Biotechnology Reports 16 (2017) 32–43

Contents lists available at ScienceDirect

Biotechnology Reports

journal homepage: www.elsevier.com/locate/btre

Review

Amino acid catabolism-directed biofuel production in *Clostridium sticklandii*: An insight into model-driven systems engineering

Sangavai C, Chellapandi P*

Molecular Systems Engineering Lab, Department of Bioinformatics, School of Life Sciences, Bharathidasan University, Tiruchirappalli, 620 024, Tamil Nadu, India

ARTICLE INFO

Keywords:
Biofuel
Amino acid catabolism
Genome-scale model
Metabolic engineering
Systems biology
ABE fermentation
Clostridium sticklandii

ABSTRACT

Model-driven systems engineering has been more fascinating process for the microbial production of biofuel and bio-refineries in chemical and pharmaceutical industries. Genome-scale modeling and simulations have been guided for metabolic engineering of Clostridium species for the production of organic solvents and organic acids. Among them, *Clostridium sticklandii* is one of the potential organisms to be exploited as a microbial cell factory for biofuel production. It is a hyper-ammonia producing bacterium and is able to catabolize amino acids as important carbon and energy sources via Stickland reactions and the development of the specific pathways. Current genomic and metabolic aspects of this bacterium are comprehensively reviewed herein, which provided information for learning about protein catabolism-directed biofuel production. It has a metabolic potential to drive energy and direct solventogenesis as well as acidogenesis from protein catabolism. It produces by-products such as ethanol, acetate, *n*-butanol, *n*-butyrate and hydrogen from amino acid catabolism. Model-driven systems engineering of this organism would improve the performance of the industrial sectors and enhance the industrial economy by using protein-based waste in environment-friendly ways.

1. Introduction

Chemical and pharmaceutical industries are manufacturing fine chemicals in use of organic solvents and acids. Acetone is a solvent in the production of cordite, a smokeless ammunition propellant. Ethanol is a gasoline additive to increase octane and improve vehicle emissions. Butanol is used as an alternative liquid transportation fuel and can be catalytically converted into jet fuels. Moreover, butanol has proved to be a better biofuel than acclaimed ethanol owing to it is less corrosive and easily blend with gasoline [1]. Isobutyric acid and *n*-butyric acid are commercially used in the production of artificial fibers, plastics and herbicides. Isovaleric acid is mainly used for perfumery production and intensive care medicine [2]. The worldwide biofuel production reached 105 billion liters in 2010 and a contribution largely made up of ethanol and biodiesel [3] (Fig. 1). Using biotechnological processes and renewable resources, the worldwide production of acetic acid exceeds 2 million metric tons per year. The worldwide annual production quantity of propionic acid was estimated to 377,000 t in 2006 [2]. Economic development of renewable chemicals and biofuel technologies rests on several important parameters. The social, environmental and technical crises and substrate availability are being the most important factors in determining bioprocess viability and economical feasibility [1].

Microorganisms are rich sources for natural products, some of which are used as fuels, commodity chemicals, fine chemicals, and polymers [4]. Genetically engineered microbial strains should have a metabolic capability of utilizing multiple substrates with variable composition and without catabolite repression, which are crucial for the development of economic processes [5]. A microbial strain that has diverse substrate utilization potential would offer a major competitive advantage to the biofuel production from various feedstock such as carbohydrates, lipids and proteins.

1.1. Carbohydrate-based substrates

Acetone–butanol–ethanol (ABE) fermentation is a known metabolic process of *Clostridium* sp. for the production of acetone, *n*-butanol, and ethanol from lignocellulosic biomass degradation [1,6,7]. Starches and simple sugars derived from sugar cane and corn are the most commonly used feedstock for the industrial production of biofuels. Microbial fermentation converts sugars produced from lignocellulosic biomass into biofuels or biorefineries using *Clostridium* sp. The theoretical maximum calculated to be 0.939 mol butanol/1 mol glucose by ABE fermentation [8] and ~1.33 mol butanol/1 mol glucose by mixotrophic fermentation [9]. *Clostridium acetobutylicum* has been studied as a model organism of biofuel production for several decades [10]. *C. acetobutylicum* ATCC 824 and *C. beijerinckii* BA101 were proved to increase *n*-butanol

* Corresponding author.
E-mail address: pchellapandi@gmail.com (P. Chellapandi).

https://doi.org/10.1016/j.btre.2017.11.002
Received 30 June 2017; Received in revised form 17 October 2017; Accepted 3 November 2017
Available online 08 November 2017
2215-017X/ © 2017 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/BY-NC-ND/4.0/).

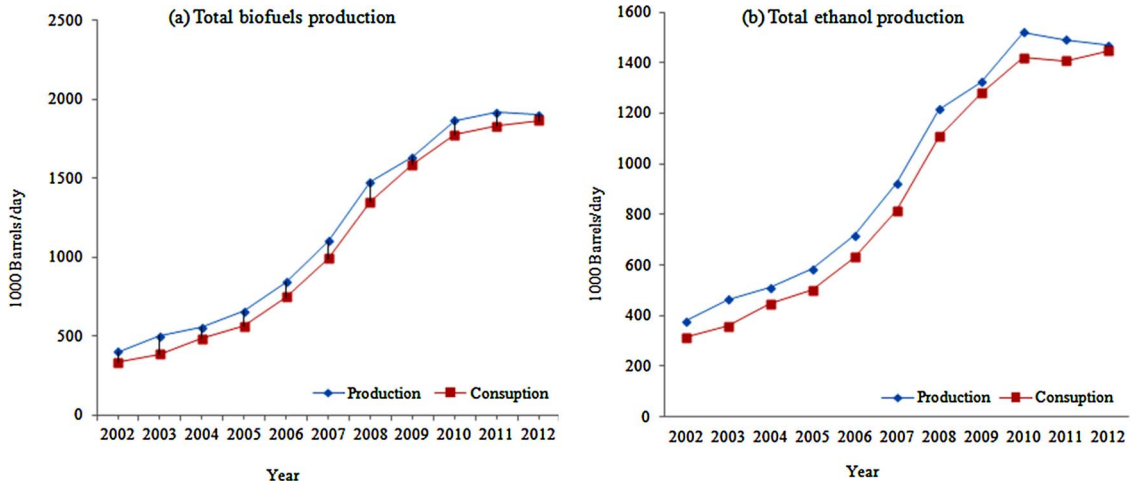

Fig. 1. International Energy Statistics for global biofuel production and consumption.

tolerance and for *n*-butanol production from carbohydrates [6,7]. Butanol production was previously commercialized from molasses produced from sugar cane industry using *C. acetobutylicum* [11]. *C. beijerinckii* BA101 mutant produced the highest concentration of *n*-butanol (17–21 g/L) from glucose across all microorganisms [12]. However, lignocellulosic biomass has to be pretreated for biofuel production under harsh conditions that requires a large amount of energy consumption [13,14].

1.2. Lipid-based substrates

Compared to cellulosic biomass, food waste holds several significant advantages to produce butanol, since it comprises with significant quantities of sugar, starch, fatty acids, proteins and minerals. The considerable amounts of these functionalized substrates can act as nutrients to proliferate the culture growth during ABE fermentation [15,16]. Even if lipids used for biodiesel generation using transesterification process, current production technology is not being economical to make biofuels through lipid fermentation [17]. However, a co-product derived from high-value fatty acids could potentially make the biorefinery process economical [18]. The maximum theoretical yield of ethanol obtained from palmitic acid (1.38 g ethanol/g palmitic acid) is significantly higher than that obtained from glucose (0.51 g ethanol/g glucose) [19,20]. Clomburg and Gonzalez [19] calculated the maximum theoretical yield of 1 mol each of ethanol and hydrogen per mole of glycerol fermented with an ethanol production of 4.6 mmol/L/h.

1.3. Protein-based substrates

Bioconversion process for releasing protein/amino acids from algal biomass or protein-rich waste may be easier than breaking down lignocellulosic to fermentable sugars [21]. The conversion efficiency of total sugar in food waste was up to 88%, but of protein was 40–70% [22]. Therefore, the conversion of proteins into biofuel is usually the rate limiting step during acidogenesis and solventogenesis phases [23]. Amino acids released by proteolysis of a protein-based substrate are catabolized to produce keto acids, which are synthesized into biofuels (ethanol, isobutanol, 2-methyl-1-butanol, and 3-methyl-1-butanol) [24]. Valine, leucine, threonine and isoleucine biosynthetic pathways have been overexpresed in *Escherichia coli* host for the production of isobutanol [25], 2-methyl-1-butanol [26], and 3-methyl-1-butanol [27]. Engineered *E. coli* achieved a yield of ~4 g/l of alcohols from a yeast extract containing 21.6 g/l of amino acids at 56% of the theoretical yield [28]. Rerouting nitrogen flux in *E. coli* was allowed to producing up to 4,035 mg/L of alcohols from *Saccharomyces cerevisiae*, *E. coli*, *Bacillus subtilis* and microalgae that can be used as protein sources containing ~22 g/l of amino acids [21]. *E. coli* carrying Clostridial butanol dehydrogenase (*bdh*) gene metabolically engineered for the conversion of protein hydrolysate to *n*-butanol and *n*-pentanol [28]. Molecular and biochemical characteristics of many Clostridium species have been extensively studied for biofuel production. However, industrial and economic importance of them on the protein-based waste are hindered due to a lack of knowledge of their complex nature of metabolic and regulatory networks at a genome-scale. Hence, systems-metabolic engineering of industrially important Clostridium would advance in the development of economically viable processes.

2. *Clostridium sticklandii* genome

C. sticklandii is a Gram-positive, anaerobic, motile and non-pathogenic bacterium isolated from mud of the San Francisco Bay Area [29]. It utilizes amino acids as an important carbon and energy sources. It also catabolizes purines, glucose, maltose and galactose through a fermentation process, but these compounds are only minor substrates for its energy and growth [30,31]. It contains a single circular chromosome of 2,715,461 bp. It encompasses 2573 coding sequences, 77 RNA genes and 38 pseudogenes. Its coding sequences contain high protein coding density of 89.2% and 2.1% repeated regions [32]. *C. sticklandii* genome consists of 199 pathways, 1103 metabolic reactions, 829 metabolites and 739 enzymes. It shares the highest number of genes that are homologous to the pathogenic *C. difficile* and hype-ammonia producing Clostridium species. In this genome, 33% coding sequences are annotated as conserved hypothetical proteins.

2.1. Comparative genomic analysis

The genomes of Clostridium genus are highly diverse in morphology, the G + C content of the DNA (24–55 mol%), and metabolic activity [33]. *C. acetobutylicum*, *C. beijerinckii* and *C. ljungdahlii* have 250 pathways, 1300 metabolic reactions and 1000 metabolites, which exhibited their complex nature of metabolic and regulatory networks (Fig. 2). *C. sticklandii*, *C. bifermentans* and *C. aminophilum* consist of a typical transporter system that is able to transport most of the amino acids and amine-derived compounds across membranes. *C. beijerinckii* has a well-established transcriptome structure, but its transcript boundaries are not well understood. It is still not clear in other Clostridial genomes [34]. Genes involved in carbohydrate, lipid and nucleotide metabolism and their annotated features are extensively available for *C. acetobutylicum*, *C. beijerinckii*, *C. cellulolyticum* and *C. ljungdahlii* to date (Fig. 3). Energetic metabolism is vastly studied to know ion-gradient driven chemiosmotic ATP generation in all Clostridial genomes excluding *C. bifermentans* and *C. aminophilum*. Genes

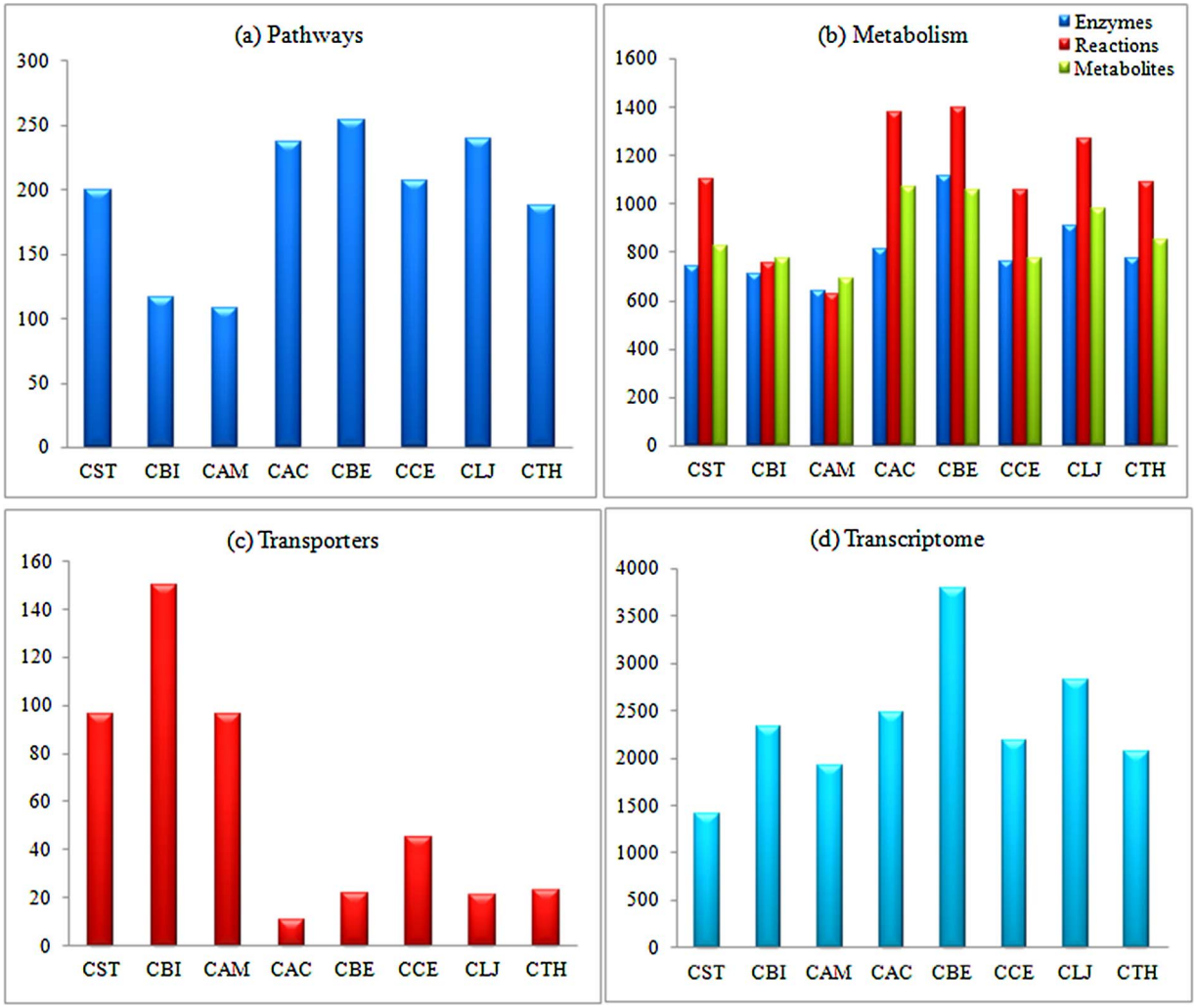

Fig. 2. Genome-scale metabolic information of *Clostridium sticklandii* DSM 519 and related Clostridium species, collected from the MetaCyc database. We compared metabolic pathways (a), metabolic properties (b), membrane transporter (c), and transcriptional units to infer genomic similarities and dissimilarities across the closely related Clostridial genomes. (CST: *C. sticklandii*; CBI: *C. bifermentans*; CAM: *C. aminophilum*; CAC: *C. acetobutylicum*; CBE: *C. beijerinckii*; CCE: *C. cellulolyticum*; CLJ: *C. ljungdahlii*; CTH: *C. thermocellum*).

involved in amino acid metabolism of many Clostridial genomes including *C. sticklandii* are greatly annotated with detailed functional information, suggesting their physiological importance in the growth and catabolism in different environmental niche.

3. General metabolism

The simultaneous presence of genes encoding for the complete Wood-Ljungdahl pathway and the glycine synthase is a particular metabolic feature of *C. sticklandii* [32]. Genes coding for sulfate assimilate pathway are absent in this genome. Biotin and pantothenate are the absolute requirement for its growth, as those vitamins biosynthesis pathways absent in this genome [35]. The genes of the non-oxidative pentose phosphate pathway are present. The Rnf complex, Na^+-dependent F0F1-ATPase and an additional V-ATPase perform ion-gradient driven chemiosmotic ATP generation via electron-transport phosphorylation [32,36]. Superoxide reductase, Mn-superoxide dismutase, PerR homolog, glutathione peroxidases, seleno-peroxiredoxin and thioredoxin-dependent peroxidase are defense systems that enhance its survival under microaerophilic conditions [37].

3.1. Amino acid catabolism

3.1.1. Protein degradation and transport

Proteins are initially hydrolyzed by exogenous proteases to small peptides and amino acids. *C. sticklandii* can be able to ferment amino acids to ammonia, CO_2, hydrogen, acetate and short chain fatty acids by the catalytic action of different metabolic enzymes as presented in Table 1. It has ABC transporters for oligopeptides, methionine, and branched-chain amino acids, serine/threonine symporter and also arginine/ornithine antiporter, which can mediate amino acid transport across the membrane [32]. It has a typical system for amino acid catabolism (Fig. 4).

3.1.2. Stickland reactions

Stickland reaction is a particular kind of fermentation of amino acids in the hype-ammonia producing Clostridium species. It is characterized by simultaneous oxidation of one amino acid and reduction of another amino acid [38,39]. Approximately 0.5 mol ATP is generated per mole amino acid transformed in Stickland reaction [40]. The δ-aminovalerate is an intermediate of arginine and proline degradation, which makes up approximately 70 percent of the theoretical propionic acid production. The theoretical stoichiometric coefficient for H_2 is 0.134, which produces about 20% H_2 from amino acid fermentation consumed by amino acids reduction [41]. C. *bifermentans*, *C. sordellii*

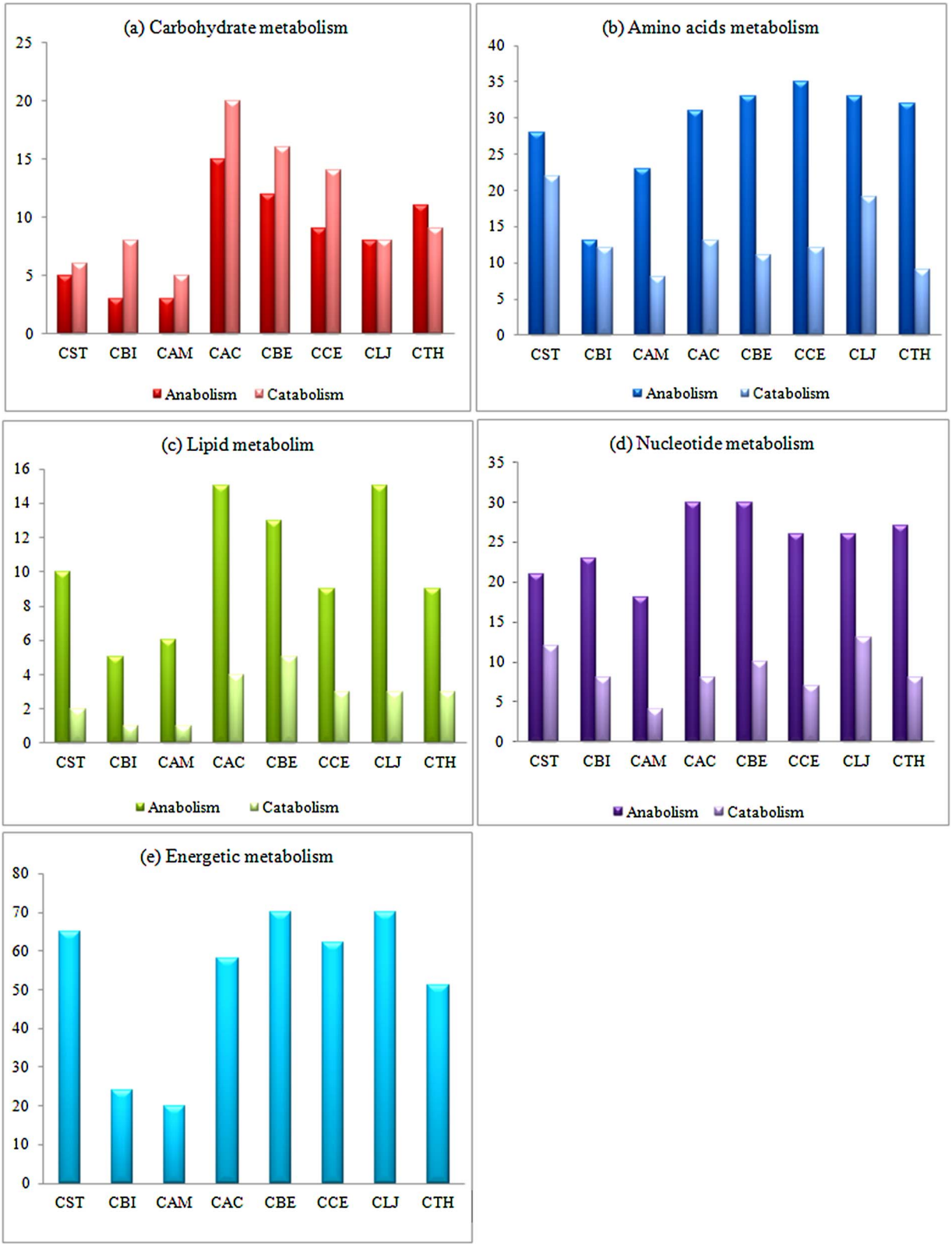

Fig. 3. Central metabolic information of *Clostridium sticklandii* DSM 519 and related Clostridium species, collected from the MetaCyc database. In our study, various subsystems involved in the metabolism of carbohydrate (a), amino acid (b), lipid (c), nucleotide (d) and energetic process (e) were compared to capture the metabolic abundance in environmental niche across the closely related Clostridial genomes (CST: *C. sticklandii*; CBI: *C. bifermentans*; CAM: *C. aminophilum*; CAC: *C. acetobutylicum*; CBE: *C. beijerinckii*; CCE: *C. cellulolyticum*; CLJ: *C. ljungdahlii*; CTH: *C. thermocellum*).

Table 1
Genes involved in amino acids metabolism of *Clostridium sticklandii* DSM 519. Information of genes and enzymes listed in this table were collected for individual amino acid catabolism from the MetaCyc database.

Amino acids	Gene	EC	Enzyme
Arginine	*speA*	4.1.1.19	Arginine decarboxylase
Arginine	*speB*	3.5.3.11	Agmatinase
Asparagine	*ansA*	3.5.1.1	Asparaginase
Aspartate	*yhdR*	2.6.1.1	Aspartate transaminase
D-Serine	*dsdA*	4.3.1.18	D-Serine ammonia-lyase
L-Serine	*sdaAA*	4.3.1.17	L-Serine ammonia-lyase
Glutamate	*gdhA*	1.4.1.2	Glutamate dehydrogenase
Glutamate	*gctA*	2.8.3.12	Glutaconate CoA-transferase
Glutamate	*gcdC*	4.1.1.70	Glutaconyl-CoA decarboxylase
Glutamate	*scad*	1.3.8.1	Short-chain acyl-CoA dehydrogenase
Glutamate	*atoA|atoD*	2.8.3.8	Acetate CoA-transferase
Glutamine	*pdxS|asnB*	3.5.1.2	Glutaminase
Glycine	*gcvPA|gcvPB|acoL*	1.4.4.2	Glycine dehydrogenase (aminomethyl-transferring)
Glycine	*gcvT|acoL*	2.1.2.10	Aminomethyltransferase
Glycine	*acoL*	1.8.1.4	Dihydrolipoyl dehydrogenase
Histidine	*hutH*	4.3.1.3	Histidine ammonia-lyase
Histidine	*hutU*	4.2.1.49	Urocanate hydratase
Histidine	*hutI*	3.5.2.7	Imidazolonepropionase
Histidine	*hutG*	3.5.3.8	Formimidoylglutamase
Isoleucine	*ilvE*	2.6.1.42	Branched-chain-amino-acid transaminase
Isoleucine	*ecm*	1.3.8.5	2-Methyl-branched-chain-enoyl-CoA reductase
Isoleucine	*paaF*	4.2.1.17	Enoyl-CoA hydratase
Isoleucine	*fabG*	1.1.1.178	3-Hydroxy-2-methylbutyryl-CoA dehydrogenase
Isoleucine	*atoB*	2.3.1.9	Acetyl-CoA C-acetyltransferase
Lysine	*speA*	4.1.1.18	Lysine decarboxylase
Methionine	*megL*	4.4.1.11	Methionine γ-lyase
Phenylalanine	*pah*	1.14.16.1	Phenylalanine 4-monooxygenase
Phenylalanine	*pcbD1*	4.2.1.96	4a-Hydroxytetrahydrobiopterin dehydratase
Threonine	*tdh*	1.1.1.103	L-Threonine 3-dehydrogenase
Threonine	*kbl*	2.3.1.29	Glycine C-acetyltransferase
Threonine	*tdh*	1.1.1.103	L-Threonine 3-dehydrogenase
Threonine	*aoc3*	1.4.3.21	Primary-amine oxidase
Threonine	*ltaE*	4.1.2.5|4.1.2.48	L-Threonine aldolase|low-specificity L-threonine aldolase
Threonine	*eutE*	1.2.1.10	Acetaldehyde dehydrogenase (acetylating)
Citrulline	*arcB*	2.1.3.3	Ornithine carbamoyltransferase
Citrulline	*arcC*	2.7.2.2	Carbamate kinase
Ornithine	*arcB*	4.3.1.12	Ornithine cyclodeaminase
Ornithine	*orr*	5.1.1.12	Ornithine racemase
Ornithine	*oat*	2.6.1.13	Ornithine aminotransferase
Ornithine	*oraE|oraS*	5.4.3.5	D-Ornithine 4,5-aminomutase
Ornithine	*ord*	1.4.1.12	2,4-Diaminopentanoate dehydrogenase
Ornithine	*proC*	1.5.1.2	Pyrroline-5-carboxylate reductase
Ornithine	*prdF*	5.1.1.4	Proline racemase

and *C. sticklandii* are belonged to Group IA strains. All strains utilize serine and threonine. The most strains produce α-amino butyrate and γ-amino butyrate [42]. Amino acid fermentation pattern is remarkably similar in *C. sticklandii*, *C. aminophilum*, *C. aminovalericum*, *C. acidiurici* and *C. barkeri,* but their growth patterns are varied in some extent [43]. These bacteria were produced up to 20-fold more ammonia than other ammonia-producing bacteria and therefore, these are considered to be ecologically important. *C. sticklandii* oxidizes ornithine, arginine, threonine, serine, and reduces proline and glycine [42,44,45]. It utilizes glycine and ornithine pair in the Stickland reaction by 2,4-diamino-pentanoate dehydrogenase (EC 1.4.1.12).

3.1.3. Aliphatic amino acids

Glycine reductase, glycine acetyltransferase, phosphotransacetylase and acetate kinase are key enzymes involved in the L-glycine catabolism in *C. sticklandii* [46,47]. Methylene-tetrahydrofolate, CO_2 and ammonia are produced from glycine by direct oxidation of NAD^+ via either glycine cleavage system or reduced to the acetyl phosphate by glycine reductase [48]. L-Alanine is deaminated to acetate and ammonia with reduction of NADH as NAD^+ by L-alanine dehydrogenase (CLOST_0512|CLOST_0519). Glycine reductase (EC 1.21.4.2) reduces L-glycine alone to acetate with the release of ammonia via intermediate acetyl phosphate. Reduced thioredoxin and ATP molecules are produced as end products by glycine reductase and acetate kinase, respectively. Acetate is formed from pyruvate via acetyl-coA and acetyl phosphate by a combined action of phosphate acetyltransferase and acetate kinase. Pyruvate-formate lyase converts pyruvate into acetyl-coA. Pyruvate dehydrogenase transfers an acetyl group with the use of electron donor NAD^+, which reduced to $NADH_2$ in the conversion of pyruvate into acetyl-CoA.

C. sticklandii has two adaptable catabolic systems such as ornithine degradation I (proline biosynthesis) and ornithine degradation II (Stickland reaction) for the degradation of L-ornithine. It contains all important genes coding for ornithine carbamoyltransferase, carbamate kinase, ornithine cyclodeaminase, ornithine aminotransferase, 2,4-diaminopentanoate dehydrogenase, and pyrroline-5-carboxylate reductase for ornithine catabolism. Ornithine racemase, adenosylcobalamin-dependent D-ornithine aminomutase and D-ornithine 4,5-aminomutase are identified and characterized for their enzymatic functions [49–53]. L-Proline is directly produced in the exponential phase from the breakdown of L-ornithine by a catalytic action of ornithine cyclodeaminase. Ornithine can be reduced to 5-aminovalerate through the formation of D-proline as an intermediate [45,54,55]. This organism has a typical operon that performs the reductive ring cleavage of D-proline into 5-aminovalerate, which is subsequently excreted [54,56]. Proline reductase, proline racemase and proline racemase are key enzymes involved in proline catabolism [57–60].

L-Alanine-L-glycine pair could not be catabolized in the Stickland

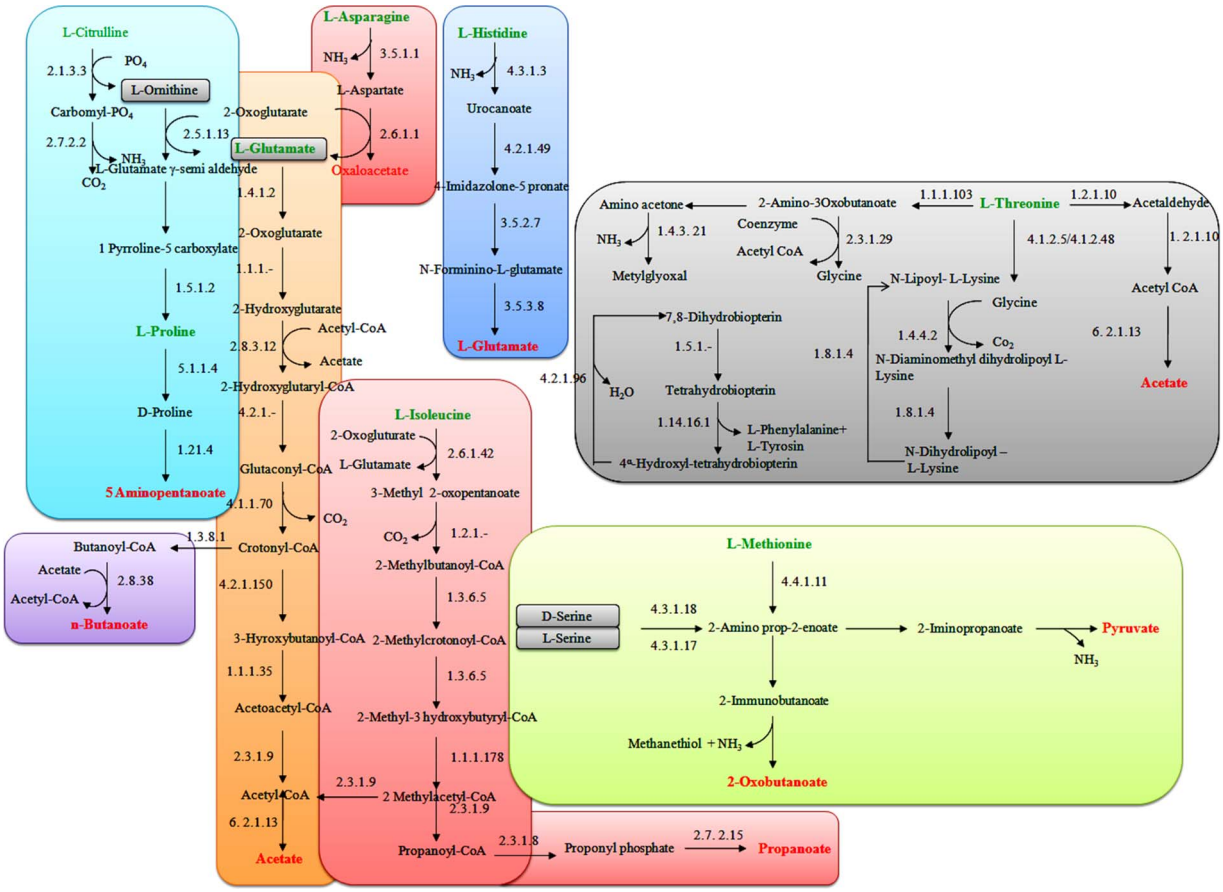

Fig. 4. The proposed pathways for the catabolism of amino acids L-asparagine, L-histidine, L-threonine, L-proline, L-glutamate, L-isoleucine, and L-methionine in *Clostridium sticklandii* DSM 519. We proposed this pathway based on the metabolic reactions collected from the MetaCyc database.

reaction as it does not have alanine dehydrogenase [61]. L-Valine, L-leucine, and L-isoleucine are probably transaminated to 2-ketoacids. These amino acids are subsequently assimilated in the primary metabolism by branched-chain-amino-acid transaminase, 2-methyl-branched-chain-enoyl-CoA reductase, enoyl-CoA hydratase, and 3-hydroxy-2-methylbutyryl-CoA dehydrogenase [62].

3.1.4. Aromatic amino acids

Aromatic amino acids are catabolized by oxidative system of *C. sticklandii*, but its catabolic pathways is still unknown [63]. However, genes coding for phenylalanine 4-monooxygenase and 4a-hydroxytetrahydrobiopterin dehydratase were recently identified from this genome, which could possibly metabolize L-phenylalanine. This genome contains all the genes (*hutH, hutU, hutI* and *hutG*) involved in the L-histidine metabolism.

3.1.5. Acidic amino acids

L-Histidine is assimilated into glutamate that is not exogenously used by *C. sticklandii* [42,64]. Glutamate is metabolized to acetyl-coA with consecutive catalytic action of glutaminase, glutamate dehydrogenase, glutaconate CoA-transferase and glutaconyl-CoA decarboxylase via glutamate degradation V (via hydroxyglutarate) or glutamine degradation I [32].

3.1.6. Neutral amino acids

Branched chain amino acids are catabolized by the oxidative system of this organism [62]. The NAD-dependent L-threonine-3-dehydrogenase and L-threonine dehydrogenase are identified and characterized from this organism. It indicates the potential of using L-threonine as carbon and nitrogen sources for its growth [65,66]. These enzymes oxidize threonine to 2-amino-3-ketobutyrate that is split into glycine and acetyl-CoA [65]. Threonine aldolase transforms threonine to glycine and acetaldehyde. Threonine dehydratase catalyzes threonine to ammonia and 2-ketobutyrate, which are oxidized to propionate and CO_2 or reductively aminated to 2-aminobutyrate [64]. L-Serine catabolism is rapidly mediated in the exponential growth phase by the presence of iron-dependent serine dehydratase and serine racemase [60,67]. This organism is able to catabolize both D-serine and L-serine due to its genome contains D-serine ammonia-lyase and D-serine ammonia-lyase. Pyruvate is an intermediate product resulted from dehydration of serine. Asparaginase is a key enzyme involved in the degradation of L-asparagine to ammonia and CO_2 via asparagine degradation I pathway.

3.1.7. Basic amino acids

L-Arginine is quickly metabolized into ornithine via citrulline and conserves energy without any redox reaction. This organism contains all the genes (*speA* and *speB*) involved in the arginine deiminase pathway as well as the ornithine oxidative-reductive pathway [54,55]. Cobamide coenzyme-dependent beta-lysine mutase, lysine-5,6-aminomutase and lysine decarboxylase are isolated and studied for understanding its L-lysine catabolism [68,69]. Lysine is catabolized into acetate, butyrate, and ammonia by exergonic reduction of crotonyl-CoA to butyryl-CoA [70–73]. After depletion of serine, arginine and cysteine, crotonyl-CoA is formed from the condensation of two acetyl-CoAs via butyrate fermentation during its exponential growth phase [32].

3.1.8. Sulfur-containing amino acids

A putative L-cysteine sulfide lyase found to degrade L-cysteine into

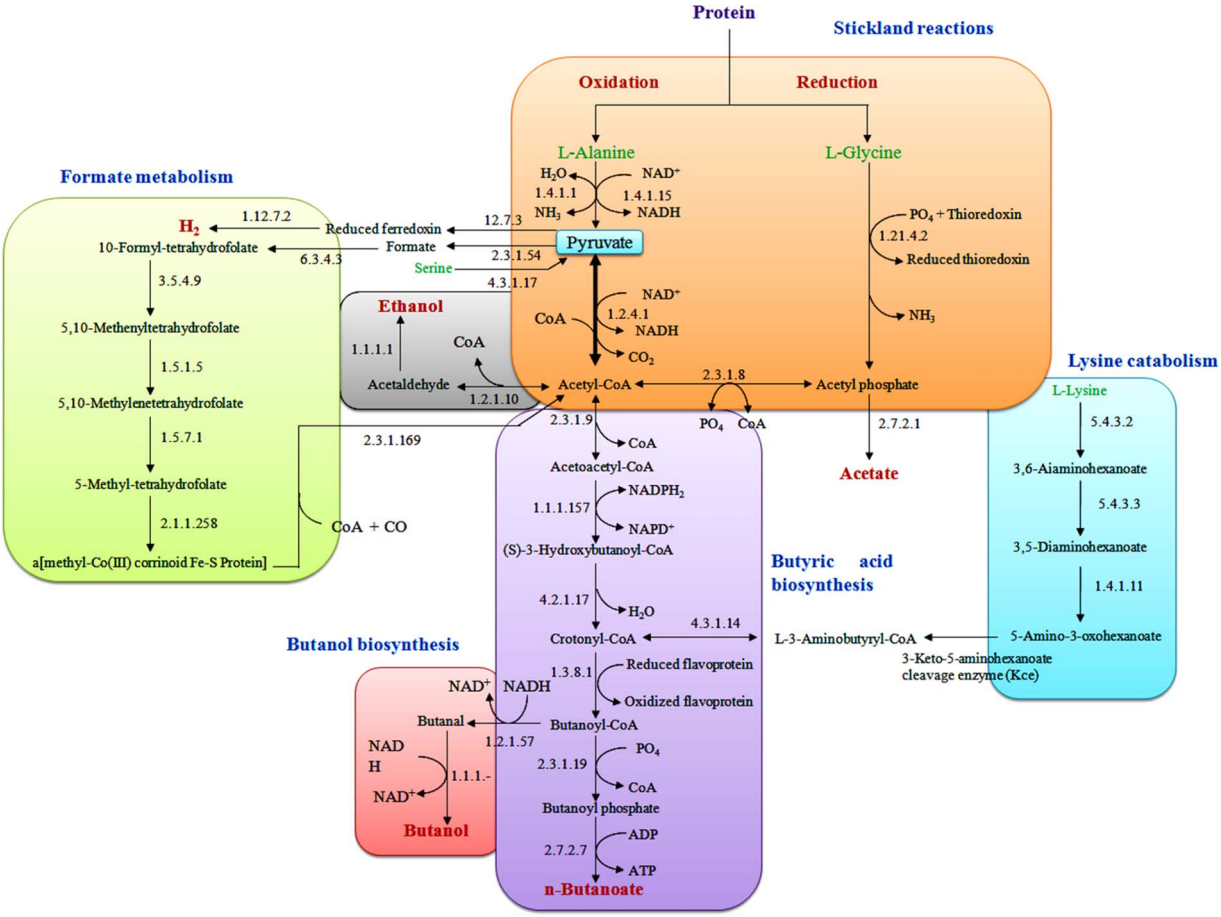

Fig. 5. The proposed pathway for protein catabolism-directed biofuel production in *Clostridium sticklandii* DSM 519. We proposed this pathway based on the metabolic reactions collected from the MetaCyc database for amino acid catabolism associated acetate, *n*-butanol, *n*-butyric acid, ethanol and hydrogen production. Enzyme commission was assigned according to the Kyoto Encyclopedia of Genes and Genomes database entries. Pyruvate was considered as a main metabolic precursor for the synthesis of organic solvents and organic acids in the proposed pathway. Ethanol is simply produced from acetaldehyde by alcohol dehydrogenase. Butanoyl-coA is a key metabolic switch to synthesis either *n*-butanol or *n*-butyric acid. Stickland reactions are a great concern of interest for amino acid catabolism by *C. sticklandii*. It has a separate pathway for lysine catabolism that directs *n*-butanol and *n*-butyric acid production. Hydrogen production is coupled with formate metabolism which may be directed by accumulation of pyruvate in the cytoplasm.

pyruvate, ammonia and hydrogen sulfide. Methionine sulfoxide reductase is involved in the repair of oxidative damaged methionine, which may enhance its growth under microaerophilic condition. Gene (*megL*) coding for methionine γ-lyase possibly degrades L-methionine through the methionine degradation II pathway. A selenium-containing $tRNA^{GIU}$ was isolated and characterized from this organism for a better understanding of the functional role of selenium in specific tRNA species that modulate the protein synthesis efficiency of certain mRNAs [74,75]. The presence of D-selenocystine alpha, beta-lyase supports selenocysteine metabolism [76].

4. Biofuels from amino acid catabolism

In the *C. sticklandii*, pyruvate is a major metabolic intermediate produced from amino acid catabolism, which further ferments into organic solvents (ethanol and *n*-butanol) and organic acids (acetate and *n*-butyrate). The proposed pathway for the solventogenesis and acidogenesis from amino acid catabolism is represented in Fig. 5. 2-Ketobutyrate formate-lyase converts pyruvate to acetyl-CoA that can be fermented in several routes. Aldehyde-alcohol dehydrogenase converts acetyl-coA to acetaldehyde, leading to produce ethanol by an alcohol dehydrogenase reaction [77].

4.1. Butanol biosynthesis

C. sticklandii has many branch points to execute solventogenesis and acidogenesis. Acetyl-CoA, acetoacetyl-CoA, and butanoyl-CoA are key intermediates produced during biofuel synthesis [78,79]. Acetyl-CoA is converted to acetate in a single step, catalyzed by the enzyme acetate-CoA ligase. Acetyl-coA acetyltransferase converts acetyl-coA to acetoacetyl-coA that forms acetoacetate. Acetone is produced from decarboxylation acetoacetate by acetoacetate decarboxylase. Acetone is reduced to 2-proponal by a reaction catalyzed by $NADP^+$-dependent oxidoreductase. Butanoyl- CoA is formed from acetoacetyl-coA via intermediates 3-hydroxybutonyl-coA and crotonyl- CoA by the consecutive action of 3-hydroxybutyryl-coA dehydrogenase, (*S*)-3-hydroxybutanoyl-CoA hydro-lyase and butyryl-coA dehydrogenase. Finally, *n*-butanote is produced from butanoyl-coA through butanoyl-coA and butanoyl phosphate by two modifying enzymes, phosphate butyryl-transferase and butyrate kinase. Butanoyl-coA is repeatedly transformed to *n*-butanol in two step mechanisms by butanal: NAD^+ oxidoreductase or butanal dehydrogenase with NAD^+ as an acceptor and oxidoreductase with the reduction of NADH. *C. sticklandii* uses lysine as a sole source of carbon and nitrogen, leading to produce *n*-butanoate and acetate via 3-keto-5-aminohexanoate pathway [45]. The (3S, 5S)-3, 5-diaminohexanoate is resulted L-β-lysine, an isomerised form of L-Lysine. Crotonyl-CoA is consecutively produced from (*S*)-3-aminobutanoyl-CoA and then is reduced to butanoyl-CoA.

4.2. Hydrogen production

C. sticklandii genome composes genes coding for periplasmic [Fe-Fe]

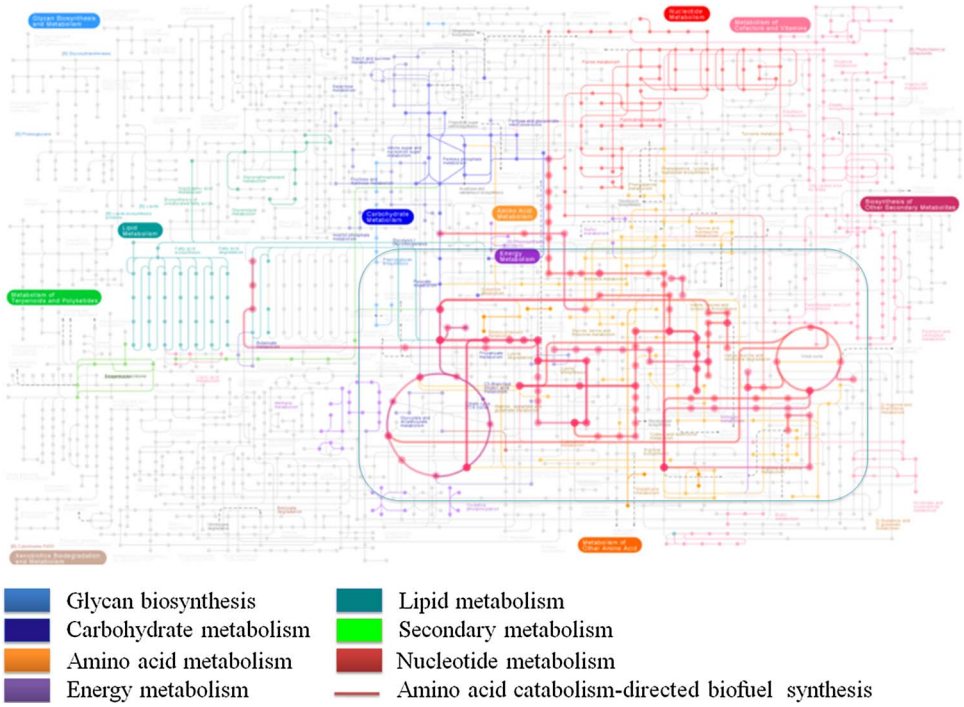

Fig. 6. Metabolic map of *Clostridium sticklandii* DSM 519 genome for the production of biofuels from amino acid catabolism using model-driven systems metabolic engineereing. It was constructed from its complete genome by using Kyoto Encyclopedia of Genes and Genomes metabolic map generator. Amino acid catabolism-directed biofuel synthesis is outlined in this map.

hydrogenase (hymC), two cytoplasmic iron-only hydrogenases (*hydA* and *hymABC*) and [Fe-Fe]-hydrogenase maturation proteins, hydF, hydG and hydE. *HydA* is clustered together with genes coding for a formate dehydrogenase (*fdhAB*). The presence of periplasmic [Fe-Fe] hydrogenase catalyzes the reduction of protons to the hydrogen along with translocates protons. The proton concentration is also lowered by the oxidation of formate to CO_2 from which released electrons are transferred directly to the hydrogenase via ferredoxin to reduce H^+ ions. Hydrogen is generated from reducing equivalents during amino acid catabolism with the proton transport and energy conservation ferredoxin oxidoreductase. It is an alternative energy-conserving system to increase its growth efficiency on formate [44].

Molecular hydrogen is produced by the reduction of protons resulted from the reversible interconversion of protons, electrons and H_2 through the action of hydrogenase. Endogenously produced formate is oxidized into CO_2 and H^+ to evolve hydrogen by formate hydrogenlyase complex formed from NAD-dependent formate dehydrogenase H (*fdhH*) and hydrogenase 3 [80,81]. FdhH catalyzes the oxidation of formate generating CO_2 and transferring the electrons to hydrogenase 3. It tranfers electrons to the catalytic subunit hydE of hydrogenase 3 via hydF and hydG subunits [82]. HydH is required for the conversion of a precursor form of hydE into the mature form. HydA is a repressor protein that represses the hyd operon [83] and increases fdhH and hydrogenase activity [81]. It is likely to control formate oxidation and the hydrogen production rate by manipulating fdhH and hydA [84]. Gene expression analysis suggested that the concentration of formate in the cell determines the biosynthesis of formate hydrogenlyase complex [85]. Endogenous selenium and molybdenum are required for the catalytic activity of formate dehydrogenase while nickel is necessary at the active site of hydrogenase 3 [86].

4.3. End-product feedback inhibition

Feedback repression is effected by amino acid end-products acting as co-repressors interfering with transcription initiation in *C. sticklandii* [87]. Nitrogen metabolite repression, nitrogen catabolite repression and ammonia repression are known nitrogen source regulation in proteolytic *Clostrdium* sp., which typically control proteases, amidases, ureases, nitrogenase, hydrogenase and those that degrade amino acids [88]. It also controls the key ammonium assimilation such as NADP-glutamate dehydrogenase, glutamine synthetase, glutamate synthase and alanine dehydrogenase. The pool sizes of one or more substrates and/or products of these enzymes may suppress the enzymatic activities [89]. TnrA becomes bound to the feedback inhibited form of glutamine synthetase, when nitrogen is excessed in the culture medium [90]. Biosynthetic intermediates of aspartate kinase can be feedback inhibited by its end product either by lysine or threonine and activated by metabolite from a competing branch as similar to *C. acetobutylicum* [91]. Engineering nitrogen flux favors the breakdown of amino acids rather than their synthesis, resulting in 2-keto-methylvalerate, 2-keto-isocaproate and 2-keto-isovalerate [28]. This approach is a great concern to overcome their feedback repression and to improve the biofuel production efficiency in *E. coli* [21].

5. Metabolic simulation for biofuel production

Mathematical models describing the overall physiology of the process, design features, mode of operation along with comparison and validation with experimental results of ABE fermentation were reviewed by Mayank et al. [92]. Ethanol production is optimized in *Synechocystis* mutant by using the flux balance analysis, method of minimization of metabolic adjustment and regulatory on/off minimization [93]. It is also improved in *C. thermocellum* by making more electrons available for acetyl coenzyme A reduction. In this approach, hydrogenase active site assembly is eliminated for hydrogen production that redirected carbon flux towards ethanol production [94]. A genetic change in *C. phytofermentans* demonstrated to improve its ethanol tolerance [95]. *E. coli* harboring engineered *C. acetobutylicum* butanol biosynthetic pathway efficiently converted *n*-butyric acid to *n*-butanol [96]. Therefore, mathematical models and metabolic simulation tools are under intense development to metabolically engineer Clostridial strains for the biofuel production [1,97–100].

5.1. Genome-scale models for biofuel production

Systems biology is the quantitative analysis of biological systems using predictive mathematical models. It is focused on interpreting biological function and behavior at a cellular level by using very large-

Table 2
Genomic information of closely related Clostridium species to *Clostridium sticklandii* DSM 519, collected from respective literatures and MetaCyc database.

Code	Genome	Strain	Size (Mbp)	Coding genes	RNA genes	References
CST	*C. sticklandii*	DSM 519	2.7	2573	77	[32]
CBI	*C. bifermentans*	ATCC 638	3.6	3176	125	[108]
CAM	*C. aminophilum*	DSM 10710	3.1	2240	67	–
CAC	*C. acetobutylicum*	ATCC 824	4.1	3778	107	[109]
CBE	*C. beijerinckii*	NCIMB 8052	6.0	5020	140	[34]
CCE	*C. cellulolyticum*	H10	4.0	3391	88	–
CLJ	*C. ljungdahlii*	DSM 13528	4.6	4184	99	[110]
CTH	*C. thermocellum*	DSM 1313	3.5	2911	71	[111]

scale datasets [101]. The *in silico* metabolic reconstruction is a common approach permitting simulations of engineered genotypes to elicit desired phenotypes, as outlined in Fig. 6. Constraint-based metabolic simulation in rational metabolic engineering requires a metabolic network reconstruction and a corresponding *in silico* metabolic model for the microorganism of interest [102]. A genome-scale metabolic model is fundamentally defined by a list of mass-balanced and charge-balanced reactions, which can simulate regulatory changes, genetic engineering effects, and dynamic cell behavior. Genome-scale metabolic networks have become more refined and complex, allowing for the expanded scopes in systematic metabolic engineering for microbial biofuel production [103–105]. However, integrated models of metabolism will become particularly important for understanding and manipulating biological behavior on a genome scale in the future. To meet industrial biotechnology process development needs, microbial cell factories are now being constructed faster and more efficiently through the use of systems biology resources [102,106].

Genome-scale models have been developed as a computational initiative for unraveling the systems-level metabolic competence of an organism and guiding metabolic engineering strategies for strain development [107]. Using these models, cofactors and energetic requirements can be metabolically balanced for the microbial growth. These models are used to predict the availability of biosynthetic precursors in response to environmental and genetic perturbations [103,104]. Genome-scale models for a range of different *Clostridium* sp. with varying metabolic capabilities have been published over the last ten years (Table 2). Several successes have been demonstrated for model-driven systems engineering of *C. acetobutylicum* [10,112–115], *C. thermocellum* [116], *C. cellulolyticum* [117], *C. beijerinckii* [105] and *C. ljungdahlii* [118] for solventogenesis and acidogenesis. Constraint-based genome-scale metabolic model has been developed in a step-wise optimization procedure by which the solvent production was predicted in the continuous culture of immobilized *C. acetobutylicum* cells [119]. A genome-scale metabolic model integrated with stress-related specific transcriptomics is used for the prediction of its focal points of gene regulation [120]. The interdependence of gene regulation, metabolism, and environmental cues can be elucidated for ABE fermentation on the basis of its systems metabolic picture [121]. Using its quantitative system-scale model, the biofuel producing steady-state can be determined for a better understanding of the functional regulation of primary metabolism [122].

5.2. Model-driven systems metabolic engineering

In this approach, genome-scale metabolic modeling and x-ome technologies are deployed an increasing extent looking into the metabolic landscape to be engineered in order to establish an economically viable process it is necessary to improve the performance of the microorganism [102,106]. Metabolic engineering of *C. sticklandii* is challenging due to the lack of an efficient gene inactivation system and the existence of complex metabolic networks. Extensive studies on protein catabolism-directed solventogenesis and acidogenesis are needed for guiding metabolic engineering of *C. sticklandii* to improve the yield of biofuel. Metabolic engineering targets for biofuel production have been evaluated from several Clostridial strains using conventional molecular approaches [1,95,100,123]. Systems-level characterization of *C. sticklandii* comprehensively examines metabolic networks to be engineered and provides a scaffold for more effective bioprocess development. Molecular complexity and the functional behavior of engineered *C. sticklandii* that plays a role in the response to alternative substrates have been efficiently explored with advances in an integrated knowledge base of its metabolic interaction networks. Current systems biological understanding of *C. sticklandii* would make it possible to expansively guide model-driven systems metabolic engineering within context of entire host metabolism, to diagnose stresses due to product synthesis, and provides the rationale to cost-effectively engineering strategies. Even if chemical processing systems are more convenient to produce fuel and refineries, these processes are very toxic to the environment and human health [15]. Concerning the green biotechnology, its systems-level modeling framework would explore the metabolic capabilities to decipher a complex genotype-phenotype relationship governing its biofuel synthesis from protein-based waste.

6. Conclusion

C. sticklandii has a great concern of utilizing proteins for producing renewable alternatives to the petroleum-based chemicals and fuels using a metabolic model at the genome-scale. Target genes for guiding metabolic engineering and novel pathways to be engineered have to be elucidated to the scientific community for competent biofuel production at industrial scale. Genome-scale model of *C. sticklandii* would provide a skeleton for predicting its metabolic behavior and emphasizing metabolic connections to biofuel synthesis. It will afford a guide for parallel reconstruction of genome-scale metabolic networks of related species. Of particular importance is the development of a regulatory circuit that allows a recombinant pathway to become part of a heterologous metabolic network for biofuel production [124] and tolerance [125]. Evolving genetic regulatory circuits would perform a self-regulating gene expression and enzyme activity in response to the precursor and or substrate supply in the host cell [126,127]. Genome-scale model of *C. sticklandii* will also provide a clue to design a synthetic pathway that needs to be integrated into the metabolic network of heterologous host in order to achieve optimal production levels. Optimized bioprocess parameters would be implemented for biofuel production from waste recycling of protein industry-based biomass in the biotechnology industry with eco-friendly manner. The present review concluded that model-driven systems metabolic engineering approach would advance the broaden applicability of using *C. sticklandii* for biofuel production in the industrial sector.

Compliance with ethical standards

The article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest

The author declares that this article's content has no conflicts of interest.

Acknowledegements

Empowerment and Equity Opportunities for Excellence in Science, Science and Engineering Research Board, Department of Science and Technology (Sanction No. SERB/F/8173/2015-16), New Delhi, India, is duly acknowledged for financial support.

References

[1] B.P. Tracy, S.W. Jones, A.G. Fast, D.C. Indurthi, E.T. Papoutsakis, Clostridia: the importance of their exceptional substrate and metabolite diversity for biofuel and biorefinery applications, Curr. Opin. Biotechnol. 23 (2012) 364–381.
[2] P. Schönicke, R. Shahab, B. Kamm, Microbial life on green biomass and their use for production of platform chemicals, in: B. Kamm (Ed.), Microorganisms in Biorefineries, Microbiol Monographs, 26 2015, pp. 21–49.
[3] A.B. Koltuniewicz, Sustainable Process Engineering: Prospects and Opportunities, De Gruyter, 2014.
[4] V. Chubukov, A. Mukhopadhyay, C.J. Petzold, J.D. Keasling, H.G. Martín, Synthetic and systems biology for microbial production of commodity chemicals, Syst. Biol. Appl. 2 (2016) 16009.
[5] A. Demain, Recombinant organisms for production of industrial products, Bioeng. Bugs 1 (2010) 116–131.
[6] J. Formanek, R. Mackie, H.P. Blaschek, Enhanced butanol production by *Clostridium beijerinckii* BA101 grown in semi-defined P2 medium containing 6 percent maltodextrin or gucose, Appl. Environ. Microbiol. 63 (1997) 2306–2310.
[7] T.C. Ezeji, N. Qureshi, H.P. Blaschek, Butanol fermentation research: upstream and downstream manipulations, Chem. Rec. 4 (2004) 305–314.
[8] E.T. Papoutsakis, Equations and calculations for fermentations of butyric-acid bacteria, Biotechnol. Bioeng. 26 (1984) 174–187.
[9] A.G. Fast, E.T. Papoutsakis, Stoichiometric and energetic analyses ofn non-photosynthetic CO2 fixation pathways to support synthetic biologynstrategies for production of fuels and chemicals, Curr. Opin. Chem. Eng. 1 (2012) 380–395.
[10] J. Lee, H. Yun, A.M. Feist, B.O. Palsson, S.Y. Lee, Genome-scale reconstruction and in silico analysis of the Clostridium acetobutylicum ATCC 824 metabolic network, Appl. Microbiol. Biotechnol. 80 (2008) 849–862.
[11] B.M. Ennis, C.T. Marshall, I.S. Maddox, A.H.J. Paterson, Continuous product recovery by *in-situ* gas stripping/condensation during solvent production from whey permeate using *Clostridium acetobutylicum*, Biotechnol. Lett. 8 (1986) 725–730.
[12] B.A. Annous, H.P. Blaschek, Isolation and characterization of *Clostridium acetobutylicum* mutants with enhanced amylolytic activity, Appl. Environ. Microbiol. 57 (1991) 2544–2548.
[13] D. Humbird, R. Davis, L. Tao, C. Kinchin, D. Hsu, A. Aden, P. Schoen, J. Lukas, B. Olthof, M. Worley, D. Sexton, D. Dudgeon, Process Design and Economics for Biochemical Conversion of Lignocellulosic Biomass to Ethanol. Dilute Acid Pretreatment and Enzymatic Hydrolysis of Corn Stover, National Renewable Energy Laboratory, 2011 (Technical Report NREL/TP-5100-47764).
[14] V. Balan, Current Callenges in Commercially Producing Biofuels from Lignocellulosic Biomass, (2014) (ISRN Biotechnol 2014: Article ID 463074).
[15] S.K. Lin, L.A. Pfaltzgraff, L.H. Davila, E.B. Mubofu, S. Abderrahim, J.H. Clark, A.A. Koutinas, N. Kopsahelis, K. Stamatelatou, F. Dickson, S. Thankappan, Z. Mohamed, R. Brocklesby, R. Luque, Food waste as a valuable resource for the production of chemicals, materials and fuels. Current situation and global perspective, Energy Environ. Sci. 6 (2013) 426–464.
[16] H. Huang, V. Singh, N. Qureshi, Butanol production from food waste: a novel process for producing sustainable energy and reducing environmental pollution, Biotechnol. Biofuels 8 (2015) 147.
[17] A. Vlysidis, M. Binns, C. Webb, C. Theodoropoulos, A techno-economic analysis of biodiesel biorefineries: assessment of integrated designs for the co-production of fuels and chemicals, Energy 36 (2011) 4671–4683.
[18] C. Ratledge, J.P. Wynn, The biochemistry and molecular biology of lipid accumulation in oleaginous microorganisms, Adv. Appl. Microbiol. 51 (2014) 1–51.
[19] J.M. Clomburg, R. Gonzalez, Biofuel production in *Escherichia coli*: the role of metabolic engineering and synthetic biology, Appl. Microbiol. Biotechnol. 86 (2010) 419–434.
[20] C. Dellomonaco, J.M. Clomburg, E.N. Miller, R. Gonzalez, Engineered reversal of the β-oxidation cycle for the synthesis of fuels and chemicals, Nature 476 (2011) 355–359.
[21] J.R. Mielenz, Biofuels from protein, Nat. Biotechnol. 29 (2011) 327–328.
[22] B. Yin, H.B. Liu, Y.Y. Wang, J. Bai, H. Liu, B. Fu, Improving volatile fatty acids production by exploiting the residual substrates in post-fermented sludge: protease catalysis of refractory protein, Bioresour. Technol. 203 (2016) 124–131.
[23] D. Shen, J. Yin, X. Yu, M. Wang, Y. Long, J. Shentu, T. Chen, Acidogenic fermentation characteristics of different types of protein-rich substrates in food waste to produce volatile fatty acids, Bioresour. Technol. 227 (2017) 125–132.
[24] K.Y. Choi, D.G. Wernick, C.A. Tat, J.C. Liao, Consolidated conversion of protein waste into biofuels and ammonia using *Bacillus subtilis*, Metab. Eng. 23 (2014) 53–61.
[25] S. Atsumi, T. Hanai, J.C. Liao, Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels, Nature 451 (2008) 86–89.
[26] A.F. Cann, J.C. Liao, Production of 2-methyl-1-butanol in engineered *Escherichia coli*, Appl. Microbiol. Biotechnol. 81 (2008) 89–98.
[27] M.R. Connor, J.C. Liao, Engineering of an *Escherichia coli* strain for the production of 3-methyl-1-butanol, Appl. Environ. Microbiol. 74 (2008) 5769–5775.
[28] Y.X. Huo, K.M. Cho, J.G. Rivera, E. Monte, C.R. Shen, Y. Yan, J.C. Liao, Conversion of proteins into biofuels by engineering nitrogen flux, Nat. Biotechnol. 29 (2011) 346–351.
[29] T.C. Stadtman, L.S. McClung, Clostridium sticklandii nov spec, J. Bacteriol. 73 (1957) 218–219.
[30] A.C. Schwartz, R. Schäfer, New amino acids, and heterocyclic compounds participating in the Stickland reaction of *Clostridium sticklandii*, Arch. Microbiol. 93 (1973) 267–276.
[31] R. Schäfer, A.C. Schwartz, Catabolism of purines in *Clostridium sticklandii*, Zentralbl. Bakteriol. Orig. A 235 (1976) 165–172.
[32] N. Fonknechten, S. Chaussonnerie, S. Tricot, A. Lajus, J.R. Andreesen, N. Perchat, E. Pelletier, M. Gouyvenoux, V. Barbe, M. Salanoubat, D. Le Paslier, J. Weissenbach, G.N. Cohen, A. Kreimeyer, *Clostridium sticklandii*, a specialist in amino acid degradation: revisiting its metabolism through its genome sequence, BMC Genomics 11 (2010) 555.
[33] E.P. Cato, E. Stackebrandt, Taxonomy and phylogeny, in: N.P. Minton, D.J. Clarke (Eds.), Clostridia, Plenum Press, New York, 1989, pp. 1–26.
[34] Y. Wang, X. Li, Y. Mao, H.P. Blaschek, Single-nucleotide resolution analysis of the transcriptome structure of *Clostridium beijerinckii* NCIMB 8052 using RNA-Seq, BMC Genomics 30 (2011) 12–479.
[35] N. Golovchenko, B. Belokopytov, N. Chuvil'skaya, V. Akimenko, Minimal synthetic culture medium for the bacterium *Clostridium sticklandii*, Appl. Biochem. Microbiol. 20 (1985) 430–434.
[36] V. Müller, G. Grüber, ATP synthases: structure, function and evolution of unique energy converters, Cell. Mol. Life Sci. 60 (2003) 474–494.
[37] F. Hillmann, R.J. Fischer, F. Saint-Prix, L. Girbal, H. Bahl, PerR acts as a switch for oxygen tolerance in the strict anaerobe Clostridium acetobutylicum, Mol. Microbiol. 68 (2008) 848–860.
[38] L.H. Stickland, Studies in the metabolism of the strict anaerobes (genus Clostridium): The chemical reactions by which *Clostridium sporogenes* obtains its energy, Biochem. J 28 (1934) 1746–1759.
[39] B. Nisman, The stickland reaction, Bacteriol. Rev. 18 (1954) 16–42.
[40] J.R. Andreesen, H. Bahl, G. Gottschalk, Introduction to the physiology and biochemistry of the genus Clostridium, in: N.P. Minton, D.C. Clarke (Eds.), Clostridia, Vol. 3 Plenum Press, New York, 1989, pp. 27–62.
[41] I.R. Ramsay, P.C. Pullammanappallil, Protein degradation during anaerobic wastewater treatment: derivation of stoichiometry, Biodegradation 12 (2001) 247–257.
[42] G.C. Mead, The amino acid-fermenting clostridia, J. Gen. Microbiol. 67 (1971) 47–56.
[43] B. Paster, J.B. Russell, C.M.J. Yang, J.M. Chow, C.R. Woese, R. Tanner, Phylogeny of the ammonia-producing ruminal bacteria *Peptostreptococcus anaerobius, clostridium sticklandii*, and *Clostridium aminophilum* sp, Int. J. Syst. Bacteriol. 43 (1993) 107–110.
[44] T.C. Stadtman, F.H. WhiteJr, Tracer studies on ornithine, lysine, and formate metabolism in an amino acid fermenting Clostridium, J. Bacteriol. 67 (1954) 651–657.
[45] H.A. Barker, Amino acid degradation by anaerobic bacteria, Annu. Rev. Biochem. 50 (1981) 23–40.
[46] N.P. Golovchenko, B.F. Belokopytov, V.K. Akimenko, Catabolism of threonine in the bacterium *Clostridium sticklandii*, Biokhimiia 47 (1982) 1159–1164.
[47] A. Graentzdoerffer, A. Pich, J.R. Andreesen, Molecular analysis of the *grd* operon coding for genes of the glycine reductase and of the thioredoxin system from *Clostridium sticklandii*, Arch. Microbiol. 175 (2001) 8–18.
[48] J.R. Andreesen, Glycine metabolism in anaerobes, Antonie Van Leeuwenhoek 66 (1994) 223–237.
[49] H.P. Chen, C.F. Lin, Y.J. Lee, S.S. Tsay, S.H. Wu, Purification and properties of ornithine racemase from *Clostridium sticklandii*, J. Bacteriol. 182 (2000) 2052–2054.
[50] H.P. Chen, S.H. Wu, Y.L. Lin, C.M. Chen, S.S. Tsay, Cloning, sequencing, heterologous expression, purification, and characterization of adenosylcobalamin-dependent D-ornithine aminomutase from *Clostridium sticklandii*, J. Biol. Chem. 276 (2001) 44744–44750.
[51] H.P. Chen, F.C. Hsui, L.Y. Lin, C.T. Ren, S.H. Wu, Coexpression, purification and characterization of the E and S subunits of coenzyme B(12) and B(6) dependent *Clostridium sticklandii* D-ornithine aminomutase in *Escherichia coli*, Eur. J. Biochem. 271 (2004) 4293–4297.
[52] K.H. Tang, A. Harms, P.A. Frey, Identification of a novel pyridoxal 5'-phosphate binding site in adenosylcobalamin-dependent lysine 5,6-aminomutase from *Porphyromonas gingivalis*, Biochem 41 (2002) 8767–8776.
[53] C.H. Tseng, C.H. Yang, H.J. Lin, C. Wu, H.P. Chen, The S subunit of D-ornithine aminomutase from *Clostridium sticklandii* is responsible for the allosteric regulation in D-alpha-lysine aminomutase, FEMS Microbiol. Lett. 274 (2007) 148–153.
[54] J. Kenklies, R. Ziehn, K. Fritsche, A. Pich, J.R. Andreesen, Proline biosynthesis from L-ornithine in *Clostridium sticklandii*: purification of delta1-pyrroline-5-carboxylate reductase, and sequence and expression of the encoding gene, proC, Microbiology 145 (1999) 819–826.
[55] N. Fonknechten, A. Perret, N. Perchat, S. Tricot, C. Lechaplais, D. Vallenet, C. Vergne, A. Zaparucha, D. Le Paslier, J. Weissenbach, A conserved gene cluster

rules anaerobic oxidative degradation of L-ornithine, J. Bacteriol. 191 (2009) 3162–3167.
[56] M.T. Madigan, J.M. Martinko, J. Parker, Brock Biology of Microorganisms, eighth edition, Prentice Hall, 1997.
[57] T.C. Stadtman, P. Elliott, Studies on the enzymic reduction of amino acids. II. Purification and properties of D-proline reductase and a proline racemase from *Clostridium sticklandii*, J. Biol. Chem. 228 (1957) 983–997.
[58] U. Kabisch, A. Gräntzdörffer, A. Schierhorn, K.P. Rücknagel, J.R. Andreesen, A. Pich, Identification of D-proline reductase from *Clostridium sticklandii* as a selenoenzyme and indications for a catalytically active pyruvoyl group derived from a cysteine residue by cleavage of a proprotein, J. Biol. Chem. 274 (1999) 8445–8454.
[59] B. Bednarski, J.R. Andreesen, A. Pich, *In vitro* processing of the proproteins GrdE of protein B of glycine reductase and *PrdA* of D-proline reductase from *Clostridium sticklandii*: formation of a pyruvoyl group from a cysteine residue, Eur. J. Biochem. 268 (2001) 3538–3544.
[60] M. Harty, M. Nagar, L. Atkinson, C.M. Legay, D.J. Derksen, S.L. Bearne, Inhibition of serine and proline racemases by substrate-product analogues, Bioorg. Med. Chem. Lett. 24 (2014) 390–393.
[61] T.C. Stadtman, On the metabolism of an amino acid fermenting *Clostridium*, J. Bacteriol. 67 (1954) 314–320.
[62] S.R. Elsden, M.G. Hilton, Volatile acid production from threonine, valine, leucine and isoleucine by clostridia, Arch. Microbiol. 117 (1978) 165–172.
[63] S.R. Elsden, M.G. Hilton, J.M. Waller, The end products of the metabolism of aromatic amino acids by Clostridia, Arch. Microbiol. 107 (1976) 283–288.
[64] S.R. Elsden, M.G. Hilton, Amino acid utilization patterns in clostridial taxonomy, Arch. Microbiol. 123 (1979) 137–141.
[65] M. Wagner, J.R. Andreesen, Purification and characterization of threonine dehydrogenase from *Clostridium sticklandii*, Arch. Microbiol. 163 (1995) 286–290.
[66] A.J. Edgar, Molecular cloning and tissue distribution of mammalian L-threonine 3-dehydrogenases, BMC Biochem. 3 (2002) 19.
[67] H. Zinecker, J.R. Andreesen, A. Pich, Partial purification of an iron-dependent L-serine dehydratase from *Clostridium sticklandii*, J. Basic Microbiol. 38 (1998) 147–155.
[68] T.C. Stadtman, P. Renz, Anaerobic degradation of lysine. V. Some properties of the cobamide coenzyme-dependent beta-lysine mutase of Clostridium sticklandii, Arch. Biochem. Biophys. 125 (1968) 226–239.
[69] F. Berkovitch, E. Behshad, K.H. Tang, E.A. Enns, P.A. Frey, C.L. Drennan, A locking mechanism preventing radical damage in the absence of substrate, as revealed by the x-ray structure of lysine 5,6-aminomutase, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 15870–15875.
[70] T. Stadtman, Lysine metabolism by clostridia, Adv. Enzymol. 38 (1973) 413–448.
[71] C.H. Chang, P.A. Frey, Cloning, sequencing, heterologous expression, purification, and characterization of adenosylcobalamin-dependent D-lysine 5, 6-aminomutase from *Clostridium sticklandii*, J. Biol. Chem. 275 (2000) 106–114.
[72] A. Kreimeyer, A. Perret, C. Lechaplais, D. Vallenet, C. Medigue, M. Salanoubat, J. Weissenbach, Identification of the last unknown genes in the fermentation pathway of lysine, J. Biol. Chem. 282 (2007) 7191–7197.
[73] H. Seedorf, W.F. Fricke, B. Veith, H. Brüggemann, H. Liesegang, A. Strittmatter, M. Miethke, W. Buckel, J. Hinderberger, F. Li, The genome of *Clostridium kluyveri*, a strict anaerobe with unique metabolic features, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 2128–2133.
[74] W.M. Ching, B. Alzner-DeWeerd, T.C. Stadtman, A selenium-containing nucleoside at the first position of the anticodon in seleno-tRNAGlu from *Clostridium sticklandii*, Proc. Natl. Acad. Sci. U. S. A. 82 (1985) 347–350.
[75] W.M. Ching, Characterization of selenium-containing tRNAGlu from *Clostridium sticklandii*, Arch. Biochem. Biophys. 244 (1986) 137–146.
[76] N. Esaki, V. Seraneeprakarn, H. Tanaka, K. Soda, Purification and characterization of *Clostridium sticklandii* D-selenocystine alpha, beta-lyase, J. Bacteriol. 170 (1988) 751–756.
[77] Eram, Ma, Decarboxylation of pyruvate to acetaldehyde for ethanol production by hyperthermophiles, Biomolecules 3 (2013) 578–596.
[78] G. Herrmann, E. Jayamani, G. Mai, W. Buckel, Energy conservation via electron-transferring flavoprotein in anaerobic bacteria, J. Bacteriol. 190 (2008) 784–791.
[79] F. Li, J. Hinderberger, H. Seedorf, J. Zhang, W. Buckel, R.K. Thauer, Coupled ferredoxin and crotonyl coenzyme A (CoA) reduction with NADH catalyzed by the butyryl-CoA dehydrogenase/Etf complex from Clostridium kluyveri, J. Bacteriol. 190 (2008) 843–850.
[80] R. Bohm, M. Sauter, A. Bock, Nucleotide sequence and expression of an operon in Escherichia coli coding for formate hydrogenlyase components, Mol. Microbiol. 4 (1990) 231–243.
[81] M. Sauter, R. Bohm, A. Bock, Mutational analysis of the operon (*hyc*) determining hydrogenase 3 formation in *Escherichia coli*, Mol. Microbiol. 6 (1992) 1523–1532.
[82] T. Maeda, V. Sanchez-Torres, T.K. Wood, Metabolic engineering to enhance bacterial hydrogen production, Microbiol. Biotechnol. 1 (2008) 30–39.
[83] S. Leonhartsberger, I. Korsa, A. Bock, The molecular biology of formate metabolism in enterobacteria, J. Mol. Microbiol. Biotechnol. 4 (2002) 269–276.
[84] A. Yoshida, T. Nishimura, H. Kawaguchi, M. Inui, H. Yukawa, Enhanced hydrogen production from formic acid by formate hydrogen lyase-overexpressing *Escherichia coli* strains, Appl. Environ. Microbiol. 71 (2005) 6762–6768.
[85] R. Rossmann, G. Sawers, A. Bock, Mechanism of regulation of the formate-hydrogenlyase pathway by oxygen, nitrate, and pH: definition of the formate regulon, Mol. Microbiol. 5 (1991) 2807–2814.
[86] J.C. Boyington, V.N. Gladyshev, S.V. Khangulov, T.C. Stadtman, P.D. Sun, Crystal structure of formate dehydrogenase H: catalysis involving Mo, molybdopterin, selenocysteine, and an Fe4S4 cluster, Science 275 (1997) 1305–1308.
[87] S. Sanchez, A.L. Demain, Metabolic regulation and overproduction of primary metabolites, Microbial. Biotechnol. 1 (2008) 283–319.
[88] E.G. ter Schure, N.A. van Riel, C.T. Verrips, The role of ammonia metabolism in nitrogen catabolite repression in *Saccharomyces cerevisiae*, FEMS Microbiol. Rev. 24 (2000) 67–83.
[89] C.J. Harper, D. Hayward, M. Kidd, I. Wiid, P. van Helden, Glutamate dehydrogenase and glutamine synthetase are regulated in response to nitrogen availability in *Myocbacterium smegmatis*, BMC Microbiol. 10 (2010) 138.
[90] K. Fedorova, A. Kayumov, K. Woyda, O. Ilinskaja, K. Forchhammer, Transcription factor TnrA inhibits the biosynthetic activity of glutamine synthetase in *Bacillus subtilis*, FEBS Lett. 587 (2013) 1293–1298.
[91] B.A. Manjasetty, M.R. Chance, S.K. Burley, S. Panjikar, S.C. Almo, Crystal structure of *Clostridium acetobutylicum* Aspartate kinase (CaAK): An important allosteric enzyme for amino acids production, Biotechnol. Rep. 3 (2014) 73–85.
[92] R. Mayank, A. Ranjan, V.S. Moholkar, Mathematical models of ABE fermentation: review and analysis, Crit. Rev. Biotechnol. 33 (2013) 419–447.
[93] T. Sengupta, M. Bhushan, P.P. Wangikar, Metabolic modeling for multi-objective optimization of ethanol production in a *Synechocystis* mutant, Photosynth. Res. 118 (2013) 155–165.
[94] R. Biswas, T. Zheng, D.G. Olson, L.R. Lynd, A.M. Guss, Elimination of hydrogenase active site assembly blocks H_2 production and increases ethanol yield in Clostridium thermocellum, Biotechnol. Biofuels 12 (2015) 8–20.
[95] A.C. Tolonen, T.R. Zuroff, M. Ramya, M. Boutard, T. Cerisy, W.R. Curtis, Physiology, genomics, and pathway engineering of an ethanol-tolerant strain of *Clostridium phytofermentans*, Appl. Environ. Microbiol. 81 (2015) 5440–5448.
[96] A.J. Mattam, S.S. Yazdani, Engineering *E. coli* strain for conversion of short chain fatty acids to bioalcohols, Biotechnol. Biofuels 6 (2013) 128.
[97] R.P. Desai, L.M. Harris, N.E. Welker, E.T. Papoutsakis, Metabolic flux analysis elucidates the importance of the acid-formation pathways in regulating solvent production by *Clostridium acetobutylicum*, Met. Eng. 1 (1999) 206–213.
[98] R.P. Desai, L.K. Nielsen, E.T. Papoutsakis, Stoichiometric modeling of *Clostridium acetobutylicum* fermentations with non-linear constraints, J. Biotechnol. 71 (1999) 191–205.
[99] H. Shinto, Y. Tashiro, M. Yamashita, G. Kobayashi, T. Sekiguchi, T. Hanai, Y. Kuriya, M. Okamoto, K. Sonomoto, Kinetic modeling and sensitivity analysis of acetone-butanol-ethanol production, J. Biotechnol. 131 (2007) 45–56.
[100] D. Amador-Noguez, X.-J. Feng, J. Fan, N. Roquet, H. Rabitz, J.D. Rabinowitz, Systems-level metabolic flux profiling elucidates a complete, bifurcated tricarboxylic acid cycle in *Clostridium acetobutylicum*, J. Bacteriol. 192 (2010) 4452–4461.
[101] F.D. Mast, A.V. Ratushny, J.D. Aitchison, Systems cell biology, J. Cell Biol. 206 (2014) 695–706.
[102] E. Simeonidis, N.D. Price, Genome-scale modeling for metabolic engineering, J. Ind. Microbiol. Biotechnol. 42 (2015) 327–338.
[103] S. Davidson, Sustainable bioenergy: genomics and biofuels development, Nature Educ. 1 (2008) 175.
[104] R.S. Senger, Biofuel production improvement with genome-scale models: the role of cell composition, Biotechnol. J. 5 (2010) 671–685.
[105] C.B. Milne, J.A. Eddy, R. Raju, S. Ardekani, P.J. Kim, R.S. Senger, Y.S. Jin, H.P. Blaschek, N.D. Price, Metabolic network reconstruction and genome-scale model of butanol-producing strain *Clostridium beijerinckii* NCIMB 8052, BMC Syst. Biol. 5 (2011) 130.
[106] J.M. Otero, J. Nielsen, Industrial systems biology, Biotechnol. Bioeng. 105 (2010) 439–460.
[107] P. Chellapandi, S. Sivaramakrishnan, M.B. Viswanathan, Systems biotechnology: an emerging trend in metabolic engineering of industrial microorganisms, J. Comput. Sci. Syst. Biol. 3 (2010) 43–49.
[108] Y.M. Wong, J.C. Juan, H.M. Gan, C.M. Austin, Draft genome sequence of *Clostridium bifermentans* strain WYM, a promising biohydrogen producer isolated from landfill leachate sludge, Genome Announc. 2 (2014) e00077–14, http://dx.doi.org/10.1128/genomeA.00077-14.
[109] J. Nölling, G. Breton, M.V. Omelchenko, K.S. Makarova, Q. Zeng, R. Gibson, H.M. Lee, J. Dubois, D. Qiu, J. Hitti, Y.I. Wolf, R.L. Tatusov, F. Sabathe, L. Doucette-Stamm, P. Soucaille, M.J. Daly, G.N. Bennett, E.V. Koonin, D.R. Smith, Genome sequence and comparative analysis of the solvent-producing bacterium *Clostridium acetobutylicum*, J. Bacteriol. 183 (2001) 4823–4838.
[110] M. Köpke, C. Held, S. Hujer, H. Liesegang, A. Wiezer, A. Wollherr, A. Ehrenreich, W. Liebl, G. Gottschalk, P. Dürre, Clostridium ljungdahlii represents a microbial production platform based on syngas, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 13087–13092.
[111] L. Feinberg, J. Foden, T. Barrett, K.W. Davenport, D. Bruce, C. Detter, R. Tapia, C. Han, A. Lapidus, S. Lucas, J.F. Cheng, S. Pitluck, T. Woyke, N. Ivanova, N. Mikhailova, M. Land, L. Hauser, D.A. Argyros, L. Goodwin, D. Hogsett, N. Caiazza, Complete genome sequence of the cellulolytic thermophile *Clostridium thermocellum* DSM1313, J. Bacteriol. 193 (2011) 2906–2907.
[112] J.Y. Lee, Y.S. Jang, J. Lee, E.T. Papoutsakis, S.Y. Lee, Metabolic engineering of Clostridium acetobutylicum M5 for highly selective butanol production, Biotechnol. J. 4 (2009) 1432–1440.
[113] J. Lee, Y.S. Jang, S.J. Choi, J.A. Im, H. Song, J.H. Cho, Y. Seung do, T. Papoutsakis, G.N. Bennett, S.Y. Lee, Metabolic engineering of Clostridium acetobutylicum ATCC 824 for isopropanol-butanol-ethanol fermentation, Appl. Environ. Microbiol. 78 (2012) 1416–1423.
[114] R.S. Senger, E.T. Papoutsakis, Genome-scale model for *Clostridium acetobutylicum*: Part I: Metabolic network resolution and analysis, Biotechnol. Bioeng. 101 (2008) 1036–1052.
[115] R.S. Senger, E.T. Papoutsakis, Genome-scale model for Clostridium

acetobutylicum: part II. Genome-scale model for *Clostridium acetobutylicum*: Part II. Development of specific proton flux states and numerically determined sub-systems, Biotechnol. Bioeng. 101 (2008) 1053–1071.
[116] S.B. Roberts, C.M. Gowen, J.P. Brooks, S.S. Fong, Genome-scale metabolic analysis of *Clostridium thermocellum* for bioethanol production, BMC Syst. Biol. 4 (2010) 31.
[117] F. Salimi, K. Zhuang, R. Mahadevan, Genome-scale metabolic modeling of a clostridial co-culture for consolidated bioprocessing, Biotechnol. J. 5 (2010) 726–738.
[118] H. Nagarajan, M. Sahin, J. Nogales, H. Latif, D.R. Lovley, A. Ebrahim, K. Zengler, Characterizing acetogenic metabolism using a genome-scale metabolic reconstruction of *Clostridium ljungdahlii*, Microb. Cell Fact. 12 (2013) 118.
[119] J. Wallenius, M. Viikilä, S. Survase, H. Ojamo, T. Eerikäinen, Constraint-based genome-scale metabolic modeling of *Clostridium acetobutylicum* behavior in an immobilized column, Bioresour. Technol. 142 (2013) 603–610.
[120] S. Dash, T.J. Mueller, K.P. Venkataramanan, E.T. Papoutsakis, C.D. Maranas, Capturing the response of *Clostridium acetobutylicum* to chemical stressors using a regulated genome-scale metabolic model, Biotechnol. Biofuels 7 (2014) 144.
[121] C. Liao, S.O. Seo, V. Celik, H. Liu, W. Kong, Y. Wang, H. Blaschek, Y.S. Jin, T. Lu, Integrated, systems metabolic picture of acetone-butanol-ethanol fermentation by *Clostridium acetobutylicum*, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) 8505–8510.
[122] M. Yoo, G. Bestel-Corre, C. Croux, A. Riviere, I. Meynial-Salles, P. Soucaille, A Quantitative system-scale characterization of the metabolism of *Clostridium acetobutylicum*, MBio 6 (2015) (e01808-15).
[123] S. Dusséaux, C. Croux, P. Soucaille, I. Meynial-Salles, Metabolic engineering of *Clostridium acetobutylicum* ATCC 824 for the high-yield production of a biofuel composed of an isopropanol/butanol/ethanol mixture, Metab. Eng. 18 (2013) 1–8.
[124] V.L. Colin, A. Rodríguez, H.A. Cristóbal, The role of synthetic biology in the design of microbial cell factories for biofuel production, J. Biomed. Biotechnol. 2011 (2011) 601834.
[125] M.J. Dunlop, Z.Y. Dossani, H.L. Szmidt, H.C. Chu, T.S. Lee, J.D. Keasling, M.Z. Hadi, A. Mukhopadhyay, Engineering microbial biofuel tolerance and export using efflux pumps, Mol. Syst. Biol. 7 (2011) 487.
[126] Chatterjee, Yuan, Directed evolution of metabolic pathways, Trends Biotechnol. 24 (2005) 28–38.
[127] J.A. Brophy, C.A. Voigt, Principles of genetic circuit design, Nat. Methods 11 (2014) 508–520.