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Applications of methylotrophs: can single carbon be harnessed for biotechnology?

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This review summarizes developments in the field of applied research involving microbial conversion of single carbon compounds (methane, methanol, CO_2). The potential of the microorganisms involved in biotechnological applications could be realized via engineering native C1 utilizers toward higher output of value-added compounds, including biofuels, or via production of value chemicals as parts of novel, heterologously expressed biochemical pathways. Alternatively, C1 metabolism could be implemented in traditional industrial platforms (*Escherichia coli*, yeast), via introduction of specific metabolic modules. Most recent research spanning both approaches is covered. The potential of C1 utilizers in biomining of rare Earth elements, as well as the potential of C1 consuming microbial consortia in industrial applications are discussed.

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Introduction

Specialized guilds of microorganisms are capable of consuming single carbon (C1) compounds, that is, compounds containing no carbon–carbon bonds, such as methane, methanol or CO_2. Microorganisms capable of oxidizing methane are known as methanotrophs, and, together with microorganisms that will not oxidize methane but will utilize other C1 compounds (methanol and so on), they belong to a broader guild of methylotrophs. Microorganisms capable of assimilating CO_2 are known as autotrophs. Some organisms are capable of both methylotropjy and autotrophy, which makes them autotrophic methylotrophs. The organisms utilizing C1 compounds are extremely attractive for biotechnological applications for two main reasons. On the one hand, value-added compounds could be produced from substrates such as methane (or derivatives) and/or CO_2, both of which are abundant in the environment. On the other hand, these compounds are also greenhouse gases, and their removal from the environment would prevent their escape into the atmosphere, decreasing their impact on global climate change. The potential of C1-utilizing microorganisms for industrial applications has been recognized early on. For example, a methylotroph-based single cell protein (SCP) production plant has been active in the 1980s [1]. Impressively, even in these early, pregenomic times, the industrial strain, *Methylophilus methylotrophus* was genetically modified for improved performance [2]. SCP production from methylotrophs is still alive and kicking [3•], and it is gaining further momentum trough employing novel prominent industrial platforms for SCP production, such as *Methylobacterium extorquens*, which, in addition to high biomass production, provides added benefits such as naturally synthesized anti-oxidant carotenoid compounds [4]. However, the future of harnessing C1 utilizing organisms for environmental biotechnology will likely involve metabolic engineering and synthetic biology. Most recent developments in this direction are discussed in this review.

Developing methylotroph catalysts

Native methylotrophs, some of which demonstrate impressively robust growth on C1 compounds, present natural platforms for biotechnology. Methylotrophs are available from different phylogenetic backgrounds, and with different temperature, salinity and pH optima. Thus, a strain with desired characteristics could be preselected as a starting platform.

Biofuels from methane

There has been renewed interest in converting methane into liquid biofuels by the way of microbial catalysis [3•], and thus a new search has been recently launched for microorganisms that could present attractive platforms for such applications. One such organism, *Methylomicrobium buryatense*, showed promise, having demonstrated attractive characteristics such as robust growth on methane and stress tolerance, including usage of natural gas of varying compositions [5]. This organism has been recently thoroughly investigated in terms of growth characteristics and responses to limitations of oxygen and methane [6], followed by the development of a metabolic model that predicts carbon distribution among different metabolic pathways during growth on methane [7]. The potential in biofuel production from this platform lies in naturally high lipid content, typical of proteobacterial methanotrophs. The lipids, after extraction, could be catalytically converted into biodiesel, via esterification. While a promising platform, methanotrophs such as *M. buryatense* would require extensive engineering, to significantly increase lipid content [8], to make the platform commercially feasible. Recently, an enhancement in conversion of methane into lipids was reported for this organism, in an engineered strain with an increased flux through the phosphoketolase pathway [9].

Other products from methane

Meantime, the same organism has been tested as a platform for production of value-added compounds other than biofuels. *M. buryatense* was genetically modified, as a proof of principle, to ferment methane into lactate, by overexpressing a recombinant lactate dehydrogenase [10]. Importantly, lactate production, at least at the level achieved in the study, did not negatively affect cellular lipid concentration. Thus, in principle, methanotrophs could be used for co-production of biofuels and other value-added chemicals [10] (Figure 1).

Employing reverse methanogenesis

Not only bacteria but also archaea present potential industrial platforms for producing value-added chemicals from methane. A model archaeon *Methanosarcina acetivorans* has been engineered to produce acetate from methane, by reversing its native methanogenesis pathway, through the heterologously expressed methyl-CoM reductase (MCR) of an uncultivated methane-oxidizing archaeon of the ANME-1 type and linking methane oxidation to solid iron ($FeCl_3$) reduction, in anaerobic conditions [11•]. This strain was further engineered by expressing a gene for 3-hydroxybutyryl-CoA dehydrogenase from *Clostridium acetobutylicum*, resulting in secreted valuable product L-lactate, of sufficient optical purity for synthesizing the biodegradable plastic poly-lactic acid [12]. This work demonstrated the utility of anaerobic methane conversion, achieving an increased lactate yield compared to aerobic methane conversion to lactate [12].

Electricity from methane

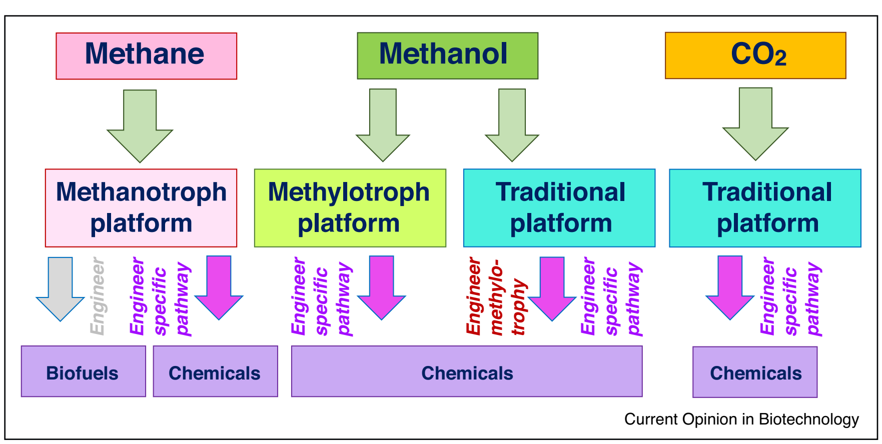
The same engineered host, *M. acetivorans* expressing MCR from ANME-1 archaeon, was employed as part of a synthetic consortium to form a microbial fuel cell converting methane into electrical current [13]. In this case, the engineered *M. acetivorans* needed to be paired with *Geobacter sulfurreducens*, a known exoelectrogen, and with a sludge adapted to methane, the latter replaceable by humic acids, proposed to act as electron shuttles [13,14].

Value-added products from methanol

One of the most developed platforms for value-added compounds from methanol remains to be the Gram-positive methylotroph *Bacillus methanolicus*. Results from experiments on engineering and validation of strains for production of L-glutamate, gamma-aminobutyric acid, L-lysine and cadaverine, through strain optimization and heterologous protein expression, have been reported over years, and these results have been recently summarized in a detailed review [15].

Another emergent bacterial platform is *M. extorquens* that has been recently engineered for *de novo* synthesis of the sesquiterpenoid α-humulene, by expressing heterologous proteins, combined with strain optimization [16]. *M. extorquens* has also been employed recently as a platform for production of 2-hydroxybutyric acid from methanol, by introducing a module that shuttles the natural intermediate of methylotrophic metabolism and polyhydroxyalkanoate synthesis, 3-hydroxybutyryl-CoA into the product of interest [17]. The same organism has also

Figure 1



A schematic summarizing general strategies in engineering microorganisms for applications in converting C1 compounds into value products. Grey arrow denotes an optional step.