POC-7-minton-C1net

Maximising reaction productivity through protein scaffolding with cohesion-dockerin domains

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Current energy and chemical needs are met by the extraction and processing of the fossil fuels. Such resources are finite and their use causes environmental pollution and greenhouse gas (GHG) emissions. The challenge facing humankind is, therefore, to identify new, sustainable and cleaner processes for chemical and energy generation. Biological routes represent a promising option, but strategies to date rely on the use of microbes to convert through fermentation the easily accessible carbohydrates (sugar and starch) of plants (such as sugar cane or corn) into chemicals and fuels. This has led to concerns over competition with the use of these carbohydrates as food, and a re-focussing of efforts on non-food, plant cell wall material (lignocellulose).

However, lignocellulose is extremely resistant to being broken down into the sugar needed for fermentation. Overcoming this recalcitrance in a cost effective manner is proving extremely challenging. There is, however, an exciting low-cost alternative which is to capture carbon directly, by harnessing the ability of certain bacteria to 'eat' single carbon GHG gases such as carbon monoxide (CO) and carbon dioxide (CO_2). The gas is injected into the liquid medium of fermentation vessels where it is consumed by the bacteria and converted into the chemicals we need. Fortunately, such gases are an abundant resource, and may be derived from non-food sources such as waste gases from industry as well as 'synthesis gas' produced from the gasification (heating) of non-food biomass and domestic/ agricultural wastes.

In this project, we aim to engineer the acetogenic bacterium *Eubacterium limosum* to produce high value, and industrially-relevant compounds, such as butanol and isopropanol. *E. limosum* has the ability to use the waste greenhouse gases CO and CO_2 as building blocks to make compounds such as acetate and butyrate, and therefore is an ideal host for the production of higher carbon chain compounds. We will use synthetic biology approaches for butanol and isopropanol pathway reconstruction and optimization. For simultaneous pathway reconstruction and optimization, we will use the design principles of macromolecular complexes known as cellulosomes. Within these complexes the enzymatic modules are docked on a scaffoldin module and their close proximity to each other allows for better reaction kinetics and thus higher reaction productivity. A combinatorial approach will be used to create the desired enzyme complexes with improved reaction productivity.

By using non-food, waste gas as a feedstock for our process organism, competition with food and land resources is avoided while at the same time providing benefits to the environment and society through a reduction in GHG emissions and the creation of a sustainable supply of essential chemical feedstocks.