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Formate production through carbon dioxide hydrogenation with recombinant whole cell biocatalysts

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HIGHLIGHTS

- *E. coli* whole-cells expressing recombinant formate dehydrogenases were engineered.
- The engineered cells efficiently catalysed the production of formate from CO_2 and H_2.
- Cells expressing *Pyrococcus furiosus* formate dehydrogenase were the most active.
- Sparging of hydrogen gas through cell suspensions improved formate yield.

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ABSTRACT

The biological conversion of CO_2 and H_2 into formate offers a sustainable route to a valuable commodity chemical through CO_2 fixation, and a chemical form of hydrogen fuel storage. Here we report the first example of CO_2 hydrogenation utilising engineered whole-cell biocatalysts. *Escherichia coli* JM109(DE3) cells transformed for overexpression of either native formate dehydrogenase (FDH), the FDH from *Clostridium carboxidivorans*, or genes from *Pyrococcus furiosus* and *Methanobacterium thermoformicicum* predicted to express FDH based on their similarity to known FDH genes were all able to produce levels of formate well above the background, when presented with H_2 and CO_2, the latter in the form of bicarbonate. In the case of the FDH from *P. furiosus* the yield was highest, reaching more than 1 g L^{-1} h^{-1} when a hydrogen-sparging reactor design was used.

1. Introduction

The generation of formic acid from CO_2 and H_2 offers a method of greenhouse gas (GHG) sequestration, a viable route to a commodity chemical and a means of hydrogen fuel storage (Leitner, 1995; Enthaler, 2008; Enthaler et al., 2010; Tedsree et al., 2011). As a result this process has recently attracted much attention (Klibanov et al., 1982; Jessop et al., 2004; Enthaler, 2008; Fellay et al., 2008; Benson et al., 2009; Enthaler et al., 2010; Yadav and Xu, 2012; Alissandratos et al., 2013a). The most common approach has been through the use of metal catalysts, both in heterogeneous and homogeneous reactions (Ikariya et al., 1994; Jessop et al., 1995, 2004; Fellay et al., 2008; Yadav and Xu, 2012). Though significant progress has been made in this area, metal catalysts generally require alkaline conditions and high gas pressures, and suffer from by-product generation. Another approach, which circumvents problems associated with metal catalysis, is biocatalysis (Klibanov et al., 1982; Alissandratos et al., 2013a). CO_2, H_2 and formate are metabolites produced and consumed by a variety of organisms, which appear to have the ability to interconvert these chemical species (Woods, 1936; Thauer, 1972; Nishio et al., 1983; Eguchi et al., 1985; Wu et al., 1993). The formate hydrogen lyase (FHL) complex in particular, composed of formate dehydrogenase (FDH) and hydrogenase, is known to convert formate to CO_2 and H_2, through a coupled formate oxidation and hydride reduction (Stephenson and Stickland, 1932, 1933; Yudkin, 1932; Seol et al., 2012). Though FDH is generally highly efficient in formate oxidation, in certain species it may also present high efficiency in CO_2 reduction, usually in accordance with the enzyme's metabolic role in those species (Thauer, 1972; Scherer and Thauer, 1978). Accordingly, we have recently reported the strong catalytic preference for CO_2 reduction by FDH from the acetogen *Clostridium carboxidivorans*, an important enzyme in the Wood–Ljungdahl carbon fixation pathway (Alissandratos et al., 2013b). When compared with FDH from *Candida boidinii*, which oxidises formate, the reverse catalytic preference of the acetogen FDH appears to be derived from its particularly low binding affinity

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for formate. More recently, the direct hydrogenation of CO_2 with an enzyme comprising an FDH as well as a hydrogenase subunit, isolated from the acetogen *Acetobacterium woodii,* was reported (Schuchmann and Müller, 2013). The transformation of H_2 and CO_2 to accumulate formate was also accomplished with the whole cells, through the addition of a sodium ionophore or under sodium-limiting conditions to block sodium-dependent formate consumption.

While the systems reported to date demonstrate the potential use of biocatalysts for formate production, they are far from ideal for biotechnological applications. Species, such as acetogens, that have FDHs with high activity for CO_2 reduction are generally troublesome to cultivate on an industrial scale, due to the requirement for impractical growth conditions such as strict anaerobicity. Further, when formate production is part of central metabolism, expensive and/or toxic additives may be required to arrest further processing, such as a sodium ionophore with acetogenesis (Schuchmann and Müller, 2013) or methyl viologen in the case of methanogenesis (Eguchi et al., 1985). Instead, the use of well understood hosts, such as *Escherichia coli*, for the production of whole cell biocatalysts is highly desirable, due to the ease of cell growth in low cost media, the high growth rates, the tolerance to high substrate and product concentrations, the wide range of genetic engineering tools available, and the high product titres obtained (Baneyx, 1999; Arbabi-Ghahroudi et al., 2005; Jana and Deb, 2005; Terpe, 2006; Jarboe et al., 2010). Therefore, the utilisation of FDHs with a catalytic preference for CO_2 reduction, in an easy to handle, oxygen-tolerant host, and where formate is not part of the central metabolism, is of considerable interest. Accordingly, here we describe the use of genetic recombination to yield easy-to-handle *E. coli* cells capable of catalysing CO_2 hydrogenation without the need for special growth conditions. Several genes from various species either known or predicted to express FDHs have been inserted into plasmids for expression in common *E. coli* strains. This has resulted in efficient whole-cell catalysts capable of generating high yields of formate when presented with H_2 gas and CO_2, the latter in the form of bicarbonate. In whole-cell systems, though formate is generally produced by fermentation through the action of formate-pyruvate lyase, the possibility of reverse action of endogenous FDHs has been suggested even in organisms where the natural function is formate oxidation (Woods, 1936; Klibanov et al., 1982). There is also a report of induction of the native FDH in *E. coli* using a high concentration of formate (100 mM) and of the induced enzyme both oxidising and producing a low level (6 mM after 24 h) of formate from bicarbonate and H_2 (Nandi et al., 1992). We have therefore compared our recombinant systems with the endogenous FDH activity of the untransformed hosts as well as hosts transformed with a blank plasmid.

2. Methods

2.1. Cloning, expression and purification of FDHs

The DNA sequences for the FDH genes of *Methanobacterium thermoformicicum* (FDH_*Mettf*, Uniprot Q50570), *E. coli* (FDH_*Ecoli*, Uniprot P07658 with the substitution Sec140Cys), *Pyrococcus furiosus* (FDH_*Pyrfu,* Uniprot Q8U0R3), *C. carboxidivorans* (FDH_*Cloca*, Uniprot E2IQB0 with the substitution Sec139Cys) and *Treponema primitia* (FDH_*Trepo*, Uniprot E2DDK9) were purchased (GeneArt, Germany) codon-optimised for expression in *E. coli*. They were reconstructed into the pETMCSIII vector (with ampicillin resistance) (Neylon et al., 2000) for subsequent expression with an *N*-terminal $(His)_6$-tag, as described previously for FDH_*Cloca* (Alissandratos et al., 2013b). After plasmid insertion through electrotransformation, protein expression was carried out in electrocompetent *E. coli* BL21(DE3) and JM109(DE3) cells. All cells were grown aerobically and the conditions were optimised in order to maximise expression of each enzyme. For FDH_*Ecoli* and FDH_*Cloca*, BL21(DE3) cells were grown in LBA medium (Luria–Bertani medium supplemented with 0.1 mg/mL ampicillin), and JM109(DE3) cells were grown in ZYP-5052 medium (autoinduction medium supplemented with 0.1 mg/mL ampicillin), in each case at 37 °C and 180 rpm for 18 h. For FDH_*Pyrfu*, FDH_*Mettf* and FDH_*Trepo*, longer expression times with slower cell growths were necessary. BL21(DE3) cells were grown in TBA (Terrific broth supplemented with 0.1 mg/mL ampicillin) and JM109(DE3) cells were grown in ZYP-5052, in each case at 30 °C and 100 rpm for 42 h. Cells transformed with pETMCSIII containing a blank insert as well as blank untransformed cells were also grown under all the sets of conditions described. Following cell growth, the cells were harvested by centrifugation at 4000*g* for 15 min. Harvested cells were resuspended and washed with sterile deionised water, then re-collected. The usual yield was approximately 3–5 g of wet cell pellet from 1 L of cell culture solution. After cell lysis, protein purification was carried out using a Ni-ion affinity chromatography column (His Gravi-Trap).

2.2. Whole-cell biocatalyst reactions

Method 1. Under an H_2 atmosphere. Wet cell pellet was weighed and resuspended in 50 mM sodium phosphate buffer, pH 7.0, containing 0.25 M sodium bicarbonate as a source of CO_2, to a final concentration of 0.5 g wet cells per mL. The mixture was placed in a two-necked flask and the overhead flask space was purged with H_2, before the mixture was gently agitated using a magnetic stirrer under a balloon of H_2 connected to one of the necks, while being incubated at 37 °C. Reaction samples were withdrawn at intervals through a suba-seal in the other flask neck. These were centrifuged at 8000*g* for 5 min and the supernatants were passed through a YM-3 centrifugation filter (Millipore), before they were injected onto a Waters Alliance HPLC-UV system, fitted with a Rezex ROA-Organic Acid H+ (8%) column (Phenomenex, 300 × 7.8 mm with 50 × 7.8 mm guard column), at 25 °C with the detector set at 210 nm for quantitation of formate. The mobile phase was isocratic 5 mN H_2SO_4 at a flow rate of 0.5 mL/min, with formate eluting at 20.7 min.

Method 2. Sparging with H_2. This procedure was directly analogous to that of Method 1, except that a three-necked flask was used and the neck used for withdrawing samples was also fitted to the inlet of a peristaltic pump (Gilson-MINIPULS 3) with PVC (Tygon) tubing (1.3 mm internal diameter) to withdraw gas from the overhead space. The pump output was passed through the third neck and a filter with a sparging frit submerged in the whole-cell mixture, at a flow rate of 6.15 mL/min. Before incubation of the mixture the tubing was purged with H_2.

3. Results and discussion

3.1. Enzyme expression

All FDH genes were recombinantly inserted into the pETMCSIII vector, transformed successfully into *E. coli* BL21(DE3) and JM109(DE3) cells, and expressed as the respective enzymes. It was found that cell growth conditions were important for protein expression. In the cases of FDH_*Pyrfu*, FDH_*Trepo* and FDH_*Mettf*, which to the best of our knowledge have not been expressed previously, it was necessary to reduce the growth rate to induce heterologous expression, by lowering the temperature (30 °C) and reducing cell aeration (100 rpm). The expressed FDHs were iso-