MANUFACTURING BIOPHARMACEUTICALS IN THE AGE OF ACCELERATION

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DEVELOPMENT OF LIVE BACTERIAL THERAPEUTICS

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"Bugs as Drugs" is an emerging therapeutic modality for treatment of diseases that are caused by or exacerbated by the bacteria inhabiting the human body. The rationale for pursuing these therapies is multi-fold, but largely revolves around the often-complex and multifactorial biology of these diseases, and poor druggability via conventional chemistry. For Seres' treatment approach via ecologies of natural commensal organisms, the challenge is not to engineer pathways in individual organisms but rather to engineer a set of organisms that will work to shift a diseased microbiome to one resembling a healthy person's. To accomplish this, a unique set of capabilities is required to identify strains of interest, screen leads and to test model ecologies. Our reverse translational approach relies on human and animal data sets, genetic sequence analysis techniques, computational models and libraries of bacteria. Finally, bioprocess, analytical and formulation know-how, along with cGMP manufacturing capability enables human clinical trial material generation and commercialization. Seres' capabilities will be discussed, along with our approaches to, and progress in, product development.

METABOLIC ENGINEERING STRATEGIES FOR PRODUCING OLEOCHEMICALS IN BACTERIA

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Key Words: Escherichia coli, oleochemical, fatty alcohol, thioesterase, thiolase

Finding a sustainable alternative for today's petrochemical industry is a major challenge facing chemical engineers and society at large. To be sustainable, routes for converting carbon dioxide and light into organic compounds for use as both fuels and chemical building blocks must be identified, understood, and engineered. Advances in metabolic engineering, synthetic biology, and other bioengineering disciplines have expanded the scope of what can be produced in a living organism. As in other engineering disciplines, synthetic biologists want to apply a general understanding of science (e.g. microbiology and biochemistry) to construct complex systems from well-characterized parts (e.g. DNA and protein). Once novel synthetic biological systems (e.g. enzymes for biofuel synthesis) are constructed, they must be engineered to function inside evolving cells without negatively impacting the host's physiology.

In this talk, I will describe pathways for producing high-value commodity chemicals derived from fatty-acids and how my group and others have combined synthetic biology and systems biology to improve oleochemical production in bacteria using sustainable feedstocks. The talk will describe the critical regulatory points in native fatty acid metabolism, strategies for deregulating the pathway, and alternatives that by-pass it altogether. I will highlight the use of heterologous plant and bacterial enzymes to alter the chain length distribution of products from common long-chain molecules to higher-value medium-chain analogs. I will also highlight strategies that we have used to produce medium-chain fatty alcohols, the highest value compounds in the class, through engineering of thioesterase and thiolase driven pathways. I will conclude with commentary on the remaining barriers to commercializing these technologies and areas where further research investment could prove fruitful.

IMPROVING BIOCHEMICAL YIELDS WITH MIXOFERM

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Key Words: Mixotrophy, Acetogen, Biochemical

Acetyl-CoA is a primary hub for metabolism and is the building block for most biochemicals of interest. However, the yields for biochemicals derived from acety-CoA are inherently limited because of the decarboxylation of pyruvate to acetyl-CoA which releases CO_2. To overcome this limitation, White Dog Labs (WDL) developed a fermentation technology call MixoFerm™ (also known as anaerobic, non-photosynthetic mixotrophy). This technology uses microorganisms capable of concurrently utilizing both organic (e.g., sugars) and inorganic (e.g., CO_2) substrates. Using MixoFerm, CO_2 can be fixed back into acetyl-CoA and thus improve biochemical yields (g product/g substrate consumed). Here, we demonstrate simultaneous utilization of both fructose and syngas by *Clostridium ljungdahlii* and *Clostridium autoethanogenum*. We next engineered *C. ljungdahlii* to produce the non-native metabolite acetone at a yield 35% greater than the theoretical maximum acetone yield without mixotrophy. Finally, we designed and generated a strain of *C. ljungdahlii* capable of consuming glucose, which the wild-type strain is unable to do. With the ability to improve biochemical yields, MixoFerm™ is a robust and flexible platform technology to improve process economics and product life-cycle analysis.

CYANOBACTERIA AS PHOTOSYNTHETIC FACTORIES: SYNTHETIC BIOLOGY METHODS IN THE DEVELOPMENT OF NEXT-GENERATION PRODUCTION PLATFORMS

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The aim of the project is to use synthetic biology to generate improved cyanobacterial cell factories for the production of desired metabolites directly from CO_2 and water using sun as the sole energy source with the emphasis specifically on the *production efficiency* of the systems. Importantly, the project generates a framework which can be used to assemble and optimize many different kinds of pathways in the cyanobacterial host *Synechocystis sp PCC 6803*. Significant effort has been placed on the development of assembly systems and characterization of carefully selected genetic components in *Synechocystis*. This system allows (i) flexible assembly of complex expression constructs in a relatively high throughput manner, (ii) quantitative evaluation of the systems *in vivo* using selected fluorescent markers, (iii) generation of optimized synthetic pathways with validated genetic regulatory systems. This study combines the power of the synthetic biology tools developed in the current work, and bioinformatics analysis of potential pathways to generate potentially interesting end-products

SYSTEMS METABOLIC ENGINEERING OF CORYNEBACTERIUM GLUTAMICUM AND BACILLUS METHANOLICUS FOR PRODUCTION OF NEW PRODUCTS FROM ALTERNATIVE CARBON SOURCES

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Amino acid production amounts to about 2 million tons of L-lysine and 3 million tons of L-glutamate per year [1]. Corynebacterium glutamicum is widely used in industry for amino acid production from sugars, while the methylotrophic Bacillus methanolicus produces L-lysine and L-glutamate from methanol with titers of about 50 g/L [2]. Both microbial hosts have been developed for production of specialty amino acids and amines. Specifically, I will present new tools for metabolic engineering of B. methanolicus and C. glutamicum including CRISPRi/dCas9, sigma factor engineering, genome reduction, induction withphotocaged IPTG, theta-type replicating vectors and biosensors [3-10]. On the other hand, I will describe examples of metabolic engineering of these hosts for the production of specialty amino acids and diamines. C. glutamicum strains for the production of L-pipecolic acid have been constructed [11] and these were improved a titer of 14.4 g L^{-1}, a volumetric productivity of 0.21 g L^{-1} h^{-1} and an overall yield of 0.20 g g^{-1} [12]. Moreover, access to production of L-pipecolic acid from glucose, glycerol, xylose, glucosamine, and starch has been enabled. Methanol-based production of the non-proteinogenic amino acid □-amino butyric acid (GABA), that finds application also as precursor for bioplastics, has been achieved with B. methanolicus [13]. Efficient production of GABA from hexose and pentose sugars has been realized and optimized in recombinant C. glutamicum strains as well [14,15]. Both for C. glutamicum and B. methanolicus characterization and engineering of product export have been instrumental [16,17].

In conclusion, the microbial cell factories B. methanolicus and C. glutamicum have been programmed for efficient and sustainable production of specialty amino acids and amines from diverse carbon feedstocks to foster their biotechnological applications.

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MOMENTUM: MICROBIAL OPTIMIZATION VIA METABOLIC NETWORK MINIMIZATION

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Key Words: Dynamic metabolic control, high-throughput metabolic engineering, metabolic network minimization, metabolic valves, robustness and scalability.

We report a high-throughput metabolic engineering platform enabling the rapid optimization of microbial production strains. The platform, which bridges a gap between current in vivo and in vitro bio-production approaches, relies on dynamic minimization of the active metabolic network and is implemented in the context of standardized 2-stage bio-processes. Dynamic metabolic network minimization is accomplished using combinations of CRISPR interference and controlled proteolysis to reduce the activity of multiple enzymes in essential central metabolism. This approach not only results in a design space with greatly reduced complexity, but also in increased metabolic fluxes and production rates as well as in strains which are robust to environmental conditions. Robustness leads to predictable scalability from high-throughput µL-scale screens, to fully instrumented L-scale bioreactors. Predictive high-throughput approaches are critical for metabolic engineering programs to truly take advantage of the rapidly increasing throughput and decreasing costs of synthetic biology. We have not only demonstrated proof of principle for this approach in two common industrial microbes: *E. coli* and *S. cerevisiae*, but also have validated this approach with the rapid optimization of *E. coli* strains producing two important industrial chemicals: alanine and mevalonic acid, at commercially meaningful rates, titers (147 g/L and 97 g/L, respectively), and yields.[1]

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NEW BIO-BASED SUPPLY CHAINS FOR PLANT-BASED MEDICINES

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Key Words: synthetic biology, metabolic engineering, plant natural products

Plants are a rich source of unique molecules, including 25% of natural-product-derived drugs. However, the discovery, synthesis, and overall material supply chains for sourcing plant-based medicines remain ad hoc, biased, and tedious. While microbial biosynthesis presents compelling alternatives to traditional approaches based on extraction from natural plant hosts, many challenges exist in the reconstruction of plant specialized metabolic pathways in microbial hosts. We have developed approaches to address the challenges that arise in the reconstruction of complex plant biosynthetic pathways in microbial hosts. We have utilized these strategies to develop yeast production platforms for an important class of plant alkaloids, which include the medicinal opioids and noscapinoids. The intersection of synthetic biology, genomics, and informatics will lead to transformative advances in how we make and discover essential medicines.

LEARNING FROM THE MAMMALIAN EXPRESSION SYSTEM TO DEVELOP A HIGH TITER HALF-ANTIBODY PROCESS IN E. COLI

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Key Words: *E. coli*, half-antibody, chaperone

While producing half-antibodies for bispecific antibody therapeutics in *E. coli* has its advantages over a mammalian expression system, it also comes with challenges associated with soluble expression and effective folding of these complex proteins. The merits and challenges of using a microbial host for half-antibody production will be discussed, with a focus on a chaperone overexpression strategy to achieve high titers in a short amount of time. To develop a high titer *E. coli* process, we looked to the mammalian expression system to better understand the mechanisms involved in antibody folding. By overexpressing the endogenous *E. coli* protein FkpA, we can facilitate similar folding mechanisms, suggesting that FkpA could be acting as an analog to both the mammalian chaperone BiP and ppiase CypB involved in antibody folding in CHO cells.

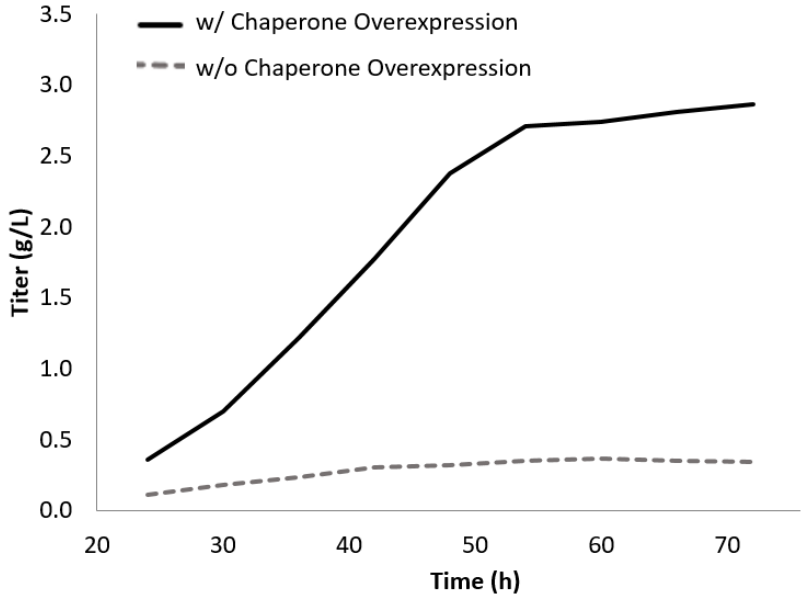

Figure 1 – Overexpression of chaperones yielded an approximate 10-fold increase in titer for this example half-antibody

MATCHING SECRETION CAPACITY VIA TRANSLATIONAL CONTROL

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Key Words: Recombinant protein production, gene expression control, RNA-based control devices, secretion.

Inducible gene expression systems commonly employed in microbial hosts are mostly composed of molecular components derived from the lactose (lac), arabinose (ara), rhamnose (rha) and tetracycline (tet) operons. These transcription-level control systems have been widely employed in research and industrial-scale protein production, however they are known to exhibit important limitations. These include; all-or-none (digital) expression profiles, stochastic transcriptional bursting, and heterogeneous expression responses at sub-maximal induction conditions. A new paradigm in genetic regulation emerged with the discovery of genetic regulatory elements within the 5'UTR of bacterial mRNA [1]. Upon binding to a specific metabolite, these so-called riboswitches change conformation, permitting differential gene regulation to occur. To allow us to utilize this alternative mechanism of genetic regulation we developed and characterized a novel recombinant expression system, termed *RiboTite* [2]. The system operates at both the transcriptional and translational level, using standard inducible promoters and translational-ON riboswitches respectively, collectively providing a multi-layered modular genetic circuit controlling both bacteriophage T7 RNA polymerase and recombinant gene(s) of interest [3]. The precise cellular-level tunable expression control afforded by this system offers a number of potential applications in terms of matching cellular expression rate to host synthetic and processing capacity. Here we will report one such application, where we used the *RiboTite* system to avoid the overload of secYEG translocon in *E. coli*, permitting expression/secretion attenuation of recombinant proteins into periplasmic space (Figure 1). Utilizing a library of different signal peptides that target the recombinant protein to secYEG, either via the post-translational (SecB/A) or the co-translational (SRP) pathway, we have demonstrated successful attenuation of recombinant protein reaching the periplasm. Finally under fed-batch fermentation conditions the system has been demonstrated to avoid the overload of the host secretion machinery and produce scFv antibody fragments at industrially relevant titers.

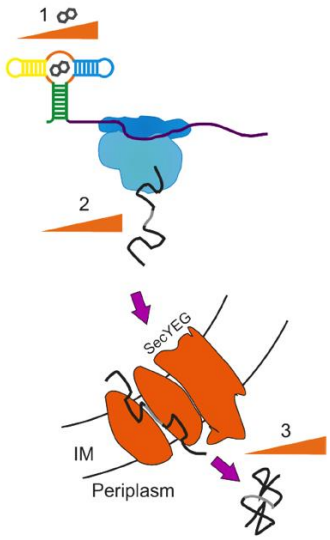

Figure 2 – Matching expression demand to secretion capacity

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C1: HOW THE C1 PLATFORM WILL CHANGE THE PRODUCTION APPROACH FOR THERAPEUTIC PROTEINS

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Key Words: C1, *Myceliopthora thermophile,* mAbs, Glycoengineering, Vaccines

For over 30 years, Dyadic has proven itself, both commercially and scientifically, to be a high quality and highly productive producer of enzymes and proteins for specialty chemical applications using a proprietary and patented expression system based on the *Myceliopthora thermophila* fungus, nicknamed C1.

The C1 platform technology, a hyper-productive fungal expression system, was used to develop & manufacture large quantities of desired proteins at industrial scale at significantly lower capital and operating expenditures. In this presentation we shall demonstrate how the benefits of C1 as a successful production host is now being harnessed by Dyadic to produce biological medicines and vaccines.

Using new and improved C1 base strains along with better molecular genetics tools that have been developed over the past several years, we demonstrate the ability of C1 to express mAbs that are secreted, folded correctly and reach high yields. MAbs produced in C1 have almost identical binding kinetics to mAbs produced using CHO cells. In addition, our research program includes comprehensive approach to identify and knock-out proteases for further enhancing protein stability and improving yields.

We have also achieved encouraging results, knowledge and experience in the rVaccine space from our prior research collaboration with Sanofi Pasteur to express rVaccines against Influenza virus. Results of a mice test that was conducted by Sanofi Pasteur, clearly demonstrated that HA produced by C1, generated high immunogenicity response against the virus without any adverse affects.

Like other filamentous fungal strains, C1 has high mannose glycoform structures. However, unlike most fungi and yeasts, C1 does not have ‘high’ mannose (branched 30-50 mannose species), but rather has ‘oligo’ mannose structure (branched 5-9 mannose species). In addition, no O-glycosylation has been observed on C1 secreted proteins, in contrast to *Pichia* that O-glycosylates all secreted antibodies, necessitating deletion of the O-glycosylation machinery. Using the benefits of those advantages, we have started Glycoengineering program aiming to develop C1 strain that produces proteins with defined human-like glycan patterns. The progress we have already made in C1 glycoengineering will also be presented.

Thus, Dyadic firmly believe that the C1 strains that we are developing with offer certain competitive advantages over other leading pharmaceutical expression systems, such as CHO cells, has the potential to become the production system of choice for therapeutic protein and vaccines manufacturing.

GRAM LEVEL scFv EXPRESSION PLATFORM OF *PICHIA PASTORIS*

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Key Words: Pichia pastrois, secretion, methanol, scFv, antibody.

The methylotrophic yeast *Pichia pastoris* secretion expression system has been developed for the antibody fragments (scFv) production platform. The platform includes three technology platforms, the first one is strain generation, the second is fermentation process development in 250 ml fermentor and the last is process scale up to 5 L. A recombinant scFv went through clone generation, include signal peptide tool box, normally yield 2.5 mg/L titer in deep well. Through the fermentation process development of induction medium composition and feeding strategy by Eppendorf Dasgip parallel 250 ml mini fermentor. During induction step, feeding 100% methanol as induction medium can only produce less than 50 mg/L scFv while feeding methanol-sorbitol mixture can significant increase the production yield to 306 mg/L in five days, about 6-folds increase in productivity. With the supply of additional nitrogen source during glycerol feeding step or at induction step, higher scFv production with 510 mg/L can be achieved. Thus, following the medium composition optimization, the production titer was improved 10 folds in 250 ml mini-fermentor stage. Moreover, when we switched the induction medium feeding strategy from DO-stat to the stepwise feeding, the titer increased form 510 mg/L to ~1000 mg/L and yielded another 2- folds improvement. During medium composition and feeding strategy optimization at 250 ml mini fermentor scale, the production titer could increase 20 folds. Overall, the production titer increased 400 folds from cell line generation to 250 ml fermentation parameter optimization. Furthermore, the process parameter can be scale-up to 5 L fernentor achieving > 1 g/L. Recent progress to include BIP in the expression vector gave at least 2 fold improvement in scFv titer in shake flask, the new clone will be optimized in our established 250 ml and 5 L fermentation platform.

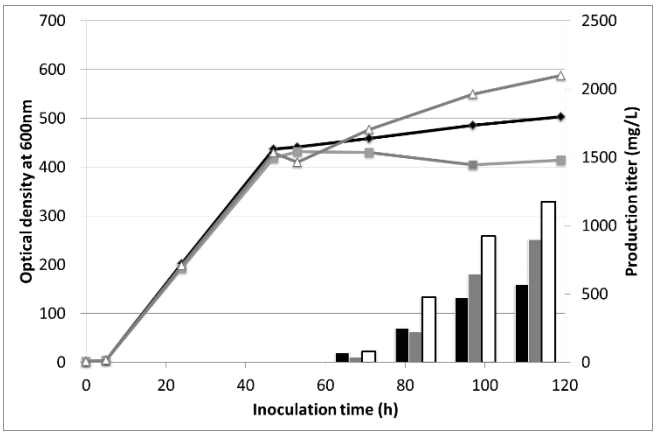

Figure 1. Biomass optical density profile and production titer using different feeding strategy. White column and triangle, methanol and sorbitol mixture feed by constant feeding rate. Gray column and squared, methanol and sorbitol i feed separately by using constant feeding rate and DO-stat feeding strategy, respectively; Black column and diamond, methanol and sorbitol mixture feed by using DO-stat feeding strategy;

MRNA VACCINES AND THERAPEUTICS: ON THE PROGRESS FROM PROMISE TO REALITY

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Messenger RNA offers unparalleled breadth and depth for the discovery and development of novel drugs and vaccines. The potential to direct specific tissue translation of both wild type and engineered intracellular, membrane-bound, and secreted proteins (and combinations thereof), coupled with rapid transition from preclinical to clinical development, has enabled Moderna and its partners to progress several development candidates into the clinic. This potential is being realized by the parallel development of different modalities (e.g. prophylactic and therapeutic vaccines, paracrine and systemic drugs). This talk will review the scientific and engineering accomplishments enabling pharmaceutical development.

NATURAL PRODUCTS BY SYNTHETIC BIOLOGY AND MICROBIAL ENGINEERING

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Key Words: Combinatorial biosynthesis, Genome mining, Microbial engineering, Synthetic biology

Natural products are made from simple building blocks, the structural diversity found in natural products is the result of Nature's intrinsic use of combinatorial biosynthesis, and recent progress in microbial genomics and synthetic biology has sparked the emergence of a suite of contemporary approaches to natural products by microbial engineering and fermentation. Current strategies are mainly based on the collective knowledge of genetics, microbiology, evolution, enzymology, and structural biology that governs the natural product biosynthetic machinery. While successful, they are limited by what information is gleaned from the above disciplines and how that information can be applied to construct the designer pathways. Nature has used evolution over billions of years to become an expert in combinatorial biosynthesis and microbial engineering, and we have only begun to tap into this knowledge. Selected examples from our current researches will be presented to highlight the opportunities in accessing natural products and expanding natural product structural diversity by exploring the vast combinatorial biosynthesis repertoire found in Nature.

AN INTEGRATED STRAIN IMPROVEMENT AND PROCESS DEVELOPMENT PROGRAM FOR THE PRODUCTION OF UK-2A, THE PRECURSOR OF THE FUNGICIDE INATREQ™ ACTIVE

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Key Words: natural product, secondary metabolite, fermentation, fungicide

Secondary metabolites produced by Actinobacteria serve a variety of functions including molecules having agricultural applications. *Streptomyces sp. 517-02* produces a novel fungicidal compound called UK-2A, which through a single step chemical reaction is converted to Inatreq™ active. Inatreq utilizes a unique target site of action, ubiquinone reductase Qi site (inner side of membrane), and is intended for use in cereals and banana markets with strong residual protectant and curative activity in wheat against *Zymoseptoria tritici*, with additional activity on rust and other diseases. To achieve a commercially viable process an integrated multidiscipline approach was applied in parallel including mutagenesis, high-throughput (HTP) screening, fermentation process optimization, and targeted genetic engineering. The presentation will review how the integrated approach contributed to a rapid acceleration in productivity gains resulting in a 75% improvement in titer over a one-year period, more than a 2 fold improvement in 4 years and successful scale-up to the final commercial production plant.

Examples of topics to be discussed:

- Development, deployment, and optimization of the mutagenesis and high-throughput screening process for the selection of improved strains.
- Deployment of targeted genetic engineering to alleviate biosynthesis bottlenecks identified using approaches such as biosynthetic gene overexpression and precursor feeding.
- Use of “omics” tools to identify native promoters which permit temporal gene expression suitable for enhanced precursor production and increased UK-2A production.
- Vetting of new strains and fermentation process improvements both in bioreactors at multiple scales and in the downstream process for product recovery.
- Use of experimental results from across the integrated program to guide prioritization of strain and process improvement targets.
- Incorporation of final product design and performance requirements into the program with a line of sight to manufacturing process constraints.

WITHDRAWN

ONE-POT SYNTHESIS OF AMINO-ALCOHOL USING A *DE NOVO* TRANSKETOLASE:TRANSAMINASE PATHWAY IN *PICHIA PASTORIS* STRAIN GS115

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Key Words: transaminase; transketolase; biocatalyst; chiral amino-alcohols, whole cell biocatalysis

Pichia pastoris (*P. pastoris*) is an attractive industrial host cell due to its ability to grow up to 60% wet cell weight (WCW) by volume, a far higher level of biomass than the typical values reached by *Escherichia coli (E. coli*) and *Saccharomyces cerevisiae*. This thesis seeks to explore how the genetic tractability and high cell densities characteristic of *P. pastoris* can be exploited to intensify whole-cell biocatalysis.

Chiral amino alcohols such as 2-amino-1,3,4-butanetriol (ABT) are key building blocks of small molecule pharmaceuticals and have previously been produced by whole-cell biocatalysis using cells engineered to overexpress a *de novo* enzyme pathway consisting of transketolase and transaminase.

Within this work, native and foreign *P. pastoris* transaminases were characterized with respect to their biocatalytic potential. Genomic data mining was performed to explore the GS115 strain genome, allowing the selection of three putative Class III transaminase genes and the construction of overexpressor strains PpTAm107, PpTAm677 and PpTAm410. The well-studied ω-transaminase CV2025 from *Chromobacterium violaceum* was also successfully engineered to generate two strains; PpTAmCV708 for single expression of CV2025, and PpTAm-TK16 strain for CV2025 co-expression alongside a native transketolase previously characterized for L-erythrulose production.

The rapid growth and high biomass characteristics of *P. pastoris* were successfully exploited for production of ABT by whole-cell biocatalysis. At high cell density, the best performance for the *de novo* pathway was obtained with the engineered PpTAm-TK16 strain, which tolerated high concentrations of substrate to achieve STY 0.57 g L^{-1} h^{-1} of ABT, 40-fold higher than levels previously achieved with *E. coli* for the same reaction.

MICROBIAL ENGINEERING OF NEW STREPTOMYCES SP. FROM EXTREME ENVIRONMENTS FOR NOVEL ANTIBIOTICS AND ANTICANCER DRUGS

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Keywords : streptomyces, antibiotics, anticancer drugs

Today there is a tremendous need for new antibiotics and novel cytotoxic compounds against cancer cells to develop efficient alternative treatment to chemotherapy. We have searched for highly active Streptomyces strains in the driest desert in the world, the Atacama desert in northern Chile. We have identified several new strains and found many novel antibiotics and anticancer agents (“Chaxamycins”, “Chaxalactins” and “Atacamycins”) from Streptomyces C34 and C38.

A genome scale model of the metabolism of *Streptomyces leeuwenhoekii* C34 has been developed from its genome sequence. The model, iVR1007, has 1726 reactions including 239 for transport, reactions for secondary metabolite biosynthesis, 1463 metabolites and 1007 genes. The model was validated with experimental data of growth in 89, 54 and 23 sole carbon, nitrogen and phosphorous sources, respectively, and showed a high level of accuracy (82.5 %). We have included reactions for desferrioxamines, ectoine, Chaxamycins, Chaxalactins and for the hybrid polyketides/non-ribosomal peptide synthesized by the halogenase cluster. A detailed Metabolic Flux Balance Analysis was carried out in order to study the metabolic pathways of Chaxalactins, Chaxamycins and the product of the halogenase cluster, by recognizing overexpression targets and useful knock-out sites to increase production of these secondary metabolites.

Alternatively we have identified the gene cluster in *S. leeuwenhoekii* C34 responsible for the biosynthesis of the Chaxamycins and Chaxalactins and have cloned the whole gene cluster in a much more efficient strain of Streptomyces, namely *S. coelicolor A3* whose heterologous expression of gene clusters from other Streptomyces strains has been successfully tested. Our recent results concerning these two alternative strategies for identification and overproduction of these important secondary metabolites will be presented and discussed in this presentation.

GENOME-GUIDED METHODS FOR DISCOVERING NEW NATURAL PRODUCT FROM FUNGI

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Key Words: Polyketide, Yeast, heterologous expression, natural product, terpenes

For decades, fungi have been an important source of medically relevant natural products (NPs). Recent advances in DNA sequencing have revealed that the biosynthetic potential of fungal genomes is much deeper than previously realized. Difficulties in culturing and genetically engineering many fungi, combined with the fact that many NP biosynthetic gene clusters (BGCs) are not expressed under standard laboratory conditions has lead to much of this biosynthetic potential remaining untapped. Here we describe the realization of a pipeline based in *S. cerevisiae* encompassing bioinformatic tools for BGC curation, genetic parts for BGC refactoring, and improved DNA assembly for BGC building.

With this pipeline, we have successfully detected novel NPs from several previously unstudied fungal BGCs, and have structurally characterized a subset of the BGC-associated compounds. We also developed activity-guided methods to discover natural products of new function, and validated the biological activity using higher-order model systems. Our pipeline demonstrates how high-throughput synthetic biology tools can facilitate the rapid discovery of complex chemical scaffolds of potential pharmaceutical relevance and their production in model fungal hosts.

AN EFFICIENT COMMERCIAL PLATFORM FOR MICROBIAL ENGINEERING OF NATURAL PRODUCTS

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Plants produce a variety of rare natural products which are used in our daily lives as flavors, fragrances, food ingredients, cosmetics, vitamins, pharmaceuticals and agricultural chemicals. Despite their intrinsic value, however, sourcing remains a bottleneck to more widespread use due to their low abundance in nature. Manus Bio has established an innovative platform technology for engineering microbial factories grounded in modular and data-driven design and developed a commercial organism which can produce a myriad of typically ultra-rare and costly ingredients used in our daily lives. These microbes are capable of converting carbon feedstock to the product at high yields and have been adapted to produce a mature pipeline of products, or "BioAssemblyLine." To engineer our “BioAssemblyLine,” we integrate three core technologies - our proprietary Multivariate Modular Metabolic Engineering (MMME), Pathway Integrated Protein Engineering (PIPE) and Integrated Multivariate Omics Analysis (IMOA) platforms. In this presentation, we will highlight several important insights and guiding principles established to engineer our “BioAssemblyLine” microbial factories.

ENGINEERED POLYKETIDE SYNTHASES FOR PRODUCTION OF COMMODITY AND SPECIALTY CHEMICALS

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Engineered modular polyketide synthases (PKSs) have the potential to be an extraordinarily effective retrosynthesis platform. Native PKSs assemble and tailor simple, readily available cellular acyl-CoAs into large, complex, chiral molecules. By successfully rearranging existing polyketide modules and domains, one can exquisitely control chemical structure from DNA sequence alone. As an example of the diverse biosynthetic potential of PKSs, we have concluded that approximately 20 of the roughly 150 commodity chemicals tracked by the petrochemical market information provider ICIS could be produced by mixing and matching naturally occurring PKS domains. To form these chemicals, engineered PKSs load acyl- CoAs, perform a programmed number of extension reactions, and then release products using previously published mechanisms. However, this potential has only just begun to be realized as the compounds that have been made using engineered PKSs represent a small fraction of the potentially accessible chemical space. In my talk, I will highlight work from our laboratory in which we have engineered polyketide synthases to produce a variety of commodity and specialty chemicals and expressed these engineered PKSs in a variety of *Streptomyces* for production of these molecules from sugars and other inexpensive starting materials.

INTEGRATED BIOENGINEERING: GENOMATICA'S APPROACH TO RAPID COMMERCIALIZATION

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Key Words: Genomatica, 1,4-butanediol, fermentation, renewable chemicals

Genomatica has developed a complete, commercial-scale bioprocess for production of 1,4-butanediol (BDO) directly from renewable carbohydrates. This process is being operated by Novamont at their MATER-Biotech plant in Bottrighe, Italy; the world's first commercial-scale biobased plant for a major intermediate chemical. The biobased BDO is being utilized within Novamont's 4th generation of MATER-BI, an innovative family of biodegradeable and compostable bioplastics.

This presentation will highlight the development of the GENO BDO™ process using the integrated approach inherent to Genomatica's bioengineering platform. This platform enables a 'whole-process' approach linking computation, microorganism design, synthetic biology, process design, techno-economics, and technology transfer. A key differentiator is an emphasis on techno-economic analysis and scale-up from the very start; using this to guide decision making on organism, fermentation, and downstream process design. Combining this integrated approach with real-world commercial expertise enabled highly reliable and predictable scale-up across multiple orders of magnitude, culminating in the successful startup of the MATER-Biotech plant.

AUTOMATING BIOENGINEERING: FIRST THE HANDS, THEN THE HEAD

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Key Words: synthetic biology, industrial biotechnology, automation, machine learning, artificial intelligence

Amyris was founded over a decade ago on the premise that synthetic biology could help address some of the world's most pressing challenges. Focusing on supply-limited molecules of societal and economic value such as medicines, nutrients, and commodity & specialty chemicals, Amyris has enabled their cost-effective and sustainable production by building a high-throughput genetic engineering platform coupled to industrial scale fermentation processes. Until 2015 this platform primarily aimed to replace the "hands" in the lab, using automation & computing to perform routine calculations and liquid manipulations for the reproducible construction of engineered microbes. Since then we have focused on replacing the "head" in the lab by implementing a suite of computational algorithms to design metabolic pathways, direct their synthesis and phenotyping, learn from the data, and iterate on the design for improved target molecule production. **In this presentation I will describe our progress on joining the hands to the head by attempting to create discrete strains that produce 450 novel molecules in 24 months.** This effort involved first creating an expert system that successfully directed the creation of >110,000 in silico strain designs, identification of >14,000 metabolic routes, ordering of >2,400 genes, assembly of >280 million bps of DNA, and generation of >32,000 distinct strains. We are now collecting >400,000 data points each week, measurements that are fed back into proprietary machine learning and optimization algorithms to drive strain re-design.

THE CIRCULAR BIO-ECONOMY AND THE CONCEPT OF THE BIOREFINERY

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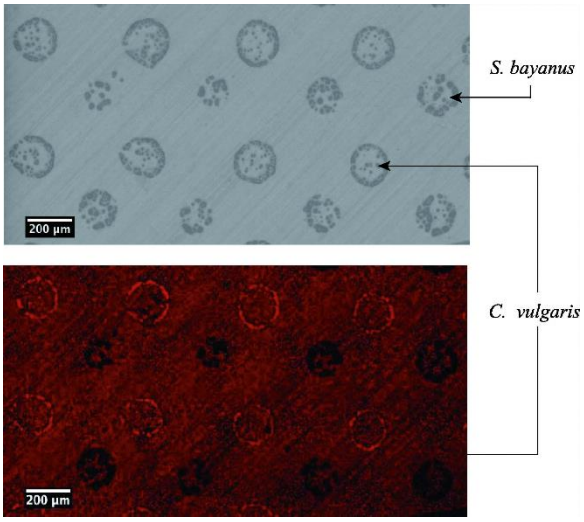
Key Words: Industrial Biotechnology, industrial ecology, Circular economy, Energetic transition,

The world is living through a biotechnological and energetic transition phase. The 200-year-old model of a linear economy with its low energy cost and waste producing economy is being challenged by the exhaustion of the planet's natural resources and by the accumulation of high levels of waste in the environment. Access to clean, renewable energy and fresh water supplies will determine the growth and development of future economies. Globalisation offers a new opportunity for addressing these challenges on a global scale.



Laser-printed micro colonies of *Saccharomyces cerevisiae* and *Chlorella vulgaris*. By printing the organisms in close proximity, they seemed to have benefited from each other's presence resulting in fast growth of the microlgae (Taidi *et al.* (2016) Colony development of laser printed eukaryotic (yeast and microalga) microorganisms in co-culture. International Journal of Bioprinting (2016)–Volume 2, Issue 2).

One model that is emerging all over the world is that of the biorefinery. This model of a circular economy with little waste production takes inspiration from the current oil based network of oil refining and distribution. The circular bio-economy takes inspiration from the agricultural model in place before humanity's access to cheap and accessible sources of fossil fuel.

Two biorefining models exist namely, the port based model and the local model. The former treats biomass as a commodity that can be imported through ports, fractioned *in situ* and distributed again though the same port. This model strongly resembles the oil-based economy. The latter approach differs in that the biorefinery is placed near the production site where the biomass is treated and used locally. This model resembles the old agricultural model.

Biotechnological research centres are being funded and developed on an international level with many countries investing in the foundation of their own national research centres on biotechnology. The Chair of Biotechnology of CentraleSupélec (CS), attached to the LGPM (Process Engineering and Materials) Department, is housed in such centre; CEBB (European Centre for the Biotechnology and the Bioeconomy).

This presentation will examine the rise of biorefineries and will outline some of the recent scientific advances of the of the Biotransformation team of the Chair of Biotechnology of CS.

EXPLOITING FATTY ACID METABOLIC PATHWAY FOR PRODUCTION OF SHORT CHAIN FATTY ACIDS IN *E. coli*

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Keywords**:** Thioesterase, Short chain fatty acids, Butyric acid, *Escherichia coli*, Fed-batch fermentation

Worldwide demand of sustainable fuels and chemicals has encouraged researchers for microbial synthesis of short chain fatty acids (SCFAs), such as such as butyric acid (C4), as they are attractive precursors to replace petroleum-based fuels and chemicals.

In this study, we explored the fatty acid metabolism for production of butyric acid in *E. coli* with the help of three thioesterases, i.e., TesAT from *Anaerococcus tetradius*, TesBF from *Bryantella formatexigens* and TesBT from *Bacteroides thetaiotaomicron*.

We found that *E. coli* strain transformed with gene for TesBT and grown in presence of 8 g/L glucose produced maximum butyric acid titer at 1.46 g/L, followed by that of TesBF at 0.85 g/L and TesAT at 0.12 g/L, showing that these thioesterases were efficiently converting short chain fatty acyl-ACP into corresponding acid. The titer of butyric acid varied significantly depending upon the strain genotype and plasmid copy number. Deletion of genes involved in initiating the fatty acid degradation such as fatty acyl-CoA synthetase and acyl-CoA dehydrogenase and overexpression of FadR, which is a dual transcriptional regulator, exerts negative control over fatty acid degradation pathway, reduced up to 30% of butyric acid titer. This observation suggested that β-oxidation pathway is working synergistically with fatty acid synthesis pathway in production of butyric acid. Moreover, accelerating the fatty acid elongation cycle by overexpressing acetyl-CoA carboxyltransferase (Acc) and 3-hydroxy-acyl-ACP dehydratase (FabZ) or by deleting FabR, the transcription suppressor of elongation, did not improve the butyric acid titer, rather favored the long chain fatty acid production. Use of chemical inhibitor cerulenin, which limits the fatty acid elongation cycle, increased the butyric acid titer by 1.7-fold in case of TesBF, while it had adverse impact in case of TesBT. *In vitro* enzyme assay showed that cerulenin also inhibited the short chain specific thioesterases, though inhibitory concentration varied according to the type of thioesterase used.

Further improvement in butyric acid was achieved by process optimization. Owing to the same pathway for both cell growth and butyric acid production, a balance was achieved between the two by growing the cells under nutrient and oxygen limiting condition. Keeping these factors in mind, a fed-batch cultivation strategy was devised for production of butyric acid in phosphorous and carbon limiting condition. Finally, we obtained 14.3 g/L of butyric acid and 17.5 g/L of total free fatty acid.

The strategy used in this study resulted in highest reported titers of butyric acid and free fatty acids in engineered *E. coli* and could be used to replace the traditional chemical methods for production of butyric acid.

ANTIBODY PRODUCTION IN MICRO-ORGANISMS

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Global demand for monoclonal antibody-based therapeutics (Mab's) far exceeds current production capacity, and is expected to continue to grow based on current development pipelines. Despite their proven efficacy in a large number of indications, equitable use of these drugs is limited by the high cost of CHO-cell based production and purification. Micro-organisms such as yeasts and filamentous fungi present an attractive alternative for antibody production, but will require extensive genetic modification to achieve both high titers and mammalian-like glycosylation patterns in a secreted product that is easily purified. Towards this end, we developed state-of-the-art genetic engineering tools for eight micro-organisms to enable the highly efficient, targeted multiplexed integrations necessary for antibody production in these hosts. We demonstrated successful antibody production in several of these micro-organisms, paving the way to low-cost microbial fermentation to replace CHO fermentation.

PROCESS OPTIMIZATION, MANUFACTURING CHANGES FROM EARLY TO LATE PHASE DEVELOPMENT, AND COMPARABILITY OF RESOLARIS

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Key Words: Manufacturing process changes, scale-up, product quality, comparability

Resolaris is an *E.coli* expressed recombinant protein derived from human histidyl-tRNA synthetase, and is currently in clinical development for the treatment of severe, rare myopathies with an immune component. The manufacturing process initially developed at laboratory scale resulted in a high degree of product purity. After process transfer to CMO#1 and scale-up to 225L, the purified material unexpectedly had significantly high levels of product related impurities. Two major impurity species were isolated and identified by peptide mapping via LC-MS/MS as norleucine for methionine misincorporation and a clipped form. The norleucine misincorporation was eliminated by amino acid supplementation during fermentation, but supplementation also increased levels of process impurities, later identified as RNA. The clipped form and increased RNA levels were minimized and controlled through modification in both fermentation and downstream procedures. Multiple GMP batches were manufactured (Process1.0) and product used in Phase 1/2a clinical studies. In order to support late phase clinical development and commercialization, the expression vector and cell line were changed to remove the protease and increase productivity. In addition to the improvement in the manufacturing process, the manufacturing site was changed (CMO#2), and further, the drug substance and drug product formulation was also changed to enhance stability. Multiple batches of Resolaris have been manufactured at 2000L scale at CMO#2 (Process 2.0), with product output per batch increased approximately 30-fold *vs.* Process 1.0. In accordance with ICH Q5E, side-by-side analytical comparability and forced degradation studies on representative batches from both processes demonstrated that products from both processes are comparable and acceptable for late phase clinical development.

PROMOTER AND PROCESS ENGINEERING FOR RECOMBINANT PROTEIN PRODUCTION IN PICHIA PASTORIS TOWARDS SIMPLE, FAST AND METHANOL-FREE CULTIVATION REGIMES AND HIGH PRODUCT TITERS

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Key Words: Recombinant protein production, promoter engineering, process engineering, *Pichia pastoris*.

Protein production in *Pichia pastoris* often applies methanol-induced gene promoters such as P_{AOX1} to drive the expression of the target gene. The use of methanol has major drawbacks, so there is a demand for alternative promoters with good induction properties independent of methanol such as the P_{GTH1} promoter which we reported recently [1]. In order to further increase its potential, we investigated its regulation in more detail by screening of promoter variants harbouring deletions and mutations. Thereby we could identify the main regulatory region and important transcription factor binding sites of P_{GTH1}. We also created a P_{GTH1} variant, called $P_{G1\text{-}3}$, with greatly enhanced induction properties compared to the wild type promoter.

Model based process engineering could successfully be implemented for $P_{G1\text{-}3}$ to outperform the P_{AOX1}-driven production in a simple feed regime, and to establish a speed fermentation with high titers after only two days total fermentation time.

[1] Prielhofer, R.; Maurer, M.; Klein, J.; Wenger, J.; Kiziak, C.; Gasser, B.; Mattanovich, D., Induction without methanol: novel regulated promoters enable high-level expression in *Pichia pastoris*. Microb Cell Fact 2013, 12 (1), 5.

TOWARDS EXTRACELLULAR SECRETION OF RECOMBINANT PROTEINS USING ANTISENSE TECHNOLOGY

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Key Words: E.coli Fermentation, Process Development, Optimization & Characterization.

Biopharmaceutical industries have been exploiting microbial organisms such as Escherichia coli to manufacture recombinant proteins that mostly are intended for therapeutic applications. For extraction of accumulated proteins in the periplasm, a method of cell lysis must take place which has a number of significant drawbacks such as numerous recovery and purification steps which leads to further reduction of the overall yield. Also, as well as the product, contaminants such as DNA and HCP are released which may be difficult to remove. Antisense technology offers a platform that once optimized can reduce the named drawbacks. Antisense RNA can target and inhibit the synthesis of proteins made by the cell, particularly outer membrane proteins in order to facilitate secretion of the recombinant product out of the cell during fermentation. This project aims to investigate the impact of inhibition of synthesis of selected outer membrane proteins on the secretion levels, thus having an effective release system. Various products such as alpha-amylase, Fab fragments have been investigated. In case of high secretion yields, the methodology can be adapted by the industry to eliminate cell lysis steps and the overall number of recovery and purification steps in the manufacturing of recombinant proteins can be reduced. The success of this project will be significantly attractive for the industry and it can lead to a new bioprocess strategy within the industry. Yields of 60% has been achieved, an increase from 10%. Yield defined as % of total product in the supernatant. Potential significance for the industry include elimination of cell disruption steps in a bioprocess, significant reduction in levels of contaminants such as HCP, DNA, proteolytic activity is greatly reduced in the culture medium, reduction in number of unit operations in a bioprocess and reduction in process running cost and time.

RATIONAL DESIGN OF EXPRESSION VECTORS FOR HIGH-QUALITY BIOLOGICS

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Key Words: Functional genomics, transcriptomics, *Pichia pastoris*, transgene cassette, product quality

Commercial proteins (e.g. antibodies, enzymes, vaccine components) for applications from biopharmaceuticals to commodity chemicals require low-cost manufacturing of high-quality products. The engineering of recombinant hosts to achieve large quantities of high-quality heterologous proteins is crucial to both minimizing costs and maximizing safety and efficacy (in the case of biopharmaceuticals). High-quality proteins are properly folded and full-length (intact), with native N-, and C-, termini and bear no significant proteolysis or other degradation (oxidation, deamidation, etc…). As most expression hosts rely on recombinant DNA technology for production of the heterologous protein, the transgene cassette provides an early, and inexpensive, opportunity for optimization of quality and protein titer. Commonly, transgene cassettes include a promoter, a heterologous gene of interest, and terminator for expression of the heterologous gene. A targeting sequence for guided recombination and selective marker for isolation of positive clones are also key elements. In engineering the transgene cassette, factors such as the promoter for heterologous gene expression, target site for transgene integration, sequence for translation initiation, and mRNA codon-optimization of the gene of interest are critical design points for a given protein-expressing strain.

Here, we demonstrate an approach to transgene cassette optimization in the methylotrophic yeast, *Pichia pastoris,* informed by functional genomics. Omics-based techniques such as RNA-Seq, ATAC-Seq and ribosomal foot-printing afford greater upfront understanding for subsequent optimized strain engineering on a product-by-product basis. These types of data are cheap and easy to acquire for yeast and can indicate host- or sequence-derived bottlenecks in transgene transcription, translation and expression. Linking these data to product quality attributes can enlighten the design of the expression vector for fast *in silico* optimization of wide-ranging factors such as gene dosage balance, translation efficiency, and balanced cell kinetics enabling high-quality protein production. Collectively, we show that these tools can enable fast vector design for new heterologous protein-producing strains, including those expressing recombinant vaccines, and robust optimization when engineering higher productivity cell lines.

HIGH YIELD PLASMID DNA PRODUCTION UNDER OXYGEN LIMITATION USING MICROAEROBICALLY INDUCED REPLICATION

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Key Words: Microaerobic Processes, Escherichia coli, Plasmid DNA, *rnaII*.

With the aim of increasing plasmid DNA (pDNA) production under oxygen limitation, a self-inducible replication system was created. An extra copy of the gene coding for *rnaII*, which is a positive control molecule for pMB1-derived replicons, was placed under control of the *lac* or *trp* promoters and cloned in plasmid pUC18. The modified plasmid pUC18-P_{trc} *rnaII* resulted in a strong overexpression of *rnaII* which in turn triggered the plasmid copy number in more than the double of that of pUC18. Based on this, a microaerobically-inducible plasmid was created by inserting an extra copy of *rnaII* under control of the microaerobic promoter from the *Vitreoscilla* hemoglobin (P_{vgb}). Such plasmid was tested in fed-batch cultures of the strain W3110 *recA*$^-$ in which dissolved oxygen was depleted for nearly 6 h. Upon oxygen depletion, *rnaII* was efficiently induced and pDNA titer increased steadily for pUC18- P_{vgb} *rnaII*, reaching nearly 400 mg/L. In contrast, only 200 mg/L of the unmodified pUC18 were obtained.

In order to improve cellular performance under oxygen limitations, engineered strains expressing the *Vitreoscilla* hemoglobin encoded in the chromosome, were created. The *vgb* gene was inserted in BL21 and W3110 strains and the performance of both strains were compared in biphasic aerobic-oxygen limited cultures. Interesting differences were observed in the kinetic behavior, metabolic fluxes distribution and gene expression levels when the vgb gene was expressed in BL21 or W3110 *recA*$^-$ *vgb*$^+$, therefore, this strain was used for production of the inducible plasmid. The amount of pUC18 produced by W3110 *recA*$^-$ *vgb*$^+$ under oxygen limitation doubled that of W3110 *recA*$^-$. However, when pUC18-P_{vgb} *rnaII* was used, the engineered strain produced only 20 mg/L. Moreover, the size of the obtained plasmid was strongly shortened. Plasmid sequencing revealed that an important fraction of the origin of replication was lost. These results demonstrate the feasibility of microaerobically-induced pDNA production, and that the performance of genetic constructions depend on the strain used. Furthermore, unexpected changes in plasmid fidelity can arise when using genetically modified strains.

A *PSEUDOMONAS FLUORESCENS* BASED PLATFORM FOR ROBUST VACCINE MANUFACTURING

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Key Words: Pseudomonas, high-throughput screening, vaccines

Pfenex Expression Technology™ has been developed specifically as a protein production platform to enable rapid development of high quality protein therapeutics including novel vaccines and biosimilars. Pfenex represents a new paradigm of microbial strain development that overcomes today's slow, iterative, error-prone process through use of a robotically-enabled, high-throughput parallel strain screening technology, delivering unprecedented speed and success in identifying protein production strains capable of producing large amounts of soluble, active product. Capitalizing on the efficiencies of our platform technology, we are able to effectively develop lower cost, higher quality vaccines including a novel rPA based anthrax vaccine.

ENHANCING THE YIELD AND QUALITY OF SUPERCOILED PLASMID THROUGH PLASMID ENGINEERING

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Key Words: Plasmid Bioprocessing; Supercoiling density; Plasmid yield; Plasmid Engineering; *E. coli*

There has been a rise in the interest of plasmid DNA as therapeutics. This is evident in the number of ongoing clinical trials involving the use of plasmid DNA. To be effective as therapeutics, high yield and high level of supercoiling are required from the bioprocessing point of view. We have approached meeting these requirements through plasmid engineering. Potentially, the level of supercoiling can have an impact on ease of downstream processing. A 7.2kb plasmid was developed by cloning of Bacteriophage-Mu Strong gyrase-binding sequence (Mu-SGS) into 6.8kb pSVβ-Gal. Four *E. coli* strains were transformed with both the modified pSVβ-Gal398 plasmid and pSVβ-Gal. Small scale fermentations and analysis were carried out in triplicate cultures to screen for best performing strains. Two of the four strains selected amplified the plasmids efficiently. There was over 20% increase in the total plasmid yield with pSVβ-Gal398 in both strains. The supercoiled topoisomer content was increased by 5% in both strains leading to a 27% increase in the overall yield. The two strains were investigated further in shake flasks. Increases in supercoiling and plasmid yield were also observed. The extent of supercoiling was examined by superhelical density quantification, with pSVβ-Gal398 maintaining a supercoil density of -0.022 and pSVβ-Gal -0.019 in both strains. The compactness of the plasmid DNA was also quantified by hydrodynamic diameter measurement using the Nanoparticle Tracking Analysis (NTA) and it was observed that pSVβ-Gal398 was more compact with a D_h of 40-59nm compared to pSVβ-Gal with D_h of 70-90nm for both strains examined. The report of this study has shown that plasmid engineered to contain the Mu-phage SGS sequence has a beneficial effect on improving not only the yield of total plasmid but also the supercoiled topoisomer content of therapeutic plasmid DNA during bioprocessing.

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COMPUTATIONAL FLUID DYNAMICS MODELING FOR FERMENTATION RISK REDUCTION DURING TECHNOLOGY TRANSER AND PROCESS UNDERSTANDING

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Key Words: Computational Fluid Dynamics, Fermentation, Commercialization, Scale-Up, Facility Fit

Computational Fluid Dynamics modeling and in-depth scaling calculations have been utilized in partnership to generate data to support equipment design and facility fit during commercialization of a fermentation and primary recovery process for a vaccine candidate across multiple technical transfers. This analysis utilizing representative computer models for tank configurations, supplemented with traditional computational scaling approaches (ungassed P/V, gassed P/V, k_La, etc.), ensures full knowledge of a tank's mixing and oxygen transfer capabilities allowing process understanding and robust manufacturing across technology transfer to multiple sites. Implementation of this approach across process steps as well as manufacturing sites allows increased knowledge prior to use in a process and/or prior to construction of a new vessel, therefore contributing to successful process transfer with reduced risks upon scale-up/scale-down and new facility introductions.

ENGINEERING OF PROBIOTIC *E. COLI* TO TREAT METABOLIC DISORDER

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Key Words: Synthetic biology, therapeutic, metabolic, Nissle

The fields of synthetic biology and microbiome research developed greatly over the last decade. The convergence of those two disciplines is now enabling the development of new therapeutic strategies, using engineered microbes that operate from within the gut as living medicines. Inborn errors of metabolism represent candidate diseases for these therapeutics, particularly those disorders where a toxic metabolite causing a syndrome is also present in the intestinal lumen. Phenylketonuria (PKU), a rare inherited disease caused by a defect in phenylalanine hydroxylase (PAH) activity, is one such disease and is characterized by the accumulation of systemic phenylalanine (Phe) that can lead to severe neurological deficits unless patients are placed on a strict low-Phe diet. As an alternative treatment, *Escherichia coli* Nissle (EcN), a well-characterized probiotic, was genetically modified to efficiently import and degrade Phe (SYN-PKU). The coupled expression of a Phe transporter with a Phe ammonia lyase (PAL) allows rapid conversion of Phe into trans-cinnamic acid (TCA) in vitro, which is then further metabolized by the host to hippuric acid (HA) and excreted in the urine. Experiments conducted in the *enu2-/-* PKU mouse model showed that the oral administration of SYN-PKU is able to significantly reduce blood Phe levels triggered by subcutaneous Phe injection. Decreases in circulating Phe levels were associated with proportional increases in urinary HA, confirming that Phe metabolism was caused by the engineered pathway in SYN-PKU. Subsequent studies have shown that SYN-PKU is similarly operative in a non-human primate model, providing a translational link to inform future human clinical studies. In addition to SYN-PKU, a second EcN strain was genetically engineered to rapidly import and degrade branched-chain amino acids (BCAAs) for the treatment of maple syrup urine disease (SYN-MSUD). MSUD, similar to PKU, is a rare genetic disorder caused by a defect in branched-chain ketoacid dehydrogenase activity leading to the toxic accumulation of BCAAs, particularly leucine, and their ketoacid derivatives. The controlled expression in SYN-MSUD of two BCAA transporters, a leucine dehydrogenase, a ketoacid decarboxylase and an alcohol dehydrogenase, result in the efficient degradation of BCAAs into branched-chain alcohols. In a mouse model of MSUD, the oral delivery of SYN-MSUD suppressed the increase in blood BCAAs level induced by a high-protein challenge and prevented the associated moribund phenotype, as measured by locomotor activity. In conclusion, the therapeutic effects observed with SYN-PKU and SYN-MSUD in pre-clinical studies support the further evaluation of engineered microbes as promising approaches for serious inborn errors of metabolism.

OPENING MICROBIAL CELLS EXPANDS THEIR CAPABILITIES

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This talk will explain how multiple advances in the technology for cell-free protein synthesis (CFPS) may be on the verge of expanding the biopharmaceutical industry.

The era of rRNA biopharmaceuticals was launched using *E.coli* in the early 1980's to produce proteins such as human insulin (Eli Lilly), human growth hormone (Genentech), granulocyte colony stimulating factor (Amgen), and alpha-interferon (Genentech and Roche). However, as the complexity of the protein pharmaceuticals increased, the industry turned to eukaryotic cells to provide more complex disulfide bond patterns and glycosylation for products such as erythropoietin (Amgen) and tissue-plasminogen activator (Genentech). Further driven by the emergence of monoclonal antibodies as a large family of potent and versatile pharmaceuticals, CHO cells then emerged as the dominant production platform.

Ironically, the need for even more complexity and precision may now be motivating a shift back to prokaryotic systems. Sutro Biopharma, independently and in collaboration with Celgene and Merck Serono, is now developing a new class of antibody drug conjugates (ADCs) produced using *E.coli* cell extracts. They have established GMP manufacturing capability and are nearly ready for human trials. CFPS is providing direct access to the protein synthesis reaction chamber, and that allows the spatially precise introduction of uniquely reactive, non-natural amino acids. This, in turn, enables the precise localization of an exact number of drug molecules per antibody. It also speeds up the production and screening of many ADC candidates so that safety and efficacy can be more effectively optimized. While the jury is still out, such products may be the wave of the future.

This presentation will summarize the technology foundation being used by Sutro. It will then go on to describe how this platform is being further strengthened so even more complex pharmaceutical targets can be approached. As an example, the development of a novel drug targeting technology will be described. It is based on a multiply-modified Virus-like Particle (VLP) based on the core capsid of the hepatitis B virus.

Even though the concept of the magic bullet for targeted drug delivery was proposed by Paul Ehrlich more than 100 years ago, we are still waiting; and there are good reasons for multiple past disappointments. We have now learned that a successful drug delivery vehicle must accept and retain hundreds of cargo molecules while remaining stable during production, storage, and administration. It must then avoid immune system surveillance, trigger internalization into only the targeted cell type, escape from the endosomal vesicles, and finally, for optimal efficacy, it must release its cargo into the cytoplasm. Combining all of these functions into a single nanoparticle has proven to be extremely difficult. This talk will explain how the design freedoms and production efficiencies offered by CFPS may finally allow Ehrlich's dream to be realized. If so, precisely targeted drug and nucleic acid delivery would allow a much broader range of molecules to be developed as therapies for many different diseases.