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Review

Pathway engineering and synthetic biology using acetogens

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ABSTRACT

Acetogenic anaerobic bacteria are defined as organisms employing the Wood–Ljungdahl pathway to synthesize acetyl-CoA from CO_2 or CO. Their autotrophic mode of metabolism offers the biotechnological chance to combine use of abundantly available substrates with reduction of greenhouse gases. Several companies have already established pilot and demonstration plants for converting waste gases into ethanol, an important biofuel and a natural product of many acetogens. Recombinant DNA approaches now opened the door to construct acetogens, synthesizing important industrial bulk chemicals and biofuels such as acetone and butanol. Thus, novel microbial production platforms are available that no longer compete with nutritional feedstocks.

1. Introduction

Acetogens (acetogenic bacteria) are anaerobes that can use the Wood–Ljungdahl pathway to (i) synthesize acetyl-CoA by the reduction of CO or $CO_2 + H_2$ (ii) conserve energy, and (iii) fix (assimilate) CO_2 for the synthesis of cell carbon [1]. A detailed characterization of acetogens and their metabolic capabilities is given by Drake et al. [1] and Ragsdale [2], important traits are summarized below.

Clostridium aceticum (spore-forming, mesophilic) was the first acetogen isolated and characterized [3]. The organism got lost soon after World War II and, thus, further investigations focused on *Clostridium thermoaceticum* (spore-forming, thermophilic), the second acetogen isolated [4]. This bacterium, later re-classified as *Moorella thermoacetica* [5], was the model organism for Harland G. Wood and Lars G. Ljungdahl for the elucidation of the biochemical and enzymological features of the acetyl-CoA pathway, which was in turn named Wood–Ljungdahl pathway. However, the organism had been isolated under heterotrophic conditions, performing a homoacetic acid fermentation, and was only shown in 1990 to be able of autotrophic growth on $CO_2 + H_2$ or CO [6]. Similarly, the acetogen *Clostridium formicoaceticum,* when isolated as a pure culture, could only be grown heterotrophically [7]. Thus, the first autotrophic acetogen investigated in detail became *Acetobacterium woodii*, which can grow on $CO_2 + H_2$ as well as on fructose and few other heterotrophic substrates [8]. Soon after, an old culture of *C. aceticum* (dated 1947) was rediscovered and reactivated [9]. Since then, this organism is again available for further investigations. Interestingly, *Clostridium scatologenes*, which was already isolated in 1927, was only in 2000 characterized as an acetogen [10].

Until now, more than 100 acetogens have been isolated. Despite their common feature of CO_2 fixation via the Wood–Ljungdahl pathway (for reviews see [1,2,11–15]), they are metabolically, ecologically, and phylogenetically diverse. The best characterized acetogenic species belong to the genera *Acetobacterium* (*A. woodii*) and *Clostridium* (*C. aceticum, C. autoethanogenum, C. ljungdahlii,* and *C. ragsdalei*), two of the known 22 genera harbouring acetogens. Species of these two genera (Table 1) will be in the focus of the review.

Under anaerobic conditions, acetogens are able to grow chemolithoautotrophically converting CO or $CO_2 + H_2$ as sole carbon sources into mainly acetate. Besides acetate, some acetogenic organisms (*Butyribacterium metylotrophicum* [16], *C. autoethanogenum* [17,18], *Clostridium carboxidivorans* [19], *Clostridium drakei* [19], *C. ljungdahlii* [20,21], *C. ragsdalei* [22,21], *C. scatologenes* [19], *Oxobacter pfennigii* (formerly *Clostridium pfennigii*) [23]) produce other products such as 2,3-butanediol, butyrate, ethanol, or butanol (Table 1).

CO and/or CO_2 are substrates for the two branches of the Wood–Ljungdahl pathway (Fig. 1), the methyl and carbonyl branch. Acetyl-CoA is the main intermediate of the pathway and serves as a precursor for the anabolism and catabolism of the respective organism. In the methyl branch, CO or CO_2 is reduced through a series of tetrahydrofolate (THF)- and cobalamin-dependent reactions into a methyl group. This methyl group is

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Table 1
Acetogenic clostridia.

Organism	Substrate	Products	Optimum temperature (°C)	Remarks	Reference
Acetitomaculum ruminis	H_2+CO_2, CO	Acetate	38		[62]
Acetoanaerobium noterae	H_2+CO_2	Acetate	37		[63]
Acetobacterium bakii	H_2+CO_2, CO	Acetate	20		[64]
Acetobacterium carbinolicum	H_2+CO_2	Acetate	27		[65,66]
Acetobacterium dehalogenans	H_2+CO_2, CO	Acetate	25		[67]
Acetobacterium fimetarium	H_2+CO_2, CO	Acetate	30		[64]
Acetobacterium malicum	H_2+CO_2	Cetate	30		[68]
Acetobacterium paludosum	H_2+CO_2, CO	Acetate	20		[64]
Acetobacterium tundrae	H_2+CO_2, CO	Acetate	20		[69]
Acetobacterium wieringae	H_2+CO_2	Acetate	30		[70]
Acetobacterium woodii	H_2+CO_2, CO	Acetate	30	Genome sequence available but not yet published; doubling time on CO [h]: 13	[8,71,72]
Acetohalobium arabicum	H_2+CO_2, CO	Acetate	38–40		[73]
Acetonema longum	H_2+CO_2	Acetate, butyrate	30–33		[74]
Blautia coccoides	H_2+CO_2		37		
Blautia hydrogenotrophica	H_2+CO_2	Acetate	35–37		[75]
Blautia producta	H_2+CO_2, CO	Acetate	37	Doubling time on CO [h]: 1.5-3	[76,77]
Blautia schinkii	H_2+CO_2	Acetate	39		[78]
Butyribacterium methylotrophicum	H_2+CO_2, CO (after adaption)	Acetate, ethanol, butyrate, butanol	37	Doubling time on CO [h]: 13.9	[16,79,80]
Clostridium aceticum	H_2+CO_2, CO	Acetate	30	Genome sequence under construction	[81–83]
Clostridium autoethanogenum	H_2+CO_2, CO	2,3-Butanediol, acetate, ethanol	37	Doubling time on CO [h]: 4	[17,21]
Clostridium carboxidivorans	H_2+CO_2, CO	Acetate, ethanol, butyrate, butanol	38	Genome sequence available; doubling time on CO [h]: 4.3	[19,84]
Clostridium drakei	H_2+CO_2, CO	Acetate, ethanol, butyrate	25–30	Doubling time on CO [h]: 5.8	[10,19,85]
Clostridium formicoaceticum	CO	Acetate, fomate	37		[7,83,86]
Clostridium glycolicum	H_2+CO_2	Acetate	37–40		[87,88]
Clostridium ljungdahlii	H_2+CO_2, CO	2,3-Butanediol, acetate, ethanol	37	Genome sequence available; doubling time on CO [h]: 3.8	[20,21,29,89–91]
Clostridium magnum	H_2+CO_2	Acetate	30–32		[92,93]
Clostridium mayombei	H_2+CO_2	Acetate	33		[94]
Clostridium methoxybenzovorans	H_2+CO_2	Acetate, fomate	37		[95]
Clostridium ragsdalei	H_2+CO_2, CO	2,3-Butanediol, acetate, ethanol	37	Doubling time on CO [h]: 4	[22,21]
Clostridium scatologenes	H_2+CO_2, CO	Acetate, ethanol, butyrate	37–40	Doubling time on CO [h]: 7.7	[19]
Eubacterium aggregans	H_2+CO_2	Acetate, formate	35		[96]
Eubacterium limosum	H_2+CO_2, CO	Acetate	39	Genome sequence available; doubling time on CO [h]: 9	[97,98]
Morellla mulderi	H_2+CO_2	Acetate	65		[99]
Morella thermoacetica	H_2+CO_2, CO	Acetate	55	Genome sequence available; doubling time on CO [h]: 9-16	[4,100–105]
Morella thermoautotrophica	H_2+CO_2, CO	Acetate	56–60	Doubling time on CO [h]: 7	[106–108]
Oxobacter pfennigii	H_2+CO_2, CO	Acetate, butyrate		Doubling time on CO [h]: 13,9	[23]
Sporomusa acidovorans	H_2+CO_2	Acetate	35		[109]
Sporomusa aerivorans	H_2+CO_2	Acetate	30		[110]
Sporomusa malonica	H_2+CO_2	Acetate	30		[111]
Sporomusa ovata	H_2+CO_2	Acetate	30		[112]
Sporomusa paucivorans	H_2+CO_2		34		[113]
Sporomusa rhizae	H_2+CO_2		35		[114]
Sporomusa silvacetica	H_2+CO_2		25–30		[115]
Sporomusa spaeroides	H_2+CO_2		35–39		[112]
Sporomusa termitida	H_2+CO_2, CO		30		[116]
Thermoacetogenium phaeum	H_2+CO_2		58		[117]
Thermoanaerobacter kivui	H_2+CO_2		66		[6,118,119]

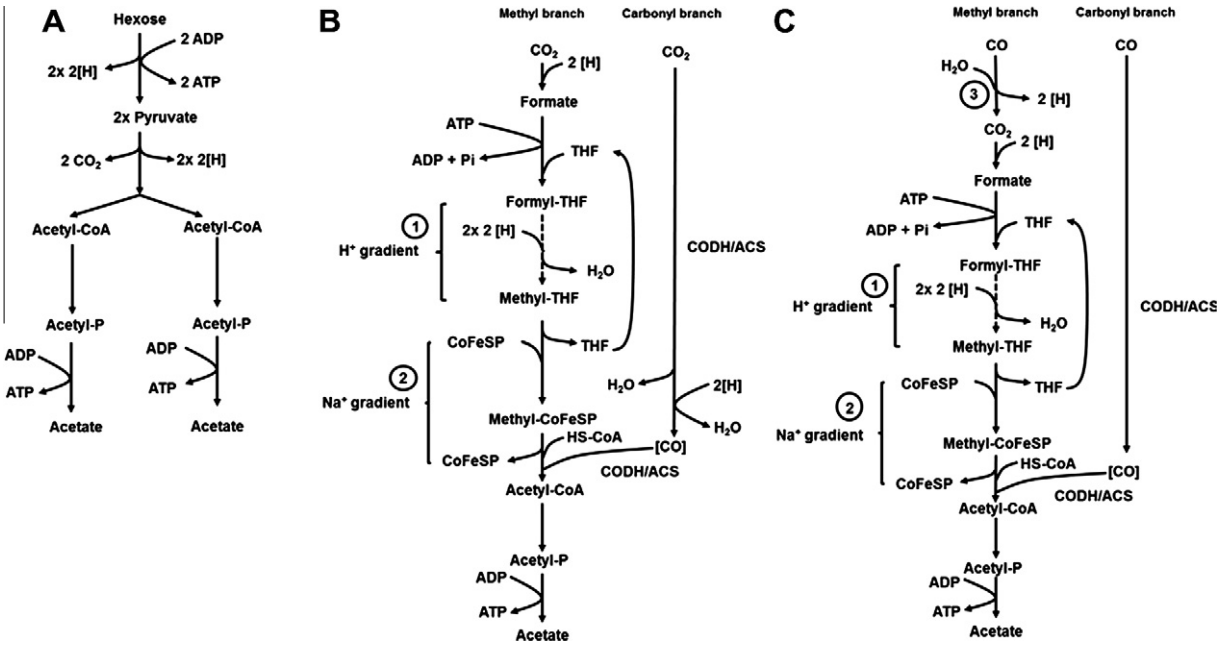

Fig. 1. Heterotrophic (A + B), autotrophic ($CO_2 + H_2$) (B), and autotrophic (CO) (C) fermentation by acetogens (Wood–Ljungdahl pathway). CODH/ACS: carbon monoxide dehydrogenase/acetyl-CoA synthase; CoFeSP: corrinoid iron–sulfur protein. (1) A proton gradient is formed during the reduction of formyl-THF to methyl-THF in organisms such as *M. thermoacetica* and *C. aceticum*. (2) A sodium gradient is formed during the transfer of the methyl group in organisms such as *A. woodii*. In this organism an Rnf complex is additionally active in generating a sodium gradient. (3) For correct stoichiometry 2 additional CO must be oxidized to CO_2 to provide the required reduction equivalents.

bound to CO, which is used directly or stems from the reduction of CO_2 in the carbonyl branch, and forms together with coenzyme A acetyl-CoA. The respective catalyzing enzyme complex is called carbon monoxide dehydrogenase/acetyl-CoA synthase (CODH/ACS). Two enzymes are responsible for the production of reducing equivalents which are required for metabolic processes: carbon monoxide dehydrogenase (CODH) and hydrogenase. CO is the substrate for the carbon monoxide dehydrogenase which generates CO_2 by the biological water-gas shift reaction ($CO + H_2O \rightarrow CO_2 + 2H^+ + 2e^-$), H_2 is the substrate for a hydrogenase ($H_2 \rightarrow 2H^+ + 2e^-$) [24]. Reducing equivalents are conserved in form of reduced ferredoxin.

Energy conservation in the Wood–Ljungdahl pathway is still not completely understood. Substrate level phosphorylation does not result in a net ATP generation, as only 1 ATP is formed in the acetate kinase reaction and 1 ATP is needed for formation of formyl-THF. Therefore, an energized membrane coupled to the pathway must be responsible for the generation of ATP. In *Moorella thermoacetica* and also *Moorella thermoautotrophica*, menaquinone and cytochromes have been detected that presumably generate a proton gradient, together with ferredoxin, flavodoxin, flavoproteins, and rubredoxin (for a model and recent reviews see [25,26]). Cytochromes have also been detected in *C. formicoaceticum* and *C. aceticum* [27]. Thus, this group of acetogens obviously uses an electron-transport chain to generate a H^+ gradient and ATP via a F_1F_0-type ATPase. A different mechanism was found in *A. woodii*. This organism is strictly dependent on sodium for growth [28]. Elegant work, mostly by the group of Volker Müller, revealed that *A. woodii* generates a Na^+ gradient, which is then used for ATP generation by a specific sodium-dependent ATPase. From analogy to methanogenic archaea it was proposed that the membrane-located methyltransferase complex, transferring the methyl group from methyl-tetrahydrofolate via the corrinoid iron–sulfur protein to CODH/ACS serves as a sodium ion pump (for a review see [26]). A third mode of energy conservation in acetogens was only discovered recently. *C. ljungdahlii* is neither Na^+-dependent nor does it contain genes required for quinone and cytochrome synthesis. Instead, it harbors an Rnf complex, which is most probably used to generate a proton gradient by simultaneous oxidation of reduced ferredoxin and transfer of reducing equivalents to NAD^+ [29]. The first acetogen found to contain an Rnf complex, however, a sodium-dependent one, was *A. woodii*, where Rnf serves as a sodium ion pump during caffeate reduction [30–34]. It might also allow an additional energy conservation during acetogenesis [34].

2. Acetogens as microbial production platforms for the production of chemicals and biofuels

In the last few years, the industrial interest in autotrophic production of bulk chemicals as well as biofuels has risen intensively. This is due to the fact that gases (CO, or $CO_2 + H_2$) as sole energy and carbon source have ecological and economical benefits. The use of gaseous substrates contributes (under certain conditions) to the reduction of the atmospheric greenhouse effect and gas fermentation is less expensive, as industrial waste gases and synthesis gas (also referred to as syngas) serve as substrates. Syngas is the product of gasification of coal, poorly degradable biomass (straw, wood), and municipal solid waste. It is also directly produced as waste gas from industrial plants, e.g. steel mills. Until now, industrial waste gases are preliminary used for heat production. The biotechnological production of chemicals and biofuels using anaerobic gaseous fermentation has important advantages compared to the corresponding chemical production. Firstly, biotechnological production is far more insensitive against variations or contaminations in the composition of the gaseous substrate and, secondly, leads to a higher product specificity [35]. Nevertheless, fermentations using gaseous substrates have not yet reached commercial scale.

The publication of genome sequences and the development of further molecular biological techniques, especially transformation protocols, pushed the use of acetogenic organisms as a production platform based on gases as sole carbon and energy source. Until

now, the genomes of the acetogenic bacteria *M. thermoacetica* [36], *C. ljungdahlii* [29], *C. carboxidivorans* [37–39], and *Eubacterium limosum* [40] have been published (Table 1). The genome sequence of *A. woodii* is completed and will be published soon; the genome sequence of *C. aceticum* is in preparation.

3. Natural products from acetogens

2,3-Butanediol, acetate, butyrate, ethanol, and butanol are natural products of acetogenic bacteria. The chemolitoautotrophic production of acetate, ethanol, and 2,3-butanediol using acetogens, especially bacteria of the genus *Clostridium* and *Acetobacterium,* is of special interest, as acetate and 2,3-butanediol are important bulk chemicals and ethanol is a promising biofuel. So far, none of the mentioned products is produced at commercial scale, but an increased academic and industrial interest towards this goal has been noticed during the last years.

3.1. Acetate production using Acetobacterium woodii

Acetate is a precursor for the synthesis of a variety of chemical products, e.g. polyvinyl acetates. The global production ranges around 10 million t/a. Generally, acetate is produced petrochemically, but it is also the main product of the acetogenic organism *A. woodii* [8]. A first attempt to increase the acetate production by *A. woodii* was already performed by Suzuki et al. [41]. Combining a bubble-column reactor, flocculated cells, and repeated batch cultures, a maximum volumetric productivity of 2.7 g acetate l^{-1} d^{-1} was achieved. Demler and Weuster-Botz [42] were able to further increase the autotrophic acetate production by changing process parameters of the anaerobic fermentation. In a batch-operated stirred-tank bioreactor, a maximum volumetric productivity of 7.4 g acetate l^{-1} d^{-1} was measured at a hydrogen partial pressure (pH_2) of 1700 mbar and a controlled pH of 7. Under these conditions, a final acetate concentration of 44 g l^{-1} was reached after a process time of 11 days. This final acetate concentration reached through autotrophic growth of *A. woodii* is the highest reported so far [42].

The pH control and the solubility of the substrate hydrogen have been identified as the most important, rate-limiting parameters of autotrophic acetate production using *A. woodii*. At a pH of 7, the produced acetate is hardly (<1%) present as acetic acid [42] and, therefore, the proven end product inhibition of acetic acid [43,44] is circumvented. Demler and Weuster-Botz [42] clearly demonstrated a linear relationship between the increasing cell specific productivity of acetate and an increasing hydrogen partial pressure in the gas phase. As hydrogen is only poorly soluble in water [45], the increasing hydrogen partial pressure enhances the hydrogen concentration in the medium.

The volumetric acetate productivity could be further improved by (i) a high pressure fermentation process resulting in an even higher hydrogen concentration in the medium, (ii) immobilization of *A. woodii*, and (iii) applying a continuous process instead of a batch process [42]. Furthermore, besides the process parameters, the strain could be improved by means of genetic and metabolic engineering. Availability of the genome sequence will allow identification of metabolic bottlenecks, which then can be overcome by metabolic modelling.

3.2. Ethanol production

Ethanol is an important biofuel, used mostly as an additive to gasoline. Three companies (IneosBio, Lisle, IL, USA; Coskata, Warrenville, IL, USA; LanzaTech, Auckland, New Zealand) develop autotrophic ethanol production using acetogenic bacteria with the aim of commercialization [18]. The companies have pilot plants and in part also demonstration plants of significant scale in operation. Thus, commercial production can be expected by 2013 or 2014.

3.3. 2,3-Butanediol production using C. autoethanogenum, C. ljungdahlii and C. ragsdalei

Besides ethanol, LanzaTech is also interested in the commercial production of 2,3-butanediol [21], a precursor for the synthesis of a variety of chemical products (solvents, methyl ethyl ketone, gamma-butyrolactone, and 1,3-butadiene) [46,47]. The potential global market of the main products produced from 2,3-butanediol is approximately 32 million t/a [21]. Generally, 2,3-butanediol is produced petrochemically, but is also a natural product of some bacteria, amongst others acetogenic bacteria of the genus *Clostridium*. *C. autoethanogenum*, *C. ljungdahlii*, and *C. ragsdalei* produce 2,3-butanediol using gases (CO or $CO_2 + H_2$) as sole energy and carbon source. Köpke et al. [21] demonstrated the production of 2,3-butanediol during the stationary growth of all three species in concentrations of 1.4–2 mM (126 mg l^{-1}). Naturally occurring autotrophic production of 2,3-butanediol using acetogenic bacteria has not been reported before.

Contrary to acetate, butyrate, ethanol, and butanol which are produced from acetyl-CoA as precursor, 2,3-butanediol is based on pyruvate as precursor. In all three organisms, pyruvate is produced via a pyruvate–ferredoxin oxidoreductase (PFOR) [21]. For 2,3-butanediol production three enzymes are required: pyruvate:acetolactate synthase, acetolactate decarboxylase, and 2,3-butanediol dehydrogenase. Respective genes have been detected in all three acetogens [21]. In contrast to other organisms (e.g. *Bacillus* species), where the genes form an operon [46,47], they are spread over the genome of *C. ljungdahlii* [21]. The same is true for the acetolactate synthase and acetolactate decarboxylase genes of *Clostridium acetobutylicum*, which is able to form acetoin, the precursor of 2,3-butanediol [46,47]. All genes probably responsible for the production of 2,3-butanediol in *C. autoethanogenum* are upregulated in the stationary growth phase, when 2,3-butanediol production takes place [21]. Thus, acetogens are also promising organisms for the production of 2,3-butanediol.

4. Artificial products from acetogens

So far, acetogens have been modified to produce acetone and butanol. The autotrophic production of acetone was achieved via a synthetic biology approach using *C. aceticum*, butanol production was established via pathway engineering using *C. ljungdahlii*. Acetone and butanol are of enormous interest for the industry, as acetone serves as precursor for the production of e.g. Plexiglas®, and butanol is both, an important bulk chemical and a promising biofuel.

4.1. Acetone production using Clostridium aceticum

Global acetone production amounts to 5.667 million tons [48]. Up to 90% of the currently used acetone is produced via the chemistry-based Hock synthesis pathway (cumene process) using propene and benzene, which are derived from crude oil [49]. Due to rising naphtha prices and an increasing demand for acetone, the acetone price has risen continuously over the last years. Fermentative production of acetone using gases as sole carbon and energy source is a promising alternative regarding both, ecological and economical aspects.

In a synthetic biology approach, *C. aceticum* was transformed with different plasmids carrying different operons for the synthesis

of acetone [50]. All plasmids were based on the *Escherichia coli–Clostridium* sp. shuttle vector pIMP1 [51]. One construct (pIMP_adc_ctfAB_thlA) contained the genes *adc*, *ctfA*, *ctfB*, and *thlA* (encoding acetoacetate decarboxylase, acetoacetyl CoA:acetate/butyrate:CoA transferase subunit A and B, and thiolase under the control of the P_{thlA} promoter (promoter of the thiolase gene)), thus representing the known acetone synthesis pathway of *C. acetobutylicum*. In a similar plasmid (pIMP_adc_atoDA_thlA), the genes *ctfA* and *ctfB* were exchanged for the genes *atoDA* encoding a acetyl-CoA/acetoacetyl-CoA transferase from *Escherichia coli*. Both constructs should mediate an acetate-dependent acetone biosynthesis, as acetate is required as a cosubstrate for the CoA transferase. A true synthetic biology approach was based on the use of thioesterases, which split off the coenzyme A-moiety from acetoacetyl-CoA, thus forming a metabolic pathway not found in nature. The gene *te*II encodes a thioesterase from *Bacillus subtilis* and the gene *ybgC* an acyl-CoA thioesterase from *Haemophilus influrenzae*. Both enzymes are able to use acetoacetyl-CoA as substrate [52]. In *C. aceticum,* acetyl-CoA thus serves as a precursor of acetate as well as acetone.

Each plasmid carrying an acetone synthesis operon was transformed separately into *C. aceticum* via electroporation, and growth as well as acetone production of all four recombinant *C. aceticum* strains were monitored. On fructose, the recombinant *C. aceticum* strains harbouring the plasmids produced acetone up to 9 mg/l [50]. Acetone was produced throughout the entire exponential growth and at the transition to stationary growth. On gas (80% H_2/20% CO_2 or 67% H_2/33% CO_2) as sole carbon and energy source, the functionality of the plasmids was also clearly demonstrated, acetone was produced up to 8 mg/l using the plasmid pIMP_adc_ctfAB_thlA [50,53]. Thus, proof of principle for using recombinant *C. aceticum* strains growing on CO_2/H_2 gas mixtures and producing important bulk chemicals has been provided. As soon as the genome sequence of the organism will become available, the whole repertoire of meanwhile developed genetic techniques can be used for further improvement of production. Inactivation of genes responsible for formation of other products (e.g. ethanol) will lead to higher product formation and easier downstream processing. Integration of the construct into the genome will get rid of antibiotic resistance genes on the plasmid, not suitable for an industrial process. Stronger promoters will increase expression. Such tools have been developed in the past for the clostridia (e.g. [54–57]). It will also be possible to transfer the constructs into other acetogens in order to find out the best suited species for industrial production.

4.2. Butanol production using Clostridium ljungdahlii

Butanol is an important industrial bulk chemical and a promising biofuel and/or biofuel additive (for reviews see [58,59]). As a biofuel, butanol has properties which are superior compared to ethanol [58–60]. Butanol has a lower vapor pressure, a higher energy content, and can be blended at any time and at any concentration with gasoline. Furthermore, butanol can be used without modifications of car engines.

As the butanol production using solventogenic clostridia depends on starchy substrates or molasses as carbon sources which are expensive and compete with nutritional feedstock, gaseous substrates and acetogenic clostridia are a promising alternative.

In this pathway engineering approach, *C. ljungdahlii* was transformed with a plasmid, carrying the *C. acetobutylicum* butanol synthesis pathway genes *thlA*, *hbd*, *crt*, *bcd*, *adhE*, and *bdhA* encoding thiolase, 3-hydroxybutyryl-CoA dehydrogenase, crotonase, butyryl-CoA dehydrogenase, butanol/butyraldehyde dehydrogenase, and butanol dehydrogenase, respectively. All genes were under the control of the promoter P_{ptb} (promoter of the phosphotransbutyrylase gene, one promoter at the beginning of the artificial operon, a second promoter after the first three genes) and transcription was terminated by the terminator T_{adc} (terminator of the acetoacetate decarboxylase gene, after the last gene) [29]. Naturally, *C. ljungdahlii* produces acetate and ethanol when grown on gases (CO or $H_2 + CO_2$). The artificial butanol production was planned to start from the central metabolite acetyl-CoA.

C. ljungdahlii was transformed with the plasmid $pSOB_{ptb}$ and growth as well as butanol production of the recombinant *C. ljungdahlii* strain were measured. Although the vector lacked the genes *etfA* and *etfB*, encoding the electron-transferring flavoproteins EtfA and EtfA, which are essential for the activity of the Bcd enzyme [61], the plasmid was functional in *C. ljungdahlii*, resulting in the production of small amounts of butanol [29]. Inspection of the genome sequence of *C. ljungdahlii* revealed five pairs of genes encoding electron-transferring flavoproteins, which could compensate for the missing genes on the plasmid [29]. At the same time, the lack of the genes *etfA* and *etfB* on the plasmid $pSOB_{ptb}$ explained why a recombinant *E. coli* strain harbouring the plasmid did not produce any butanol. The recombinant *C. ljungdahlii* strain produced butanol in the middle of the exponential growth phase (up to 150 mg/l butanol). However, then butanol was consumed by the organism and the concentration dropped to almost zero (0–15 mg/l) until the end of growth. As a new product, butyrate was detected (53–71 mg/l) [29]. Two pairs of aldehyde/alcohol dehydrogenase genes have been found in the genome, the products of which are most probably responsible for butanol oxidation [29]. Again, this will allow strain improvement by identification and inactivation of these genes, placement of the construct into the chromosome, usage of stronger promoters and genes encoding more efficient enzymes, and streamlining the construct by removal of hairpin-loop structures, resulting from the original construction.

5. Conclusions

The examples of recombinant *C. aceticum* producing acetone and *C. ljungdahlii* producing butanol clearly show that alternative fermentation processes will become available soon, which no longer depend on substrates (sugar, starch), competing with nutritional feedstocks. In addition, gas fermentation will offer an ecological advantage as greenhouse gases such as CO_2 and CO will be converted into industrial products. As bulk chemicals, they will be found eventually in long-lasting products, thus in effect reducing CO_2 and CO emissions. And even if used as biofuels and burned again, uptake and conversion of gases during the fermentation will be almost equal to the amount released as CO_2 during driving. It will be important now to upscale the specific production rates in order to meet the economic requirements. Then, a bright future can be predicted for gas fermentation, as acetogenic production platforms will be able to synthesize the whole repertoire of microbially-formed metabolites.

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