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METABOLIC ENGINEERING OF SOLVENTOGENIC *Clostridia*

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Literary data on the organization of the genome, the connection of genes with the production and regulation of solvents, and the metabolic engineering of solventogenic clostridia are presented. The transition from the phase of the formation of acids to the phase of the formation of alcohols and the relationship of the latter with sporogenesis are analyzed. The main key genes (*ak, pta, buk, ptb, thl, crt, bcd, BdhAB, ctfAB, adc, rub, Spo0A, adhE, hdb*) that affect their course have been identified. The possibility of improving strains by genetic manipulations (inactivation of genes, entering of genes of other microorganisms, etc.) is shown. The effect of gene inactivation on solvent production is shown. An analysis of methods for increasing the accumulation of butanol showed the need to create effective recombinant producer strains for butanol supra synthesis using renewable raw materials.

Key words: butanol, producer strains, clostridia, solventogenesis.

Today, the microbiological conversion of biosphere renewable resources for the purpose of receiving of commercial products, in particular, the biofuel, is one of the recent problems of biotechnology [1]. Anaerobic bacteria of the *Clostridiaceae* family are known as producers of one of the most promising biofuels, the biobutanol. Currently, the microbiological synthesis of butanol during the classical acetone-butanol-ethanol (ABE) fermentation is economically unprofitable. The creation of commercial biobutanol technology requires high-yielding strains and an affordable, cheap and preferably renewable raw material — the plant biomass [2].

Industrial production of butanol based on microbiological synthesis was established in the early twentieth century and is related to the production of acetone (as a concomitant process) by *Clostridium acetobutylicum* with the production of acetone, butanol and ethanol on a 3:6:1 ratio and the using of corn flour as a substrate [3, 4]. In the classical ABE fermentation, the oil, propionic, lactic and acetic acids (acid formation stage) were produced by *C. acetobutylicum* at the initial stages, and eventually, the hydrogen index decreased and the production stage of butanol, acetone, and ethanol began (alcohol formation stage). The increasing demand for butanol and the sharp increase in petrochemical production have led to the fact that the biotechnological process for the butanol production became economically unprofitable and has been replaced by a more efficient chemical synthesis. In recent years, there has been renewed interest in butanol biotechnology not only as a raw material in the plastic, paint and varnish production and in printing and pharmaceutical applications, but also as an alternative fuel [5]. Today, the biofuels account for only 2% of all fuels used. Biofuel consumption volume in the fuel market is projected to reach 30% in the next 5–7 years [6].

The recent studies were related to the search for new productive strains that produce butanol and cheap non-food raw materials as a substrate [7, 8] and the optimization of butanol biotechnology. The following stages of butanol biotechnology optimization can be identified: 1 — primary selection of producer strains; 2 — determination of preferable

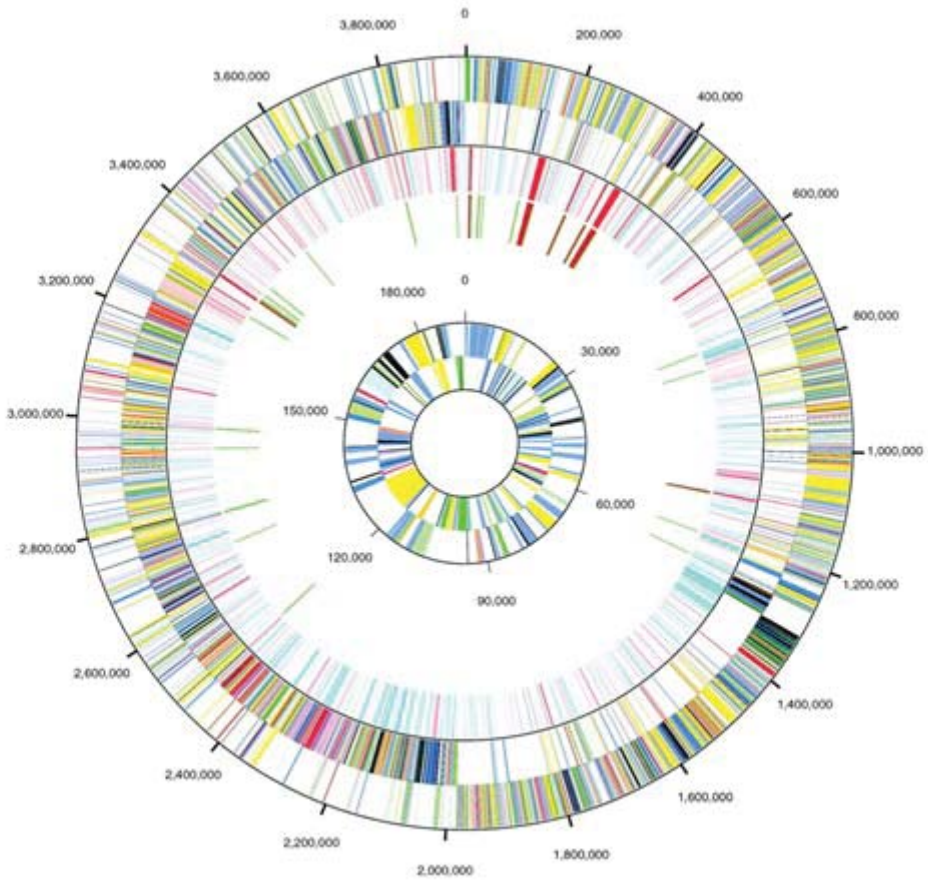

Fig 1. ***Clostridium acetobutylicum*** **ATCC 824 genome and megaplasmid [9]**

technological parameters (pH, temperatures, nutrient requirements) and preferable mode of nutrition and biomass accumulation, yield of the product in terms of the consumed substrate, rate of product formation; 3 — immobilization of producing cells; 4 — changes in genetic structure of a microorganism for increase in the accumulation of butanol.

Genetic manipulations with the *Clostridia* were made possible thanks to the determination of the nucleotide sequence/decoding of the complete genome of the *Clostridium acetobutylicum* ATCC 824 strain (Fig. 1).

Circular representation of the *C. acetobutylicum* genome and megaplasmid. The outer two rings indicate the positions of genes on the forward and reverse strands of the genome, respectively, color-coded by function. Moving inward, the third ring indicates the G1C content of each putative gene: turquoise (27%), gray (27 to 35%), pink-red (35%); the fourth ring indicates the positions of tRNA (green) and rRNA genes (dark red). The inner rings show the positions of genes on the forward and reverse strands of pSOL1, respectively, color-coded by function (the distance scale for the inner rings differs from the scale of the outer rings, as indicated). The functional color-coding is as follows: energy production and conversion, dark olive; cell division and chromosome partitioning, light blue; amino acid transport and metabolism, yellow; nucleic acid transport and metabolism, orange; carbohydrate transport and metabolism, gold; coenzyme metabolism, tan; lipid metabolism, salmon; translation, ribosome structure, and biogenesis, pink; transcription, olive drab; DNA replication, recombination, and repair, forest green; cell envelope biogenesis, outer membrane, red; cell motility and secretion, plum; posttranslational modification, protein turnover, and chaperones, purple; inorganic ion transport and metabolism, dark sea green; general function prediction only, dark blue; conserved protein, function unknown, medium blue; signal transduction mechanisms, light purple; predicted membrane protein, light green; hypothetical protein, black [9].

The genome size of *C. acetobutylicum* ATCC 824 is 4.13 Mbp, of which the chromosome consists of 3940880 bp and the megaplasmid pSOL1 is 192000 bp. The ratio

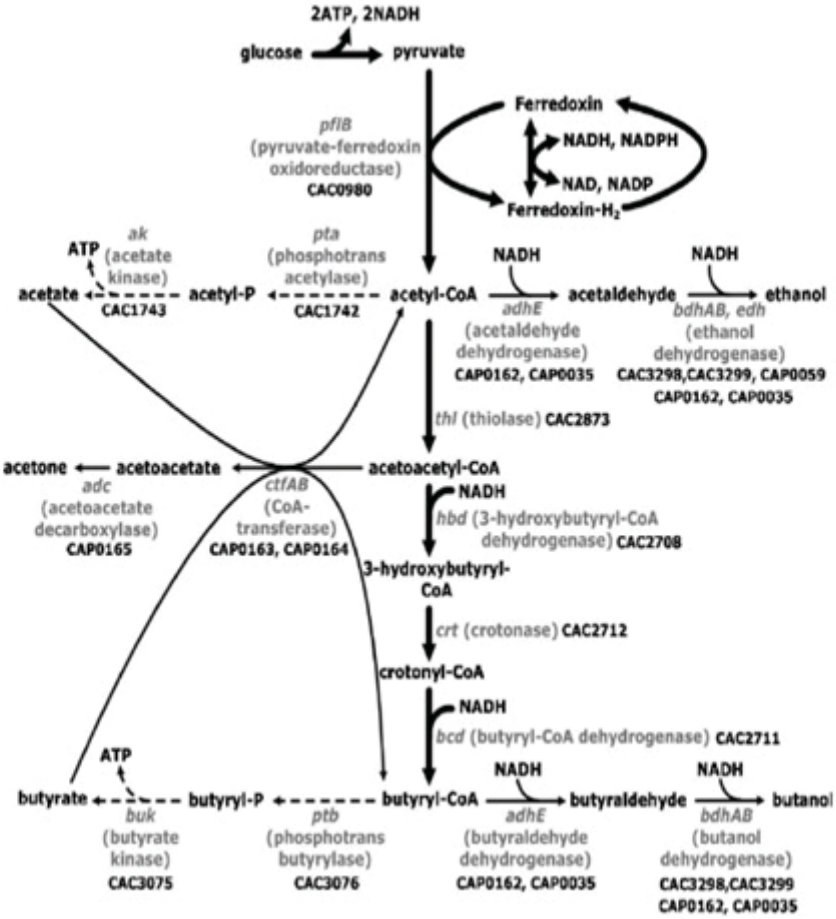

Fig. 2. **The metabolic pathway of *Clostridium acetobutylicum* [10]**

of G-C pairs is 30.8%. The genome consists of 3778 genes. The genetic information of the chromosome contains the major genes responsible for alcohol formation and encodes 178 polypeptides. It is these genes and the genes that encode the multifermental complex that is located on the cell surface (cellulosome) that is a unique metabolic profile of the *Clostridia* [9]. They demonstrate the role of horizontal gene transfer in the evolutionary development of these bacteria and determine the pathway for the synthesis of metabolites (Fig. 2).

The ABE process is discussed in detail in [11]. The genes responsible for the ABE process in *C. acetobutylicum* ATCC 824 may or may not be the part of certain groups (clusters). Two enzymes are involved in the formation of acetate-acetate kinase (*ak*) and phosphotransacetylase (*pta*), the genes of which are the part of acetate operon [12]. Butyrate kinase (*butK*) and phosphotransbutyrylase (*ptb*), which are the part of butyrate operon, are associated with the formation of butyrate. Both kits (sets) of these genes coexist in tandem on the chromosome and form operons in which *ptb* is located on the 5′-direction from *butK* and *pta* — from *ak*. The conversion of acetyl coenzyme A (*CoA*) involves the enzyme thiolase (*thl*), a gene of which is not the part of the functional units of organizations of genetic material in which there are several open reading frames (operons). The major genes responsible for converting 3-hydroxybutyryl-CoA to butyryl-CoA are the butyryl-CoA synthesis operon (BCS), namely crotonase (*crt*), butyryl-CoA dehydrogenase (*bcd*), the genes of two electron (*etfA, B*) transport flavoprotein subunits, β-hydroxybutyryl-CoA dehydrogenase (β*hbd*) [13].

Genes responsible for the formation of butanol dehydrogenase (*bdhAB*) are not clusters. Genes of acetaldehyde dehydrogenase (*aad*), acetyl-CoA acetate/butyrate CoA transferase (*ctfAB*), acetone

acetate decarboxylase (*adc*) make up the solventogenesis operon (*sol*) contained in the megaplasmid pSOL1 [14–16]. Genes of basic metabolism enzymes such as hydrogenase (*hydA*), flavodoxin (*flav*), rubredoxin (*rub*), the transcription factor responsible for the activation of sporulation (*Spo0A*) do not belong to clusters at all [17]. It should be noted that depending on the species and strain of bacteria of the genus *Clostridium*, the number of genes and their location in the clusters might vary [18–23]. Thus, the selective use of the positive effect of *Spo0A* on solvent formation is necessary to improve the productivity of the solvent. This requires *Spo0A* functioning as a gene for the activation of solventogenesis without the function of sporulation activation.

Sporulation is a major factor in solvent synthesis

The solvent synthesis process is closely related to the sporulation process (Fig. 3).

Some clostridia strains can convert acetone to isopropanol, producing a mixture of isopropanol, butanol and ethanol (IBE process), each of which can be used as a biofuel. The natural strain producer of isopropanol *C. beijerinckii* BGS1 accumulated 10.21 g/l of butanol and 3.41 g/l of isopropanol [25]. During normal vegetative growth, *C. acetobutylicum* cells asymmetrically divide by double division and exhibit typical bacillar morphology during glucose assimilation and acid accumulation [26]. During acid accumulation, the pH drops sharply and the medium becomes toxic to the cells. In response to changes in cell pH, the mechanisms of survival — sporulation and solventogenesis — are initiated [27].

Solventogenesis provides the cells with protection from low pH through the secondary consumption of acids and their conversion into solvents, which reduces the toxicity of the environment. Sporulation leads to high culture stability and provides a long-term survival mechanism until favourable conditions occur [28]. In the process of sporulation, various morphological changes of the cells occur [29]. The first morphological change that occurs in the cells is called the clostridial form. The cell acquires a cigar-shaped or swollen form with granulosa (vesicles of amylopectin) inside [30].

Previously, it was believed that cells with clostridial form produce solvents, but solventogenesis is started before they acquire it. Solventogenesis depends on three aggregate factors: the environment, the metabolic responses of a cell, and the activation of regulatory genes [31]. More than 256 genes that are differentially expressed during the transient phase are involved in solventogenesis [32].

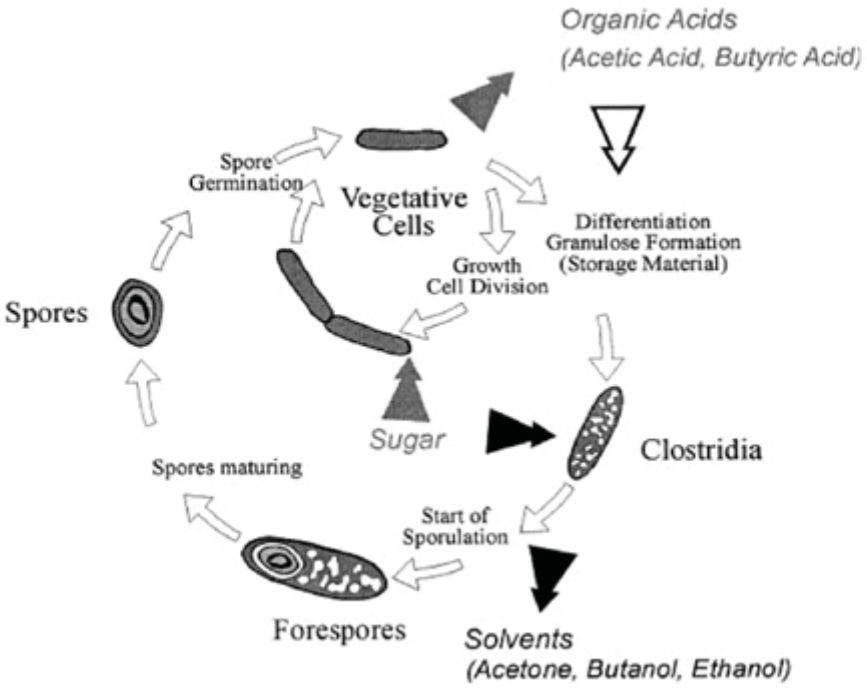

Fig 3. **Life cycle and sporulation of *Clostridia* [24]**

The transcription factor responsible for the activation of sporulation (*Spo0A*) also initiates the solvent synthesis in *C. acetobutylicum* by activating the transcription genes of acetoacetate decarboxylase (*adc*), alcohol dehydrogenase (*adhE*) and coA transferase (*ctfAB*). *Spo0A* is activated by phosphorylation, which is provided by the two-component signal transduction system. Phosphorylation is carried out by histidine kinase, which is encoded by the *ca_c0323, ca_c0903, ca_c2730, ca_c0437 and ca_c3319* genes in *C. acetobutylicum,* in *C. botulinum,* CBO1120 (CBO0336, CBO0340 and CBO2762) is responsible for it, and in *C. thermocellum* — *clo1313_0286, clo1313_2735* and *clo1313_1942*. Sigma factors that affect the transcription of sporulation genes in *C. acetobutylicum* are encoded by the *spoIIGA, sigE,* and *sigG* genes (Fig. 4).

The transcription factors encoded by sinR and abrB also regulate the onset of sporulation. The authors of [35] have suggested that abrB310 may also be a regulator during the transition between acidification and solvent phases.

For instance, in the case of the *C. beijerinckii* NCIMB 8052 mutant strain, the production of the solvents was controlled by the transcription factor *Spo0A*, which was the main regulator of stationary phase gene expression. The enzymes involved in solventogenesis have not been encoded in the plasmid, and therefore, are less sensitive to removal or loss [35].

Bacteria of the genus *Clostridium*, including *C. acetobutylicum*, are prone to degeneration (loss of increased solvent production) during cultivation on rich and easily digestible (carbon source) media.

The loss of a 210-tpn plasmid fragment containing the genes of the solvent synthesis enzymes leads to the emergence of *C. acetobutylicum* mutants that could not produce the solvent enzymes — degenerate, by definition, and could occur at the loss of 210 kbp of the plasmid where a few several genes are situated encoding several enzymes, solvent production [36, 37].

For *C. beijerinckii* NCIMB 8052 mutants, in addition to the abnormalities and degeneration caused by genetic alterations, the rapid cell growth in a rich medium with a high sugar concentration caused the inability to form solvents and spores. During the rapid growth of volatile fatty acids (acetic and oily) products that have been forming and accumulating in the medium quite intensively, simultaneously, the pH of the medium has also decreased. The rate of acid formation was so high that cells could not effectively induce solvent production [37–39].

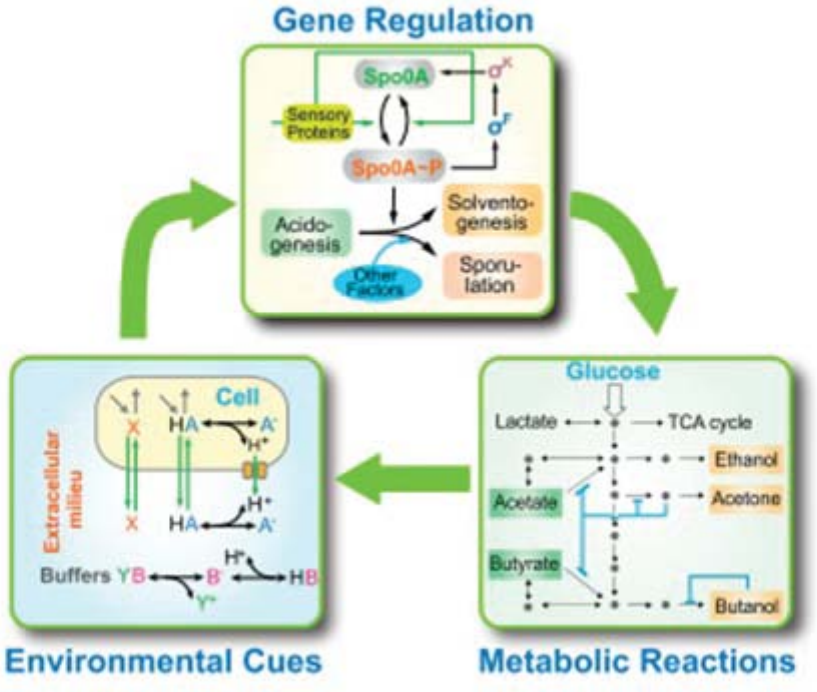

Fig. 4. **A system-level view of the acetone-butanol-ethanol (ABE) fermentation of *C. acetobutylicum* [34]**

Vectors for metabolic engineering

One of the major problems of genetic engineering of any microorganisms is the selection and development of vectors for the transfer of genetic information. The most promising vectors are plasmid-based — the extrachromosomal autonomous replication double-stranded ring DNA molecules. There are several types of plasmids: autonomous (not related to the bacterial chromosome) and integrated (built into the chromosome) plasmids.

F-plasmids carry information that ensures their own conjugative movement from one cell to another. R-plasmids carry antibiotic resistance genes. There are plasmids that have a specific set of genes that are responsible for the disposal of non-specific metabolites (degradation plasmids). There are plasmids that contain genes with unknown functions. If two or more plasmids cannot exist in the same cell, they are assigned to the same incompatibility group. Some plasmids carry a specific point of origin of replication (origin) and can replicate only in cells of a particular species, while others make this replication initiation site less specific and replicate in a variety of bacterial cells. Thus, the plasmids can be distinguished by the spectrum of hosts — wide or narrow.

For effective use in genetic engineering, the plasmids must have three characteristics.

1. Plasmids must be small in size because the transfer of exogenous DNA to *Escherichia coli* significantly reduces if plasmid lengths are greater than 15 kbp [40].

2. Each plasmid must have restriction sites at which insertion can occur [40].

3. A plasmid must have one or more selective markers to identify recipient cells carrying recombinant DNA [40].

In view of the above facts, pFNK1 vectors have been developed to increase the transformation efficiency of *C. acetobutylicum* ATCC 824 strain [41]. One of the first used was the integrative plasmid technology for metabolic pathway inhibition aimed for obtaining of acetate and butyrate through *C. acetobutylicum* ATCC 824. The copy number of plasmids commonly used for *C. acetobutylicum* was approximately 7–20 copies per cell [42–43].

Integration of the plasmid into the chromosome homologous region deactivated the *ack* and *pta* genes and formed a mutant with the deleted genes. Non-replicative integration plasmids (pAN1, pJC4, pJC7, pPUC-PTAK) that could be inserted into the *C. acetobutylicum* ATCC 824 genome were selected. Such plasmids required a host DNA fragment and an identified genetic marker. After the transfer, the plasmids can be anchored by integration in homologous regions. It were the integration plasmids that were introduced into the of *C. acetobutilicum* ATCC 824 chromosome [5]. Inactivation of *buk* and *pta* on the chromosome occurred by using the non-replicative integration plasmids containing the *buk* and *pta* genes. By inactivating the genes involved in the formation of the acid, it was possible to redirect the carbon stream to produce the solvent and increase its concentration.

Inactivation of the *buk* and *pta* genes allowed reducing energy costs for the production of acetate and butyrate. This resulted in a more than 10% increase in butanol production and 50% decrease in acetone production. Strains with changes in these genes accumulated more biomass and remained stable for 30 generations. The results of genetic engineering studies have demonstrated the possibility of using a gene inactivation method to control the metabolic pathway of glucose butyrate formation [44].

The *C. beijerinckii* BA 101 mutant (superproducer) was obtained from the original *C. beijerinckii* strain NCIMB 8052 by using the *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (NTG) chemical mutagen. Subsequently, the selection of the treated culture for resistance to 2-deoxyglucose was performed. This mutant produced twice as much solvent and utilized carbohydrates better. For the study of genetic mutations, the pJT 297 and pBUT 23 plasmids were used as compared to the original strain. These plasmids were used to study the activity of the fermentation genes of the *C. beijerinckii* NCIMB 8052 and *C. beijerinckii* BA 101 strains at different cultivation periods. It is the activity of the *ptb* and *ctfAB* genes, which was determined by the mRNA accumulation level, which is the distinguishing difference between the strains. The activity of *ptb* and *ctfAB* genes in the *C. beijerinckii* BA 101 strain was 2-fold higher than in the *C. beijerinckii* NCIMB 8052 strain, and this coincided with the increased level of solvent production. Based on these results, it was concluded that genetic mutations occurred in these genes and they were essential in butanol synthesis increase [45].

It was shown that the placement of a transposon between restriction sites *Sna*BI

and *Eco*RI resulted in decrease in the rate of growth of the culture, decrease in the tendency to degeneration and increase in viability. Mutants actively grew in environments with high sugar content, had long-term stability of solvent production and the ability to grow at 42 °C according to phenotype and, as a result, the target product accumulation increased 2-fold [46].

To improve the *C. cellulovorans* strain, a shuttle plasmid pYL001 with pMRL83151 was developed by moving the 1138 bp fragment containing the *Cce*7431/743 II restriction site and changes in ColEI and in the non-coding region. This plasmid enabled to incorporate genes that encode butanol synthesis (*adhE1, adhE2, bdhB, aorm*) [47].

Several metabolic pathways were also altered by integration plasmid technology [48].

As an alternative to plasmids, a λ phage vector was used. Typically, this vector is used to create genomic libraries that require work with DNA fragments larger than possible to clone in a plasmid (greater than 10 kbp). Such vector makes it possible to work with large genetic constructs and incorporate them into clostridial cells [49].

Genetically modified strains

Genetic manipulations with clostridia were aimed at the altering of selected strain genes to increase the accumulation of butanol, to increase the tolerance of the strain, to expand the range of raw material (substrate) for cultivation and industrial production of butanol not mixed for obtaining of other solvents and acids [50]. The first effective result of metabolic engineering was an increase in acetone production. Acetoacetate decarboxylase of the recombinant *C. acetobutylicum* strain with amplified *adc* (encodes acetoacetate decarboxylase) and *ctfAB* (encodes COA transferase) genes that was involved in the synthesis of acetone, became much more active in the process of cultivation then usually, which caused the early induction of acetone formation. As a result, the concentrations of acetone, butanol and ethanol increased by 95%, 37% and 90% respectively, compared to the parent strain [51].

Most of the works were focused on the inactivation of genes responsible for the accumulation of acetate, butyrate and acetone and enhancing the expression of solventogenesis genes. High production of butanol (16.7 g/l) was obtained by inactivation of the butyratkinase (*buk*) gene. Metabolic pathway analyses showed a 300% increase in the butanol formation flow in the recombinant strain. Cumulative silencing of phosphatacetyltransferase (*pta*) butyratkinase (*buk*) genes and *adhE1* overexpression (encodes mutant aldehyde/alcohol dehydrogenase) resulted in a 60% increase in butanol production (from 11.8 to 18.9 g/l) with a 145% increase in bioconversion butanol (0.71 vs. 0.29 mol/mol glucose) [52, 53].

Inhibition of acetyl-CoA acetate/butyrate coenzyme transferase (CoAT) ratio increases the ratio of butanol to acetone, but decreases the accumulation and titer of butanol [54, 55]. Various strategies were used to increase the accumulation of butanol by *C. acetobutylicum* strains by overexpression of 6-phosphofructokinase (*pfkA*) and pyruvate kinase (*pykA*) glycolytic pathways [56]. The engineered strain showed an increase in the intracellular level of nicotinamidudinucleotide (NADN) and adenosine triphosphate (ATP) and an increase in butanol tolerance in connection with increase in butanol concentration from 19.12 g/l to 28.02 g/l.

Genetic analyses has shown that minor alcohol dehydrogenase (*sadh*) was responsible for the conversion of acetone to isopropanol [57–59]. *Sadh* overexpression shows the change of ABE to IBE processes in solventogenic clostridial strains. Overexpression of *sadh* and *hydG* (encodes the electron transfer protein) led to an increase in the total amount of IBE production (27.9 g/l) [60–65].

The silencing of histidine kinase in the *C. acetobutylicum* ATSC 55025 asporogenic strain has increased its tolerance to butanol and its accumulation by 44% (18.2 g/l vs. 12.6 g/l) and its productivity by 90% (0.38 g/lh vs. 0.20 g/lh) [66, 67].

A butanol producing strain was also constructed based on *C. tyrobutyricum* with overexpression of *adhE2*, which was asporogenic and accumulated butanol (20 g/l) by cultivation with nutrition [68]. The *C. tyrobutyricum* strain with inactivated *ack* and *adhE2* overexpression accumulated 16 g/l butanol versus 10 g/l in the original strain by using mannitol as a substrate [69].

To increase the yield of butanol and ethanol from lignocellulosic raw material, a *C. cellulolyticum* producer strain was created with overexpression of solventogenesis genes and inhibited function of acidogenesis genes. Alcohol dehydrogenase (*adh*) and pyruvate decarboxylase (*pdc*) genes from *Zymomonas mobilis* were incorporated into *C. cellulolyticum* H10 strain to produce

cellulose ethanol. Malatdehydrogenase (*mdh*) and lactate dehydrogenase (*ldh*) genes were added to increase ethanol accumulation by the *C. cellulolyticum* H10 strain [70–72].

In [73], a strain of *C. cellulolyticum* was improved by integrating thiolase (*atoB*) with *E. coli* and a CoA-dependent butanol synthesis pathway (*adhE2, hbd, crt, bcd*) with *C. acetobutylicum*. However, the resulting strain produced only 0.12 g/l of butanol from cellulose after 20 days of cultivation.

In order to obtain butanol by using cellulose, the *C. cellulovorans* strain was enhanced by the integration of alcohol dehydrogenase (*adhE2*) with *C. acetobutylicum*. Thus obtained *C. cellulovorans* producing strain has been producing butanol (3.47 g/l) from cellulosic raw materials [47, 74–76].

In order to obtain butanol from the thermotolerant strain of *C. thermocellum*, a 2-keto acid strain was created for the biosynthesis of isobutanol, which produced 5.4 g/l of solvent during periodic fermentation with use of cellulose [77].

Synthetic gases may be the alternative substrates for butanol. To obtain butanol from gas, the pathway of butanol biosynthesis was transferred to *C. ljungdahlii* from *C. acetobutylicum*. Butanol accumulation of 0.15 g/l was obtained by using gas as a carbon source, which eventually decreased to 0.015 g/l [78].

By silencing the phosphatacetyltransferase and acetoaldehyde dehydrogenase and integrating the part of the genes from the butanol synthesis pathway (*thl, hbd, crt, bcd,* butyraldehyde dehydrogenase and NAD-dependent butanol dehydrogenase), the *Clostridium* sp. MTButOH1365 mutant strain was obtained from *Clostridium* sp. MT1962 into the chromosome. The resulting strain accumulated 22 g/l of butanol from the CO/H_2 gas mixture during periodic cultivation [79].

The complexity of regulation of clostridium genes has prompted the researchers to explore other organisms (for example, *E. coli*) as hosts for butanol producing genes [80]. Metabolic engineering of *E. coli* produced a strain that accumulated butanol at a concentration of 18.3 g/l. The formation of by-products such as acetate and butyrate can be avoided in the process of improving the activity of *adhE2* [81].

Despite some advances in producing strain genetic engineering researches, the use of genetically modified butanol producing strains in butanol accumulation increase remains imperfect and complex.

Recently, the attention of researchers has returned to butanol, as butanol as a fuel has significant advantages over traditional ethanol-based biofuels. Butanol is already starting to be used as a liquid biofuel for current vehicles without engine modification. However, there are some limitations in the process of microbiological synthesis and commercial butanol technology — low productivity of producing strains and inhibition of synthesis products in the cultivation process. This review provides examples of genetic modifications of butanol producing strains by overexpression or inactivation of the respective genes to enhance butanol accumulation. The gene of solventogenic clostridia, genes involved in the transition between acidification and solvent production, and the influence of sporulation on the accumulation of alcohols are described. The review literature can serve as a basis for further research and the creation of genetically modified butanol-accumulated strains.

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МЕТАБОЛІЧНА ІНЖЕНЕРІЯ СОЛВЕНТОГЕННИХ *Clostridia*

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Наведено дані літератури щодо організації геному, участі генів у продукуванні та регуляції синтезу розчинників і метаболічної інженерії спиртоутворювальних клостридій. Проаналізовано перехід від фази утворення кислот до фази утворення спиртів та зв'язок останньої зі спорогенезом. Визначено основні ключові гени (*ak, pta, buk, ptb, thl, crt, bcd, BdhAB, ctfAB, adc, rub, Spo0A, adhE, hdb*), які впливають на їх перебіг. Показано можливість удосконалення штамів шляхом генетичних перетворень (інактивації генів, уведення генів інших мікроорганізмів тощо). Установлено вплив інактивування генів на продукування розчинників. Аналіз методів підвищення накопичення бутанолу засвідчив необхідність створення ефективних рекомбінантних штамів-продуцентів для надсинтезу бутанолу в разі використання відновлювальної сировини.

Ключові слова: бутанол, штами-продуценти, клостридії, солвентогенез.

МЕТАБОЛИЧЕСКАЯ ИНЖЕНЕРИЯ СОЛВЕНТОГЕННЫХ *Clostridia*

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Приведены данные литературы относительно организации генома, участия генов в продуцировании и регуляции синтеза растворителей и метаболической инженерии спиртообразующих клостридий. Проанализирован переход от фазы образования кислот до фазы образования спиртов и связь последнего со спорогенезом. Определены основные ключевые гены (*ak, pta, buk, ptb, thl, crt, bcd, BdhAB, ctfAB, adc, rub, Spo0A, adhE, hdb*), которые влияют на их ход. Показана возможность усовершенствования штаммов путем генетических преобразований (инактивации генов, введения генов других микроорганизмов и др.). Установлено влияние инактивации генов на продуцирование растворителей. Анализ методов повышения накопления бутанола показал необходимость создания эффективных рекомбинантных штаммов-продуцентов для надсинтеза бутанола при использовании возобновляемого сырья.

Ключевые слова: бутанол, штаммы-продуценты, клостридии, солвентогенез.