	NA	0.87
<i>ylbE1</i>	Predicted protein	A → G	114	Coding	547694	Glu → Glu	1.00
		1-bp insertion (G)	253	Coding	547833	X (GNG) → Gly (GGG)	1.00
Intergenic	Between <i>fucAO</i> and <i>fucPIKUR</i> operons	IS5 insertion	–175 from <i>fucP</i>	Promoter	2932181	NA	1.00

^a DNA from single colonies isolated from the end point of the *E. coli* cultures adapted to grow on minimal medium containing L-1,2-PDO M9 were screened for mutations using Solexa technology. Mutations were confirmed by Sanger sequencing of the DNA isolated from single colonies using primers flanking the mutated site. The mutation frequency was estimated using 15 clones of the eBOP12 population at the end point of evolution. NA, not available.

plemental material). The eBOP12 population was grown on solid minimal medium supplemented with L-1,2-PDO, and 10 random colonies were isolated for analysis. Of the 10 clones isolated, a single clone, eBOP12-6, was selected because it had the highest growth rate on minimal medium containing L-1,2-PDO (see Table S3 in the supplemental material). Genomic DNA of the eBOP12-6 clone was extracted and sequenced by using the Solexa sequencing system. When SNPs and indels were accounted for, a total of eight putative mutations were found to have accumulated in the evolutionary lineage from the GC strain to eBOP12-6. Six of these mutations were confirmed by PCR amplification and Sanger sequencing, which revealed three SNPs, one deletion, and two insertions, including a transposable element. Five of the six mutations were in coding regions, and there was an IS5 insertion in the *fuc* operon. All confirmed mutations are listed in Table 1.

An SNP was found in the *fucO* gene encoding the POR (Table 1), which catalyzes the first step of L-1,2-PDO catabolism in *E. coli* (see Fig. S1 in the supplemental material). It seems likely that this mutation allowed the evolved population to utilize L-1,2-PDO by overcoming metal-catalyzed inactivation (39). An insertion sequence (IS5) was found in the region between the *fucAO* and *fucPIKUR* operons (see Fig. S1 in the supplemental material), which might have caused constitutive activation of the *fucAO* operon (9, 41). The L-1,2-PDO-evolved eBOP12-6 clone contained two genes (*ilvG* and *ylbE1*) that are not expressed in WT *E. coli* K-12 MG1655 due to internal frameshift mutations (22). The *ilvG* and *ylbE1* genes, which encode acetohydroxy acid synthase (AHAS) II and a predicted protein, respectively, were expressed in the eBOP12-6 clone by deletion and insertion of single nucleotides in their coding regions, respectively. In addition, a synonymous mutation occurred in the coding region of the *ylbE1* gene (Table 1). The *ilvG* gene is known to be involved in biosynthesis of valine and isoleucine by conversion of pyruvate to 2-acetohydroxy acid. Because L-1,2-PDO enters central metabolism as pyruvate, it seems likely that the mutation in *ilvG* improves the flow of carbon for amino acid biosynthesis. The evolved eBOP12-6 clone also had a mutation in the *rrlD* gene encoding 23S rRNA, which is the RNA component of the large subunit (50S subunit) of the *E. coli* ribosome. The *rrlD* mutation found in the eBOP12-6 clone is located in domain V (U2016 to G2625) of 23S rRNA of *E. coli*, which has been identified as the peptidyl

transferase center that acts as a general protein folding modulator by binding to unfolded proteins (45). Furthermore, the mutation is close to one of the five recently identified sites in domain V (G2553 to C2556) where proteins bind during refolding (44).

Analysis of the mutations found. To determine where the six mutations that accumulated in the eBOP12-6 clone were fixed in the eBOP12 population at the end point of adaptive evolution, the genotypes of 15 randomly selected clones were determined for each mutation locus. Five mutations appear to have been fixed during the 700-generation evolution, whereas one locus (*rrlD*) was fixed at a frequency of nearly 0.87 (Table 1). In addition, we screened the six mutations that were found in the eBOP12 population in the eBOP13 and eBOP14 populations by Sanger sequencing. The synonymous mutation in *ylbE1* was not detected in the eBOP13 and eBOP14 populations at the end point of adaptive evolution. However, four other mutations (*fucO*, *ilvG*, *ylbE1*, and IS5 insertion) were all fixed in both populations (see Table S4 in the supplemental material).

In order to determine the approximate time of appearance of each mutation in the eBOP12 population, the frozen stock samples obtained at intermediate times during laboratory evolution were screened for the appearance of each mutation found in the end point population by Sanger sequencing of PCR-amplified mutated regions (Fig. 3). An SNP was considered present if the dominant signal peak resulting from Sanger sequencing indicated that the mutation was present, although at times lower levels of SNPs were observed in the population as nondominant peaks in the sequencing trace. Sequencing of intermediate points showed that mutations occurred over the course of adaptive evolution. The increase in the growth rate was greatest during the first approximately 100 generations (250 to 350 generations in Fig. 3) after the evolved populations started to utilize L-1,2-PDO as the sole carbon source, which corresponded to the appearance of two major mutations (in *fucO* and its promoter region) that are directly involved in L-1,2-PDO catabolism in the eBOP12-6 clone. The eBOP13 and eBOP14 populations also had two mutations during the first approximately 100 generations after the evolved populations began to take up L-1,2-PDO (see Fig. S2 in the supplemental material). It was reasonable to expect that the *fucO* mutation and IS5 insertion would be detected simultaneously when the eBOP12 population started to utilize only L-1,2-

FIG. 3. Timeline showing the temporal order of appearance of acquired mutations in the eBOP12 population. Sanger sequencing was used to screen the mutations that we found after whole-genome resequencing of the eBOP12-6 clone. The first day that a mutation was identified is indicated by an arrowhead. Once a mutation was present, it was found on all subsequent days of the experiment. The line shows the growth rate trajectory over the course of experimental evolution. **ylbE1* indicates a synonymous mutation in *ylbE1*.

PDO. However, the *fucO* mutation was not dominant in the sequence trace in this screen, suggesting that it was not fixed yet in the eBOP12 population when L-1,2-PDO utilization started (~250 generations). For the unfixed *fucO* mutation, we screened several individual colonies of the eBOP12 population after ~250 generations. Of 15 eBOP12 colonies, 10 had only the IS5 insertion, 1 had only the *fucO* mutation, 1 had both the *fucO* and IS5 mutations, and the remaining 3 had neither mutation, suggesting that the *fucO* mutation was present at a very low frequency in the adaptive culture after ~250 generations.

To evaluate the contributions of individual mutations that enabled organisms to grow on minimal medium containing L-1,2-PDO and that improved fitness, we introduced each mutation into the WT and GC strains using a site-directed mutagenesis strategy called gene gorging (29). The growth rate recovery of the mutants constructed using M9 minimal medium containing L-1,2-PDO is shown in Fig. 4. A growth rate recovery of 0% indicates that the mutant did not grow in minimal medium containing L-1,2-PDO, while a mutant with a growth rate recovery of 100% grew at the same rate as the eBOP12-6 clone. We found that mutations in the *fucO* gene and its promoter region resulted in the largest growth rate recoveries in the GC (61%) and WT (53%) strains (Fig. 4). Furthermore, these mutations were essential for utilization of L-1,2-PDO in *E. coli* because a single *fucO* or IS5 insertion mutation did not allow growth of the WT or GC strain in minimal medium containing L-1,2-PDO (Fig. 4). In addition to the GC strain mutant, the WT strain mutant with two mutations in *fucO* and its promoter region could grow in minimal medium containing L-1,2-PDO. This result suggests that adaptation to growth with L-1,2-PDO as the only carbon source did not occur with a background of previous glycerol adaptation. Besides single mutants, we also made multiple mutants to

FIG. 4. Growth rates of site-directed mutants. The gene gorging method was used to introduce mutations individually and in combination into WT and GC strain backgrounds. For each mutant of the WT and GC strains, the designations of the mutated genes are indicated. The growth rate recovery was calculated by dividing the growth rate of the mutant by the growth rate of the evolved eBOP12-6 clone in minimal medium containing L-1,2-PDO. A single mutation of *fucO* or the IS5 insertion did not allow the WT or GC strain to grow in minimal medium containing L-1,2-PDO.

reconstruct the eBOP12-6 clone isolated from the eBOP12 population. Mutations in *ilvG* and/or *ylbE* with a background of *fucO* and its promoter mutation also resulted in growth rate recoveries from 11% to 22%. The effect of *rrlD* mutation on the growth rate was not significant. The growth rates of reconstructed strains with all mutations almost matched the growth rate of the evolved eBOP12 clone in L-1,2-PDO-supplemented minimal medium. This indicates that we were able to identify all of the important mutations in this evolved clone through whole-genome sequencing and that there was not an influential epigenetic component of the adaptation to L-1,2-PDO.

Functional characterization of mutations. To verify the exact nature of the mutations and their phenotypic consequence, the *fucO* alleles were amplified by PCR using genomic DNA of the GC and eBOP12-6 strains and cloned into pGEX-6P-1 containing GST as a fusion partner. WT or mutant POR expressed in *E. coli* BL21 was purified by affinity chromatography. When kinetic constants were investigated by using reciprocal plots for saturating concentrations of the corresponding enzymes, the K_m values of the mutant POR for L-1,2-PDO and glycoaldehyde were 1.2-fold and 2.1-fold higher than those of the WT POR, indicating that the mutant POR had low affinity for substrates of the reduction and oxidation reaction. However, the V_{max} of the mutant POR for L-1,2-PDO was 10-fold

TABLE 2. Comparison of kinetic constants of wild-type and mutant POR^a

Substrate varied	Substrate fixed	K_m (mM)		V_{max} (U/mg)	
		FucO	FucO ^{Leu-8 → Met-8}	FucO	FucO ^{Leu-8 → Met-8}
L-1,2-PDO oxidation					
L-1,2-Propanediol	NAD ⁺	1.07 ± 0.03	1.31 ± 0.06	4.6 ± 0.3	47 ± 3.2
NAD ⁺	L-1,2-Propanediol	0.023 ± 0.004	0.018 ± 0.002	139 ± 5.1	144 ± 5.4
L-Lactaldehyde reduction					
Glycoaldehyde	NADH	0.038 ± 0.004	0.081 ± 0.006	24.6 ± 2.8	26.1 ± 4.1
NADH	Glycoaldehyde	0.028 ± 0.005	0.031 ± 0.003	148 ± 3.6	151 ± 2.4

^a The activity of POR for oxidation and reduction was measured by monitoring NAD(H) release at 340 nm. The L isomer of 1,2-PDO was used for the POR assay. Glycoaldehyde was used as a substrate because it has been used as an alternative for L-lactaldehyde.

greater than that of the WT POR, while the V_{max} values of the two enzymes for glycoaldehyde were similar. No difference in the K_m and V_{max} values for NAD and NADH was found between the two enzymes (Table 2). Thus, the ability to grow and to grow rapidly on L-1,2-PDO can be partially attributed to altered kinetic properties of POR.

To determine the effect of IS5 inserted between the *fucAO* and *fucPIKUR* operons on *fucAO* expression, the gene expression of the *fucAO* operon in the eBOP12-6 clone was analyzed using minimal medium containing glycerol or L-1,2-PDO and compared to that of the parental GC strain grown on glycerol-containing M9 minimal medium (Fig. 5). In glycerol-containing minimal medium, the expression of the *fucAO* operon in evolved clone eBOP12-6 was increased 11-fold. The *fucAO* expression of the eBOP12-6 clone in minimal medium containing L-1,2-PDO was also upregulated 12-fold compared with that of the GC strain in glycerol-containing minimal medium,

indicating that the *fucAO* operon was constitutively expressed in the evolved eBOP12-6 strain regardless of the carbon source. In order to determine the effect of *ilvG* and *ylbE1* mutations in this study, the gene expression of the *ilvGMEDA* and *fdrA-ylbE-ylbF-ybcF* operons in the eBOP12-6 clone in glycerol-containing minimal medium was analyzed and compared to that in the parental GC strain (Fig. 5). It is known that mutations that restore the reading frame of the *ilvG* gene result in a 5- to 10-fold increase in expression of the *ilvEDA* genes (37, 54). Consistent with previous studies, the *ilvG* mutation (single nucleotide deletion at bp 977) resulted in 3- to 4-fold upregulation of the expression of the distal genes (*ilvE* and *ilvA*) in the same operon of the eBOP12-6 clone (Fig. 5). Because L-1,2-PDO enters central metabolism via pyruvate, it seems likely that the mutation in the *ilvG* gene improves the flow of carbon for amino acid biosynthesis. However, there was no difference in expression of the genes downstream of *ylbE* (*ylbF* and *ybcF*) (data not shown).

DISCUSSION

Laboratory adaptive evolution can provide key information to address a wide range of issues in evolutionary biology. In this study, we used whole-genome sequencing to obtain an integrative understanding of the genetic and phenotypic changes during evolution of *E. coli* in response to a nonnative carbon source, L-1,2-PDO. We found that (i) experimental evolution of *E. coli* in 1,2-PDO-supplemented minimal medium revealed that preevolved *E. coli* acquired the ability to use L-1,2-PDO as a sole carbon and energy source; (ii) six mutations detected by whole-genome sequencing accumulated in the evolved *E. coli* clones over the course of adaptive evolution with L-1,2-PDO; (iii) five of the six mutations were in the coding region, and the IS5 insertion was between two *fuc* regulons; (iv) two major mutations (*fucO* and IS5 insertion) involved in L-1,2-PDO catabolism appeared early during adaptive evolution; and (v) multiple defined knock-in mutant strains with all of the mutations had growth rates essentially matching that of the evolved strain.

We found that *E. coli* could acquire the ability to grow on L-1,2-PDO through laboratory evolution without a mutagen. Although an *E. coli* mutant able to utilize L-1,2-PDO was reported previously, this mutant was isolated after mutagen treatment (48). Later, a spontaneous L-1,2-PDO-utilizing mutant was isolated from an *E. coli* K-12 derivative pregrown on fucose (24), suggesting that POR-induced *E. coli* K-12 was

FIG. 5. Relative transcription levels of *ilvEA* and *fucAO* determined by real-time quantitative PCR. The GC strain was grown on minimal medium containing glycerol (GG), and the eBOP12-6 clone was cultured on minimal medium containing glycerol (PG) or minimal medium containing L-1,2-PDO (PP). The *ilvE* and *ilvA* gene and *fucAO* operon were upregulated in the eBOP12-6 clone cultured on minimal medium containing glycerol compared to the GC strain grown on minimal medium containing glycerol. When the eBOP12-6 clone cultured on minimal medium containing L-1,2-PDO was compared to the eBOP12-6 strain grown on minimal medium containing glycerol, the *fucAO* operon was still upregulated, suggesting that the *fucAO* operon was constitutively expressed in the evolved *E. coli* regardless of the carbon source.

used to generate the L-1,2-PDO-evolved *E. coli* strain. In contrast to our assumption concerning catabolite repression and gradual adaptation, Hacking and Lin (24) cultured WT cells pregrown on fucose with glucose to support cell growth in minimal medium containing 1,2-PDO. In addition, evolutionary trajectories of fitness during the overall evolution were not shown in previous studies. We used a natural non-PTS substrate without catabolite repression, glycerol, to support the initial growth of glycerol-evolved *E. coli* in minimal medium containing 1,2-PDO. The amount of glycerol was decreased gradually for ~250 initial generations, while the concentration of 1,2-PDO was increased. During adaptation using this two-substrate strategy, *E. coli* could utilize L-1,2-PDO as a sole carbon source, and there was a significant increase in the growth rate on L-1,2-PDO over the course of experimental evolution (Fig. 2). This result indicates that experimental approaches to studying evolution with microorganisms can be expanded to nonnative carbon sources. Based on fitness, the best representative (clone eBOP12-6) of the eBOP12 population at the end point of evolution was selected to determine genetic mechanisms of evolution with a nonnative carbon source.

Mutations can be found using new whole-genome resequencing technology. Recent next-generation DNA sequencing technology allows us to determine how many mutations are acquired during experimental evolution, where the mutations occur, the order of acquisition of mutations, and the contribution of mutations to fitness. Whole-genome sequencing with targeted Sanger sequencing revealed that only a small number of mutations accumulated during adaptation to L-1,2-PDO. The first study of *E. coli* that adaptively evolved in glycerol-containing minimal medium found that only a small number of mutations (two or three mutations) are required for adaptation to the new environment (30). This result suggests that marked changes in phenotype can be mediated by as few as two mutations, in agreement with results for *Saccharomyces cerevisiae*, maize, and the influenza virus (49, 53, 56). None of the mutations were found to occur in the intergenic or promoter region.

In contrast, the number of mutations found in the present study of L-1,2-PDO-evolved *E. coli* was higher (6 mutations); one of the mutations occurred in the promoter region, although the rest of the mutations were found in coding regions. The results suggested that adaptive evolution of *E. coli* with a nonnative carbon source, L-1,2-PDO, is more complex than adaptive evolution with a native carbon source. No large-scale genomic duplication or deletion distinguished the evolved strain from its ancestor. The synonymous mutation rate should equal the expected mutation rate (36) because synonymous sites are not a selective constraint. In *E. coli*, the point mutation rate is very low if the mutations are selectively neutral (4.5×10^{-9} mutation per synonymous site per year, 4.1×10^{-10} mutation per bp, or 1.6×10^{-10} mutation per bp per generation) (2, 17, 40). Our finding that only one synonymous substitution (*ylbE1*) was fixed during the evolution with L-1,2-PDO for ~700 generations is consistent with a low neutral mutation rate. We identified two major genetic events that specifically targeted L-1,2-PDO catabolism in the evolved population (Table 1). The first mutation that we found was the IS5 insertion between the *fucAO* and *fucPIKUR* operons, which resulted in constitutive activation of the *fucAO* operon (Fig. 5)

(49, 53, 56). Mobile genetic elements, particularly insertion sequence elements, excise from and insert into DNA at a relatively high frequency. Movement of these elements is one of the most common types of genetic changes and is considered an important factor in evolution (25). The *E. coli* K-12 MG1655 genome contains seven different IS elements (a total of 26 copies), the most prevalent of which are IS1 and IS5 (46). In the case of the *bgl* operon, insertion of IS1 or IS5 in the *cis* regulatory region, *bglR*, can allow induction expression (42). The levels of activity of the *tdh* operon enzymes are too low to allow WT *E. coli* cells to utilize threonine as a carbon source. However, IS3 insertion activated the *tdh* promoter constitutively, resulting in *E. coli* cells that utilize threonine as a sole carbon source (1). The second event was a single nucleotide alteration ($C^{22} \rightarrow A^{22}$) in the *fucO* gene that resulted in an N-terminal hydrophobic amino acid substitution (Leu⁸ $\rightarrow$ Met⁸). It is well known that POR is irreversibly inactivated by a metal-catalyzed oxidation system (8). An *E. coli* mutant able to use L-1,2-PDO aerobically was reported previously (48), and it expressed constitutively a POR mutant enzyme with resistance to MCO due to an Ile⁷-to-Leu⁷ or Leu⁸-to-Val⁸ substitution (39). This suggests that the mutation found in this study also might confer MCO resistance with POR in the evolved population. The purified POR protein from cells expressing the mutant gene showed a 10-fold increase in the reaction rate compared with the protein of the ancestral GC strain, suggesting that rapid growth on L-1,2-PDO can be partially attributed to the altered kinetic and regulatory properties of POR.

The *ilvGMEDA* operon of *E. coli* K-12 is an amino acid biosynthesis operon required for synthesis of the branched-amino acids isoleucine, leucine, and valine (51). The first two genes of this operon, *ilvG* and *ilvM*, encode the large and small subunits of AHAS II, respectively. AHAS catalyzes the first step in the biosynthesis of the branched amino acids. The reaction involves decarboxylation of pyruvate, followed by condensation with a second molecule of pyruvate or with 2-oxobutyrate. The *ilvG* gene in WT *E. coli* K-12 contains a frameshift mutation which results in termination of translation in the middle of the gene. Given that *E. coli* K-12 does not contain a functional version of AHAS II, the presence of excess valine represses synthesis of isoleucine even during isoleucine starvation (37). This phenomenon, termed valine toxicity, is relieved by depletion of valine to levels that enable isoleucine synthesis, presumably as a result of valine catabolism.

Recently, a *ylbE1* mutation in *E. coli* K-12 resulted in anaerobic resistance to nitric oxide (35). *E. coli* K-12 MG1655 populations generated by ionizing radiation were evolved, and analysis showed that there was a 1-nucleotide insert mutation in *ylbE1* that restored the full-length reading frame of an annotated pseudogene (27). Although the crystal structure of the *ylbE* product (oxidoreductase) from *Lactococcus lactis* (PDB accession code 3DQP) was determined recently, there is no similarity between *E. coli ylbE* and *L. lactis ylbE*. We tried to characterize the *ylbE* product of the evolved *E. coli*; however, there was no evidence that *ylbE* is involved in L-1,2-PDO metabolism.

The IS5 insertion mutation appeared first when we determined the order of acquisition of mutations in the eBOP12 population. Although the *fucO* mutation or IS5 insertion, which was introduced into the chromosomes of the WT and

GC strains, resulted in no growth in L-1,2-PDO-supplemented minimal medium, two major mutations (*fucO* and IS5 insertion) involved in L-1,2-PDO catabolism appeared early during adaptive evolution, which corresponded to the increase in the growth rate during the first ~100 generations (250 to 350 generations) after the evolved population started to utilize L-1,2-PDO. The evolved *E. coli* showed stepwise increases in the growth rate during adaptation as additional mutations were acquired (Fig. 3). It was reasonable to expect to see simultaneous mutations in *fucO* and IS5 insertion when the evolved *E. coli* started to utilize L-1,2-PDO during laboratory evolution. One possible interpretation is that the *fucO* mutation was present at a very low frequency in that time point.

The mutations identified can be used to reconstruct the phenotypic changes. The reconstructed strain with mutations in *fucO* and its promoter grew in minimal medium containing L-1,2-PDO, suggesting that these mutations are a prerequisite for utilization of L-1,2-PDO and provide a great fitness advantage (53 to 61%). Several studies, beginning with the classic studies on β -galactosidase (28), on amidases (26), and on ribitol dehydrogenase (10) in which bacteria were exposed to substrates that they normally cannot use, have shown that regulatory mutations that cause increased expression of an enzyme with marginal activity with the substrate are important in the early stages of acquiring new catabolic functions. Although we were able to relate some of the observed adaptive mutations (mutations in *fucO* and its promoter) to known features of the relevant metabolic pathways, other mutations pointed to genes not previously associated with the relevant physiology. Since reconstruction of the evolved strains with the *ilvG*, *ylbE1*, and *rrlD* genes with a background of mutations in *fucO* and its promoter using mutagenesis almost accounted for the phenotypic change based on the growth rate (Fig. 4), the effects of *ilvG*, *ylbE1*, and *rrlD* mutations on the growth of *E. coli* on minimal medium containing L-1,2-PDO will be investigated in the future.

The application of whole-genome sequencing to the study of experimental evolution using microbes, especially with a non-native carbon source, allowed determination of the genetic basis that underlies adaptive evolution of bacteria. Because of the potential of this method, we anticipate that there will be many more studies of the genetic basis of adaptation in the near future and that these studies will provide further insight into the molecular mechanisms of adaptive evolution in bacteria.

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