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Dissemination Level		
PU	Public	
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	X
CO	Confidential, only for members of the consortium (including the Commission Services)	

SB4.0:
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Background information

The ***Synthetic Biology*** series of conferences is the flagship meeting of this field. These conferences have brought together a diverse group of participants from a variety of disciplines, including the leading experts in biological engineering, biochemistry and biophysics, molecular and cellular biology, computer science, electrical engineering, bioethics, policy and governance and the biotech industry. Each meeting has included approximately 30 invited speakers and 400 academic and industrial participants, including Nobel Laureates.

The mission of ***Synthetic Biology 4.0*** was to bring together researchers who are working to i) design and build biological parts, devices and integrated biological systems, ii) develop technologies that enable such work and iii) place this scientific and engineering research within its current and future social context. The conference has been a coordinated effort between HKUST, Hong Kong University, and Chinese University. Hong Kong provided an ideal location to explore the commercialization of Synthetic Biology in Asia as well as the launching of regional research and educational programs. Further, the meeting aimed at facilitating connections between researches and leaders in government, industry, and civic organizations.

The session ***Industrial Biotechnology*** provided examples for the manipulation of microorganisms for production purposes with special emphasis on energy consumption and pathway design.

George Guo-Qiang - Chen Tsinghua University: Application of Synthetic Biology in Industrial Biotechnology

George Guo-Qiang begun his presentation with the statement that one of the biggest impacts of Synthetic Biology will be on “Industrial Biotechnology” (or white Biotechnology) that exploits microorganisms for the production of energy, materials and chemicals. The limitations in yield and density, and slower production rates resulting from the competition to chemical production process, however, require a lot of energy input. Accordingly, he identified the following areas of Synthetic Biology as the ones that have the highest potential of impacting industrial biotechnology: i) ***Construction of synthetic pathways*** that allow cells to grow to high densities resulting in an enhancement of production yield, and ii) Construction of synthetic pathways that turn aerobic processes into ***micro- or anaerobic processes*** offering dramatic energy savings for microbial processing. These two principles were exemplified by the microbial production of the biopolymer PHA (intracellular polyester Polyhydroxyalkanoate), which is normally produced in inclusion bodies (accumulation up to 96% of cell weight). In economic terms, high cell density and allowing cells to continuously produce energy also during the stationary phase is crucial for cost reduction. Guo-Qiang’s group is working with *Pseudomonas putida* as production organism. Since it growth strictly aerobic, fermentation is associated with heavy oxygen consumption and due to the need for rigorous stirring during fermentation it has in general high energy consumption. The main objective of the presented work was to ***reduce oxygen consumption*** by introducing a facultative anaerobic

pathway to improve the energy balance. The ***synthetic anaerobic pathway*** was designed from six enzymes derived from an acetate pathway, an ethanol pathway, and a NADH oxidase gene in combination with anaerobic promoters. The resulting facultative anaerobic pseudomonas strain demonstrated the feasibility of constructing synthetic pathways that turn aerobic processes to micro- or anaerobic processes. As a result the PHB density increased from 29% to 48% (off dry weight). In addition to reduced energy consumption, there is a demand for an increased substrate to product transformation rate. In order to address this limitation, a ***reversed TCA cycle*** has been introduced. One way to improve the utilization of glucose is the recovery of CO_2 using this rTCA cycle. The first step in the construction of an *E. coli* strain containing an rTCA cycle was the deletion of the existing pathway of mixed acid fermentation, and subsequently exchanging TCA with five rTCA key enzymes. Using this approach 11 modifications were introduced in total, resulting in an increase of glucose to PHB (Polyhydroxybutyrate) conversion from 48% in the strictly aerobic environment to 64% using the engineered rTCA strain. The construction of gene clusters within the presented developments was performed according to biobrick guidelines. The commercial aspects were evaluated as such that the use of PHA as plastic is to date at least twice the price of conventional plastics like PE or PP. However, the use as biofuels should be cost competitive. This aspect is of special relevance since combustion heat is comparable to Ethanol. In addition, a production process to reduce costs (sludge-based) was presented, resulting in calculated costs as biodiesel at app. US$ 1500 per ton.

Qingsheng Qi - Shandong University: Exploiting the Novel Potential of Escherichia coli, an Industrialized Microorganism

In his introduction Qingsheng Qi characterized *E. coli* as a broadly applicable organism, with important features in i) basic research as a ***"model" bacterium*** and ii) in Synthetic Biology in terms of a ***"protein factory"*** and further also as a potential ***"chemical factory".*** Advantageous features include good growth behavior in minimal medium, the use of many kinds of carbon sources, and the setup of metabolic pathways for many chemicals. His research objective is the development of economic production strains by the substitution of expensive nutrition sources with ***cheaper biomass***. Metabolic approaches, including rational metabolic engineering, inverse metabolic engineering and random engineering strategies (based on random mutagenesis) were applied for the generation of a base strain. Improving substrate utilization capabilities were exemplified for xylose and fatty acids consumption, based on FadD and Acyl-CoA Synthetase introduction. By rational design a base strain has been developed that is able to consume sugars such as glucose, arabinose, xylose simultaneously. In addition, fatty acids, even short chain length fatty acids can be consumed simultaneously, and acetic acid and propionic acid can be incorporated more efficiently. ***Acid resistance capability*** could also be improved. In an effort to maximize the biomass production the ***reduction of acetate production*** was achieved by mutating four genes, resulting in an OD (optical density) shift from four to eighteen. Performing a screening for the regulator that might affect the biomass in order to shut down the side carbon flow, and regulating the flow path and flow channel resulted in a ***4.6-fold biomass increase***. Another aspect of industrial use relates to an improved induction system. Since IPTG is too expensive for industrial application another system based on rpoS genes (stress regulated sigma factor) was explored and resulted in expression, stronger than lac promoter, in the middle exponential growth phase. Taken together, this work has demonstrated that it is feasible and valuable to optimize *E. coli* for industrial applications. In this example the naturally occurring catabolite repression could be diminish, resulting in a strain that is able to consume a wide range of substrates (lactose, xylose, sucrose)

simultaneously, thereby producing maximized biomass and product accumulation. The substrate consumption was complemented by the development of a cheap and simple fermentation process including a suitable induction system for efficient recovery. Utilizing the modifications as a whole the PHA production by an engineered *E. coli* was shown to make up to 90% of cell dry weight (CDW).

Kristy Hawkins – Caltech: Metabolic Engineering of Saccharomyces cerevisiae for the Production of Benzylisoquinoline Alkaloids

Benzylisoquinoline alkaloids (BIAs) play a natural role in plant defense. BIAs represent more than 2500 elucidated structures, many of which possess ***therapeutic activities*** (anti-microbial, anti-cancer, anti-HIV), and the pathways and enzymes are in general well-characterized. Due to complex secondary metabolism and costly purification processes, metabolic engineering strategies are limited in plants. However, yeast is regarded a well suited production host for natural products (history of an industrial microbe, GRAS organism, ease of genetic manipulation) and displays additional advantages, like the functional expression of e.g. membrane associated proteins, or the production of rare intermediates and non-natural alkaloids. The overall goal of the presented work is the construction of yeast strains for the total biosynthesis of ***complex alkaloids from tyrosine***. The combination of *in vivo* conversion and chemical synthesis is regarded important for drug discovery and manufacturing. Different BIA production developments were presented, starting with the production of ***Reticulin*** from a commercially available substrate based on three methyltransferases. In a combinatorial testing approach the enzyme variants were structured in three selected combinations for chromosomal integration. In order to tune enzyme expression, the enzyme activity has been titrated individually using an engineered GAL promoter system (keeping the two other enzymes constant). The metabolite production could be regulated as a function of GAL concentration. After the tuning process the promoters have been replaced by a TEV7 promoter system. Additional examples of alkaloid production in yeast included production of ***berberine*** (using three enzymes), the ***morphinan alkaloids*** branch (CYP2D6 activity has been demonstrated), ***salutaridinol*** production (applying codon optimization of SalR has been shown to be beneficial since high level expression is required), ***thebaine*** production (developing a 2-stage process with demonstrated production of a precursor), and dopamine to ***norlaudanosoline*** (increased conversion to up to 35-fold based on knock-out mutants). The long term goal in biosynthesis of BIA backbone is the synthesis from tyrosine, including activities from other organisms. In conclusion the presentation provided foundational work in the engineering of yeast strains to produce all types of BIAs, supported by new methods of combinatorial testing of enzyme variants and a novel tuning strategy.