Functional cooperation of the glycine synthase-reductase and Wood–Ljungdahl pathways for autotrophic growth of Clostridium drakei

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Among CO2-fixing metabolic pathways in nature, the linear Wood–Ljungdahl pathway (WLP) in phylogenetically diverse acetate-forming acetogens comprises the most energetically efficient pathway, requires the least number of reactions, and converts CO2 to formate and then into acetyl-CoA. Despite two genes encoding glycine synthase being well-conserved in WLP gene clusters, the functional role of glycine synthase under autotrophic growth conditions has remained uncertain. Here, using the reconstructed genome-scale metabolic model iSL717 based on the completed genome sequence, transcriptomics, 13C isotope-based metabolite-tracing experiments, biochemical assays, and heterologous expression of the pathway in another acetogen, we discovered that the WLP and the glycine synthase pathway are functionally interconnected to fix CO2, subsequently converting CO2 into acetyl-CoA, acetyl-phosphate, and serine. Moreover, the functional cooperation of the pathways enhances CO2 consumption and cellular growth rates via bypassing reducing power required reactions for cellular metabolism during autotrophic growth of acetogens.

CO2 fixation | acetogen | Wood–Ljungdahl pathway | systems biology | glycine synthase-reductase pathway

The linear Wood–Ljungdahl pathway (WLP) in anaerobic acetogens is considered the most energetically efficient pathway to convert CO2 to formate and then into acetyl-CoA. With this advantage, acetogens are considered to be the most promising industrial platform to produce biofuels and chemical commodities through synthesis gas fermentation (1–4). Although gene composition and arrangement of the WLP vary among acetogens, the WLP-coding genes are well-conserved, along with two genes encoding a partial glycine synthase; the glycine cleavage system H protein (gcvH) and dihydrolipoyl dehydrogenase (lpdA) genes (5–8). The glycine synthase pathway was initially proposed for the utilization of CO2 under autotrophic growth conditions (2). While the two genes are well-conserved in the gene cluster, other genes in the glycine synthase pathway are missing in many acetogen genomes, which raises questions regarding a potential functional role of these enzymes under autotrophic growth conditions. Following synthesis, glycine can be reduced to acetyl-phosphate (acetyl-P), which is likely to be converted into acetate by acetate kinase (ackA), thereby producing one ATP, termed the glycine synthase-reductase pathway (GSRP). Alternatively, serine hydroxymethyltransferase (SHMT) converts the produced glycine to serine, which then becomes transformed to pyruvate and biomass (9–11). Recently, an artificial metabolic pathway constructed with glycine synthase and SHMT, termed the reductive glycine pathway (RGP), has shown the capability of fixing CO2 using alternative electron donors (12, 13). Despite sharing common reactions and the presence of genes encoding a partial glycine synthase, the functional role of the pathway in the presence of intact WLP has remained uncertain.

In this study, we elucidated the role of the GSRP and RGP in pure-cultured Clostridium drakei SL1 during autotrophic growth. Initially, assembly of the C. drakei genome revealed the coexistence of the GSRP, RGP, and WLP, which were then utilized for reconstruction of a genome-scale metabolic model (GEM) to predict metabolic flux through the core carbon pathways. Subsequently, transcriptome analysis revealed the transcriptional activation of genes encoding the pathways; this was then validated by using 13C isotope-based metabolite-tracing experiments, biochemical assays, and genetic engineering. Based on the integration of the data, we concluded that C. drakei co-utilizes the GSRP, the linear Wood–Ljungdahl pathway (WLP) with the glycine synthase-reductase pathway (GSRP) and reductive glycine pathway (RGP) to fix C1 compounds has remained unknown. In this study, using Clostridium drakei, we elucidated the role of the GSRP and RGP in the presence of the WLP, via a genome-scale metabolic model, RNA-seq, 13C isotope-based metabolite-tracing experiments, biochemical assays, and heterologous expression. Overall, the data suggested the pathways are functional under autotrophic conditions. Along with the WLP, GSRP and RGP convert CO2 to glycine and then to acetyl-phosphate and serine, which then obtain ATP by producing acetate and operate with limited reducing power. This is a unique co-utilization of the pathways under autotrophic conditions in acetogens.

Significance

Despite sharing the first four reactions, co-utilization of the Wood–Ljungdahl pathway (WLP) with the glycine synthase-reductase pathway (GSRP) and reductive glycine pathway (RGP) to fix C1 compounds has remained unknown. In this study, using Clostridium drakei, we elucidated the role of the GSRP and RGP in the presence of the WLP, via a genome-scale metabolic model, RNA-seq, 13C isotope-based metabolite-tracing experiments, biochemical assays, and heterologous expression. Overall, the data suggested the pathways are functional under autotrophic conditions. Along with the WLP, GSRP and RGP convert CO2 to glycine and then to acetyl-phosphate and serine, which then obtain ATP by producing acetate and operate with limited reducing power. This is a unique co-utilization of the pathways under autotrophic conditions in acetogens.
RGP, and WLP to fix CO2, and then converts it into acetyl-CoA or acetate during autotrophic growth.

Results

Genes and Metabolic Pathways for Carbon Fixation in the *C. drakei* Genome. The *C. drakei* genome was assembled completely, resulting in a 5.7-Mbp complete genome with 29.7% GC content (SI Appendix, Figs. S1 and S2 and Datasets S1 and S2) (14). Following the assembly, we identified 5,144 genes, which were composed of 5,024 coding sequences, 30 ribosomal RNAs, and 90 transfer RNAs (Dataset S3; see SI Appendix, Text S2 for details). The *C. drakei* genome encodes three formate dehydrogenase (FDH) genes, one WLP gene cluster, one ATP synthase gene cluster, and one CO dehydrogenase (CODH) gene cluster (Fig. L4 and Dataset S3) (15, 16). The third FDH (B9W14_20090) was located downstream of hydrogenase- and NAD(P)H-coding genes, indicating a similar genomic composition to that found in *Clostridium autoethanogenum*, which was reported to reduce formate using charged ferredoxin (Fd) and NAD(P)H (17). A single WLP gene cluster consisting of 15 genes associated with the carbonyl and the methyl branches was identified, which is identical to the gene composition in the *Clostridium* species (Fig. 1B; see SI Appendix, Text S3 for details) (6, 7). Energy conservation systems play essential roles in the autotrophic growth of acetogens by orchestrating the required reduction power for the WLP reactions and generating ATP (18, 19). In the *C. drakei* genome, hydrogenase
complex, Fd–NADH oxidoreductase complex (Rnf), ATP synthase complex, electron transfer flavoprotein (ETF), and electron-bifurcating transhydrogenase (Nfn)-coding gene clusters were identified, which are similar to those in C. autoethanogenum and Clostridium ljungdahlii (Fig. 1B and SI Appendix, Fig. S3; see SI Appendix, Text S4 for details).

In the C. drakei genome, the glycine synthase cluster consists of glycine cleavage system T protein (gcvT) and two glycine dehydrogenase subunits (gcvPA and gcvPB), along with gcvH and lpdA, which are located in the WLP gene cluster (Fig. 1B and Dataset S3). The first four reactions of the glycine synthase pathway are similar to the methyl branch of the WLP, converting CO2 to methylene-THF (tetrahydrofolate), which then forms glycine from methylene-THF using the first four reactions of the methyl branch of the WLP during autotrophic growth. Subsequently, methylene-THF diverges to form glycine or acetyl-CoA by using glycine synthase or the remaining WLP enzymes, respectively. Next, the formed glycine is either converted into serine or acetyl-P via the RGP or GSRP, respectively (Fig. 2B). Although the pathways are not conserved across most acetogens, the functionality of the genes substantiates that the pathways could be utilized during autotrophic growth, which needs to be validated.

Reconstruction of a Genome-Scale Metabolic Network of C. drakei

To predict the functionality of the metabolic pathways, we reconstructed a GEM (iSL771) that mathematically calculates

Fig. 2. Construction of the genome-scale metabolic network model of C. drakei (iSL771). (A) C. drakei flux distribution obtained from a Markov chain Monte Carlo. The top boxes represent the flux value from the heterotrophic condition. The bottom boxes represent the flux value from the autotrophic condition. (B) Flux distributions of CO2-fixing pathway-associated reactions in wild-type and knockout strains. (C) Acetyl-CoA production ratio in wild-type and knockout strains.
cellular metabolism in a given condition (SI Appendix, Fig. S4A; see SI Appendix, Text S3 for details) (23–26). The model iSL711 represents an acetogen genome-scale metabolic model with a genome size exceeding 5.0 Mbp, which is composed of 771 genes, 922 reactions, and 854 metabolites. Following reconstruction, the iSL771 model was then experimentally validated for the functional capability for growth and production rates in a heterotrophic condition using fructose and an autotrophic condition using H2/CO2. The experimental and simulated growth rates were 0.184 and 0.178 h−1 for the fructose condition and 0.044 and 0.046 h−1 for the H2/CO2 condition, respectively (SI Appendix, Fig. S4B). The respective experimental and simulated acetate production rates were 4.524 and 4.490 mmol·g dry weight (gDW)−1·h−1 and 3.278 and 3.291 mmol·gDW−1·h−1 for the fructose and the H2/CO2 conditions, respectively (SI Appendix, Fig. S4B). The comparisons between the experimental and in silico data indicate that the model predicted fairly accurate growth and production rates under both conditions.

Then, the genome-scale metabolic flux states of C. drakei under the growth conditions were predicted with the GEM iSL771 and Markov chain Monte Carlo sampling with 100,000 sampling points (Dataset S4). The predictions of growth conditions were predicted with the GEM model by removing the CODH/ACS, GLYR, and 5.08 (DESeq P < 10−10) fhs (B9W14_02200) that was located in the WLP cluster, with a minimum fold change of 7.48 (DESeq P < 10−41) for gcvT, along with the gene expression of gcvH and lpdA located in the WLP cluster, with a minimum fold change of 2.52 and 2.55 (DESeq P < 1.26 × 10−56), respectively (Fig. 3A and Dataset S8 and S10). Moreover, gene expression of the second and third GLYR clusters (B9W14_09375–09410 and B9W14_09465–09495) was significantly up-regulated with a minimum fold change of 1.86 (DESeq P < 2.81 × 10−12) for cooC (B9W14_22005). For the methyl branch of the WLP, all of the genes were significantly up-regulated with a minimum fold change of 2.14 (DESeq P < 5.04 × 10−14) for gcvH and lpdA located in the WLP cluster, with a minimum fold change of 2.52 and 2.55 (DESeq P < 1.41 × 10−19), respectively (Fig. 3A and Dataset S8 and S10). Consistent with the expression levels of the WLP-coding genes, a transcriptional abundance of the glycerine synthase-coding gene (B9W14_22245) that was located in the WLP gene cluster was increased by 2.52 (DESeq P < 5.02 × 10−18).

Transcriptional Response of C. drakei in the Autotrophic Condition. To validate the in silico prediction, we measured the transcriptomic changes induced by the heterotrophic and autotrophic conditions using RNA-seq (sequencing) (SI Appendix, Figs. S5 and S6 and Dataset S5; see SI Appendix, Text S6 for details). Following RNA-seq experiments, differentially expressed gene (DEG) analysis identified 693 up-regulated and 651 down-regulated genes (SI Appendix, Fig. S7 and Datasets S6 and S7) (30). Gene expression of FDH- and WLP-encoding genes was significantly up-regulated in the H2/CO2 condition (Fig. 3A). Of the three FDHs, the first (B9W14_06825) and third (B9W14_20090) were transcriptionally up-regulated with fold changes of 3.02 and 4.04 (adjusted P value for DESeq2 package) (DESeq P < 7.10 × 10−36), respectively. A minimum fold change of 5.10 (DESeq P < 1.26 × 10−56) for gcvT (B9W14_02207) located upstream of the third FDH (B9W14_02209) suggested that the FDH utilizes nearby hydrogenase genes (B9W14_20060–B9W14_20085) to reduce CO2 into formate (Fig. 3A, SI Appendix, Fig. S8, and Datasets S8 and S9). All of the genes associated with the carbonyl branch of the WLP were up-regulated with a minimum fold change of 1.86 (DESeq P < 2.81 × 10−12) for cooC (B9W14_22005). For the methyl branch of the WLP, all of the genes were significantly up-regulated with a minimum fold change of 2.14 (DESeq P < 5.04 × 10−14) for fhs (B9W14_22300). Consistent with the expression levels of the WLP-coding genes, a transcriptional abundance of the glycerine synthase-coding gene (B9W14_22245) that was located in the WLP gene cluster was increased by 2.52 (DESeq P < 5.02 × 10−18).

Notably, we observed that transcription levels of the GSRP-associated genes were significantly up-regulated in the H2/CO2 condition (Dataset S6). For glycerine synthase, all of the associated genes were up-regulated with a minimum fold change of 1.87 (DESeq P < 3.25 × 10−13) for gcvT, along with the gene expression of gcvH and lpdA located in the WLP cluster, with a minimum fold change of 2.52 and 2.55 (DESeq P < 1.41 × 10−19), respectively (Fig. 3A and Datasets S8 and S10). Moreover, gene expression of the second and third GLYR clusters (B9W14_09375–09410 and B9W14_09465–09495) was significantly up-regulated with a minimum fold change of 7.48 (DESeq P < 1.88 × 10−41) and 5.08 (DESeq P < 3.24 × 10−14) for gcvC (B9W14_04940) and gcvD (B9W14_09410), respectively. In the first GLYR cluster
(B9W14_00350–00380), five genes were transcriptionally upregulated, with the other three genes remaining unchanged. Taken together, the DEG analysis results showed that C. drakei transcriptionally activates the WLP cluster along with the GSRP-associated genes, indicating that fixed CO2 formed into acetyl-CoA by using the WLP whereas the synthesized glycine, via the GSRP, is likely re-derived to acetyl-P under the autotrophic condition.

13C-Labeling Experiment Confirmed Activation of the Glycine Synthesis Metabolic Flux under the Autotrophic Growth Condition. Based on the in silico analysis and the transcriptional analysis, C. drakei was strongly expected to cotranslute the WLP and the GSRP to fix CO2. To confirm the functional incorporation of the two pathways, we examined the path of 13C quantitatively using [U-13C]fructose or 13CO2 as a carbon source (Fig. 3B). [U-13C]Fructose-supplemented C. drakei showed a 100% fraction of unlabeled carbon for glycine (Fig. 3C). The basal medium for C. drakei contained 2 g/L yeast extract, which is required for the bacterium to proliferate. It is possible that the presence of the yeast extract supplemented unlabeled amino acids to C. drakei, resulting in unlabeled glycine in the heterotrophic condition.

In contrast, 47.3% of detected serine was labeled, demonstrating that C. drakei actively biosynthesizes serine in the presence of fructose (Fig. 3C). In C. drakei, serine can be synthesized from glycine synthase or the glycolytic pathway by converting 3-phosphoglycerate to 3-phosphohydroxypyruvate and then finally to serine. With a number of charged carbons, serine synthesis likely derived from the glycolytic pathway that utilized [U-13C]fructose, rather than from glycine, which was unlabeled (Fig. 3C). Conversely, labeled glycine was observed in the presence of 13CO2.
demonstrating that C. drakei generates glycine using glycine synthase (Fig. 3C). Subsequently, the direction of glycine was checked by investigating the $^{13}$C labeling of serine, which demonstrated that 7.8% of serine was labeled, much lower than in the heterotrophic condition. In addition, a fold change of glycine over serine in the strain under the autotrophic condition was 4.48, higher than the change detected under the heterotrophic condition with 1.25-fold change (Fig. 3D). Decreases in the labeled serine fraction and glycine-to-serine ratio indicate that serine synthesis may be limited in the autotrophic condition. Taken together, consistent with the genome-scale metabolic network prediction, the $^{13}$C-labeling experiment results demonstrated that the glycine synthase reaction fixes CO$_2$ and synthesizes glycine.

**Glycine Reductase Pathway Converts the Synthesized Glycine to Acetyl-P.** The $^{13}$C-labeling experiments validated the role of glycine synthase; however, the downstream direction of glycine during autotrophic growth remained unclear. For the GSRP, thioredoxin reductase activity plays an important role in reducing glycine. We hypothesized that if the activity of thioredoxin reducetase in autotrophically grown cells increased compared with that in the heterotrophic condition, more thiooxidore would be available to potentially reduce glycine into acetyl-P, as demonstrated in the RNA-seq results. To test this hypothesis, thiooxidone reductase activity in cells grown under both conditions was measured using a colorimetric assay, resulting in average activity units of 3.76 $\times$ 10$^{-4}$ and 2.82 $\times$ 10$^{-2}$ for the fructose and the H$_2$/CO$_2$ conditions, respectively. This result demonstrates a significant difference between the two conditions by a minimum change of fourfold ($P = 1.3 \times 10^{-3}$) (Fig. 3E). Taken together, the results validated that C. drakei activates not only the WLP but also the GSRP during the autotrophic growth condition, and then utilizes thiooxidore for reduction of glycine into acetyl-P, rather than serine.

**Glycine Reductase Pathway Enhances the CO$_2$ Consumption Rate under the Autotrophic Growth Condition.** Despite the validations, a phenotypical effect of coexistence of the pathways remains unclear. To confirm the functional role of a pathway, a reconstruction of C. drakei to modify the pathways is required to confirm the prior result, but, unfortunately, developing a genetic modification tool for the strain was infeasible. As an alternative, a genetically modifiable acetogen, the *Eubacterium limosum* ATCC 8486 strain with the absence of GSRP-coding genes in its genome, was examined for the heterogeneous introduction of the pathway (§31). To confirm the functional role, the GSRP-coding gene cluster from the C. drakei genome was cloned into a plasmid, which was then introduced into E. limosum (the GSRP strain), and the same plasmid backbone without the gene cluster was introduced into E. limosum as a control strain (SI Appendix, Fig. S9A). Using the control and the GSRP strain, growth, consumption, and production profiles were measured to understand the effect of the pathway in the presence of the WLP. According to the growth profile, the maximum cell densities of the control and the GSRP strains were 0.241 and 0.243, respectively, indicating the introduction of the GSRP does not enhance maximum biomass produced under the condition (Fig. 4.4). Similarly, a difference of total consumed H$_2$ and CO$_2$ between the strains was insignificant, and likewise for amounts of acetate produced by the strains (Fig. 4 B-D). Despite the similar biomass production, the growth rate of the GSRP strain was faster than that of the control strain, with growth rates of 0.00719 and 0.00523 h$^{-1}$, respectively (Fig. 4E). Similar to the growth rates, the consumption rates of H$_2$ and CO$_2$ by the GSRP strain were 2.291 and 0.276 mmol gDW$^{-1}$ h$^{-1}$, respectively, which were much higher than the consumption rates of 0.943 and 0.191 mmol gDW$^{-1}$ h$^{-1}$ by the control strain, respectively (Fig. 4F). Consistent with the results, the acetate production rate by the GSRP strain was higher than the rate by the control strain, with 1.191 and 0.559 mmol gDW$^{-1}$ h$^{-1}$, respectively (Fig. 4G). To validate the result, gene expression of the GSRP and the WLP-coding genes in the strains was measured using qRT-PCR. As expected, gene expression of the GSRP was observed only in the GSRP strain and not in the control strain, indicating genes encoding the GSRP were transcriptionally active under the autotrophic growth condition (SI Appendix, Fig. S9B). In addition, the WLP gene expression in the control strain was transcriptionally more active than in the GSRP strain (Fig. 4F), likely due to the absence of genes encoding the GSRP leading to more energy available for WLP gene expression (SI Appendix, Fig. S9C).

Taken together, although the GSRP did not affect the autotrophic growth capacities in the acetogen, with an additional CO$_2$-fixing route the introduction of the pathway enhanced CO$_2$ and H$_2$ consumption rates that led to an increase of the production rate of acetate, which provided energy available for the cell that eventually altered the growth rate of the acetogen.

**Discussion**

Among phylogenetically and physiologically diverse acetogens, several contain the GSRP and the RGP coupled with the WLP (6, 22, 32). Despite sharing the first four reactions, coutilization of the WLP with the GSRP and RGP in acetogens to fix C1 compounds has remained unknown. In this study, to ensure the functional role of the GSRP and RGP in the autotrophic growth condition, the genome-scale metabolic network model of *C. drakei* was reconstructed and predicted metabolic flux values of the reactions from methylene-THF to glycine to acetyl-P, along with all WLP reactions. Furthermore, transcriptomic analysis, $^{13}$C-label metabolite-tracing experiments, biochemical activity measurements, and heterologous expression of the GSRP-coding genes further revealed that the GSRP in the autotrophic condition was fully functional in the presence of the WLP.

In terms of energy conservation, similar to the WLP, the GSRP gains ATP by converting acetyl-P into acetate, thereby recouping the ATP invested at the beginning of the pathway. For creating a chemical gradient that leads to ATP synthesis, the GSRP is predicted to be less efficient than the WLP due to bypassing methylene-THF, which translocates ions across the membrane, resulting in a lower chemical gradient generated (33). However, the GSRP helps to maintain the organism even at low reduction potential, such as fixing CO$_2$ in low-ATP environments during the actinomycete reaction (2). The WLP and the GSRP utilize similar electron carriers, but additional reduced Fd for the WLP and NADPH is used for the GSRP. Comparing the reduction power difference, NADPH has a higher redox potential than reduced Fd, indicating under the limited reduced Fd available condition the strain is likely to operate the GSRP to fix CO$_2$. Another advantage of utilizing the identified pathways is the synthesis of pyruvate using the RGP. For producing pyruvate, the conventional WLP catalyzes por using reduced Fd, but the RGP circumvents the usage of reduction and converts serine to pyruvate. Overall, we hypothesized that C. drakei balances the reduction potential, which is critical during autotrophic growth, by coutilizing the WLP, GSRP, and RGP, which were calculated by in silico and RNA-seq analysis.

Further, to balance reduction power, Nfn-, ETF-, Rnf-, and ATP synthase-coding genes clusters were identified in the genome, which are responsible for energy conservation in C. drakei. Among these, the Nfn complex, which utilizes reduced Fd to reduce NADP$^+$, showed transcriptional abundance during autotrophic growth (Dataset S9). This result is consistent with a previous report that mutation of the Nfn complex in *C. autoethanogenum* retards growth in autotrophic conditions (34). The NADPH generated from the complex is likely to reduce thiooxidore to cooperate with glycine reductase in C. drakei. In addition, among all ETFs, only lactate dehydrogenase (LDH)-bound ETF, which reduces oxidized Fd from NADH, was transcriptionally
activated (Dataset S9). It is unclear why the gene expression increased despite the absence of lactate. One speculation is that acetogen is capable of bifurcating various metabolites using the LDH complex, suggesting that in the presence of energy-deficient conditions the cluster is transcriptionally activated to receive electrons from other metabolites (35). Accordingly, the energy conservation-associated proteins mentioned are vital in net ATP-gaining environments to balance redox couples to generate energy.

Notably, among the seven hydrogenase clusters, two were significantly up-regulated. Prior to the transcriptional analysis, it had been speculated that in the presence of high-level hydrogen the majority of the hydrogenases would be activated to utilize available hydrogen. However, the other five hydrogenases exhibited low gene expression levels in both conditions, indicating that they may not oxidize hydrogen in the autotrophic condition. Of the three FDH clusters, only two were up-regulated, whereas the oxidoreductase-coding gene cluster was inactive in the autotrophic condition. Although the 13C-label metabolite-tracing experimental data validate the synthesis of glycine from CO2 using the pathways, lowering the unlabeled glycine proportion would improve the quality of the data and strongly support the proposed conclusion, which requires a yeast extract-independent C. drakei strain. Overall, we confirmed the cointilization of the pathways during autotrophic conditions, which represents genetic modules to guide strain engineering for advancing the CO2-fixing capability of the strain.

Materials and Methods

Bacterial Strains and Growth Conditions. C. drakei SL1” was obtained from the Leibniz Institute DSMZ (German Collection of Microorganisms and Cell Cultures). Culture was performed anaerobically at 30 °C in 100 mL DSM 135 medium (pH 7.0). Medium composition and culture conditions are described in Supplementary Material. A purged H2/CO2 (80:20) at a pressure of 200 kPa in 50 mL of
headspace provided autotrophic growth condition and 5 g/L fructose provided heterotrophic growth conditions. All of the experiments were done with biological duplicates.

DNA Isolation. At exponential growth, the cell pellet was collected by an aerobic centrifugation at 3,000 × g for 15 min. Cells were ground using liquid nitrogen in a mortar; detailed information on isolating DNA is described in SI Appendix.

Genome Sequencing. The obtained C. drakei genome was sequenced using the PacBio system with an average size of 20 kb based on polymerase version PS3 and C3 chemistry. Detailed descriptions of genome sequencing, assembly, and annotation are described in SI Appendix. The assembled genome information is deposited in the DNA Database of Japan/European Molecular Biology Laboratory/GenBank (DBJ/EMBL/GenBank) database under accession no. CP020953.

RNA-Seq Library Preparation. C. drakei was cultivated under heterotrophic and autotrophic conditions, and then sampled at the midexponential phase. A description of extracting total RNA from the collected cells is in SI Appendix. Subsequently, ribosomal RNAs (rRNA) in the obtained RNA were removed using the Ribo-Zero rRNA Removal Kit for Metabacteria (Epizentrum) according to the manufacturer’s instruction. The rRNA-depleted RNA was used to construct the RNA libraries; detailed information is described in SI Appendix.

Genome- scale Model Reconstruction. C. drakei annotated information was used to construct metabolic networks using COBRA Toolbox v3.0.3 and COBRApy v0.14.1 software. Detailed information on the construction is described in SI Appendix.

Detailed materials and methods conducted in this study are described in SI Appendix. Information on reagents, detailed culture conditions, DNA isolation, genome sequencing, genome annotation, RNA-seq library preparation, RNA-seq analysis, GEM reconstruction, 13C isotope-labeling assay, biochemistry assay, and plasmid construction is described in SI Appendix, Materials and Methods.

Data Access and Availability. The accession number for the associated data is GSE118519 in the Gene Expression Omnibus. The complete genome sequence of C. drakei SL1 has been deposited in the DDBJ/EMBL/GenBank database under accession no. CP020953.

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