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Research review paper

Impact of synthetic biology and metabolic engineering on industrial production of fine chemicals

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ABSTRACT

Industrial bio-processes for fine chemical production are increasingly relying on cell factories developed through metabolic engineering and synthetic biology. The use of high throughput techniques and automation for the design of cell factories, and especially platform strains, has played an important role in the transition from laboratory research to industrial production. Model organisms such as *Saccharomyces cerevisiae* and *Escherichia coli* remain widely used host strains for industrial production due to their robust and desirable traits. This review describes some of the bio-based fine chemicals that have reached the market, key metabolic engineering tools that have allowed this to happen and some of the companies that are currently utilizing these technologies for developing industrial production processes.

Contents

1. Introduction

Cell factories created by engineering metabolic pathways are capable of converting renewable feed-stocks into fuels, chemicals, food ingredients and pharmaceuticals (Keasling, 2010). With increasing climate change awareness alternative transportation fuels are needed. Furthermore, many food, pharmaceuticals and cosmetic ingredients are extracted from plants where seasonal dependent growth can cause supply depletion and extraction methods can be expensive. There is therefore much interest in developing cellular biocatalysts to produce direct replacements for specific chemicals as well as new advanced bioproducts that have properties superior to existing products. Many companies, both biotech and traditional chemical companies, are now translating research successes from both academic and industrial groups to industrial processes. These ventures are motivated by consumer demand for chemical products that are environmentally friendly, less expensive, and possess superior properties compared with those generated by traditional chemical synthesis. Advances in industrial biotechnology and bioengineering over the last two decades and several successful implementations of novel industrial processes have led to significant growth of the field of so-called white biotechnology. Continued advances in DNA synthesis, synthetic biology, and systems biology will only encourage further interest in using genetically engineered cell factories for production of many different chemicals.

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Cell factories are designed and assembled using metabolic engineering and synthetic biology. Tools from these two fields can be used to redirect fluxes towards desired metabolites. The two fields share the same basis in bioengineering with the use of quantitative, model-driven methods for predicting cellular phenotypes (Nielsen et al., 2014), and a common in silico to in vivo approach. Metabolic engineering involves enhancing or redirection of flux through metabolic pathways by making genetic modifications that alter the activity of enzymatic reactions. Genetic modifications include deletion of genes, replacement of gene expression signals, and/or introduction of recombinant DNA cassettes encoding foreign enzymes. Metabolic engineering also includes a detailed analysis of the metabolic pathways to identify targets for manipulation (Nielsen and Jewett, 2008; Ostergaard et al., 2000). These strategies often comprise elimination of unwanted activities, increasing activity at flux controlling steps, and introduction of irreversible reactions to drive the flux in desired directions. Synthetic Biology is the study of how to perform these manipulations in a quantitatively predictive way and how engineering principles can be applied to the design and construction of biological systems. A pillar of synthetic biology is the use of assembly standards for assembling genetic materials. This approach is adapted from other engineering disciplines such as electrical engineering wherein complex systems can be built by combining separate, well-characterized parts. The scope of synthetic biology has grown from construction of codon optimized genes to assembling a complete synthetic genomes (e.g. Mycoplasma "Synthia" (Gibson et al., 2010)), assembling a designer eukaryotic chromosome (Annaluru et al., 2014) and incorporation of new synthetic nucleotides (Malyshev et al., 2014) to increase the information content. The synergy between the two fields will be further exploited to advance research in pathway engineering. However, the two fields can be distinguished as metabolic engineering being a top-down approach, i.e. retrofitting of the metabolism of a cell factory, and synthetic biology being a bottom-up approach, i.e. the reconstruction of a new synthetic cell, is considered (Nielsen et al., 2014).

The performance of cell factories is typically evaluated through three chemical production metrics: titer, rate (a.k.a. productivity), and yield (TRY). In addition, a high performance cell factory should have the qualities needed to thrive in an industrial setting including high osmotic tolerance, broad pH tolerance, and minimal impact on the environment (i.e. generally regarded as safe—GRAS). The yeast *Saccharomyces cerevisiae* meets most of these criteria, and it is therefore widely used as a cell factory for production of food and beverages. Yeast has been in the service of humans since the neolithic period. *S. cerevisiae* has thus been accepted for industrial chemical production and has been assigned to GRAS status. *S. cerevisiae* has also a low risk of contamination due to its low pH tolerance. Homologous recombination, which also occurs to a high degree in yeast, allows for incorporation of genetic fragments into the genome resulting in a more stable strain. *Escherichia coli* is another well-studied cell factory. This model Gram-negative prokaryote is easy to genetically manipulate, an enormous knowledgebase is available, and has been used to develop many of the underlying principles of synthetic biology and metabolic engineering. *E. coli* has been used to produce chemicals commercially and remains a highly studied system in the academic community. Success with model organisms and the difficulties encountered in conferring complex traits to them has motivated interest to develop cell factories from non-model microorganisms that possess desired abilities. For example, photosynthetic and methanotrophic organisms are being developed as cell factories to gain the advantage of using CO_2 or natural gas as carbon source. Adaptation of synthetic biology tools and metabolic engineering strategies for these and other organisms will enable the deployment of future cell factories.

2. From system understanding to design of function

Over the past decades our knowledge about biological systems has increased dramatically. To a large extent this is due to the many techniques that have arisen, not only from biology, but from physics and mathematics and that have proven useful in the service of biology. This is illustrated in Fig. 1, summarizing major technologies and tools in a historical context for the past 20 years combined with upcoming trends. During the past 20 years, technologies have enhanced the scale of bioengineering efforts from individual genes and gene products, through pathways and traits, to complex systems encoded by genome scale DNA—including organisms. Technologies targeting small scales have matured, but much remains to be learned when it comes to designing, constructing, and modifying chromosomes and entire genomes. The technique that has influenced molecular biology the most is the invention of Polymerase Chain Reaction (PCR) in the 1980s (Mullis et al.,

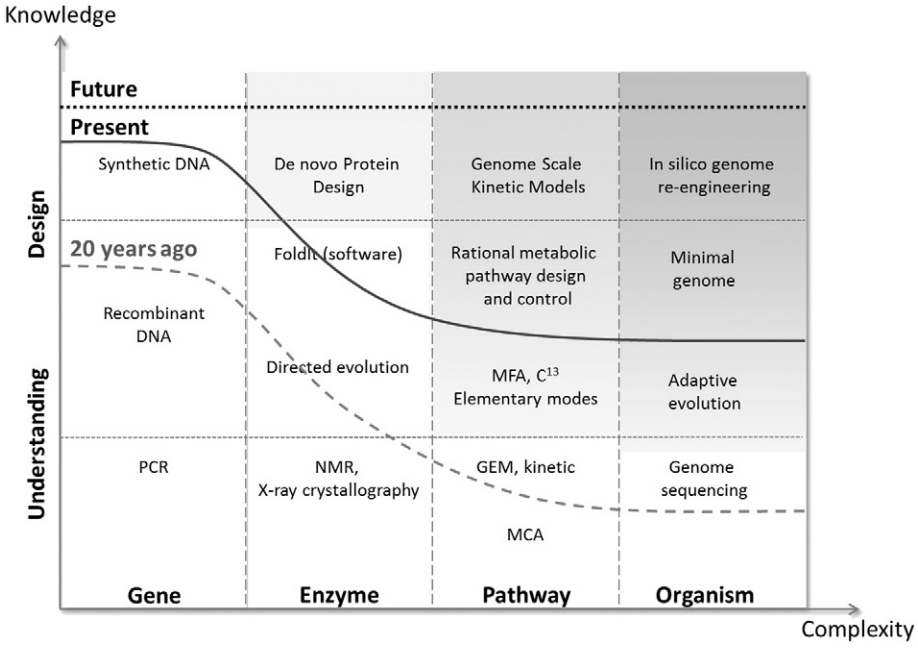

Fig. 1. The graph of knowledge and complexity. At present, we have reached a level of understanding, compared to 20 years ago, that allows us to design parts in a system but we lack a systematic overview to fully understand what makes a system function appropriately.

1987). A decade earlier, recombinant DNA technology (Lobban and Kaiser, 1973) was invented as a tool to fuse multiple sources of DNA fragments into one, these techniques allowed for recombinant protein production in a host cell. In the 1980s solid-phase phosphoramidite chemistry was invented and is now the most prominent way of creating synthetic DNA (Kosuri and Church, 2014). DNA synthesis has also been optimized by the use of automated instruments, which have revolutionized the gene design field. This automation allowed a reduction in the purchase prize of completely synthesized genes from a price of 50 $/bp 20 years ago to <0.2 $/bp today. This has enabled a design-of-function or bottom-up-approach rather than the trial and error approach, which was common in the first attempts to create new genes. The field of physics provided X-ray crystallography (Drenth, 2007) and nuclear magnetic resonance (Wishart et al., 1991), which have been instrumental in studies of proteins and enzyme functions. These two technologies have given rise to over 100,000 enzyme structures and enzyme compositions (Bank, 2014). Earlier it was extremely computationally demanding to calculate the 3D structure and active sites of enzymes as the algorithmic landscape for optimizing the lowest energy is too great for a computer to calculate. Today, computer tools like FoldIt (Khatib et al., 2011) have enabled fast structure prediction as the algorithm learns in ingenuities from the users how to fold the peptide chains in a more natural and correct manner. Directed evolution (Crameri et al., 1998), which is also used for enzyme maturing, is a high throughput technique where diversity is created by inserting random mutations into the genetic sequence encoding the enzyme. These different variants are then analyzed to select one with desired properties (Voigt et al., 2000). Tools have been developed for altering enzyme activity by controlling gene expression and therefore the amount of a particular enzyme. Synthetic biology tools for controlling transcription (Redden et al., 2014; Rhodius et al., 2012), translation (Na and Lee, 2010; Salis et al., 2009), and RNA turnover (Pfleger et al., 2006) have been instrumental in balancing gene expression in metabolic pathways. Most of these tools optimize expression from genes in *cis*, but recent tools such as small RNAs, CRISPR/Cas, and TAL effectors offer the ability to regulate genes in *trans* (Copeland et al., 2014; Yoo et al., 2013). Cloning methods such as Ligase Chain Reaction (LCR) (Kok et al., 2014) or Gibson assembly (Gibson et al., 2009), have greatly enhanced the ability to assemble libraries of DNA constructs and enabled high throughput exploration of possible sequence space. Unfortunately, our ability to predict the optimal level of gene expression and our understanding of the pathways in a metabolic process remains limited. As a metabolic flux of interest is likely to be controlled by several enzymes, altering one specific enzyme might not be enough. To identify flux control the concept of metabolic control analysis (MCA) is useful, even though it requires extensive information about the kinetics of the individual enzymes. Other modeling approaches like genome metabolic models (GEM) combined with flux balance analysis (FBA) and analysis of elementary flux modes are easier to implement as these models require only a few parameters, but they cannot provide specific information about flux control (Kerkhoven et al., 2014). C^{13}-labeled flux analysis is a technique that allows a more accurate model based estimation of metabolic fluxes and is used to detect flux changes and potential metabolic bottlenecks (Blank et al., 2005; Soons et al., 2013). Through combination with elementary flux mode analysis it is even possible to identify flux control at branch points in the metabolic network (Bordel and Nielsen, 2010). In the future, genome scale kinetic models may give a systemic view that allows for precise identification of bottlenecks and targets for manipulation.

With the Human Genome Project (HUGO) project and complete genome sequences of several microorganisms hopes were high to find the formula to create complete synthetic in silico organisms. However, this turned out to be harder to be realized than anticipated, particular as our knowledge about molecular interactions, both in terms of connectivity and in terms of kinetics, are limited. Thus, even though it has been possible to create synthetic chromosomes (Annaluru et al., 2014) a milestone of full understanding of the complete function of minimal genomes has yet not been achieved. However, many approaches to generate a minimal genome have been employed and this is likely to reveal a set of key genes required for cellular function (Kumagai et al., 2014; Moran and Bennett, 2014). These techniques, and in the future even better and more reliable techniques, will aid us in reaching a deeper systematic understanding that will allow for a design-of–function, not only of single genes and proteins but rather of whole microorganisms with desired functionalities.

3. Platform strains and their role in fine chemical production

One of the most applied concepts when it comes to fine chemical production is the usage of platform strains that have desired traits (Fig. 2). This approach allows easy insertion of different product formation pathways and thereby significantly reduces the development time, which is of great importance for commercial success. Feedstocks that are utilized by platform strains can vary, but platform strains have been evolved to utilize several different raw materials. The platform strain is engineered to have robust properties such as stress tolerance, fermentation performance and substrate utilization. This enables the strain to be used under industrial conditions, where the environment can be rather harsh for the organism to exclude contaminations and reach high cell densities. Products produced by platform strains can range from high volume chemicals, such as biofuels and commodity chemicals, to high value chemicals such as fine chemicals and proteins.

At the center of cellular metabolic networks a set of twelve chemicals, so-called precursor metabolites, resides from which all cellular building blocks and chemical products can be derived (Neidhardt et al., 1992; Nielsen, 2003). Metabolic reactions can therefore be organized into one of three categories: 1.) catabolic reactions, 2.) anabolic reactions, and 3.) central metabolic reactions. Catabolic reactions comprise pathways that convert feedstock (e.g. carbon source) into precursor metabolites, reducing power, and energy. Anabolic reactions comprise pathways that

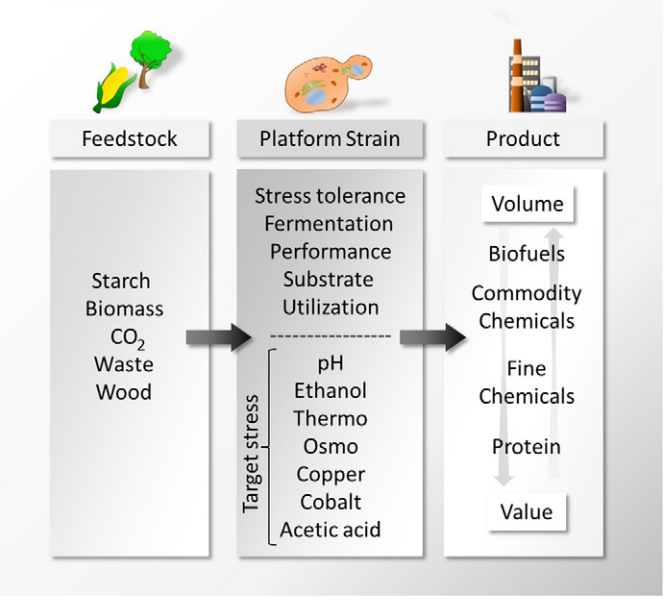

Fig. 2. The concept of a platform strain as a plug-and-play solution for industrial production. A platform strain is robust and possesses desirable traits thus enabling easy upstream development of new processes. Platform strains possessing desired abilities can be selected to process a desired feedstock and convert it into one of several different products. In the chemical market, market volume is typically inversely proportional to the product selling price. Cell factories have long been used for high value products such as proteins and are increasingly being used for molecules at the other end of the spectrum.

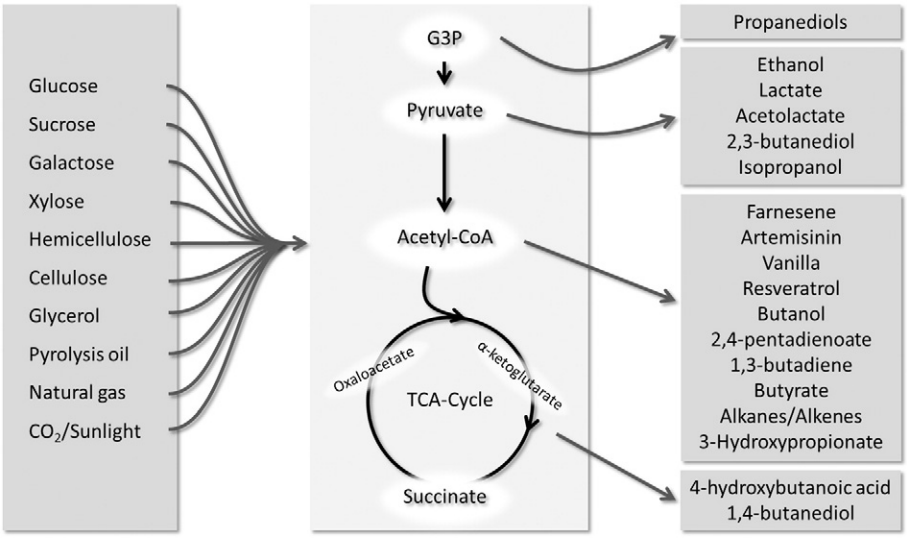

Fig. 3. The bow tie structure of metabolism with the main metabolic precursor metabolites residing in the center of the three super-pathways: catabolic reactions, central metabolism and anabolic reactions. Key precursor metabolites are: G3P (glyceraldehyde 3-phosphate), pyruvate, acetyl-CoA (acetyl-Coenzyme A), oxaloacetate, a-ketoglutarate, succinate, fatty acids, prenyl-pyrophosphates, and acyl-thioesters.

consume reducing power and energy to produce cellular components (e.g. lipids, nucleic acids, cell wall) or desired chemical products. Central metabolic reactions are those that enable the cell to interconvert between the twelve precursor metabolites thereby permitting production of all cellular components from a single catabolic pathway. This structure is akin to the "unit operation" framework for chemical process design. In this way, a biocatalyst can be designed by combining technologies for consuming a desired feedstock with an anabolic pathway for producing a desired chemical product.

From this perspective, metabolism is shaped like a bow tie with a large number of pathways funneling into a small number of central metabolites that then branch out into a wide range of anabolic pathways. Given this structure, the strongest intellectual property position is obtained by controlling the branch points. For this reason, groups seek to develop platform strains for producing metabolites at the branch points of pathways within specific chemical families. For example, isoprenoids are a large family of compounds (>50,000) made from two, 5-carbon building blocks (isopentylpyrophosphate (IPP) and dimetylallylpyrophosphate (DMAPP) (Kirby and Keasling, 2008)), and further from acetyl-CoA (Chen et al., 2012, 2013). Technologies that enable high volume production of these two precursors can then be used to produce any or all of the higher-value downstream products. Amyris has used this strategy to develop platform strains for producing isoprenoid precursors and applied the technology to a wide range of markets including fuels (e.g. farnesene), drugs (e.g. artemisinin), flavors and fragrances (e.g. patchouli oil) and other chemicals (e.g. squalene). Similar efforts by other groups have targeted branch metabolites such as malonyl-CoA, acetyl-CoA, pyruvate, succinate, with the goal of ultimately producing oleochemicals, polyketides, fusel alcohols, amino acids, diols, flavonoids, or other fine chemicals. Other groups have developed platform technologies for consuming specific feed-stocks such as pentose sugars, cellulose, and/or CO_2 that they plan to use in combination with various anabolic pathways. Lastly, groups have screened, evolved, and engineered platform strains that possess specific traits such as pH tolerance, solvent tolerance, thermal stability (Caspeta et al., 2014) with the hypothesis that superior (cheaper, more stable, more efficient) processes can be run under these extreme conditions. For example, the production of lactic acid was greatly enhanced by development of biocatalysts based on yeasts that could tolerate low pH thereby circumventing the costs associated with neutralization and purification of lactic acid at neutral pH (Ilmén et al., 2007) (Fig. 3).

4. Industrial strain design

Cell factories are developed using an iterative design, build, test cycle. Projects often start by selecting a platform strain possessing a large portion of required traits. The design phase adds genes that confer the missing traits and enables production of a target compound from a target feedstock. The design phase can be accomplished with different strategies. These range from establishing entirely new metabolic pathways, to optimizing gene expression of existing pathways, to replacing specific genes. Designs must optimize the essential metabolic pathways, but must also consider strain physiology (e.g. product tolerance, energy management, robustness) (Steensels et al., 2014). Here, techniques for whole genome engineering come into play comprising rational site-directed and more untargeted techniques (David and Siewers, 2014). The most classical tool for strain improvement is by inducing random mutations through UV light or chemicals and selecting for a desired phenotype. In many cases a genetic basis for desired traits or optimal pathway function is unknown. Therefore high throughput techniques capable of screening large libraries are used to process thousands of new strains each week. Tools for creating these libraries include combinatorial genetics where tens to hundreds of genes derived from various species are randomly combined and tested. Standardized tools like genetic circuits (Brophy and Voigt, 2014) with well characterized properties enable rational approaches and fast developments. Using a directed evolution approach specific enzymes of the pathway are typically in focus of the optimization. Here, diversity is created by gene mutations combined with an appropriate screening approach to find a beneficial variant. The final functionality of a component of interest sometimes has to be enhanced by specific modifications. Here, decoration technologies like glycosylation and transfer of molecular oxygen to –CH, –NH or –SH bonds (Renault et al., 2014; Weitzel and Simonsen, 2013) are of great importance. In this context, libraries with different enzyme variants for modifying glycosylation patterns and cytochrome P450 enzymes are used.

For high throughput screenings it is essential to have a suitable read-out signal to speed up the optimization cycle. These can be based on so called function-led screens were the main parameter is e.g. cell survival or GFP expression using transcription factors or riboswitch based biosensors (Schallmey et al., 2014; Yang et al., 2013). These function-led screens can have as much as 1 billion screening events per day. For structure-led screens LC-MS and NMR can be used for evaluating the performance of

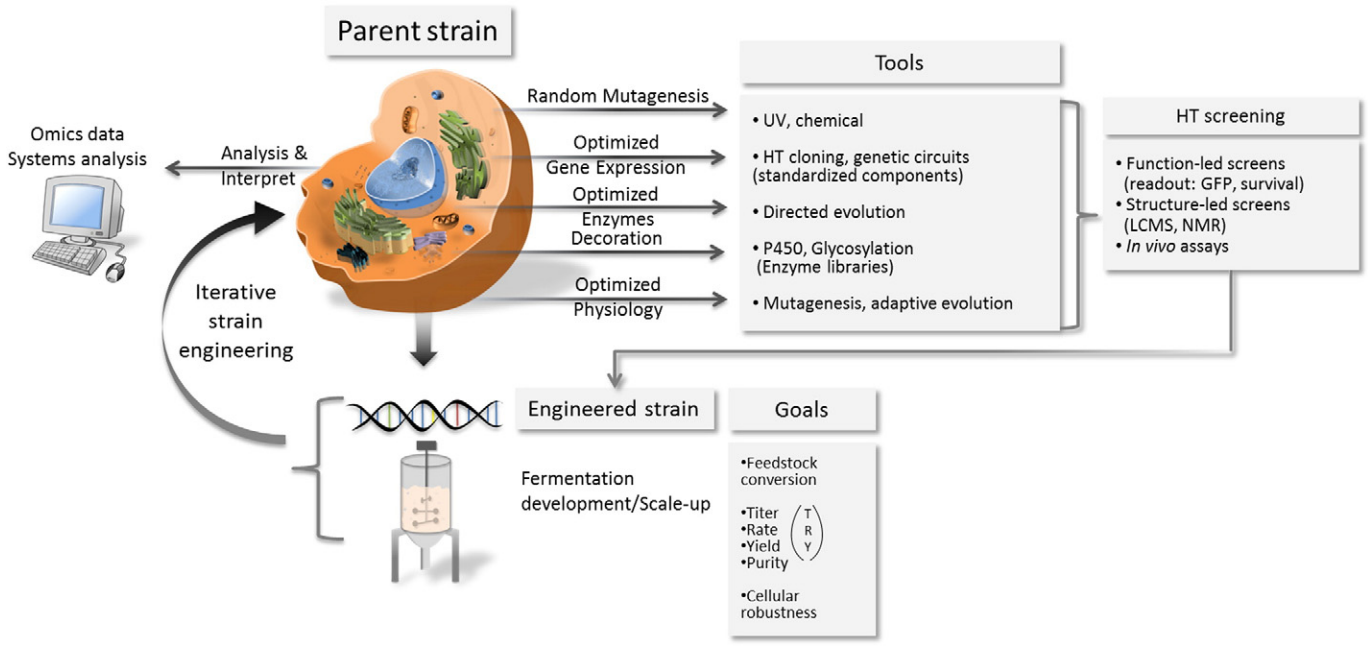

Fig. 4. The development of industrial strains for chemical production undergoes several iterative cycles where desired traits and scale-up are guided by the many available engineering tools and analysis techniques.

pathways leading to the desired product, but the throughput of these screens is much lower. When evaluating the engineered strain, after establishing the pathway and the design, there are some main goals that the strains must achieve to be ready for industrial production. These goals are: reduced by-product formation, variety and efficiency of feedstock conversion (yield), speed of production (rate), a high final titer and robustness of the cell factory. When the strain is evolved to an intermediate strain, the fermentation development and scale-up are of outmost importance as the chosen strain often dictates the design of the full scale production process. Scale-up typically emerges late in the process and any problems occurring at this stage with the chosen organism may have serious impact on the development time and can significantly increase costs. Following screening the chosen strain is often analyzed in detail through systems analysis and omics techniques (Kim et al., 2012), and based on this new engineering strategies are implemented. This cycling optimization of the cell factory forms the so-called iterative loop for strain development, or the metabolic engineering cycle (Nielsen, 2001), that following many cycles finally results in a cell factory that meets the TRY requirement for implementing a commercially viable production process. (Fig. 4)

5. Impact in industrial applications

As previously mentioned, metabolic engineering and synthetic biology have an impact on multiple industrial sectors ranging from production of chemicals, biofuels, food ingredients and supplements, and pharmaceuticals. Fig. 5 provides a representative overview of the current market situation related to companies, their target products and the particular current market status. These grouped products range from low-value, high market volume products like biofuels and commodity chemicals to high-value small market volume products like food additives and pharmaceuticals. Companies generally aim for producing chemical building blocks, or strategic intermediates that can be further used by chemical processes to create even higher value-added products. Three stages of development will be considered in the following discussion to highlight the stage of current development projects: small-scale laboratory development (1), pilot-scale evaluation (2) and commercial scale production (3). Here we primarily focus on metabolite based products produced by yeast, bacteria or algae, and will discuss some products that have made it to commercial scale.

Hydrocortisone was one of the first complex products demonstrated to be produced in yeast through metabolic engineering and synthetic biology. The recombinant pathway involves 13 engineered genes including a P450 system (Szczebara et al., 2003). Sanofi is currently working on an industrial production based on this system (Brocard-Masson et al., 2012). Another commercial scale product that has been launched in recent yeasts is D-lactic acid for use in production of polylactic acid (PLA), a bioplastic. A main advantage of the bioprocess is that the production of only the D-lactic acid is permitted to ensure proper PLA formation and to prevent expensive separation of the two forms. Cargill (Carlson and Peters, 2002) was the first to develop a novel process for lactic acid production using a low pH tolerant yeast strain, and they have been the key driver of introducing PLA on the market. Today there are, however, several industrial producers of lactic acid, e.g. Myriant that produces lactic acid from non-food cellulosic feedstock since 2008. In the biofuel space a good example of a commercial product is Gevo's production of isobutanol from renewable feedstock for the chemical and fuel market. Existing ethanol-producing plants were successfully retrofitted with a production capacity of 38 million gallons of isobutanol per year. Another example of biofuel production is Solazyme's production of biodiesel based on engineered microalgae. This biofuel is fully compliant with Fatty Acid Methyl Ester (FAME) standards. There is much interest in advanced biofuels that resemble traditional fuels, and Amyris has established a successful production of farnesene (Keasling et al., 2005). Farnesene can be dehydrated to farnesane that can be used as a diesel fuel, but also be in surfactants, lubricants or personal care products. Another commercial scale compound based on the farnesene platform strain is squalene, which is a high value ingredient, used in cosmetics and personal care products and produced on a commercial basis since 2011. Succinic acid is another important product in the chemical field and Bioamber produces this chemical using a genetically engineered *E. coli* or a low pH tolerant yeast strain licensed from Cargill. According to the Environmental Protection Agency (EPA) Bioamber produces succinic acid at costs 40%

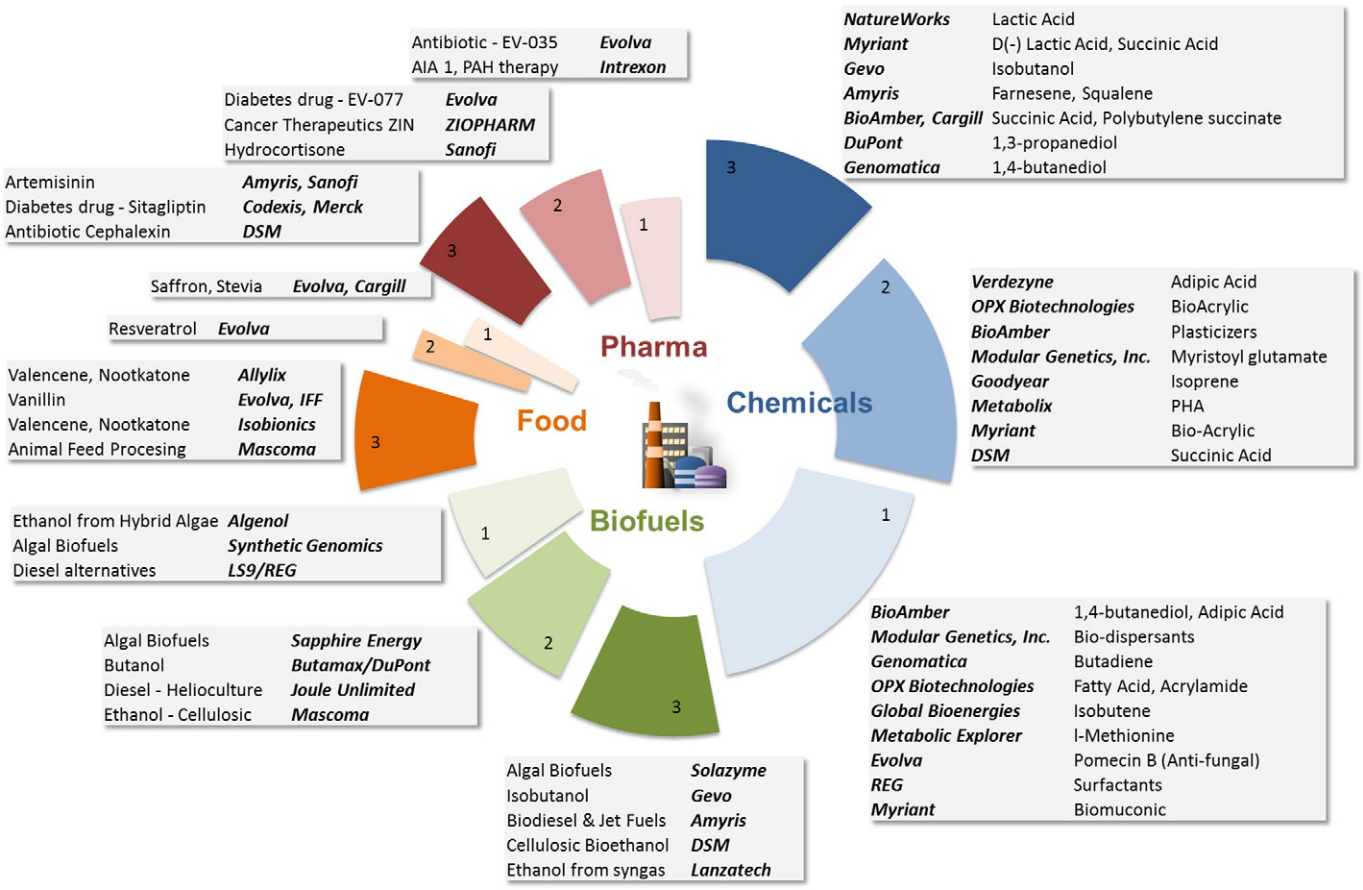

Fig. 5. Companies in the field of microbial engineering for fine chemical production (Allylix, 2014; Amyris, 2014; BioAmber, 2014; Bioenergies, 2014; Biofuels, 2014; Biotechnologies, 2014; Butamax, 2014; Cargill, 2014; Codexis, 2014; Digest, 2014; DSM, 2014; DuPont, 2014; Energy, 2014; Evolva, 2014; Explorer, 2014; Genomatica, 2014; Gevo, 2014; International Flavors and Fragrances, 2014; Intrexon, 2014; Isobionics, 2014; Mascoma, 2014; Merck, 2014; Metabolix, 2014; Modular Genetics, 2014; Myriant, 2014; Natureworks, 2014; Project, 2014; REG, 2014; Sanofi, 2014a; Solazyme, 2014; Synthetic Genomics, 2014; Unlimited, 2014; Verdezyne, 2014; Ziopharma Oncology, 2014), data also generated from Biofuels Digest (2014) and Synthetic Biology Project (2014). Four major areas are visualized: Food, Pharma Chemicals, and Biofuels. The number corresponds to the stage in progress that the company has reached: 1) small-scale laboratory development, 2) over pilot-scale, and 3) commercial scale.

less than petroleum based production. It can be widely applied as a chemical building block for production of various chemicals and polymers as well as food, drugs and cosmetic ingredients. Another example of commercial production of a chemical building block is Genomatica's commercially feasible production of 1,4 butandiol (Burk et al., 2011), an important precursor compound for plastics and the textiles industry with a value of 2000$/ton and global market sales of $4 billion per year. In five years Genomatica successfully optimized the production strain and the associated bioprocess and succeeded in licensing it to Novamont and BASF, the largest BDO producer in the world. Similar type chemicals are produced by Lanzatech, that is producing ethanol and 2,3-BDO from syngas by using microbial conversion. A first commercial facility is operating at a steel mill in China, producing 30 million gallons of ethanol per year based on waste industrial flue gases.

Having a closer look at the field of food additives, companies like Isobionics (Sonke and de Jong, 2012) and Allylix (recently acquired by Evolva) found a niche in producing the citrus flavors nalencene and nootkatone based on engineered terpene producing yeast cells. Another product that recently entered commercial scale production is vanillin, which was launched by Evolva in collaboration with International Flavor & Fragences Inc. (Hansen et al., 2013). A major product candidate from the pharma sector is artemisinin, an anti-malaria drug that is traditionally derived from plants. Here, Sanofi/Amyris recently launched large scale industrial artemisinin production and entered the market based on original development of the cell factory by Amyris (Sanofi, 2014b).

6. Discussion

Here we provide an overview of the current impact of synthetic biology and metabolic engineering in the industrial area where numerous companies have developed commercially viable cell factories. Also established big players in the chemical sector like BASF, Cargill, Dupont, DSM and Sanofi are increasingly involved in the field of biochemical production and typically enter collaborations with smaller biotech companies, through establishing partnerships, formation of joint-ventures or by licensing their technologies. We have seen in the last years how synthetic biology and metabolic engineering have delivered novel and innovative solutions to ensure process robustness and utilization of different substrates at the same time reaching targets on high titer, rate, yield and purity of the cell factory that can be used for industrial production. All this has been possible through many years of tool and technique development where non-automated and labor intense techniques have been replaced by fully automated systems for high throughput screening. DNA sequencing, DNA synthesis and modeling have all gone through rapid changes as computer power has increased. Could our knowledge in in silico genome re-engineering be boosted by artificial intelligence and neural networks or is it our ideas and lack of understanding that is the bottleneck in the final frontier of creating cell factories?

In addition, when it comes to industrial production and developing new platform strains; the host strain matters. Shall one rather go for a few well-studied main workhorses or rather go for multiple hosts

with different special production properties? Through bio-prospecting novel strains can be found that are more suitable for specific compound production. However, these strains need to be evolved to strains that are applicable for industrial fermentation and if needed go through GMP assessment. Furthermore, lack of suitable tools for genetic manipulation for such strains often hinders their use for metabolic engineering. However, with further research novel host strains can be adapted to industrial fermentation by altering just a few key regulators in the strains' metabolic machinery (Damkiær et al., 2013).

Looking at the product spectrum of the current bio-based industry we see a clear trend focusing on chemicals rather than biofuels but also food additives and pharmaceuticals are of increasing interest. One has to keep in mind that these products still have to compete with the current petroleum or plant based production methods, meaning that besides the argument of being more sustainable the process also has to be cost efficient. This has been a main driver for companies solely dedicated to biofuels to shift towards a broader spectrum of more valuable products, but still rely on platform strains as an opportunity to produce biofuels.

When it comes to process economics the feedstock is of great importance. Most bioprocesses still use glucose as carbon source, which makes the discussion of "food versus fuel" still relevant. Currently, many efforts are made to enable 2nd generation production of fuels and chemicals. Here one aims to use biomass derived cellulosic material as a direct carbon source. So far established processes still rely on chemical and enzymatic pretreatment but the final aim is to engineer microorganisms to break down cellulosic polymers. The ultimate goal is to use photosynthetic organisms as production host only relying on CO_2 and light for growth. Though there are much interest in this space there are still no industrial processes established, and an in depth system understanding is required, in particular to reach a sufficient high cell density to ensure high productivities, but also novel scale-up technologies are limiting industrial implementation.

The biggest bottleneck for industrial implementation of novel bioprocesses is often in the scale-up step, where the host strain has to be chosen definitely as it will generally be too expensive to change at a later stage. From an industrial perspective *S. cerevisiae* is besides others benefits as mentioned above, a robust host strain with many desired traits such as high osmo-tolerance, low pH-tolerance and a permissive insertion of recombinant DNA through homologous recombination. *S. cerevisiae* is widely used both in academia and in industry as illustrated by several of the examples discussed here. The wide use of *S. cerevisiae* in academic research is important, as academia and industry have in many cases joined forces where we are starting to see the fruits of these joint ventures in products that have reached the market.

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