METABOLIC ENGINEERING OF SOLVENTOGENIC CLOSTRIDIA O. Tigunova, N. Beyko, G. Andrijash, S. Shulga

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METABOLIC ENGINEERING OF SOLVENTOGENIC Clostridia
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.
1. Abd-Alla M. H., Zohri A.-N. A., El-Enany A.-W. E., Ali S. M. Acetone-butanol-ethanol production from substandard and surplus dates by Egyptian native Clostridium strains. Anaerobe. 2015, V. 32, P. 77–86. https://doi.org/10.1016/j.anaerobe.2014.12.008

2. Menon N., Paszotor A., Menon B. R. K., Kallio P., Fisher K., Akhtar M. K., Leys D., Jones P. R., Scrutton N. S. A microbial platform for renewable propane synthesis based on a fermentative butanol pathway. Biotechnol. Biofuels. 2015, 8 (61), 1–12. https://doi.org/10.1186/s13068-015-0231-1

3. Linggang S., Phang L. Y., Wasoh H., Abd-Aziz S. Acetone-butanol-ethanol production by Clostridium acetobutylicum ATCC 824 using sago pith residues hydrolysate. BioEnergy Res. 2013, 6 (1), 321–328. https://doi.org/10.1007/s12155-012-9260-9

4. Sreekumar S., Baer Z. C., Pazhamalai A., Gunbas G., Grippo A., Blanch H. W., Clark D. S., Toste D. F. Production of an acetone-butanol-ethanol mixture from Clostridium acetobutylicum and its conversion to high-value biofuels. Nature Protocols. 2015, V. 10, P. 528–537. https://doi.org/10.1038/nprot.2015.029

5. DeJaco R. F., Bai P., Tsapatsis M., Siepmann J. I. Absorptive separation of 1-butanol from aqueous solutions using MFI- and FER- type zeolite frameworks: a Monte Carlo study. Langmuir. 2016, 32 (8), 2093–2101. https://doi.org/10.1021/acs.langmuir.5b04483
6. Bruant G., Levesque M.-J., Peter C., Guiot S. R., Masson L. Genomic analysis of carbon monoxide utilization and butanol production by *Clostridium carboxidivorans*.

Stain p7

*Plos* ONE

2010, 5 (9), 1–12. https://doi.org/10.1371/journal.pone.0013033

7. Abo B. O., Gao M., Wang Y., Wu C., Wang Q., Ma H. Production of butanol from biomass: recent advances and future prospects.

*Environm. Sci. Pollut. Res.*

2019, 26 (20), 20164–20182. https://doi.org/10.1007/s11356-019-05437-y

8. Qureshi N., Ezji T. C. Butanol, ‘a superior biofuel’ production from agricultural residues (renewable biomass): recent progress in technology.

*Biofuels, Bioprod. Bioref.*

2008, 2 (4), 319–330. https://doi.org/10.1002/bbb.85

9. Nolling J., Breton G., Omelchenko M. V., Makarova K. S., Zeng Q., Gibson R., Lee H. M., Dubois J., Qiu D., Hitti J., Wolf I. Y., Tatusov R. L., Sabathe F., Stamm L. D., Soucaille P., Daly M. J., Bennett G. N., Koonin E. V., Smith D. R. Genome Sequence and Comparative Analysis of the Solvent-Producing Bacterium *Clostridium acetobutylicum*.

*J. Bacteriol.*

2001, 183 (16), 4823–4838. https://doi.org/10.1128/JB.183.16.4823–4838.2001

10. Lee S. Y., Park J. H., Jang S. H., Nielsen L. K., Kim J., Jung K. S. Fermentative butanol production by *Clostridia*.

[Review].

*Biotechnol. Bioeng.*

2008, 101 (2), 209–228. https://doi.org/10.1002/bit.22003
11. Tigunova O., Shulga S., Blume Y. Biobutanol as an Alternative Type of Fuel. *Cytolo. Genet.* 2013, 47 (6), 51–71. https://doi.org/10.3103/S0095452713060042

12. Boynton Z. L., Bennett G. N., Rudolph B. F. Cloning, sequencing and expression of genes encoding phosphotransacetylase and acetate kinase from *Clostridium acetobutylicum* ATCC 824. *Appl. Envir. Microbiol.* 1996, 62 (8), 2758–2766. https://doi.org/10.1128/AEM.62.8.2758-2766.1996

13. Boynton Z. L., Bennett N. G., Rudolph B. F. Cloning, sequencing and expression of cluster genes encoding β-hydroxybutyryl-Coenzyme A (CoA) dehydrogenase, crotonase, and butyryl-CoA dehydrogenase from *Clostridium acetobutylicum* ATCC 824. *J. Bacteriol.* 1996, 178 (11), 3015–3014. https://doi.org/10.1128/JB.178.11.3015-3024.1996

14. Nair V. R., Papoutsakis E. T. Expression of plasmid-encoded *aad* in *Clostridium acetobutylicum* M5 restores vigorous butanol production. *J. Bacteriol.* 1994, 176 (18), 5843–5846. https://doi.org/10.1128/JB.176.18.5843-5846.1994

15. Nair V. R., Green E. M., Watson D. E., Bennett N. G., Papoutsakis E. T. Regulation of the *sol* locus genes for butanol and acetone formation in *Clostridium acetobutylicum* ATCC 824 by a putative transcriptional repressor. *J. Bacteriol.* 1999, 181 (1), 319–330. https://doi.org/10.1128/JB.181.1.319-330.1999
16. Nair V. R., Bennett N. G., Papoutsakis E. T. Molecular characterization of an aldehyde/alcohol dehydrogenase gene from *Clostridium acetobutylicum* ATCC 824. *J. Bacteriol.* 1994, 176 (3), 871–885. https://doi.org/10.1128/JB.176.3.871-885.1994

17. Cornillot E., Croux C., Soucaille P. Physical and genetic map of the *Clostridium acetobutylicum* ATCC 824 chromosome. *J. Bacteriol.* 1997, 179 (23), 7426–7434. https://doi.org/10.1128/JB.179.23.7426-7434.1997

18. Parades C. J., Rigoutsos I., Papoutsakis E. T. Transcriptional organization of the *Clostridiu m acetobutylicum* genome. *Nucleic Acids Res.* 2004, 32 (6), 1973–1981. https://doi.org/10.1093/nar/gkh509

19. Fischer R.-J., Oehmcke S., Meyer U., Mix M., Schwarz K., Fiedler T., Bahl H. Transcription of the *pst* operon of *Clostridium acetobutylicum* is depend on phosphate concentration and pH. *J. Bacteriol.* 2006, 188 (15), 5469–5478. https://doi.org/10.1128/JB.00491-06

20. Borden J. R., Papoutsakis E. T. Dynamics of genomic-library enrichment and indentification of solvent tolerance genes for *Clostridium acetobutylicum*. *Appl. Envir. Microbiol.* 2007, 73 (9), 3061–3068. https://doi.org/10.1128/AEM.002296-06
21. Tomas C. A., Welker N. E., Papoutsakis E. T. Overexpression of groESL in Clostridium acetobutylicum results in increased solvent production and tolerance, prolonged metabolism and changes in the cell’s transcriptional program. Appl. Envir. Microbiol. 2003, 69 (8), 4951–4965. https://doi.org/10.1128/AEM.69.8.4951-4965.2003

22. Tummala B. S., Welker N. E., Papoutsakis E. T. Development and characterization of a gene expression reporter system for Clostridium acetobutylicum ATCC 824. Appl. Envir. Microbiol. 1999, 65 (9), 3793–3799. https://doi.org/10.1128/AEM.65.9.3793-3799.1999

23. Paredes C. J., Senger R. S., Spath I. S., Borden J. R., Sillers R., Papoutsakis E. T. A general framework for designing and validation oligomer-based DNA microarrays and its application to Clostridium acetobutylicum. Appl. Envir. Microbiol. 2007, 73 (14), 4631–4638. https://doi.org/10.1128/AEM.00144-07

24. Berezina O. V., Zakharova N. V., Yarotsky C. V., Zverlov V. V. Microbial producer of butanol. Appl. Biochem. Microbiol. 2012, 48 (7), 625–638. https://doi.org/10.1134/S0003683812070022

25. Zhang C., Li T., Ye J. Characterization and genome analysis of a butanol–isopropanol-producing Clostridium beijerinckii strain BGS1. Biotechnol. Biofuels. 2018, 11 (280), 1–11. https://doi.org/10.1186/s13068-018-1274-x

26. Dürr P. Physiology and Sporulation in Clostridium. In Driks A., Eichenberger P. (ed), The Bacterial Spore: from Molecules to Systems. ASM Press, Washington, DC
27. Patakova P., Branska B., Sedlar K., Vasylikvsa M., Jureckova K., Kolek J., Koscova P., Provaznik I.
Acidogenesis, solventogenesis, metabolic stress response and life cycle changes in Clostridium beijerinckii NRRL B-598 at the transcriptomic level.
Sci Rep.
2019, 9 (1371). https://doi.org/10.1038/s41598-018-37679-0

28. Poehlein A., Solano J. D. M., Flitsch S. K., Krabben P., Winzer K., Reid S. J., Jones D. T., Green E., Minton N. P., Daniel R. Dürrre.
Microbial solvent formation revisited by comparative genome analysis.
Biotechnol Biofuels.
2017, 10 (58). https://doi.org/10.1186/s13068-017-0742-z

29. Alsaker K. V., Papoutsakis E. T. Transcriptional program of early sporulation and stationary-phase events in Clostridium acetobutylicum.
J. Bacteriol.
2005, 187 (20), 7103–7118. https://doi.org/10.1128/JB.187.20.7103–7118.2005

30. Al-Hinai M. A., Jones S. W., Papoutsakis E. T. The Clostridium sporulation programs:
diversity and preservation of endospore differentiation.

Microbiol
Mol
Biol
Rev

2015, 75 (1), 19–37. https://doi.org/10.1128/MMBR.00025-14

31. Shi Z., Blaschek H. P. Transcriptional analysis of Clostridium beijerinckii NCIMB 8052 and the hyper-butanol-producing mutant BA 101 during the shift from acidogenesis to solventogenesis.

Appl. Envrir. Microbiol.

2008, 74 (24), 7709–7714. https://doi.org/10.1128/AEM.01948-08

32. Harris L. M., Welker N. E., Papoutsakis E. T. Northern, morphological, and fermentation analysis of spo0A inactivation and overexpression in Clostridium acetobutylicum ATCC 824.

J. Bacteriol.

2002, 184 (13), 3586–53597. https://doi.org/10.1128/JB.184.13.3586-3597.2002

33. Lee S. Y., Park J. H., Jang S. H., Nielsen L. K., Kim J., Jung K. S. Fermentativebutanol production by Clostridia [Review].

Biotechnol. Bioeng.

2008, V. 101, P. 209–228. https://doi.org/10.1002/bit.22003

34. Liao C., Seo S.-O., Celik V., Liu H., Kong W., Wang Y., Blaschek H., Jin Y.-S., Lu T. Integrated, systems metabolic picture of acetone-butanol-ethanol fermentation by Clostridium acetobutylicum.

PNAS

2015, 112 (27), 8505–8510. https://doi.org/10.1073/pnas.1423143112
35. Scotcher M. C., Bennett G. N. Activity of abrB310 promoter in wild type and spo0A-deficient strains of *Clostridium acetobutylicum*. *J. Ind. Microbiol. Biotechnol*. 2008, 35 (7), 743–750. https://doi.org/10.1007/s10295-008-0341

36. Ravagnani A., Jennert K. C., Steiner E., Grünberg R., Jefferies J. R., Wilkinson S. R., Young D. I., Tidswell E. C., Brown D. P., Youngman P., Morris J. G., Young M. Spo0A directly controls the switch from acid to solvent production in solvent-forming clostridia. *Mol. Microbiol*. 2000, 37 (5), 1172–1185.

37. Cornillot E., Nair R. V., Papoutsakis E. T., Soucaille P. The genes for butanol and acetone formation in *Clostridium acetobutylicum* ATCC 824 reside on a large plasmid whose loss leads to degeneration of the strain. *J. Bacteriol*. 1997, 179 (17), 5442–5447. https://doi.org/10.1128/JB.179.17.5442-5447.1997

38. Kosaka T., Nakayama S., Nakaya K., Yoshino S., Furukawa K. Characterization of the sol Operon in Butanol-Hyperproducing *Clostridium saccharoperbutylicum* Strain N1-4 and Its Degeneration Mechanism. *Biosci. Biotechnol. Biochem*. 2007, 71 (1), 58–68. https://doi.org/10.1271/bbb.60370

39. Chen C.-K., Blaschek H. P. Effect of Acetate on Molecular and Physiological Aspects of *Clostridium beijerinckii* NCIMB 8052 Solvent Production and Strain Degeneration. *Appl. Environ. Microbiol*. 1999, 65 (2), 499–505. https://doi.org/10.1128/AEM.65.2.499-505.1999
40. Kompanets T. A. Virusy yak vektorni systemy. Kyiv. 2007, 84 p. (In Ukrainian).

41. Merzelstein L. D., Welker N. E., Bennett G. N., Papoutsakis E. T. Expression of cloned homologous fermentative genes in Clostridium acetobutylicum ATCC 824. Biotechnol. (N Y). 1992, 10 (2), 190–195. https://doi.org/10.1038/nbt0292-190

42. Lee S. Y., Merzelstein L. D., Papoutsakis E. T. Determination of plasmid copy number and stability in Clostridium acetobutylicum TCC 824. FEMS Microbiol. Lett. 1993, 108 (3), 319–323. https://doi.org/10.1016/0378-1097(93)90562-G

43. Friehs K. Plasmid copy number and plasmid stability. Adv. Biochem. Eng. Biotechnol. 2004, V. 86, P. 47–82. https://doi.org/10.1007/b12440

44. Green E. M., Boynton Z. L., Harris L. M., Rudolph F. B., Papoutsakis E. T., Bennett G. N. Genetic manipulation of acid formation pathways by gene inactivation Clostridium acetobutylicum ATCC 824. Microbiol. 1996, V. 142, P. 2079–2086. https://doi.org/10.1099/13500872-142-8-2079

45. Lee J., Mitchell W. J., Tangney M., Blaschek H. P. Evidence for the presence of an alternative glucosetransport system in Clostridium beijerinckii NCIMB
8052 and the solvent-hyperproducing mutant BA
101.

*Appl. Environ. Microbiol.*

2005, 71 (6), 3384–3387. https://doi.org/10.1128/AEM.71.6.3384-3387.2005

46. *Evans V. J., Liyanage H., Ravagnani A., Young M., Kashket E. R.* Truncation of Peptide Deformylase Reduces the Growth Rate and Stabilizes Solvent Production in *Clostridium beijerinckii* NCIMB 8052.

*Appl. Environ. Microbiol.*

1998, 64 (5), 1780–1785. https://doi.org/10.1128/AEM.64.5.1780-1785.1998

47. *Bao T., Zhao J., Zhang Q., Yang S.-T.* Development of a shuttle plasmid without host restriction sites for efficient transformation and heterologous gene expression in *Clostridium cellulovorans.* *Appl. Microbiol. Biotechnol.*

2019, 103 (13), 5391–5400. https://doi.org/10.1007/s00253-019-09882-0

48. *Zheng J., Tashiro Y., Wang Q., Sonomoto K.* Recent advances to improve fermentative butanol production: Genetic engineering and fermentation technology.

*J. Biosci.*

2015, 119 (1), 1–9. https://doi.org/10.1016/j.jbiosc.2014.05.023

49. *Heap J. T., Ehsaan M., Cooksley C. M., Ng Y.-K., Cartman S. T., Winzer K., Minton N. P.* Integration of DNA into bacterial chromosomes from plasmids without a counter-selection marker.

*Nucleic Acids Res.*

2012, 40 (8), e59:1–10. https://doi.org/10.1093/nar/gkr1321

50. *Cheng C., Bao T., Yang S.-T.* Engineering *Clostridium* for improve solvent production: recent progress and perspective.

*Appl. Microbiol. Biotechnol.*

2019, 103 (14), 5549–5566. https://doi.org/10.1007/s00253-019-09916-7
51. Mermelstein L. D., Welker N. E., Bennett G. N., Papoutsakis E. T. Expression of cloned homologous fermentative genes in Clostridium acetobutylicum ATCC 824. 
BioTechnology . 1992, V. 10, P. 190–195. 
https://doi.org/10.1038/nbt0292-190

52. Green E. M., Bennett G. N. Inactivation of an aldehyde/alcohol dehydrogenase gene from Clostridium acetobutylicum ATCC 824. 
Appl. Biochem. Biotechnol. 1996, V. 57–58, P. 213–221. 
https://doi.org/10.1007/BF02941702

53. Jang Y. S., Lee J. Y., Lee J., Park J. H., Im J. A., Eom M. H., Lee J., Lee S. H., Song H., Cho J. H., Seung D. Y., Lee S. Y. Enhanced butanol production obtained by reinforcing the direct butanol-forming route in Clostridium acetobutylicum. 
MBio. 2012, 3 (5), e00314–312. https://doi.org/10.1128/mBio.00314-12

54. Sillers R., Al-Hinai M. L., Papoutsakis E. T. Aldehyde-alcohol dehydrogenase and/or thiolase overexpression coupled with CoA trasferase downregulation lead to higher alcohol titers and selectivity in Clostridium acetobutylicum fermentati on. 
Biotecnol Bioeng. 2009, 102 (1), 38–49. https://doi.org/10.1002/bit.22058

55. Yu L., Zhao J., Xu M., Varghese S., Yu M., Tang I. C., Yang S. T. Metabolic engineering of Clostridium tyrobutyricum for
56. Ventura J.-R., Hu H., Jahng D. Enhanced butanol production in *Clostridium acetobutylicum* ATCC824 by double overexpression of 6-phosphofructokinase and pyruvate kinase genes. *Appl. Microbiol. Biotechnol.* 2013, 97 (16), 7505–7516. https://doi.org/10.1007/s00253-013-5075-7

57. Yang Y., Hoogewind A., Moon Y. H., Day D. Production of butanol and isopropanol with an immobilized *Clostridium*. *Bioprocess Biosyst. Eng.* 2016, 39 (3), 421–428. https://doi.org/10.1007/s00449-015-1525-1

58. Youn S. H., Lee K. M., Kim K. Y., Lee S. M., Woo H. M., Um Y. Effective isopropanol-butanol (IB) fermentation with high butanol content using a newly isolated *Clostridium sp.* A1424. *Biotechnol. Biofuels.* 2016, 9 (1), 1–15. https://doi.org/10.1186/s13068-016-0650-7

59. Zhang C., Li T., He J. Characterization and genome analysis of a butanol-isopropanol-producing *Clostridium beijerinckii* stain BGC. *Bio technol. Biofuels.* 2018, V. 11, P. 280. https://doi.org/10.1186/s13068-018-1274-x

60. De Gerando H. M., Wasels F., Bisson A., Clement B., Bidard F., Jordier E., Lopez-Contreras A.-M., Ferreira N. L. Genome and transcriptome of the natural isopropanol producer *Clostridium beijerinckii* DSM6423. *BMC Genomics.* 2018, 19 (1), 242. https://doi.org/10.1186/s12864-018-4636-7

61. Dai Z., Dong H., Zhu Y., Zhang Y., Li Y., May Y. Introducing a single secondary alcohol dehydrogenase into butanol-tolerant *Clostri*
*Clostridium acetobutylicum*
Rh8 switch ABE fermentation to high level IBE fermentation.
*Biotechnol. Biofuels.*
2012, V. 5, P. 44. https://doi.org/10.1186/1754-6834-5-44

62. *Dusseaux S., Croux C., Soucaille P., Meynial-Salles I.* Metabolic engineering of *Clostridium acetobutylicum* ATCC 824 for the high-yield production of a biofuel composed of an isopropanol/butanol/ethanol mixture.
*Metab. Eng.*
2013, V. 18, P. 1–8. https://doi.org/10.1016/j.ymben.2013.03.003

63. *Collas F., Kuit W., Clement B., Marchal R., Lopez-Contreras A.-M., Monot F.* Simultaneous production of isopropanol, butanol, ethanol and 2,3-butanediol by *Clostridium acetobutylicum* ATCC 824 engineered stains.
*AMB Express.*
2012, 2 (1), 45. https://doi.org/10.1186/2191-0855-2-45

64. *Lee J., Jang Y. S., Choi S. J., Im J. A., Song H., Cho J. H., Seung D. Y., Papoutsakis E. T., Bennette G. N., Lee S. Y.* Metabolic engineering *Clostridium acetobutylicum* ATCC 824 for isopropanol-butanol-ethanol fermentation.
*Appl. Environ. Microbiol.*
2012, 78 (5), 1416–1423. https://doi.org/10.1128/AEM.06382-11

65. *Jang Y. S., Malaviya A., Lee J., Im J. A., Lee S. Y., Lee J., Eom M. H., Cho J. H., Seung D. Y.* Metabolic engineering of *Clostridium acetobutylicum* for the enhanced production of isopropanol-butanol-ethanol fuel mixture.
*Biotechnol. Progr.*
2013, 29 (4), 1083–1088. https://doi.org/10.1002/btpr.1733

66. *Xu M., Zhao J., Yu L., Tang I. C., Xue C., Yang S. T.* Engineering *Clostridium acetobutylicum* with a histidine kinase knockout for enhanced n -butanol tolerance and production.
67. Xu M., Zhao J., Yu L., Yang S. T. Comparing genomic analysis of *Clostridium acetobutylicum* for understanding the mutations contributing to enhanced butanol tolerance and production. *J. Biotechnol*. 2017, V. 263, P. 36–44. https://doi.org/10.1016/j.jbiotec.2017.10.010

68. Yu M., Du Y., Jiang W., Chang W. L., Yang S. T., Tang I. C. Effects of different replicons in conjugative plasmids on transformation efficiency, plasmid stability, gene expression and n-butanol biosynthesis in *Clostridium tyrobutyricum*. *Appl. Microbiol. Biotechnol.* 2012, 93 (2), 881–889. https://doi.org/10.1007/s00253-011-3736-y

69. Yu M., Zhang Y., Tang I. C., Yang S. T. Metabolic engineering of *Clostridium tyrobutyricum* for n-butanol production. *Metab. Eng*. 2011, 13 (4), 373–382. https://doi.org/10.1016/j.ymben.2011.04.002

70. Guedon E., Desvaux M., Petitdemange H. Improvement of cellulolytic properties of *Clostridium um cellulolyticum* by metabolic engineering. *Appl. Environ. Microbiol*. 2002, 68 (1),53–58. https://doi.org/10.1128/AEM.68.1.53-58.2002
71. Li Y., Tschaplinski T. J., Engle N. L., Hamilton C. Y., Rodriguez M. J. r, Liao J. C., Schadt C. W., Guss A., Yang Y., Graham D. E.
Combined inactivation of the Clostridium cellulolyticum lactate and malate dehydrogenase genes substantially increases ethanol yield from cellulose and switchgrass fermentations. Biotechnol Biofuels. 2012, 5 (1), 2. https://doi.org/10.1186/1754-6834-5-2

72. Higashide W., Li Y., Yang Y., Liao J. C. Metabolic engineering of Clostridium cellulolyticum for production of isobutanol from cellulose. Appl Environ Microbiol. 2011, 77 (8), 2727–2733. https://doi.org/10.1128/AEM.02454-10

73. Gaida S. M., Liedtke A., Jentges A. H., Engels B., Jennewein S. Metabolic engineering of Clostridium cellulolyticum for the production of n-butanol from crystalline cellulose. Microb Cell Fact. 2016, 15 (6). https://doi.org/10.1186/s12934-015-0406-2

74. Yang X., Xu M., Yang S. T. Metabolic and process engineering of Clostridium cellulovorans for biofuel production from cellulose. Metab Eng. 2015, V. 32, P. 39–48. https://doi.org/10.1016/j.ymben.2015.09.001
75. Ou J., Xu N., Ernst P., Ma C., Bush M., Goh K., Zhao J., Zhou L., Yang S. T., Liu X. Process engineering of cellulosic n-butanol production from corn-based biomass using Clostridium cellulovorans. *Process Biochem*. 2017, V. 62, P. 144–150. https://doi.org/10.1016/j.procbio.2017.07.009

76. Wen Z., Ledesma-Amaro R., Lin J., Jiang Y., Yang S. Improved n-butanol production from Clostridium cellulovorans by integrated metabolic and evolutionary engineering. *Appl Environ Microbiol*. 2019, 22, 85 (7), e02560–18. https://doi.org/10.1128/AEM.02560-18

77. Lin P. P., Mi L., Moriok A. H., Yoshino M. M., Konishi S., Xu S. C., Papanek B. A., Riley L. A., Guss A. M., Liao J. Consolidated bioprocessing of cellulose to isobutanol using Clostridium thermocellum. *Metab Eng*. 2015, V. 31, P. 44–52. https://doi.org/10.1016/j.ymben.2015.07.001

78. Köpke M., Held C., Hujer S., Liesegang H., Wiezer A., Wollherr A., Ehrenreich A., Liebl W., Gottschalk G.
Clostridium ljungdahlii represents a microbial production platform based on syngas.

PNAS 2010, 107 (29), 13087–13092. https://doi.org/10.1073/pnas.1004716107

79. Berzin V., Tyurin M., Kiriukhin M. Selective n-butanol production by Clostridium sp. MTButOH1365 during continuous synthesis gas fermentation due to expression of synthetic thiolase, 3-hydroxy butyryl-CoA dehydrogenase, crotonase, butyryl-CoA dehydrogenase, butyraldehyde dehydrogenase, and NAD-dependent butanol dehydrogenase.

Appl Biochem Biotechnol 2013, 169 (3), 950–959. https://doi.org/10.1007/s12010-012-0060-7

80. Kumar M., Gayen K. Development in biobutanol production: new insights. Appl. Energy. 2011, 88 (6), 199–2012. https://doi.org/10.1016/j.apenergy.2010.12.055

81. Ohtake T., Pontrelli S., Laviña W. A., Liao J. C., Putri S. P., Fukusaki E. Metabolomics-drive n approach to solving a CoA imbalance for improved 1-butanol production in Escherichia coli

Metab. Eng. 2017, V. 41, P. 135–143. https://doi.org/10.1016/j.ymben.2017.04.003

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