Advances in
Biotechnology & Microbiology
ISSN 2474-7637

Juniper Publishers
key to the Researchers

Mini Review
Volume 9 Issue 3 - May 2018
DOI: 10.19080/AIBM.2018.09.555763

Adv Biotech & Micro

Current Trends in Methylotrophy -based Biotechnology

Yuri A Trotsenko* and Maria L Torgonskaya

GK Skryabin Institute of Biochemistry and Physiology of Microorganisms of Russian Academy of Sciences, Russia

Submission: April 28, 2018**; Published:** May 24, 2018

***Corresponding author:** Yuri A Trotsenko, Laboratory of Methylotrophy, GK Skryabin Institute of Biochemistry and Physiology of Microorganisms of Russian Academy of Sciences, Russia, Tel: (++7) 495 956-33-70; Email: trotsenko@ibpm.pushchino.ru

Abstract

The review briefly summarizes the viewpoints on biotechnological potential of methylotrophic bacteria and yeasts, present state of art and new trends of application of their unique metabolism for development of novel technologies for biodegradation, bioanalytics and biosynthesis.

Keywords: Methylotrophy; Biotechnology; Bioremediation; Biosynthesis; Biocatalysis; Bioanalytics

Introduction

Methylotrophy is an amazing lifestyle, which allows prokaryotic and eukaryotic microorganisms to use single-carbon compounds as the sole sources of carbon and energy. Specialized group within methylotrophs is represented by methanotrophs capable of oxidizing a greenhouse gas methane, whereas the majority of methylotrophic bacteria and yeasts use methanol and other methylated compounds for their growth [1]. The presence of natural sources of C1-compounds such as plants, biomass burning, volcanic activity, etc. is evidently responsible for the almost ubiquitous distribution of microorganisms implementing methylotrophic metabolism [2-5]. Being biological sink for toxic C1-compounds methylotrophs prevent their release into the atmosphere and decrease the impact (as greenhouse gases) to global climate. At the same time C1-utilizers are able to synthesize a wide spectrum of valuable products from these abundant and potentially cheap substrates [6,7]. Together with a well-studied metabolism, availability of genomic data and actively developing methods of metabolic engineering, this peculiarity makes methylotrophs an attractive instrument for biotechnologists. In the present review current directions and modern tendencies of biotechnological application of methylotrophs and their specific metabolic abilities are listed.

Biodegradation and bioremediation

The most obvious application of methylotrophic metabolism lies in use of corresponding bacterial degraders and their enzymes in processes of biodegradation of industrially produced C1-compounds and bioremediation of polluted ecosystems. Methylotrophic bacteria are usually capable of oxidizing methanol, formaldehyde and formate utilizing these substances without formation of undesirable by-products [8-10]. High phenotypic plasticity allows to methylotrophs occupy different (soil, water, sediments), even extreme habitats, colonize plants and animals [11,12]. These microorganisms are phylogenetically diverse and adapt to environmental challenges by tuning of metabolic modes, therefore strains from different ecological niches can be used for selection of starting platforms for further development of desired technologies and products based on single-carbon substrates [7]. Some C1-utilizing bacteria can effectively remove methylated amines, methylated sulfur compounds (dimethylsulfide, dimethyldisulfide, methanesulfonic acid, etc.) [5,13] and halogenated methanes (chloro, bromo- and iodomethanes, DCM) [4,14]. However, the latter metabolic possibilities are rather rare and likely represent a result of evolution under conditions of long selective pressure of specific C1-compounds [15,16]. The ability to mineralize C1-compounds can be further improved by genetic engineering. For example, it was shown, that heterologous expression of methylotrophic genes encoding key enzymes of the ribulose monophosphate C1-assimilation pathway hexulose phosphate synthase (EC 4.1.2.43) and 6-phospho-3-hexuloisomerase (EC 5.3.1.27), increase the efficiency of removing formaldehyde by bacterial strains and transgenic plants [9,17,18].

Besides C1-compounds some methylotrophic bacteria can degrade a variety of other organic toxicants. *Pseudomonas esterophilus* 27RD, *Pseudomonas esterovorus* 24RA utilize methyl- and ethyl-acetates [19]. Two strains of *Methylobacterium populi* (V2 and BJ001) were shown to destruct polyaromatic

hydrocarbons [20] and even toxic explosives, such as 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazene (hexogen) and octahydro-1,3,5,7-tetranitro-1,3,5-tetrazocine (octagen) [21]. *Methylobacterium* sp. GPE1 degrades a heterocyclic constituent of tobacco smoke carbazole [22]. Bacterial consortia, containing methylotrophic bacteria, were demonstrated to efficiently remove phenol [23] and also common soil pollutants methyl-tert-butyl ether [24,25] and trichloroethylene [26]. Genomic prediction of methylotrophic lifestyle, basing on the concept of minimal metabolic modules [1], constantly expands our understanding of distribution and environmental roles of methylotrophic bacteria. In particular, available genomic data of newly discovered poly (ethylene terephtalate)-degrading bacteria *Ideonella sakaiensis* 201-F6 [27], suggest that this strain can also employ autotrophic methylotrophy.

Bioanalytics

Whole cells and enzymes of methylotrophs are also used in bioanalytics for detection and monitoring of the content of toxic C1-compounds in the environment (e.g., industrial gas emissions, wastewaters, and indoor air), drinking water, various products, foods, therapeutic drugs, and biological fluids. Highly sensitive and selective biosensor technology is an accessible alternative to the expensive and complex physicochemical methods of detection. Biosensors are hybrid devices containing a bioelement (immobilized bioactive substance), which defines a degree of selectivity, and a physical transformer (transducer). To date a number of biosensors and bioreporter systems on the basis of cells, enzymes and even specific cofactors of methylotrophs were designed for methanol, ethanol, dichloromethane, formaldehyde, mono-, di-, and trimethylamines, glucose, lactate, aspartam, n-butanol, benzyl alcohol [6, 28,29].

Another type of bioreporter systems is represented by genetically modified cells demonstrating visible signals in response to chemical stimuli. Such a system constructed by introducing into intact cells of the chloromethane destructor *Methylobacterium chloromethanicum* CM4 of a plasmid with the gene of the yellow fluorescent protein under control of the promoter of the chloromethane dehalogenase (EC 2.1.1.-) *cmuA* gene allowed marking of the natural sources of methyl halides by biofluorescence [30].

Biosynthesis and biocatalysis

Historically, methylotrophic microorganisms represent a widely used platform for bioproduction of chemicals for agricultural and chemical industry from non-sugar substrates. Methylotroph-based single cell protein (SCP) production has been active already in the 1980s, but has not yet exhausted its potential and can progress with use of new perspective strains [7,31]. Remarkable outcomes were reached for methane- and methanol-based production of biopolymers - polyhydroxyalkanoates, which have a wide range of applications in packaging, medicine, or as textile and household materials, and represent a fully biodegradable environmentally friendly alternative to traditional petroleum-based plastics [6,32]. Stiffness and brittleness of natural product poly-3-hydroxybutyrate (PHB) can be overcomed by thorough selection of strains (≥3000 kDa for *Methyloligella halotolerans* C2 polymer vs. 50-200 kDa for PHB from *M. extorquens*) [6,33] and carbon sources ensuring the synthesis of high-molecular polymer or by functionalizing of PHB by addition of copolymers with other hydroxyalkanoate co-monomer(s), like 3-hydroxyvalerate or 3-hydroxyhexanoate [34]. Second way needs an addition of precursors increasing production cost, therefore as a less expensive scenario an artificial limitation of flux through ethyl-malonyl-CoA pathway (EMCP) was suggested, thus providing propionyl- and butyryl-CoA precursors for synthesis of functionalized PHB [34]. Methylotrophic bacteria and yeasts are also commonly used as natural producers in methanol-based synthesis of a series of chemicals for pharmaceutic, cosmetic, perfume and chemical industry like aldehydes, formate, glycerol, hydrogen peroxide, pyruvate, citrate, amino acids (L-serine, L-glutamate, L-lysine), glyoxylate, gamma-aminobutyric acid, cadaverine, dicarboxylic acids from EMCP ((2S)-methylsuccinic and mesaconic acid), cofactors (FAD, GSH, ATP), homological methylotrophic and antioxidant enzymes, exopolysaccharides, bioprotectant ectoine, antioxidant carotenoid compounds [6,12,35-37]. The possibility of additional increase of the naturally high lipid content in cells of proteobacterial methanotrophs by genetic engineering makes these bacteria promising platform for biodiesel production from methane [7].

Heterologous expression of proteins represents another way of implementation of biotechnological potential of methylotrophs. At present, the most used methylotrophic expression systems suitable for investigations and production of industrial recombinant enzymes include well described yeast platforms of *Pichia pastoris*, *Hansenula polymorpha*, *Pichia methanolica* and *Candida boidinii* [35,38], which were subjected to adaptive laboratory evolution, resulting in populations with improved growth and production characteristics [39]. Examples of use of methylotrophic bacteria *M. extorquens* for methanol-based expression of heterological proteins (green fluorescent protein, insecticidal protein Cry1Aa, a haloalkane dehalogenase, and Enterocin P) were also reported, but the achieved yields were not sufficient for commercial use. Facilitation of product recovery procedures by using of minimal media and relatively "clean" supernatant of methylotrophic cultures can be noted as advantage of methanol-based expression systems. However, the necessity to synthesize products for food and pharmaceutical industry often makes consider the use of toxic substrate as a shortage of these platforms and avoid it. Furthermore, bacterial hosts lack necessary posttranlational modification machinery and secretion machinery for these strains is not sufficiently studied [36].

Heterologous expression also opens new possibilities for synthesis of chemicals. Recently, this approach was used

How to cite this article: Yuri A T, Maria L T. Current Trends in Methylotrophy -based Biotechnology. Adv Biotech & Micro. 2018; 9(3): 555763. DOI: 10.19080/AIBM.2018.09.555763.

for engineering of methylotrophic bacteria for production of 2-hydroxybutyric acid, 3-hydroxypropionic acid, mevalonate, lactate and sesquiterpenoid a-humulene [7,36], and also for reversion of the native methanogenic pathway and addition of supplementary enzymes in archaeon *Methanosarcina acetivorans* for synthesis of acetate and L-lactate from methane [40]. An alternative way to gain benefit from using of simple C1-substrates is represented by so-called "synthetic methylotrophy" and implies an introduction of a minimal set of well studied methylotrophic modules/enzymes enabling methylotrophy into usual heterotrophic industrial platforms like *E. coli* and *Corynebacterium glutamicum*. Nowadays, the available instruments of this direction of synthetic biology include design of improved and even artificial variants of methylotrophic enzymes and pathways and also biosensors for monitoring of some C1-fluxes in heterologous hosts [7].

The ability of methylotrophic bacteria to form stable associations with non-methylotrophic microorganisms, supplying them with carbon sources [7], makes economically attractive the use of microbial communities for the synthesis of valuable products, which are not specific for C1-utilizers. It was shown, that in consortium with exoelectrogen *Geobacter sulfurreducens* and in the presence of humic acids the engineered *Ms. acetivorans* with reversed methanogenic pathway can convert methane into electrical current. Biotechnological potential of several platforms involving synthetic consortia of methanotrophs and non-methanotrophic methylotrophs or cyanobacteria for production of chemicals and biofuels is under analysis [7].

The last extensive area of application of methylotrophic bacteria lies in agricultural use for increasing of plant growth due to production of phytohormones (auxins, cytokinines, gibberelins) and siderophores, nitrogen fixation, ethylene production decreasing, solubilization of phosphorus, inhibition of plant pathogens and induction of systemic resistance in plants [11,12]. Interestingly, for some methylotrophic phytosymbionts the ability to increase production of furanoid compounds, responsible for fruit flavor, was also demonstrated [41]. Furthermore, the C1-utilizers decrease environmental stress by degrading toxic organic compounds and immobilizing heavy metals [12].

Recent unexpected discovery in methylotrophs of enzymes requiring rare Earth elements (REEs) - lanthanides for their functioning [42] suggest that they possess yet unknown specific mechanisms for sensing, transport and binding of these elements [7]. Identification of such systems can be potentially useful for engineering of new bioprocesses of REEs' mining for industrial purposes basing on methylotrophic strains.

Conclusion

Broad functional diversity, unpretentiousness in terms of substrates and culture media, profoundly studied metabolism, cultivation technologies, and well-developed systems of genetic modification make methylotrophs increasingly popular agents of modern green biotechnologies. Conversion to valuable products of universally available renewable C1-substrates such as methane, methanol and carbon dioxide, is able not only reduce the cost of synthesis, but also decrease the harmful effects of these sustainable compounds on the ecosystem and the global climate by reducing their anthropogenic emissions. The use of C1-compounds, synthesized as by-products, as a raw material for other industrial processes, obviously represents an element potentially important for the development of technologies of non-waste production.

Acknowledgement

This work was supported by the Russian Foundation of Basic Research (no. 18-04-01148).

Conflict of Interest

The authors declare no conflicts of interest.

References

1. Chistoserdova L (2011) Modularity of methylotrophy. Environ Microbiol 13(10): 2603-2622.
2. Galbally IE, Kirstine W (2002) The production of methanol by flowering plants and the global cycle of methanol.J Atmos Chem 43(3): 195-229.
3. Kolb S (2009) Aerobic methanol-oxidising bacteria in soil. FEMS Microbiol Lett 300(1): 1-10.
4. Schaefer JK, Miller LG, Oremland RS, Murrell JC (2007) Bacterial cycling of methyl halides. Adv Appl Microbiol 61: 307-346.
5. Schaefer H, Myronova N, Boden R (2010) Microbial degradation of dimethylsulfide and related C1-sulfur compounds: organisms and pathways controlling fluxes of sulfur in the biosphere. J Exp Bot 61(2): 315-334.
6. Doronina NV, Torgonskaya ML, Fedorov DN, Trotsenko YA (2015) Aerobic methylobacteria as promisimg objects of modern biotechnology (Review). Appl Biochem Microbiol 51(2): 125-134.
7. Chistoserdova L (2018) Applications of methylotrophs: can single carbon be harnessed for biotechnology? Curr Opin Biotechnol 50: 189-194.
8. Jakobsen OM (2006) Upregulated transcription of plasmid and chromosomal ribulose monophosphate pathway genes is critical for methanol assimilation rate and methanol tolerance in the methylotrophic bacterium *Bacillus methanolicus*. J Bacteriol 188(8): 3063-3072.
9. Koopman FW, De Winde JH, Ruijssenaars HJ (2009) C1 compounds as auxiliary substrates for engineered *Pseudomonas putida* S12. Appl Microbiol Biotechnol 83(4): 705-713.
10. Qiu LW (2014) Formaldehyde biodegradation by immobilized *Methylobacterium sp*. XJLW cells in a three phase fluidized bed reactor. Bioprocess Biosyst Eng 37(7): 1377-1384.
11. Fedorov DN, Doronina NV, Trotsenko YA (2011) Phytosymbiosis of aerobic methylobacteria: new facts and views. Microbiology 80(4): 443-454.
12. Dourado MN, Neves AAC, Santos DS, Araújo WL (2015) Biotechnological and agronomic potential of endophytic pink-pigmented methylotrophic *Methylobacterium* spp. BioMed Res Int 2015: 909016. doi: 10.1155/2015/909016.

How to cite this article: Yuri A T, Maria L T. Current Trends in Methylotrophy -based Biotechnology. Adv Biotech & Micro. 2018; 9(3): 555763. DOI: 10.19080/AIBM.2018.09.555763.

13. Nanda S, Sarangi PK, Abraham J (2012) Microbial biofiltration technology for odour abatement: an introductory review. Journal of Soil Science and Environmental Management 3(2): 28-35.

14. Flanagan WP (1998) Biodegradation of dichloromethane in a granular activated carbon fluidized-bed reactor. Water Environment Research 70(1): 60-66.

15. Goodwin KD, Varner RK, Crill PM, Oremland RS (2001) Consumption of tropospheric levels of methyl bromide by C1 compound-utilizing bacteria and comparison to saturation kinetics. Appl Environ Microbiol 67(12): 5437-5443.

16. McDonald IR (2002) A review of bacterial methyl halide degradation: biochemistry, genetics and molecular ecology. Environ Microbiol 4(4): 193-203.

17. Orita I, Sakamoto N, Kato N, Yurimoto H, Sakai Y (2007) Bifunctional enzyme fusion of 3-hexulose-6-phosphate synthase and 6-phospho-3-hexuloisomerase. Appl Microbiol Biotechnol 76(2): 439-445.

18. Sawada A (2007) Purification capability of tobacco transformed with enzymes from a methylotrophic bacterium for formaldehyde. Int J Phytoremediation 9(6): 487-496.

19. Rakov DY, Doronina NV, Trotsenko YA, Alieva RM (1990) Pathway of methylacetate metabolism in methylotrophic bacteria. FEMS Microbiol Lett 67(1-2): 67-72.

20. Ventorino V (2014) *Methylobacterium populi* VP2: plant growth promoting bacterium isolated from a highly polluted environment for polycyclic aromatic hydrocarbon (PAH) biodegradation. Scientific World Journal 2014: 1-12.

21. Van Aken B, Yoon JM, Schnoor JL (2004) Biodegradation of nitro-substituted explosives 2,4,6-trinitrotoluene, hexahydro-1,3,5-trinitro-1,3,5-triazine, and octahydro-1,3,5,7-tetranitro-1,3,5-tetrazocine by a phytosymbiotic *Methylobacterium* sp. associated with poplar tissues (Populus deltoides×nigra DN34). Appl Environ Microbiol 70(1): 508-517.

22. Pasternak G, Kolwzan B (2013) Surface tension and toxicity changes during biodegradation of carbazole by newly isolated methylotrophic strain *Methylobacterium* sp. GPE1. Int Biodeterior Biodegradation 84: 143-149.

23. Khongkhaem P, Intasiri A, Luepromchai E (2011) Silica-immobilized Methylobacterium sp. NP3 and *Acinetobacter sp*. PK1 degrade high concentrations of phenol. Lett Appl Microbiol 52(5): 448-455.

24. Zhang LL, Chen JM, Fang F (2008) Biodegradation of methyl t-butyl ether by aerobic granules under a co substrate condition. Appl Microbiol Biotechnol 78(3): 543-550.

25. Müller RH, Rohwerder T, Harms H (2008) Degradation of fuel oxygenates and their main intermediates by *Aquincola tertiaricarbonis* L108. Microbiology 154(5): 1414-1421.

26. Fernandes VC, Albergaria JT, Oliva-Teles T, Delerue-Matos C, de Marco P (2009) Dual augmentation for aerobic bioremediation of MTBE and TCE pollution in heavy metal-contaminated soil. Biodegradation 20(3): 375-382.

27. Yoshida S (2016) A bacterium that degrades and assimilates poly(ethylene terephthalate). Science 351(6278): 1196-1199.

28. Gülce H, Guelce A, Kavanoz M, Coscun H, Yildiz A (2002) A new amperometric enzyme electrode for alcohol determination. Biosens Bioelectron 17(6-7): 517-521.

29. Sibirny V (2011) Formaldehyde oxidizing enzymes and genetically modified yeast Hansenula polymorpha cells in monitoring and removal of formaldehyde. In: Einschlag FSG (Ed.), Waste water - evaluation and management. In Tech Publishers, Rijeka, Croatia, pp. 115-154.

30. Haque MFU, Nadalig T, Bringel F, Schaller H, Vuilleumier S (2013) Fluorescence-based bacterial bioreporter for specific detection of methyl halide emissions in the environment. Appl Environ Microbiol 79(21): 6561-6567.

31. Strong PJ, Xie S, Clarke WP (2015) Methane as a resource: can the methanotrophs add value? Environ Sci Technol 49(7): 4001- 4018.

32. Keshavarz T, Roy I (2010) Polyhydroxyalkanoates: bioplastics with a green agenda. Curr Opin Microbiol 13(3): 321-326.

33. Poroshina MN, Doronina NV, Ezhov VA, Trotsenko YA (2014) Comparative characteristics of biosynthesis of polyhydroxybutyrate from methanol by *Methylobacteria extorquens* G10 and *Methyloligella halotolerans* C2. Appl Biochem Microbiol 50(3): 253-258.

34. Orita I, Nishi kawa K, Nakamura S, Fukui T (2014) Biosynthesis of polyhydroxyalkanoate copolymers from methanol by *Methylobacterium extorquens* AM1 and the engineered strains under cobalt-deficient conditions. Appl Microbiol Biotechnol 98(8): 3715-3725.

35. Sakai Y, Tani Y, Kato N (1999) Biotechnological application of cellular functions of the methylotrophic yeast. J Mol Catalysis B Enzymatic 6(3): 161-173.

36. Ochsner AM, Sonntag F, Buchhaupt M, Schrader J, Vorholt J (2015) *Methylobacterium extorquens*: methylotrophy and biotechnological applications. Appl Microbiol Biotechnol 99(2): 517-534.

37. Hou CT, Laskin AI, Patel RN (1979) Growth and polysaccharide production by *Methylocystis parvus* OBBP on methanol. Appl Environ Microbiol 37(5): 800-804.

38. Adrio JL, Demain AL (2010) Recombinant organisms for production of industrial products. Bioeng Bugs 1(2): 116-131.

39. Moser JW (2017) Implications of evolutionary engineering for growth and recombinant protein production in methanol-based growth media in the yeast *Pichia pastoris*. Microb Cell Fact 16: 49.

40. McAnulty MJ (2017) Metabolic engineering of *Methanosarcina acetivorans* for lactate production from methane. Biotechnol Bioeng 114(4): 852-861.

41. Verginer M (2010) Monitoring the plant epiphyte *Methylobacterium extorquens* DSM21961 by real time PCR and its influence on the strawberry flavor. FEMS Microbiol Ecol 74(1): 136-145.

42. Chistoserdova L (2016) Lanthanides: new life metals? World J Microbiol Biotechnol 32(8): 138.

How to cite this article: Yuri A T, Maria L T. Current Trends in Methylotrophy -based Biotechnology. Adv Biotech & Micro. 2018; 9(3): 555763. DOI: 10.19080/AIBM.2018.09.555763.

DOI: 10.19080/AIBM.2018.09.555763

How to cite this article: Yuri A T, Maria L T. Current Trends in Methylotrophy -based Biotechnology. Adv Biotech & Micro. 2018; 9(3): 555763. DOI: 10.19080/AIBM.2018.09.555763.