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LU CHEN | DIVISION OF BIOTECHNOLOGY | LUND UNIVERSITY

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Lu Chen

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LUND UNIVERSITY

Department of Chemistry
Box 124
SE–221 00 LUND
Sweden

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Abstract
Concerns regarding environmental issues have led to increasing efforts for investigating sustainable production routes for the future industry that is based on renewable resources. Among the biobased chemicals, the C3-chemicals 3-hydroxypropionaldehyde (3-HPA), 1,3-propanediol (1,3-PDO) and 3-hydroxypropionic acid (3-HP), have been identified as top candidates for the chemical industry. *Lactobacillus reuteri* is a heterofermentative probiotic microorganism that possesses the metabolic pathway called the propanediol utilization (Pdu) pathway that catalyzes dehydration of glycerol to 3-HPA by glycerol dehydratase (PduCDE) and further to 3-HP and 1,3-PDO by a series of reactions catalyzed by propionaldehyde dehydrogenase (PduP), phosphotransacylase (PduL), and propionate kinase (PduW), and 1,3-propanediol oxidoreductase (PduQ), respectively. The Pdu pathway is operative inside Pdu microcompartment (MCP) that encapsulates different cofactors and enzymes needed for metabolizing glycerol or 1,2-propanediol, and protects the cells from the toxic effect of the aldehyde intermediate.

This thesis focuses mainly on the theoretical investigation of different aspects of the Pdu pathway in *L. reuteri*, and the MCP shell made up of several proteins. Two of the major shell proteins, PduA and PduJ were investigated, and the effect of small alterations in the pore structures in the respective proteins by generating mutants of the key amino acid residues lining the pores was studied (Paper I). Enzymes of the Pdu pathway, i.e. PduCDE, PduQ and PduP, were characterized with respect to their substrate selectivity (Paper II). In all cases, C3 substrates were the most optimal for the enzyme activities. The substrate range for PduCDE could be increased up to C6 diols by site-directed mutagenesis of two residues of *pduC* gene, however the passage of the longer substrates through the MCP shell in *L. reuteri* seemed to be limited. While PduQ has been known to reduce the 3-HPA to 1,3-PDO inside the MCP, the possible role of other alcohol dehydrogenases (ADHs) in transformation of 3-HPA outside the MCP and to maintain redox balance was investigated. It was discovered that while PduQ was the active enzyme in reducing the 3-HPA in the resting cells, it was ADH7, a cytoplasmic enzyme that reduced the hydroxyaldehyde in the growing cells to provide the cofactor NAD^+ required for glucose metabolism (Paper III). The heterologously expressed PduQ enzyme was characterized with respect to its activity against its optimal substrates 3-HPA and 1,2-propanediol (Paper IV). Exposure to air resulted in significant loss of PduQ activity and its bound iron content. The identity of the residues on the enzyme binding iron were confirmed by mutational studies.

Key words
L. reuteri, Pdu pathway, MCP, shell proteins, glycerol, 3-hydroxypropionaldehyde, 3-hydroxypropionic acid, 1,3-propanediol, glycerol dehydratase, 1,3-propanediol oxidoreductase, propionaldehyde dehydrogenase

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Cover illustration front: Red Chinese door with a gold lion handle. Red in Chinese culture means good luck, happiness and joy.

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Dedicated to my family
Hong and Leah

Contents

List of Publications

This thesis is based on the following publications, referred to by their Roman numerals:

I **Microcompartment shell proteins in *Lactobacillus reuteri*: selective engineering of a pore residue influences glycerol biotransformation**
L. Chen, P.D. Bromberger, R. Hatti-Kaul
Submitted

II **Exploring *Lactobacillus reuteri* DSM20016 as a biocatalyst for transformation of longer chain 1,2-diols: Limits with microcompartment.**
L. Chen, R. Hatti-Kaul
PLOS ONE 12 (9): e0185734 (2017)

III **Redox balance in *Lactobacillus reuteri* DSM20016: Roles of iron-dependent alcohol dehydrogenases in glucose/glycerol metabolism**
L. Chen, P.D. Bromberger, G. Nieuwenhuiys, R. Hatti-Kaul
PLOS ONE 11(12): e0168107 (2016)

IV ***Lactobacillus reuteri* 1,3-propanediol oxidoreductase (PduQ): Kinetic characterization and molecular modeling**
L. Chen, G. Nieuwenhuiys, Tarek Dishisha, R. Hatti-Kaul
Manuscript

Paper not included in the thesis:

Lactic acid bacteria: from starter cultures to producers of chemicals

R. Hatti-Kaul, **L. Chen**, T. Dishisha, H. El Enshashy
FEMS Microbiology Letters 2018

Paper II and III are reproduced with permission of their respective publishers. Fig. 6&7 (drawn by Tarek Dishisha) are obtained from Paper (not included) with permission from FEMS. Parts of the results in Paper I (PduA mutant) and Paper IV (PduQ activities) were presented at conference 4th Applied Synthetic Biology in Europe (October, 2018) and also reported in Master theses of Paul David Bromberger and Gavin Nieuwenhuiys.

Author Contributions

Co-authors are abbreviated as follows:
Rajni Hatti-Kaul (RHK), Paul David Bromberger (PDB), Gavin Nieuwenhuiys (GN), Tarek Dishisha (TD)

Paper I: Microcompartment shell proteins in *Lactobacillus reuteri*: selective engineering of a pore residue influences glycerol biotransformation

I designed and performed all the experiments, data analysis and writing of the manuscript. PDB (Master student) participated in performing analysis on glycerol consumption and 3-HPA prodcution by mutant *L. reuteri* (H40A). RHK was involved in the manuscript editing.

Paper II: Exploring *Lactobacillus reuteri* DSM20016 as a biocatalyst for transformation of longer chain 1,2-diols: Limits with microcompartment.

I designed and performed all the experiments, data analysis and writing of the manuscript which was revised by RHK.

Paper III: Redox balance in *Lactobacillus reuteri* DSM20016: Roles of iron-dependent alcohol dehydrogenases in glucose/glycerol metabolism

I designed and performed all the experiments, data analysis and writing of the manuscript. PDB (Master student) did the experiment on 3HPA analysis and GN (Master student) involved in alcohol dehydrogenase gene cloning and protein expression. The work was performed under the supervision of RHK.

Paper IV: *Lactobacillus reuteri* 1,3-propanediol oxidoreductase (PduQ): Kinetic characterization and molecular modeling

I designed and performed all the experiments, data analysis and writing of the manuscript which was revised by TD and RHK. GN (Master student) participated in gene cloning and protein expression work.

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Thank you **Mum** and **Dad**, for your love and unconditional support. Love you!

Thank you **Hong**, you know that You Are the Apple of My Eye. "Father, Smith, Warrior, Mother, Maiden, Crone, Stranger, I am his, and he is mine, from this day until the end of my days." **Leah**, sometimes when I feel so down and out, I only need to look at you and be reminded that you are my miracle. Daddy love you, baby girl.

For the ancestors who pave the path before me upon those shoulders I stand. Thank you all! Your stories make my day! " Any sufficiently advanced technology is indistinguishable from magic".

Finally, I thank myself, for having positive attitude and love inside my natural being, and for this beautiful life I have.

Popular Summary in English

Around four billion years ago the earth saw the emergence of life in the form of bacteria. Since then, these single-cell organisms have populated our planet. The reason they have been able to survive for such an incredibly long time–despite dramatically varying conditions such as volcanic eruptions and ice ages–is their fantastic ability to adapt. Research on bacteria and their importance for our health has literally exploded in recent years and new theories and insights on exploiting microorganisms as production hosts or cell factories are being discussed extensively. These developments have been facilitated by availability of tools and technologies that provide better understanding of a microbial system and their metabolic capabilities and possibilities to modify their synthetic power.

Cell factories have been largely exploited for the controlled production of substances of interest for food, pharma and biotech industries. The physiological diversity of the microbial world offers an intricacy of metabolic pathways from which novel bioproducts, including nano- or microstructured materials, offer promises in even more diverse applications.

Lactic acid bacteria are a diverse group that have been used for centuries for fermentation of foods and their preservation, and today they are clearly the most important group of industrial microorganisms with a market in the range of multibillion dollars. LAB are used as starter cultures for fermentation of milk, vegetables, meat, fish and cereals, to make yogurt, cheese, cultured butter, sour cream, sausage, cucumber pickles, olives, sauerkraut, etc., and also animal feed in the form of silage. But some species of lactic acid bacteria may spoil beer, wine and processed meats. Among the important features of lactic acid bacteria are their tolerance to environmental stresses, simple metabolism and their ability to metabolise several carbon sources. Currently, scientists are fuelling attention towards novel uses of these microorganisms from food fermentations and probiotics to therapeutic agents for animals, plants and humans as well as platform production strains for chemicals and fuels. A very well known product is lactic acid, which besides being used in foods and cosmetics, has found increasing interest in the production of biodegradable plastic, polylactic acid (PLA).

Throughout evolution *Lactobacillus reuteri*, as one kind of LAB, has made its home in the specific environment found in the human gut. It is the first bacterial species that has been found to be established in the microbiota of newborn babies. *L. reuteri* has also been found in breast milk, mouth, stomach, small intestine, vagina, etc. It has even been found to inhibit pathogens like *Escherichia coli* and *Salmonella*, and has other beneficial health effects because of which it is used as a probiotic for infants, children and adults. Its name derives from "reuterin"–3-hydroxypropionaldehyde (3-HPA)–a chemical with antimicrobial activity produced by *L. reuteri* from glycerol. *L.*

reuteri can itself tolerate high concentrations of reuterin, which is useful for its antimicrobial function. The bacterium does not use glycerol for cell growth but helps to improve cell growth when provided together with sugars. My thesis is focused on *Lactobacillus reuteri*, a bacteria with excellent probiotic features. In the thesis, I have tried to explore the metabolic pathway, a so-called propanediol utilization pathway, for metabolism of glycerol to different products including 3-HPA, 3-hydroxypropionic acid and 1,3-propanediol, all of which are considered to be valuable platform chemicals for the chemical and material industry of the future based on renewable feedstocks instead of fossil resources (Fig. 1). Glycerol is an important by-product of manufacture of soaps and biodiesel from plant oils, and thus forms a highly useful renewable feedstock.

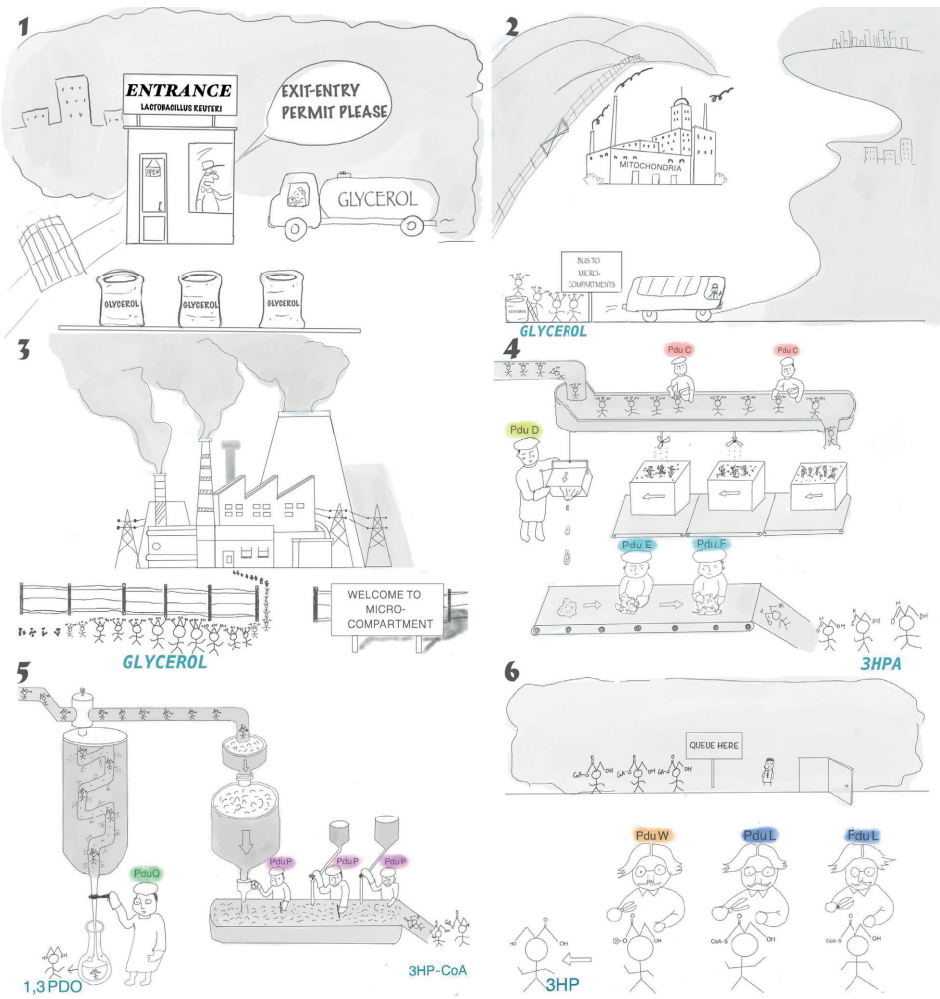


Figure 1 – Major players within the propanediol utilization pathway.

Popular Summary in Chinese

亲爱的们：

这应该是一封特别特别的信，信的内容将是有关我的博士课题，和那些背后的故事。我明白你们中的大多数会且仅会读完下面的文字，那我就认真写，你请慢慢听。

至今我依然清晰的记着我能说上来的第一份人生梦想—成为一名博士。在你看到这封信的时候我已经鬼使神差地把它实现了。我从来不算是一个有任何天赋的人，但唯独能在关键选择前看清机会：万幸地上本科，侥幸地考研，再加上稀里糊涂地读完博士，我也开始把自己当成个尽人事听天命的宠儿。

2013 年 8 月，来到瑞典隆德大学生物技术系读博，与我而言不算是挑战，更像是一种自我奖励。印度裔瑞典籍的导师不算大牛，学术能力一般但却足够穷力，实验室氛围消极压抑却都保持着彬彬有礼的克制。2-3 名相似课题方向的博士即将毕业，无心指导。那一刻，我就明白这将是一场孤独，坚忍，却充满机会的旅程。平常心，不抱怨，相信遇见就是最好的安排，成了那段时间里我经常的自我独白。

我的第一个课题和一种叫乳酸菌的微生物有关，它是一种大量存在于人类体内的益生菌，能够将碳水化合物发酵成乳酸，因而得名。益生菌能够帮助消化，有利于肠脏健康。在女儿出生的三个月，每天蒜口服防止肠绞痛的药里，就含有该微生物，这也让我瞬间对我的工作充满使命感。我感兴趣的是一条特殊的代谢通路，简称 PDU 通路，它天然的存在于这类乳酸菌形成的由多种壳蛋白构成的微颗粒体中，由于各类酶的作用，甘油可以通过该通路高效转化为 3 羟基丙醛，3 羟基丙酸和 1,3 丙二醇，这些都是在工业界价值极高的平台小分子，相比传统的化学冶炼，通过生物工程菌发酵的方式大量生产将会极具市场竞争力。我的工作就围绕着研究其间的分子工作机理展开。第二个课题从 2016 年暑假开始，很遗憾我无法在这本书里展示，我将会在博士结束后的一年时间里陆续发表这类的工作成果。简而言之，它涉及所谓的合成或系统生物学，主要致力于研究工程化的生物体系，通过对酵母或棒状杆菌的基因组改造，实现高效生产平台分子的功能。

最后还是要说点虚的，所谓的经验和教训。

导师向别人介绍我时最常用的一个词：ambitious，英文解释叫 having or showing a strong desire and determination to succeed。中文叫有抱负，志向远大，野心勃勃。我把它看成是一个中性词。自以为这应该是大多数中国学生给导师的基本印象：能干活，却不善交际；仰望星空的多，脚踏实地地少。看得到从小到大升学压力带来的影子，像机器一样的工作，对于梦想，呈懵逼状。现在回头来审视自己博士做的事情，迷迷怔怔，内心没法真正的慢下来，就匆匆地随着人潮去做了。

以后的日子，会为自己的内心而活。

最后，还是要矫情地感谢命运，因为足够幸运，2015 年，有了心爱的妻子，2017 年，有了健康的女儿。也许那是人生最爽快的 5 年，都留在了瑞典。

最近几年，爱读书了，是个好事。我的人生后 40 年一定会是位马尔克斯那样的作家，即便写不出《百年孤独》，但就算一辈子读本《道德经》，也会有更深的体会：

天之道，利而不害；圣人之道，为而不争。

2018 国庆，

于瑞典隆德

Populärvetenskaplig Sammanfattning på Svenska

Cellfabriker har länge utnyttjats för att producera intressanta ämnen åt livsmedels-, läkemedels- och bioteknik-industrin. Den mikrobiella världens fysiologiska mångfald erbjuder ett otal biosyntetiska produktionsvägar från vilken nya bioprodukter, inklusive nano- eller mikro-strukturerade material, kan utvinnas samtidigt som de erbjuder löften om fler applikationer.

Mjölksyrabakterier är en fylogenetiskt varierad grupp, vars användning snabbt expanderar från traditionella fermenterade livsmedel och probiotika till läkemedel för djur, växter och människor samt som produktionsstammar för kemikalier och bränslen. Fördelarna som mjölksyrabakterier erbjuder dels i a) säkerhet för mänsklig och djurkonsumtion, b) metabolisk mångsidighet, c) bred ekologisk nischanpassning, i kombination med d) deras långa användningshistoria i olika former av bioteknik, har gjort att forskare nu undersöker nya användningsområden för dessa mikroorganismer.

I den pågående utvecklingen att använda mjölksyrabakterier som cellfabriker för att effektivt producera många industriellt viktiga kemikalier och material, undersökte min avhandling mekanismerna *Lactobacillus reuteri*, en typ av mjölksyrabakterie som, kan använda för att producera värdefulla plattformskemikalier från glycerol.

List of Abbreviations

L. reuteri	*Lactobacillus reuteri*
E. coli	*Escherichia coli*
G. thermoglucosidasius	*Geobacillus thermoglucosidasius*
1,3-PDO	1,3-Propanediol
3-HP	3-Hydroxypropionic acid
3-HPA	3-Hydroxypropionaldehyde
GDH	Glycerol dehydratase
PduP	Propionaldehyde dehydrogenase
PduQ	1,3-Propanediol oxidoreductase
PduL	Phosphotransacylase
PduW	Propionate kinase
Nox	NADH oxidase
ADHs	Alcohol dehydrogenases
LDHs	Lactate dehydrogenases
EM pathway	Embden-Meyerhof pathway
PK pathway	Phosphoketolase pathway
GIT	Gastrointestinal tract
CAGR	Compound annual growth rate
PTT	Polytrimethylene terephthalate
PET	Polyethylene terephthalate
PA	Polyamide
MCP	Microcompartment
LAB	Lactic acid bacteria
BMC	Bacterial microcompartment
MCF	Microbial cell factory
Pdu	1,2-Propanediol utilization
Etu	Ethanol utilization
Eut	Ethanolamine utilization
GC content	Guanine-cytosine content
NICE system	Nisin controlled gene expression system

Exploring the Propanediol Utilization Pathway in *Lactobacillus reuteri*

1 Introduction

Concerns regarding environmental issues have led to intense efforts within the research community for investigating sustainable routes for production for the future industry that is independent of fossil feedstocks and instead based on renewable resources. Much of these efforts have so far been directed towards addressing the energy challenge by developing processes for production of bioethanol, biodiesel and other advanced biofuels from biomass in order to reduce the amount of fossil based gasoline and diesel currently used as transportation fuels. Fuel is however a low cost product, and as in petrochemical refineries integrated production of chemicals and materials with energy and transportation fuels, should provide higher added economic value to biobased production [25].

The challenges for the biobased chemicals production are the large number of targets, including those already made by the chemical industry as well as new structures, and the need for conversion technologies. Building a biobased chemical industry requires transformation of the biomass components to platform chemicals that serve as building blocks for other chemicals and so on. A number of C2-C6 chemical products have been identified as top candidates for production from biomass. Among these are the C3-chemicals, 3-hydroxypropionaldehyde (3-HPA), 3-hydroxypropionic acid (3-HP) and 1,3- propanediol (1,3-PDO), which are covered in this thesis.

Microorganisms offer versatile tools for manufacture of biobased products. The enormous diversity of microorganisms in nature provides large number of metabolic pathways and enzymes for large variety of chemical transformations. Moreover, the availability of genomics, proteomics and genetic tools allows deeper understanding and

designing of micoorganisms with desired activity and selectivity.

1.1 Scope of the Thesis

This thesis is based on the studies performed on *Lactobacillus reuteri*, a heterofermentative probiotic microorganism that possesses a metabolic pathway called the propanediol utilization (Pdu) pathway that catalyzes dehydration of glycerol to 3-HPA and then branches into an oxidative and reductive routes to give 3-HP and 1,3-PDO, respectively, as end products. The Pdu pathway is operative inside Pdu microcompartment that encapsulates different cofactors and enzymes needed for metabolizing glycerol or 1,2-propanediol, and protects the cells from the toxic effects of the aldehyde intermediate. The work described in this thesis focuses mainly on fundamental investigations to increase the understanding of the components of Pdu pathway and the microcompartment shell housing the pathway in *L. reuteri*.

The thesis is based on 4 papers. To date, two of which are published.

Paper I deals with a study on the major shell proteins of the microcompartment, PduA and PduJ to understand their role in passage of metabolites in and out of the microcompartment.

In Paper II, the key enzymes of the pathway, glycerol dehydratase (PduCDE), 1,3-PDO oxidoreductase (PduQ) and propionaldehyde dehydrogenase (PduP) were characterized with respect to their substrate selectivity, and the dehydratase enzyme was mutated to broaden the substrate scope of the enzyme.

Paper III investigates the roles of PduQ and other two iron-dependent alcohol dehydrogenases (ADHs) located in cytoplasm in 3-HPA reduction to 1,3-PDO and in establishing cofactor homeostasis.

Paper IV deals with further characterization of PduQ with respect to its activity with its substrates, glycerol and 1,2-propanediol, and structural considerations especially with respect to metal binding.

The following sections in the thesis provide background information concerning bioeconomy, potential of industrial biotechnology and microbial cell factories, lactic acid bacteria, particularly *Lactobacillus reuteri*, summary of the investigations performed and the results achieved in the different papers, and finally conclusions and future perspectives based on the results presented.

2 Bioeconomy–Green but not Panacea

The consequences of climate change and the finite nature of fossil resources constitute two broadly acknowledged challenges for society in the decades to come. Deploying green raw materials (biomass and wastes) as feedstocks for chemical products and energy opens a whole new world of possibilities for the use of biomass and the gradual replacement of fossil resources (coal, oil, natural gas). Bioproducts, which are derived fully or at least in part from renewable resources, have the unique advantage over conventional product of being carbon-neutral and in reducing the dependence on non-renewable feedstock such as crude oil and in reducing greenhouse gas (GHG) emissions [98].

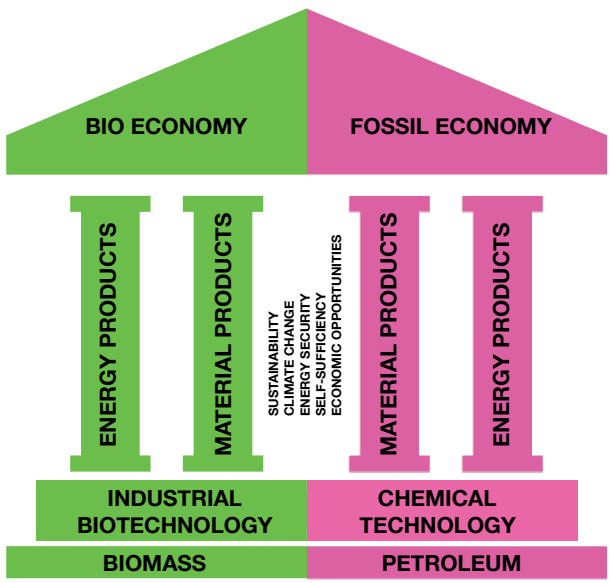


Figure 2 – Bio economy and fossil economy–Co-construction of the future.

Bioeconomy is an economy in which food, feed, chemicals, materials, transport fuels, electricity, heat, etc. are produced economically and sustainably from green resources: resources that are renewable [151][215]. The analysis of the Eurostat data from 2013 showed that the total turnover of bioeconomy (including food, beverages, the primary sectors agriculture and forestry) in the EU-28 was valued at 2.1 trillion euros. Roughly half of this is accounted for by the food and beverages sector, almost a quarter of the turnover is created by the primary sectors (agriculture and forestry), while the other

quarter is created by the so-called bio-based industries (such as chemicals and plastics, pharmaceuticals, paper and paper products, forest-based industries, textile sector, biofuels and bioenergy). In addition, a significant proportion of 10% (22 million jobs) of the total employment in the Union came from bio-based industries [186][14].

The development of the bio-based economy needs optimised cooperation between the production of food, feed, bio-based chemicals, materials, biofuels and energy. Originally, we believed that production of bulk chemicals or fuels using bio-based approaches will provide low cost and probably environmentally friendly products to partially replace petro-chemicals products. Yet, the move towards the bio-based economy is not as fast and smooth as we thought, which is subject to critical discussions around stability of petroleum price, remote reality of depletion of petroleum, unbalanced regional economic situation, feedstock availability, and need for efficient, green conversion technologies [51][38][183][182][31].

Indeed, the last decade has witnessed a tremendous increase in exciting new products with increased bio-based content, but these products have tended to come with high costs, maintenance or durability issues, or inconsistent availability. It seems like non-fossil based materials should be inherently green and preferable, but that doesn't mean they always are. For example, when wind (or solar) power replace fossil energy, they cut CO_2 emissions, reducing GHG-driven global climate change, while at the same time causing climatic impacts elsewhere. For example, generating today's US electricity demand (0.5 TWe[1]) with wind power would warm Continental US surface temperatures by 0.24°C as reported by Miller and his colleagues [154].

The controversies concerning the various steps in the biomass production and processing chain demonstrate that the development of a bio-economy involves a complex process of change fraught with uncertainties [5]. There are many different opinions as to how we should be dealing with this process of change. Should we simply tackle the challenge and learn by doing, or should we ensure that we have the right, i.e. sustainable methods before we increase the demand for biomass?

Despite considerable successes and potential risks, a lot of research and development still needs to be done. Facing challenges including the emergences of alternative energy sources (e.g. shale gas, gas hydrate and sand oil), many opportunities are still there and the answers to make industrial biotechnology processes more competitive are being proposed by scientists.

[1]Terawatt eletricity. The terawatt is equal to one trillion watts

2.1 Puzzle pieces of Biorefinery System

The replacement of fossil-based carbon with renewable carbon from biomass leads to the development of biorefinery facilities, where transportation biofuels, bioenergy, biochemicals, biomaterials, food and feed are efficiently coproduced. Currently, a broad spectrum of different biorefinery systems are under study, some of which are already competitive in the market while others are still under development [86][116].

There are a very large number of possible combinations of feedstock, pre-treatment options, conversion technologies and downstream processes that can be followed as potential pathways to make biofuels and biochemicals.

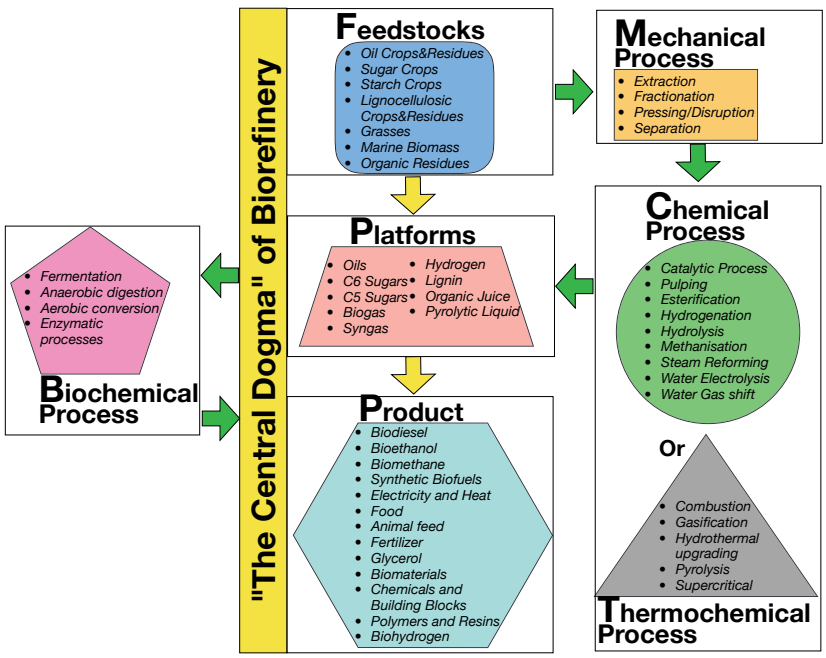


Figure 3 – Biorefinery system.

The International Energy Agency (IEA) Bioenergy Task 42 classified each individual biorefinery system with the following four main features as listed in order of importance [37](Fig. 3):

- **Platforms.** Platforms are intermediates which link feedstocks and final products. They can be reached via various conversion processes applied to different raw materials. Conversion of these platforms to marketable products can be carried

out using different processes as well.

- **Products.** Biorefineries produce both energy and material products, which are classified as energy-driven biorefinery systems and material-driven biorefinery systems, respectively.

- **Feedstock.** Feedstock is the renewable raw material, also called biomass that is converted to marketable products in a biorefinery. The biomass feedstock can be generally subdivided into primary (from forest or agricultural land), secondary (process residues) and tertiary (wastes).

- **Processes.** In order to produce biofuels, biochemicals or biomaterials, the feedstock is transformed into final products using different conversion processes, e.g. physical, biochemical, chemical and thermochemical processes.

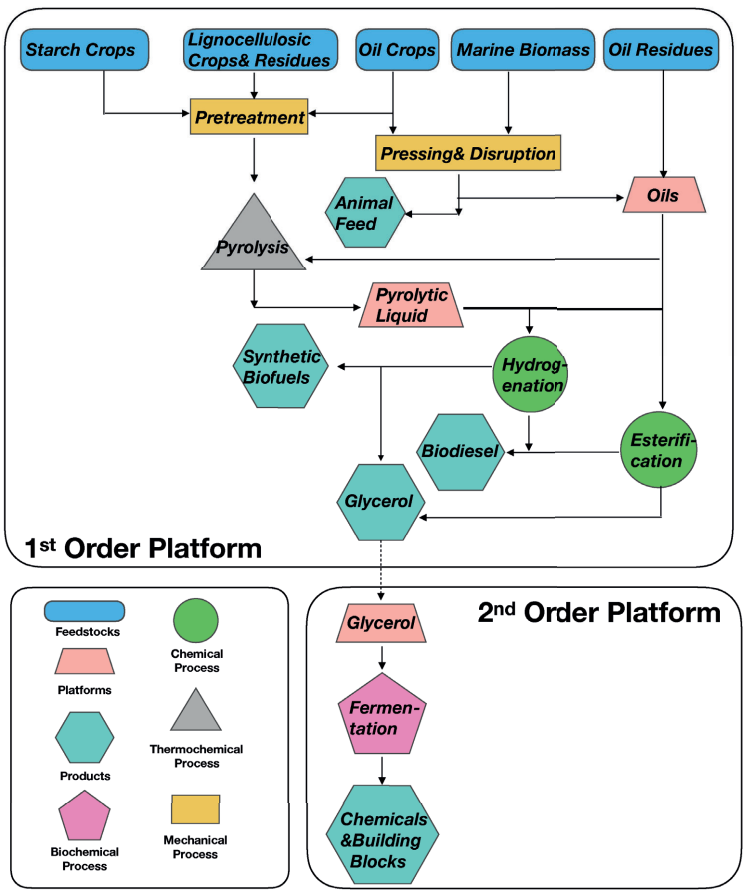


Figure 4 – Two-order platform biorefinery for glycerol.

Based on the classification of biorefinery systems described above, the biorefinery network of Fig. 4, illustrated as an example, shows a two-order biorefinery system for glycerol production, which is further converted to building block chemicals (e.g. 3-HPA, 3-HP, 1,3-PD). This network description does provide a valuable overview of the systems, identifying possible alternative pathways for exploiting all different biomass components and leading to the possibility to replace fossil-based products in the most efficient way.

3 Industrial Biotechnology for the Production of Platform Chemicals

Industrial biotechnology is one of the most promising new approaches to pollution prevention, resource conservation, and cost reduction, which is often referred to as the third wave in biotechnology, and is distinct from the first two waves (medical or red biotechnology and agricultural or green biotechnology). Industrial biotechnology makes use of enzymes and microorganisms or plant/animal cells to make products in a wide range of industrial sectors including chemicals, pharmaceuticals, food and feed, detergents, pulp and paper, textiles, energy, materials and polymers [207]. Compared to chemical technology, bioprocesses using living cells or enzymes, offer several advantages over conventional chemical methods of production: *(i)* they usually require moderate temperature, pressure and pH, *(ii)* they can use renewable resources as well as synthetic compounds as raw materials, and *(iii)* relatively large quantities can be produced with less energy consumption (Table 1).

Table 1 – Industrial biotechnology vs. Chemical technology.

Points	Industrial biotechnology	Chemical technology
Substrates	Bio-based	Petroleum derivates
Conversion efficiency	Relatively low	Relatively High
Process selectivity	Often high	Often low
Product concentration	Low	High
Reaction system	Mostly water	Mostly organic solvent
Reaction temperature	30-40°C	>100°C generally
Reaction rate	Relatively slow	Relatively fast
Reaction pressure	Normal	High
Recovery cost	High	Medium
Sterilization	Necessary	No need
Waste	Not toxic	Generally toxic

Already, there are some major industrial fermentation products of biotechnology like citric acid (1,200,000 mt/y[2]), ethanol (31,600,000 mt/y), glutamate (1,000,000 mt/y), lactic acid (259,000 mt/y), lysine (1,200,000 mt/y), penicillin (215,000 mt/y), xanthan gum (110,000 mt/y), etc. [207] produced by microbial fermentations. However, there are many challenges to enable greater use of biological processes in various areas, including the production of platform chemicals, polymers and biofuels (table 2). Among the popular strategies being pursued to overcome some of the challenges in industrial biotechnology is the engineering of microorganisms to develop robust and selective microbial cell factories that can be used in a biorefinery systems for the transformation of biomass components or derivatives [57][41].

[2]metric tons per year

Table 2 – Challenges for industrial biotechnology.

Problems	Possible solutions
Low density of growing cells	Developing high cell density system
Product inhibition	Continuous process, *in situ* product recovery
Low efficiency in mixed substrates utilization	Assembling multiple pathways
Formation of by-products	Engineering microbial cells
Limited biocatalyst stability	Protein engineering, process design
Large amount of water consumption	Utilization of recycle water
High energy demand	Integrating with suitable process to capture wasted energy, developing anaerobic bioprocess
Discontinuous processing	Develop continuous processes
Unknown environmental impact of the developed process	Life-cycle assessment (LCA)

Economical production of platform chemicals from the (pretreated) biomass is key to the success of chemicals production in bioeconomy. A number of chemical structures including several organic acids and alcohols/polyols have been recommended as platform candidates by the United States Department of Energy (US DOE) based on their potential for further valorization to a spectrum of products [239][106].

In recent years, several publicly funded research centers associated with industrial biotechnology, e.g. B-Basic (The Netherlands), ACIB (Austria), JBEI (U.S.), DTU Biosustain (Denmark), MBEL (South Korea), etc., have integrated academic research with industrial need for bio-based products. With all the efforts, white and green biotechnology will grow more closely entwined as the bioeconomy continues to develop.

3.1 Microbial Cell Factories–a Biochemical Process Way

In the traditional chemical processes as mentioned above, the processed starting material is converted into the product of interest in the presence of a catalyst through a series of unit operations. Advancement and prospect of catalysis technologies, together with the process optimization plays crucial role in maximizing conversion efficiency and overall profitability. Microbial cell factories (MCFs) have emerged as a revolutionary platform, which is of considerable interest to convert low value renewable substrates to high value chemicals and biofuels through combining traditional unit operations and complex multi-step catalysis into a single self-replicating microbe [75]. Substrates can be selectively taken up by cells and converted into desired products via metabolic networks (native or artificial) inside the living organisms [57].

Design of a MCF is a multifaceted optimization problem that consists of various challenges in defining the approach (native, heterologous, or artificial) [65], selecting the

MCF chassis (*Escherichia coli*, *Saccharomyces cerevisiae*, or others) [135], designing biosynthetic pathway (tools) [6], enzyme engineering (catalytic rate, stability, specificity, or cofactor requirement) [222] and metabolic engineering (experimental or computational tools) [205]. It is always clear that designing and optimizing of microbial cell factories originates with critical selection of product of interest, which will be a fundamental factor determining profitability, and *(i)* the substrates selected, *(ii)* the nature of product (e.g. secretion machinery, toxicity to the microbial host) and process (downstream), *(iii)* pathway activity and cell growth considerations, are critical in chassis selection for MCFs.

Table 3 – Cell factory engineering.

Strategies& Challenges	References
Native metabolites production–strategies	
Pathway overexpression	[249]
Transporter engineering	[68]
De-branching	[179]
Product degradation	[136]
Co-factor engineering	[138][4][250]
Removal of feedback inhibition	[189][134]
By-product elimination	[235]
Precursor/substrate enrichment	[167][256]
De-regulation of carbon catabolism	[173][187]
Signal transduction engineering	[119]
Heterologous expression of biosynthetic pathways–challenges	
Compartmentalization or steric proximity	[206][67][237][33]
Co-factor availability	[32][22]
Substrate and co-substrate availability	[9][12][122]
Product efflux pumps	[184][247][34]
Biosynthesis of functional groups	[30][78]
Transcription engineering	[185]
Protein expression–strategies	
Promoter engineering	[76][149]
Gene fusion for enhanced secretion	[47]
Stability of heterologous gene transcripts	[96][28]
Improved translocation to the endoplasmic reticulum	[28][28]
Protein secretion stress engineering	[233][85][203]
Post-translation modification machinery engineering	[48]
Improved vesicle trafficking	[110]
Protein glycosylation engineering	[233][143]
Protease deletions	[238]
By-product removal	[142]

Metabolic engineering seeks to reprogram the metabolic wiring in a cell in order to expand, improve or modify its natural properties for useful purposes, for example,

maximizing the production of a target compound or degrading a pollutant in an efficient way. Enzyme- and metabolic engineering can improve the flux and stability, and develop a productive MCF [49](Table 3).

Recent progress in engineering microorganisms as a platform for the production of substances of interest for food, pharma and biotech industries from renewable resources enables varied choice of MCF chassis via redesigning microbial cellular networks and fine-tuning physiological capabilities. While the platform strains of the bacterium *E. coli* [135][49] and the yeast *S. cerevisiae* [73][168][169] still dominate as popular hosts mainly according to *(i)* availability of various toolkits, *(ii)* controllable physiological traits, *(iii)* fast growth, etc. This is expected to change, in the near-term.

The biological diversity in nature offers an intricacy of biosynthetic pathways which will allow the MCF chassis to take advantage for efficient production of chemicals and materials via innovative bio-approaches [139][42]. Mammalian and insect cells are intensively reported for production of high quality proteins [74][188], and other unconventional systems including algae [91][103], fungi [152][19], *Pseudomonas* sp. [16][241], *Corynebacterium* sp. [101][13], *Bacilli* sp. [240][93], etc. are under continuous exploration for proteins and chemicals production.

3.2 Lego Bricks–Shaping the Perception of Synthetic Biology

During the last decade, a steady increase of synthetic biology-inspired approaches for metabolic engineering has enabled researchers in the field to adopt a truly rational methodology to reprogram the cell behavior. Synthetic biology (SynBio) aims at making biology an engineering discipline by turning molecular and cellular behaviour into a more controllable, standardized and predictable set of features. Based upon the scientific rationale of designing and constructing new biological parts, devices and systems, synthetic biology links the knowledge from the "omics" tools, transcriptomics, proteomics, systems biology to metabolic engineering. Indeed, SynBio has brought together biologists and engineers to work on a common goal of engineering organisms to behave according to design specifications. Synthetic biologists follow a rather simple approach in which different living systems can be created by engineering and assembling essential, foundation bricks of life–DNA.

Handling the DNA in terms of Lego bricks conveys ideas of playfulness and toys because the word LEGO is mainly associated with such expressions, but is doing synthetic biology something quite different than playing around with toys?

Consider the ubiquitous Lego toy system [11], the signature feature of which is the patented snap connection for easy but stable assembly of components. The snap is the basic Lego protocol, and Lego bricks are its basic modules [45]. Similarly, The Cent-

ral Dogma is the basic bio-protocol. Similarly, the present developments in synthetic biology allow gene fragments, cassettes or operons to be synthesized by even complete genomes [87][88]. More exciting is to generate bio-bricks as basic bio-modules (e.g. plasmids, transposons, genomic islands, gene cassettes or other discrete genomic sequences) with various gene functions that can be incorporated into different host cells and, in varying combinations, tested in high throughput systems for functionality.

While there are many bottlenecks, varying from identifying multiple bio-bricks functions to exploring new basic bio-modules, synthetic biology is the ultimate engineering approach that builds upon the biological understanding of systems biology that offers a set of methodological and strategic tools for applying systems approaches for understanding complex interactions among the metabolic network at the cell level, which are collectively represented as overall system performance [139]. Industrial strain development requires system-wide engineering and optimization of cellular metabolism while considering industrially relevant fermentation and recovery processes [138](Fig. 5).

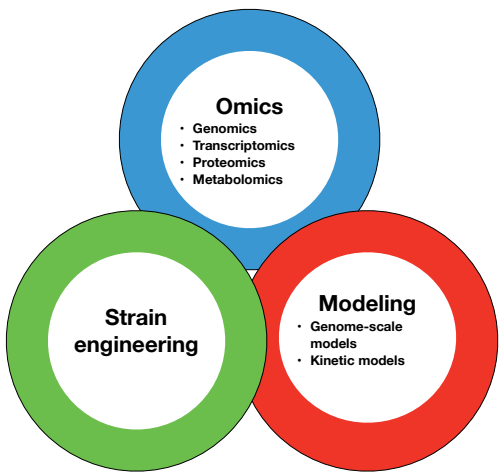


Figure 5 – General scheme of systematic approaches.

4 Lactic Acid Bacteria (LAB) as Cell Factories

Lactic acid bacteria are among the most important group of microorganisms used industrially as starter cultures and also as probiotics with a market in the range of multibillion dollars [54]. Among the features that favor widespread industrial applications of LAB are the GRAS (Generally Regarded As Safe) status of most members of this group of bacteria and their tolerance to various stress environments [150]. Because of these features, LAB are being developed for *in vivo* delivery of therapeutic proteins and DNA vaccines. Presently, they are under development as emerging platforms for production of both food and nonfood products from renewable feedstocks in a biobased economy.

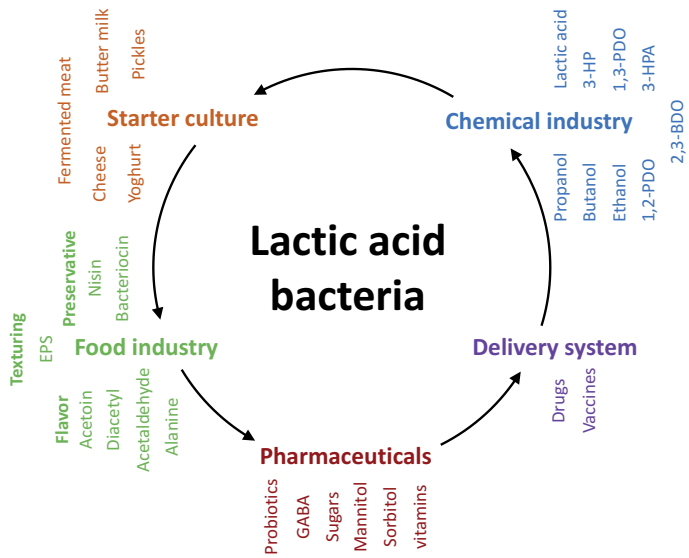


Figure 6 – Lactic acid bacteria: from starter cultures to producers of chemicals (drawn by Tarek Dishisha, Hatti-Kaul *et al.* 2018).

Lactic acid bacteria are a diverse group of gram-positive, low GC content, facultative anaerobic rods or cocci bacteria. Most of the known microaerophilic LAB belong to the phylum Firmicutes under several genera including *Enterococcus*, *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Oenococcus*, *Lactococcus*, *Streptococcus*, *Weissella*, etc. The anaerobic LAB belong to *Bifidobacterium* genus under the phylum Actinobacteria. Most commercially available starter cultures are represented by *Lactobacillus*, *Lactococcus*, *Streptococcus* species, while *Lactobacillus* and *Bifidobacterium* genera are the most commonly reported probiotics [43].

Until now, more than 75 complete LAB genomes have been sequenced. The genomes

of lactic acid bacteria are generally relatively small (1.23 Mb in *L. sanfranciscensis* to 4.91 Mb in *L. parakefiri*)[63][216][123]. Reductive evolution of the genomes involving loss of several metabolic genes and related functions, and also fewer higher-level genetic control systems as compared to many other microbes has been commonly observed in LAB. This is said to be due to their adaptation to nutrient rich niches [204]. Since lactic acid bacteria have a relatively simple metabolism, they require growth media with a high content of nutrients resulting in added costs for their cultivation in large scale.

Based on their metabolic capabilities, LAB are basically classified into three groups:*(i)* obligate homolactic fermenters (lactic acid as the single fermentation product), *(ii)* obligate heterolactic fermenters (produce lactic acid, ethanol/acetate and CO2), and *(iii)* facultative heterolactic organisms [80][109].

4.1 Central Carbon Metabolism

LAB metabolize sugars by fermentation and generate ATP by substrate level phosphorylation. The homofermentative LAB (*Streptococcus*, *Lactococcus*, *Enterococcus*, *Pediococcus*, and some *Lactobacillus* species) produce lactic acid as the main product, formed via the Embden-Meyerhof (EM) pathway that yields 2 moles of pyruvate per mol glucose. The pyruvate acts as an electron acceptor and is reduced to 2 mol lactic acid in a reaction catalyzed by lactate dehydrogenase (LDH). Phosphofructokinase enzyme was identified to have an important role in the EM pathway flux, decreased or increased activity of the enzyme results in proportionally lowered or improved flux and lactate formation. Under conditions of carbon limitation, low growth rate and change in oxygen concentration, the homofermentative bacteria have been found to shift to mixed acid fermentation. In the presence of oxygen, NADH oxidase (Nox) activity of the cells is increased that leads to competition for the available NADH and hence shift in metabolism to yield a mixture of products. The heterofermentative strains (*Leuconostoc*, *Weissella* genera and certain *Lactobacillus* species) produce also ethanol/acetate and CO2 (1 mol/mol sugar) besides lactate (1 mol) via the phosphoketolase (PK) pathway (Fig. 7).

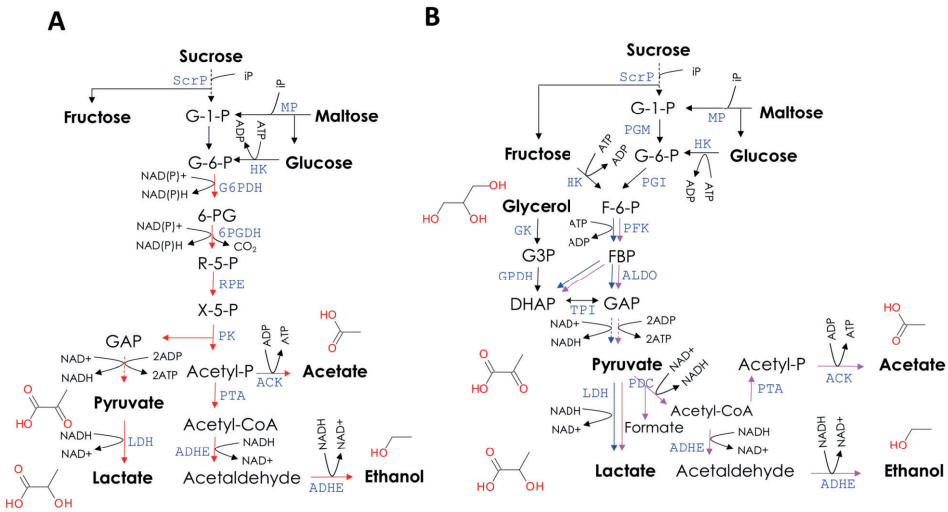


Figure 7 – Overview of the central carbon metabolic pathways of glucose, maltose, sucrose and glycerol in lactic acid bacteria. (A) Phosphoketolase (PK) pathway in heterofermentative bacteria (red); (B) Embden-Meyerhof (glycolytic) pathway in homofermentative (blue) and heterofermentative bacteria (purple). Enzyme abbreviations (Blue capitals): HK: hexokinase, MP: maltose phosphorylase, PGM: phosphoglucomutase, PGI: phosphoglucose isomerase, PFK: phosphofructokinase, GK: glycerol kinase, GPDH: glycerol-3-phosphate dehydrogenase, ALDO: fructose bisphosphate aldolase, TPI: triose-phosphate isomerase, LDH: lactate dehydrogenase, PDC: pyruvate dehydrogenase complex, PTA: phosphotransacetylase, ACK: acetate kinase, ADHE: bifunctional aldehyde and alcohol dehydrogenase, G6PDH: glucose-6-P dehydrogenase, PGL: phosphoglucolactonase, 6PGDH: 6-P-gluconate dehydrogenase, RPE: ribulose epimerase, PK: phosphoketolase. Intermediates abbreviations: G-6-P: glucose-6-phosphate, G-1-P: glucose-1-phosphate, F-6-P: fructose-6-phosphate, FBP: fructose-1,6-biphosphate, GAP: glyceraldehyde-3-phosphate, DHAP: dihydroxyacetone phosphate, G3P: glycerol-3-phosphate, 6-PG: 6-phophogluconate, R-5-P: ribulose-5-phosphate, and X-5-P: xylulose-5-phophate (drawn by Tarek Dishisha, Hatti-Kaul *et al.* 2018).

The homofermentative bacteria, mainly some *Lactobacillus* species, are used for industrial production of optically pure lactic acid that has found extensive application in the production of polylactic acid (PLA), a biodegradable and thermoplastic polyester [150]. The optical purity of the lactic acid produced depends on the type of lactate dehydrogenase (LDH) present, L- or D-LDH or both and also the presence of lactate racemases that interconvert the two isomers [146].

LAB are able to utilize several compounds as electron acceptors besides pyruvate and

oxygen, e.g. fructose, citrate, glycerol, 1,2-propanediol and 1,2-ethanediol. Fructose is reduced to mannitol by mannitol dehydrogenase [242], while citrate is split into acetate and oxaloacetate, which is converted to pyruvate and then lactate [111]. The latter three polyhydric alcohols are dehydrated to their corresponding aldehydes and further reduced to alcohols with regeneration of NAD^+.

4.2 *Lactobacillus reuteri*

Within the lactic acid bacteria, *Lactobacillus* species, which have been isolated from varied environments (e.g. from human gastrointestinal tract (GIT) to soil) is a highly diversified genus with over 160 different species displaying a large panel of catabolic activities [113][50]. *Lactobacillus* species can be divided into three groups including: *(i)* Obligately homofermentative (*L. acidophilus*, *L. delbrueckii*, *L. helveticus*, *L. salivarius*; *(ii)* Facultatively heterofermentative (*L. casei*, *L. curvatus*, *L. plantarum*, *L. sakei*); *(iii)* Obligately heterofermentative (*L. brevis*,*L. buchneri*, *L. fermentum*, *L. reuteri*).

Table 4 – Probiotic traits of *L. reuteri*

Probiotic properties	Ref.
In terms of metabolites production:	
Reuterin	[92]
Histamine	[58]
Vitamins	[220]
Exopolysaccharide	[196]
In terms of roles on modulation:	
Host microbiota modulation	
Gut microbiota	[100]
Oral microbiota	[232]
Vaginal microbiota	[147]
Immunomodulation	[236]
Neuromodulation	[255]
Leaky gut	[160]
In terms of roles on attenuating human diseases:	
Early-life disorders	[153]
Systemic lupus Erythematosus	[53]
Obesity	[17]
Neurodevelopmental disorder	[27]
Stressor exposure and enteric infection	[81]

Lactobacillus reuteri is a non-pathogenic, obligate heterofermentative bacterium that inhabits the gastrointestinal tract of humans and other animals [10]. Due to its properties, such as production of an antimicrobial agent called reuterin (a mixture of mono-

meric and dimeric forms of 3-hydroxypropionaldehyde) via glycerol fermentation, *L. reuteri* can inhibit the growth of pathogenic bacteria such as *E. coli* and *Salmonella*, which is utilized in its function as a probiotic [62][210][161]. *L. reuteri* based probiotic products for infants, children and adults are developed and marketed by a Swedish company BioGaia (www.biogaia.com). In addition to the GIT, *L. reuteri* has also been isolated from both the vagina and breast milk, and strains sequestered from these environments have been widely associated with probiotic health benefits, including the synthesis of vitamins such as B12 and folate (B9), as well as prevention and treatment of infectious diarrhea [225]. Several probiotic properties of *L. reuteri* that contribute to its diverse beneficial effects on host health and disease prevention from reported literature are listed in Table 4. However, many of the probiotic functions of *L. reuteri* are strain-dependent [161].

4.3 Glycerol as a Feedstock for Chemicals

Glycerol is a chemical which has multitude uses in pharmaceuticals, cosmetics, and food industries [2]. It can be produced as a by-product from saponification and hydrolysis reactions in oleochemical plants as well as transesterification reaction in biodiesel production [219]. Purification of glycerol as well as the conversion of glycerol into valuable products using both chemical and biotechnology processes have attained growing interest in recent years due to a glut in the glycerol market [131]. Global glycerol market is expected to reach 6261.75 thousand metric tons by 2024 from 3551.00 thousand metric tons in 2016, at compound annual growth rate (CAGR) of 7.5% [131][157]. The major factors driving the growth of this market are bio-renewable chemicals, growth in biodiesel production and wide range of applications in various industrial sectors [230][144]. Indeed, glycerol is also used as the raw material in the bio-production of valuable chemicals including 1,3-propanediol, *n*-butanediol, 2,3-butanediol, citric acid, polyunsaturated fatty acids, lipids, PHA, and others [2].

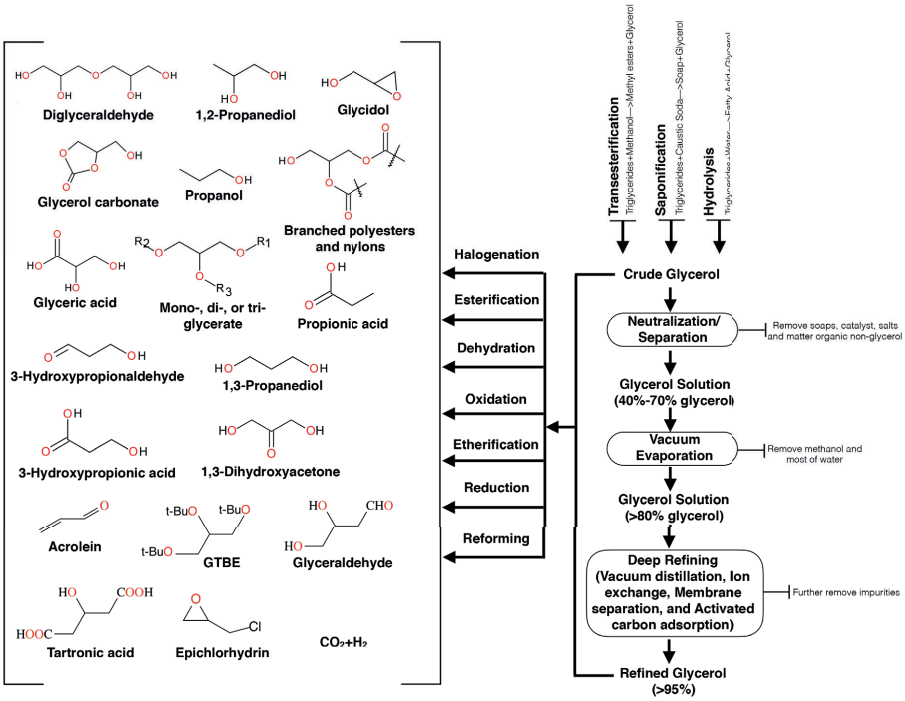


Figure 8 – Glycerol as a raw material for several valuable chemicals of industrial importance.

1,3-Propanediol (1,3-PDO) has attracted interest due to its applications in manufacture of polytrimethylene terephthalate (PTT) used in making fibers (Sorona®), which is expected to partially replace polyethylene terephthalate (PET) and polyamide (PA) [202]. 1,3-PDO has been synthesized from fossil based acrolein by Degussa and from ethylene oxide by Shell and is currently produced from glucose by DuPont using a patented metabolically engineered microorganism [257][125][70]. There has been a long-standing interest in the possible microbial production of 1,3-PDO from glycerol by anaerobic bacteria *Citrobacter*, *Klebsiella* and *Clostridia* species, but for a good economy it is preferable that all glycerol should flow into the 1,3-PDO forming pathway instead of entering the central metabolism of the bacteria [163][18][162][257][158].

3-Hydroxypropionic acid (3-HP) is a valuable platform chemical that can produced from glucose or glycerol by several microorganism, e.g. *L. reuteri*, *L. collinoides* and *Alcaligenes faecalis* [128]. The bifunctionality of 3-HP owing to the presence of a carboxyl and hydroxyl group (at the β position), makes it a versatile agent for organic synthesis. The compound can be used for the production of chemicals, such as acrylic acid, 1,3-PDO, methyl acrylate, acrylamide, ethyl 3-HP, malonic acid, propiolactone and acrylonitrile and can be used as a cross-linking agent for polymer coatings, metal

lubricants and antistatic agents for textiles [90][197]. Despite the diverse applications, the commercial production of 3-HP is limited due to the low yield and high production cost [115][55][217].

4.4 Propanediol Utilization(Pdu) Pathway

Glycerol is used as a carbon source for growth by some LAB, in which it enters the EM pathway at dihydroxyacetone phosphate (Fig. 7). It also acts as an electron acceptor in several heterofermentative LAB like *L. reuteri*, *Lactobacillus brevis*, *Lactobacillus buchneri*, and *Lactobacillus diolivorans* when present together with glucose and enables regeneration of NAD(P)H, being itself converted to 1,3-PDO as the end product and also resulting in increased growth rate and biomass yield [140]. Among the LAB, *L. diolivorans* and *L. reuteri* have been shown to be promising microbial hosts for the production of 1,3-PDO [140][257][158].

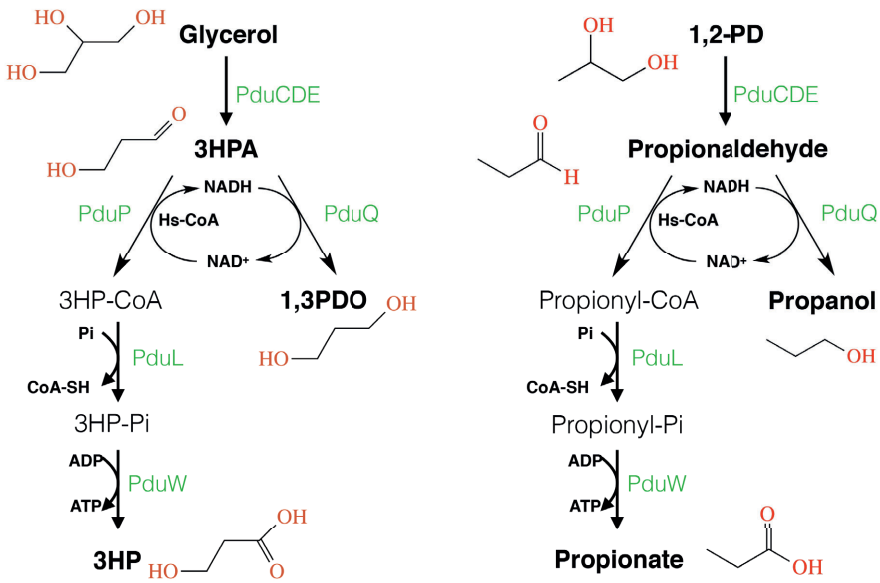


Figure 9 – Propanediol utilization (Pdu) pathway (glycerol and 1,2-propanediol as substrates, respectively).

Like the other anaerobes, *L. reuteri* converts glycerol to 1,3-PDO in two steps involving dehydration to 3-hydroxypropionaldehyde (3-HPA) followed by reduction to 1,3-PDO (Fig. 9). The enzymes catalyzing the glycerol (and 1,2-PDO) metabolism in *L. reuteri* belong to the propanediol utilization (Pdu) pathway, which takes 3-HPA

through reductive and oxidative routes, the flux through these routes being determined by the level of NAD^+/NADH inside the microbial cell [61]. Higher levels of NAD^+ would lead to oxidation of 3HPA to 3-HP, while accumulation of NADH would result in reduction of 3-HPA to 1,3-PDO catalysed by 1,3-propanediol oxidoreductase (PduQ) [213]. The oxidative route for 3-HPA metabolism involves 3 consecutive reactions catalyzed by coenzyme-A acylating propionaldehyde dehydrogenase (PduP), phosphotransacylase (PduL) and propionate kinase (PduW), respectively, to give 3-HP [61][194][195].

The enzymes of Pdu pathway are housed inside microcompartments that protect the cells from the toxic effects of the aldehyde intermediate [213].

4.5 Microcompartment

In 1956, Drews and Niklowitz observed an intracellular structure within the cyanobacterium *Phormidium uncinatum*. The organelles appeared similar to viral capsids which led to the speculation that they might be phages [40]. In 1969, Gantt and Conti found similar intracellular polyhedral bodies in many other blue green alga. Using electron microscopy, they described the polyhedral structures as highly ordered array of crystalline bodies. In 1973, these polyhedral inclusions were isolated and discovered to contain an enzyme known as ribulose bisphosphatecarboxylase (RuBisCO) [254]. The investigators proposed to name the bacterial organelle as carboxysome. It was later discovered that these structures exist in many other prokaryotic organisms. As opposed to membrane-bound eukaryotic organelles, the polyhedral bodies consist of proteinaceous shell and the structure is generally known as "bacterial microcompartment" (BMC) [77]. Further investigation of these polyhedral bodies revealed that there are at least seven different types of bacterial microcompartments [252]. Several BMCs are far more complex than the carboxysome. They consist of various functional metabolic enzymes within the proteinaceous shell, and thus these microcompartments were proposed to be called as "metabolosomes" –structural bodies that enclose metabolic activities. Four types of bacterial microcompartments have well-established functions and will be discussed briefly here: carboxysome, ethanol utilisation (Etu), ethanolamine utilisation (Eut), and 1,2-propanediol utilisation (Pdu) bacterial microcompartments [118].

4.6 Pdu Operon

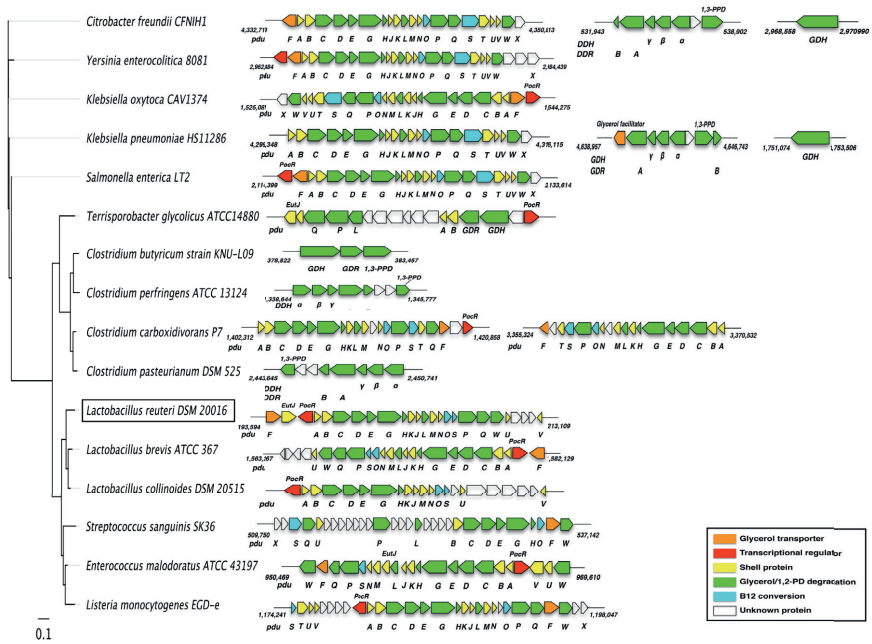


Figure 10 – Comparison of the genomic location that contains the *pdu* gene cluster. Genes in the *pdu* gene cluster are depicted by arrows indicating the transcription direction with the same color codes as decribed in the legend.

Pdu enzymes are encoded by the pdu-operon, which also contain genes encoding the shell proteins of the microcompartment housing the metabolic pathway [253]. In *Lactobacillus reuteri*, the genes required for glycerol/1,2-PDO metabolism form a contiguous cluster in the *pdu* locus at position ranging from 193 594 bp to 213 109 bp of the chromosome. This locus includes all the structural proteins and lumen enzymes that comprise the Pdu MCP as well as the regulatory genes that control production (Fig. 10). The *pdu* operon includes at least 18 *pdu* genes, *pduABCDEGHJKLMNOSPQWU*, that encode 7 of glycerol/1,2-PDO degradative enzymes (PduCDELPQW, colored green), 4 of B12 recycling proteins (PduGHOS, cyan), as well as 7-8 of Pdu shell proteins (Pdu ABB'JKNUM) and other unknown proteins. The *pocR* (red) and *pduF* (orange) encode a positive transcriptional regulatory protein and a glycerol diffusion facilitator, respectively. The stimulatory effects of 1,2-PDO on the transcription of the cobalamin (vitamin B12) biosynthetic (*cob*) operon and the 1,2-PD utilization (*pdu*) genes was demonstrated by Rondon and his colleagues [191].

4.7 Challenges and Strategies in Exploiting LAB as MCF

The main traits of lactic acid bacteria that are important for being exploited as MCF in industrial microbiology are depicted as *(i)* high sugar uptake rates(various hexoses and pentoses), *(ii)* simple carbon metabolism and flexibility of electron acceptors, *(iii)* low biomass yield and *(iv)* resistance against several stresses (pH, alcohol concentration, osmotic stress, etc.)[201]. These attributes make LAB attractive cell factories for the industry.

However, many phenotypic differences exist between the lactic acid bacteria even at species level, despite their relatedness [244]. This phenotypic variation has not really been used for chemicals production due to potential challenges listed below.

- **Growth requirements.** Can we wean lactic acid bacteria? This is an interesting question proposed by Michael Sauer and colleagues [201]. The strong adaptation of LAB to nutrient-rich niches have led to a significant reduction of their metabolic capabilities as a beneficial trait for downstream process in industry. On the contrary, the requirement of complex media for these fastidious microorganisms is difficult in terms of higher cost. In order to be competitive with other established microbial systems, the LAB hosts should be optimized for prototrophic growth on simple media obviating the need for yeast extract or other additions. That said, even though the carbohydrate metabolism of these organisms is efficient with higher fluxes and fewer defined products, strategies should be applied based either on *(i)* genome editing [124][66] or *(ii)* selection of other carbon- and nitrogen sources as a substitute [156][1][52].

  Efforts are ongoing to increase the substrate scope of LAB in order to directly utilize the complex carbohydrates present in the biomass and thus lower the dependence on pretreatment and enzymatic hydrolysis. These strategies include the use of consortia of cellulolytic microorganism and LAB, or engineering cellulolytic activities from aerobic and anaerobic microorganisms in LAB [221]. Increasing number of reports are emerging on developing strains expressing more than one polysaccharide degrading enzymes, which allow the recombinant strains to grow on the feedstocks being treated [82][165].

- **Toolkit for genetic engineering.** Strain development of LAB is continuously done to develop starter cultures with improved properties. This is done using natural strategies such as random mutagenesis, adaptive evolution, dominant selection, and even natural transduction and conjugation systems [56]. Increased knowledge of the genetic elements including plasmids, conjugative transposons and bacteriophages [54] has led to the development of various gene cloning, and expression systems (Table 5), which are being applied for metabolic engineering

of the bacteria for improving the production titers, modifying substrate utilization, making novel compounds, etc. in biotechnological applications [83][23]. While some of them, such as *Lactobacillus plantarum*, *L. reuteri*, *Lactococcus lactis* or *Lactobacillus casei*, are easy to transform and many tools have been described, others are still difficult to handle with transformation, and more research effort is needed to improve efficiency. Optimization of the growth medium (sugars and salts added), washing buffer and electroporation protocols could enable efficiencies for gene editing purpose [24][190][107][7].

Table 5 – Genetic toolbox for gene modifications in LAB.

Tools	Species	Ref.
Expression system		
Nisin system	*L. lactis*, *L. helveticus*, *L. plantarum*	[127][108][84][164][64]
Sakacin system	*L. sakei*, *L. casei*, *L. plantarum*	[8][148][166]
Promoters development	*L. lactis*, *L. plantarum*	[114][102][192]
Reporter development	*L. lactis*, *L. reuteri*, *L. plantarum*	[94][79][117]
Chromosomal modification		
pORI system	*L. lactis*	[132][141]
pTRK system	*L. lactis*, *L. acidophilus*, *L. gasseri*, *L. casei*	[193][89][212]
Cre-loxP system	*L. plantarum*, *L. reuteri*	[29][130]
Single stranded recombineering	*L. lactis*, *L. reuteri*, *L. gasseri*, *L. plantarum*	[228][229]
CRISPR-Cas9	*S. thermophilus*, *L. reuteri*, *L. gasseri*	[171][105]

The gene cloning systems (Table 5), especially the NICE (Nisin controlled gene expression) system, have been applied for engineering LAB, especially *L. plantarum* amd *L. lactis*, for re-routing carbon metabolism from lactate formation to production of food ingredients and chemicals like L-alanine [108], diacetyl [111][112], acetaldehyde [21] and 2,3-butanediol [84], and biofuel products including ethanol [211], *n*-propanol [234] and butanol [15]. Enhanced production of polyhydric alcohols like mannitol [178], sorbitol [170] and 1,2-propanediol [159] by engineering LAB has also been achieved.

- **Bioprocess engineering.** Reducing the processing costs and finding alternative solutions for enhancing the titres, yields and productivities will ensure the long-term growth of industrial biotechnology [59]. Approaches that can be taken are: *(i)* high cell density fermentation, *(ii)* whole-cell biocatalysis, *(iii)* co-factor recycling engineering, *(iv) in situ* product complexation and removal, *(v)* intergrated refineries, etc.

- **Stress tolerance engineering.** Environmental stresses (e.g. oxidative, acid, cold and osmotic) are commonly encountered during the application of LAB [244]. These bacteria have developed various strategies for countering environmental

stresses in both industrial milieu and gastrointestinal tract, e.g. by maintaining cell membrane functionality, regulating cellular metabolism, exopolysaccharide production and expression of stress response proteins [246][243][177]. Different non-specific approaches such as genome shuffling, adaptive evolution, and error-prone whole genome amplification have been used to obtain strains that are able to grow at substantially lower pH than the wild type strains [181][259][251]. Recently, system biology approaches (e.g. genomics, transcriptomics, proteomics, and metabolomics analyses) have facilitated the understanding of molecular mechanisms underlying the stress resistance of LAB and many efficient strategies have been proposed as: *(i)* addition of protectant [120], *(ii)* adaptive evolution [259], *(iii)* pre-adaption [121], *(iv)* cross-protection [72][226], *(v)* amino acid metabolism engineering [224], *(vi)* cell membrane engineering [26], *(vii)* exogenous biosynthesis pathway engineering [95] and *(viii)* stress response proteins expression [245].

5 Microcompartment Shell Proteins in *L. reuteri*(Paper I)

The Pdu microcompartment shell encapsulates the Pdu pathway enzymes and also sequesters some metabolites and cofactors. However, other metabolites and cofactors are allowed to cross the shell in order for the MCP to function. For example, the shell must be permeable to propanediol, propanol, glycerol, 1,3-PDO, propionyl phosphate and also Vitamin B12 and perhaps ATP.

From earlier reports, it is known that significant amount of 3-HPA, the dehydration product of glycerol, is transported out of the MCPs and even crosses the cell envelope of *L. reuteri* (Paper I, S1 Fig.). On the other hand, propionaldehyde formed from 1,2-PDO is sequestered to prevent cell damage [20]. Also, coenzyme A and NAD^+ needed for further reaction with the aldehyde need to be recycled inside the microcompartment, which is achieved by a phosphotransacylase enzyme and an alcohol dehydrogenase, respectively.

5.1 Glycerol Transport

Glycerol, like other small uncharged molecules, can enter the cytoplasm by passive diffusion [218]. Nevertheless, it was shown that uptake of glycerol is induced by glycerol or glycerol-3-phosphate (G3P), and repressed by growth in the presence of glucose [198]. Because of the membrane permeability of glycerol through the cell membrane, if actively accumulated in the cytoplasm it would then be free to move down the concentration gradient, out of the cell. Instead, cytoplasmic glycerol is phosphorylated by glycerol kinase and thus trapped as G3P inside the cell [258][46].

In *L. reuteri*, it was reported that *pduF*, the gene encoding the glycerol facilitator, is in the operon of *pdu* cluster. Based on homology modelling, the structure of Lr-PduF shows a potential gating mechanism caused by the conformational change of Leucine 61, a nonpolar side chain, allows transient penetration of water molecules, which greatly increases the polarity of the redox site environment and provides a source of protons as discussed also by Min and coworkers in *Clostridium pasteurianum* [155](Fig 11).

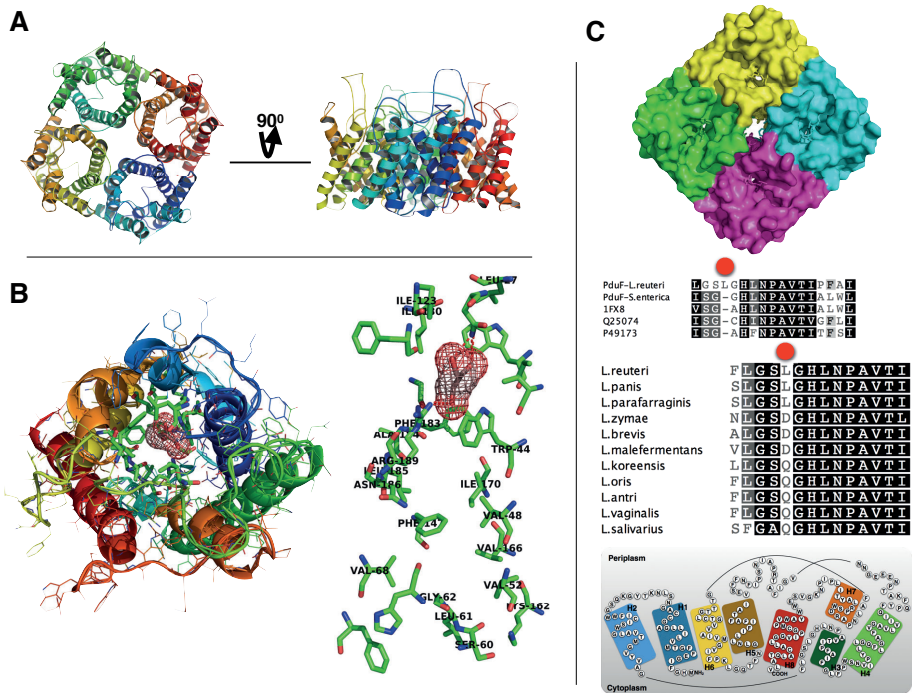


Figure 11 – PduF A) 3D structure of PduF. B) Proposed glycerol transport through PduF. C)Multiple sequences alignment of glycerol transport proteins from several organisms.

5.2 Shell Proteins

Studies with *Salmonella enterica* have shown that Pdu MCP assembly is a polyhedron 150-200 nm in diameter with irregular hexagonal or pentagonal tiles, and is enclosed by a shell composed of several thousand copies of shell proteins of several types [209][39]. Nine different Pdu shell proteins paralogs, i.e. PduABB'JKMNTU are present in the final assembly in distinct and reproducible ratios [40]. The *pduB* gives rise to both a full-length and truncated polypeptide via alternate translation start sites, termed PduB and PduB', respectively [176][99][180]. The full length PduB is 25 amino acid longer at the N-terminal end than PduB'. Crystal structures of some of the shell proteins (e.g. PduA, PduU and PduN) have shown that they are assembled in a way to form a central pore, which could be used for mediating the transport of substrates, products and enzyme cofactors between the interior of the microcompartment and cytoplasm of the cell [35][44][208]. For instance, PduA is abundant in the shell and is assumed to be involved in the selective transport of 1,2-PDO. Occlusion of its pore by mutations, PduA^{S40GSG}, PduA^{S40L}, PduA^{S40C}, and PduA^{S40Q}, resulted in growth defects that are overcome by increased 1,2-PDO concentrations in the feed, while escape of the intermediate propionaldehyde was enhanced in the PduA^{S40A} [39].

In *L. reuteri*, PduMCP has seven different shell proteins, PduA, PduB, PduB', PduJ, PduK, PduU, PduN, and possibly an eighth PduM [208], which seem to confine the propionaldehyde intermediate formed. PduT, a proposed single-electron channel, was shown to be missing in *L. reuteri pdu* operon. Approximately 85% of the total shell protein in *L. reuteri* belongs to PduABB'J, which is consistent with the shell protein composition of *Salmonella* Pdu MCP determined by proteomics analysis [40][118]. Crystal structure of *L. reuteri* strain DSM20016 PduB revealed a trimeric structure with pseudo-hexagonal symmetry formed by tandem structural repeats within the subunit [176]. Three pores are present between the tandem repeats, each of which bind three glycerol molecules, suggesting their involvement in channeling glycerol into the MCP (Fig. 12).

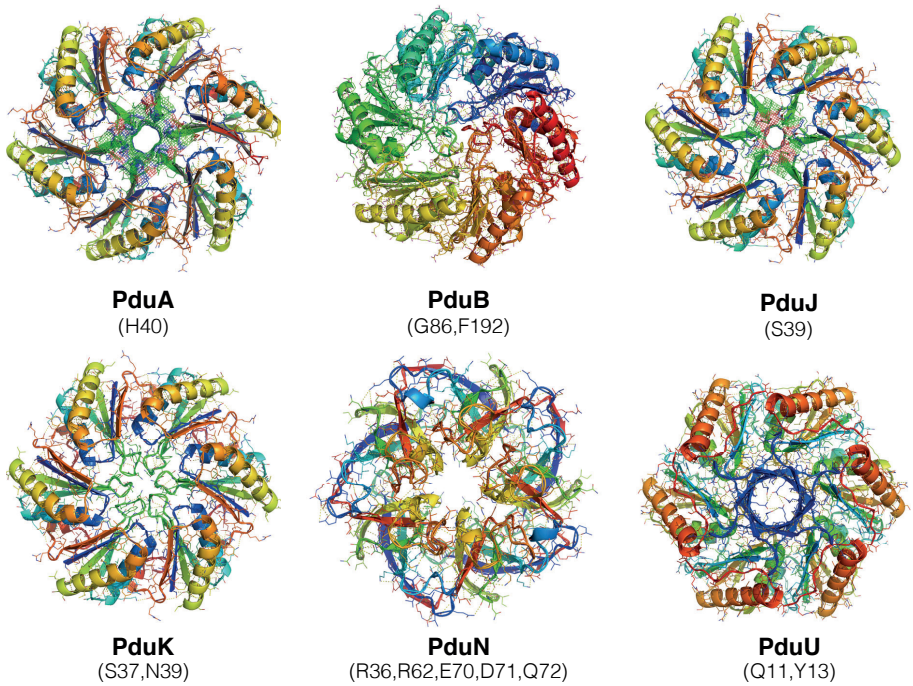


Figure 12 – Shell proteins with key amino acids at pore sites.

The focus of the **Paper I** was on PduA and PduJ, the two other major components of the MCP shell in *L. reuteri*, with the aim to determine the involvement of their pores in the transport of glycerol and its metabolic product across the shell. The study comprised identification of the critical amino acid residue(s) around the pores in the two proteins, selective engineering of the residues by single strand DNA recombineering, and its effect on glycerol consumption, 3-HPA formation as well as cell growth. The *pduA* gene encodes a small 93-amino-acid protein, which shares 74%

amino acid sequence identity with PduJ (96 aa). According to homology model analysis, both are expected to form a hexameric tile complex containing a central pore approximately 6Å and 8Å, respectively. Multiple sequence alignment of PduA/PduJ shell homologs highlighted the importance of the locus 40 of the sequence, showing that *Lactobacillus* sp. PduA-H40 is widely conserved in the diverse genera of lactobacilli, while the analogous residue in PduJ is S39. Mutants of the major shell proteins PduA (H40) and PduJ (S39) were generated to test their effects on cell growth and glycerol transformation. The PduA^{H40L} and PduA^{H40S} mutants showed slower consumption rate to glycerol while PduA^{H40A} excreted increased amount of 3-HPA. Also, PduJ^{S39H} mutant consumed glycerol at faster rate compared to the wild-type (Fig. 13).

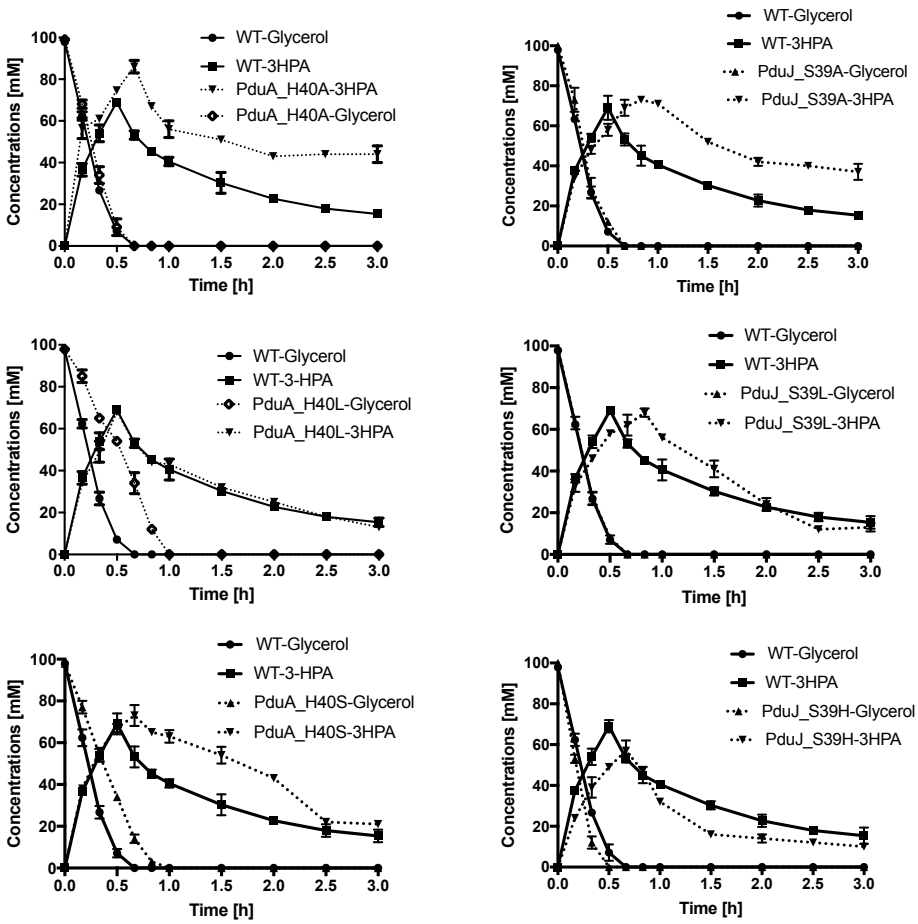


Figure 13 – Profiles of glycerol consumption and 3-HPA production by wild-type and mutant *L. reuteri* strains during biotransformation of glycerol. The reaction was initiated with 100 mM glycerol. Error bars represent standard error from three independent measurements.

The results showed that a single amino acid substitution of a pore-lining residue, particularly PduA (H40) and PduJ (S39) could cause changes on transport properties (i.e. polarity and hydrophobicity) of Pdu microcompartment, leading to varied growth and biotransformation phenotypes of *L. reuteri* with glycerol as feedstock, hinting that PduA and PduJ could facilitate the influx of glycerol and egress of intermediates in a dissimilar way (Fig. 13).

6 Transformation of Different 1,2-Diols(Paper II)

The Pdu pathway is known to utilize 1,2-PDO and glycerol as substrates. Co-feeding of these polyols along with glucose during cultivation of *L. reuteri* helps to maintain the recycling of NAD^+ for glucose metabolism by using the NADH for reduction of 3-HPA/propionaldehyde intermediate in the Pdu pathway to 1,3-PDO/*n*-propanol. In the absence of glucose, glycerol/1,2-PDO are metabolized by *L. reuteri* without being used for growth, and the cofactor recycling is maintained by processing of the aldehyde intermediate by the oxidative and reductive branches of the Pdu pathway simultaneously, resulting in co-production of 3-HP/propionate and 1,3-PDO/*n*-propanol [213].

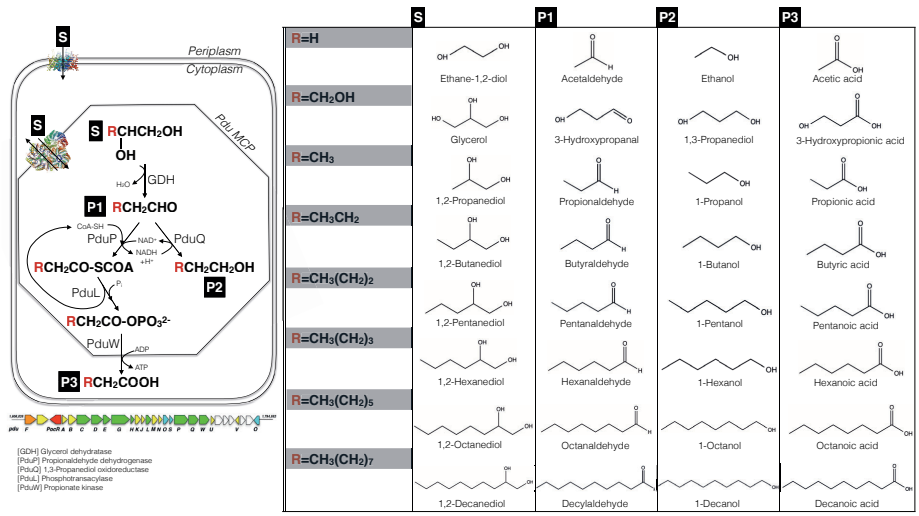


Figure 14 – General reaction scheme for glycerol/1,2-diols conversion to corresponding alcohol and acid by lumen enzymes of Pdu-microcompartment.

Expanding the repertoire of diol substrates for transformation by the Pdu pathway could result in a novel synthetic route for the production of interesting alcohols and organic acids (Fig. 14). However, establishing routes for microbial production of chemicals often requires overcoming pathway bottlenecks by tailoring enzymes to accept non-native substrates. Diols are important chemicals with applications as building blocks for polymers, in cosmetics and pharmaceuticals, and as fuels [231][223]. 1,2-Diols, namely 1,2-propanediol, 1,2-butanediol, 1,2-pentanediol, 1,2-hexanediol can be biotechnologically produced by microbial bioconversion of renewable feedstocks [69][137][97].

In **PaperII**, key enzymes of the Pdu pathway (i.e. glycerol dehydratase, PduP and

PduQ) of *L. reuteri* DSM20016 were characterized with respect to the scope of the substrates accepted by them and the corresponding reaction kinetics. The genes encoding the lumen enzymes were cloned and expressed in *E. coli* BL21(DE3) separately and the enzymes were purified. Substrates of varying chain lengths were used for reaction with each enzyme. GDH exhibited activity against C2-C4 polyols (1,2-ethanediol, 1,2-propanediol, glycerol and 1,2-butanediol), with the highest activity against glycerol and 1,2-PDO. PduQ and PduP, on the other hand, were able to transform aldehydes of chain lengths up to C10, however the best substrate for both was 3HPA (Fig. 15).

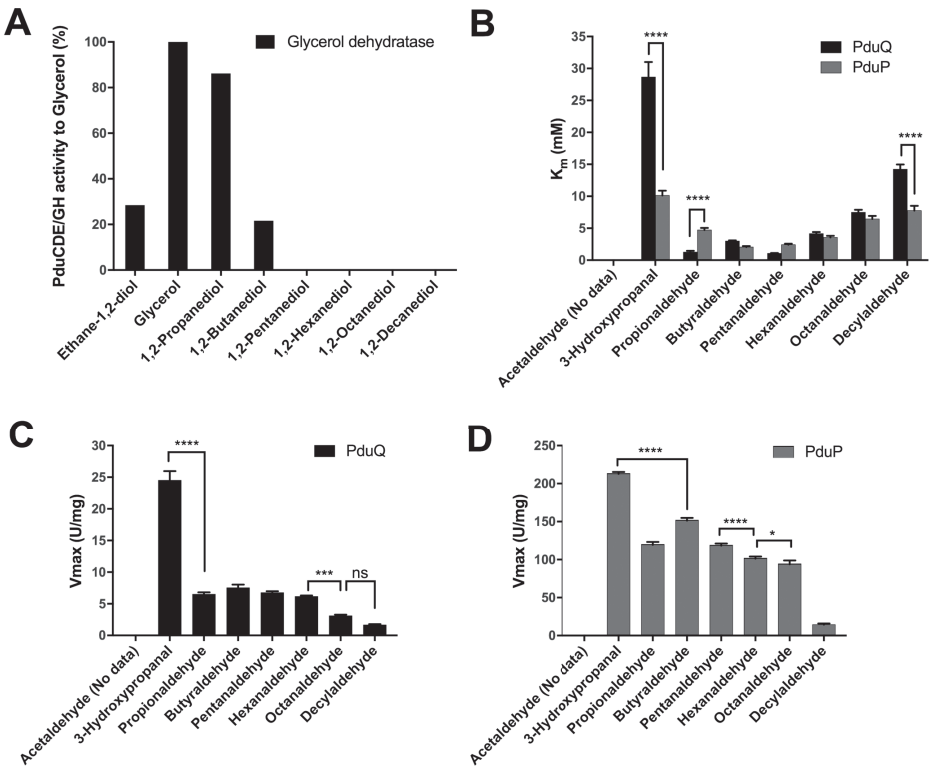
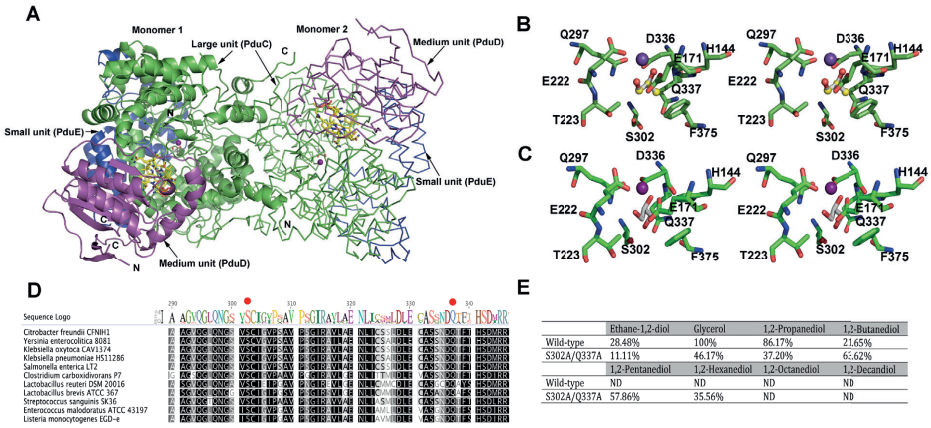


Figure 15 – Kinetic analysis of lumen enzymes on different substrates. A) Relative activities of cell free extracts of recombinant *E. coli* expressing wild type glycerol dehydratase toward glycerol/1,2-diols, using the activity towards glycerol as 100 %. B) K_m values for PduQ and PduP towards different aldehydes. V_{max} values for C) PduQ and D) PduP.

As the activity of glycerol dehydratase was found to be the limiting factor in bioconversion of different diols by *L. reuteri*, broadening its substrate selectivity to longer chain diols by rational engineering was considered. Docking the model of glycerol/1,2-PDO to glycerol dehydratase homology model based on the crystal structure of diol

dehydratase-cyanocobalamin complex from *Klebsiella oxytoca* was drawn (Fig. 16). GDH is a heterotrimer (PduCDE) encoded by three genes *pduCDE* (together with two genes *pduGH* encoding the two subunits of GDH activase). The model showed the GDH to be assembled as a dimer of the heterotrimer. It was observed from the model that on one hand, 3-OH group of glycerol forms a hydrogen bond with the side chain of S302, while the side-chain amide nitrogen atom of Q337 formed hydrogen bonds with the main chain carbonyl oxygen atoms of D336 and F375, both of which are key residues for interacting with the substrate molecule. Based on the assumption that loss of these hydrogen bonds may alter the conformation and flexibility of the enzyme, redesign of glycerol dehydratase was performed in which both of the residues (S302 and Q337) were substituted by alanine. The S302A/Q337A double mutations resulted in decrease in activity towards 1,2-ethanediol, glycerol and 1,2-PDO, while activity towards 1,2-butanediol increased almost three fold. Moreover, activities towards longer chain 1,2-diols, i.e. 1,2-pentanediol and 1,2-hexanediol, were also detected with relative increase by around 58% and 36%, respectively.



	Ethane-1,2-diol	Glycerol	1,2-Propanediol	1,2-Butanediol
Wild-type	28.48%	100%	86.17%	21.65%
S302A/Q337A	11.11%	46.17%	37.20%	6.62%
	1,2-Pentanediol	1,2-Hexanediol	1,2-Octanediol	1,2-Decandiol
Wild-type	ND	ND	ND	ND
S302A/Q337A	57.86%	35.56%	ND	ND

Figure 16 – Redesign of glycerol dehydratase from *L. reuteri*. A) Overall fold of the glycerol dehydratase monomer and the proposed biological dimer; Stereodrawing of the B) 1,2-PDO or C) glycerol bound form of the glycerol dehydratase. Homology modeling for the prediction of the tertiary structure of glycerol dehydratase (PduCDE) resulted in a model based on the crystal structure of diol dehydratase-cyanocobalamin complex from *Klebsiella oxytoca* (1DIO), with sequence similarities and coverages above 42% and 95%, respectively. Quality model assessment of the homology model revealed a QMEAN6-score of 0.83 and a Z-score of -0.53. Ramachandran plot revealed that none of residues were present in the disallowed regions. Color codes for carbon atoms: yellow for adenosylcobalamin (AdoCbl), white for glycerol/1,2-PDO, purple for calcium ion. The model figure was generated using Pymol. D) Sequence alignment of large subunit of glycerol dehydratase (PduC) from different bacteria. The numbering scheme follows the amino acid sequences of PduC. Identical residues in all sequences are highlighted in black and conserved in grey. E) Relative activities of WT/mutant glycerol dehydratases towards different 1,2-diols/glycerol. The data is normalized by taking the activity of the wild-type enzyme towards glycerol as 100%.

To study the effect of mutations on GDH activity in whole cells, engineered *L. reuteri* with GDH variant were used for transformations, resulting in no detectable activity towards 1,2-ethanediol, 1,2-butanediol, 1,2-pentanediol and 1,2-hexanediol. However, recombinant *E. coli* cells bearing the glycerol dehydratase variant showed activity with substrate chain length up to C6 and highest activity with C4. This may suggest that the protein shell of the microcompartment in *L. reuteri* poses a barrier to the passage of longer chain substrates (Fig. 14).

7 Role of PduQ and Other Iron-dependent Alcohol Dehydrogenases in Glycerol Metabolism in *L. reuteri* (Paper III)

Earlier reports have shown that *L. reuteri* is unique in excreting 3-HPA out of the cells at rather high concentration and the organism exhibits relatively high tolerance to the 3-hydroxyaldehyde [60][199][200][145]. This is of course advantageous for its use as probiotic so that it can survive while inactivating any pathogenic bacteria. 3-HPA is reduced to 1,3-PDO inside the microcompartments by PduQ, a $NAD(P)^+$–dependent alcohol dehydrogenase. This suggests that *L. reuteri* is likely to have other routes of 3-HPA transformation outside the microcompartment.

Hence in **Paper III**, diversity of alcohol dehydrogenases in *L. reuteri* was investigated. Nine ADH enzymes were found that were all metal-ion dependent, out of which 3 (PduQ, ADH6 and ADH7) belong to the group of iron-dependent enzymes (group III; Zinc-dependent and metal-free enzymes being groups I and II, respectively) that are known to transform aldehydes/ketones to alcohols. ADH6 is a bifunctional alcohol/aldehyde dehydrogenase. The three enzymes showed conserved regions for binding of substrate, NADH and iron as in the iron-dependent ADHs. *L. reuteri* mutants were constructed in which ADH6, ADH7 and PduQ were deleted individually. The slow growth phenotype of these deletion mutants indicated that NAD^+ recycling becomes limiting for growth in the absence of the respective ADHs. The addition of glycerol during cultivation of the wild type and mutant *L. reuteri* showed the highest increase (66%) in the maximum growth rate in case of ADH6 deletion mutant, which indicates that ADH6 is not likely to be involved in 3-HPA reduction. All the cells showed almost similar consumption behaviour for glucose and glycerol. The end products, lactate, ethanol and 1,3-PDO were also measured. Interestingly, the PduQ deletion mutant displayed similar production behaviours as the wild type. ADH6 deletion mutant showed increase in lactate indicating the use of lactate dehydrogenase for regeneration of NAD^+, but ethanol production was significantly lowered that is in line with the idea that the enzyme is bifunctional alcohol/aldehyde dehydrogenase participating in cofactor regeneration during conversion of acetyl-CoA to ethanol. ADH7 deletion mutant showed increased ethanol production perhaps due to resorting to ADH6 activity to regenerate NAD^+, and much lower 1,3-PDO production (5.3 mM compared to 13 mM by the other mutants), which meant that PduQ was not very active under these conditions. These observations indicated that ADH7 was most likely involved in reduction of 3-HPA to 1,3-PDO outside the microcompartment.

Production of 3-HPA, 3-HP and 1,3-PDO was then studied in a two-step process involving cultivation of *L. reuteri* under anaerobic conditions in the medium supplemented with 20 mM glycerol, followed by using the resting cells for transformation of 300 mM glycerol. The PduQ deletion mutant produced the highest amount of 3-HPA (152.5 mM) but very low amounts of 1,3-PDO (7.7 mM) and 3-HP (16.2 mM)

were produced compared to 18.9 mM and 23.7 mM, respectively, by the wild type cells. This confirms that PduQ is active in generating NAD^+ during glycerol metabolism within the microcompartment by resting cells, while ADH7 functions to balance NAD^+/NADH by converting 3-HPA to 1,3-PDO outside the microcompartment by the growing cells. ADH6 does not seem to have a role in 3-HPA reduction. Fig. 17 shows the pathways for glucose and glycerol metabolism with proposed functions of the iron-dependent ADHs.

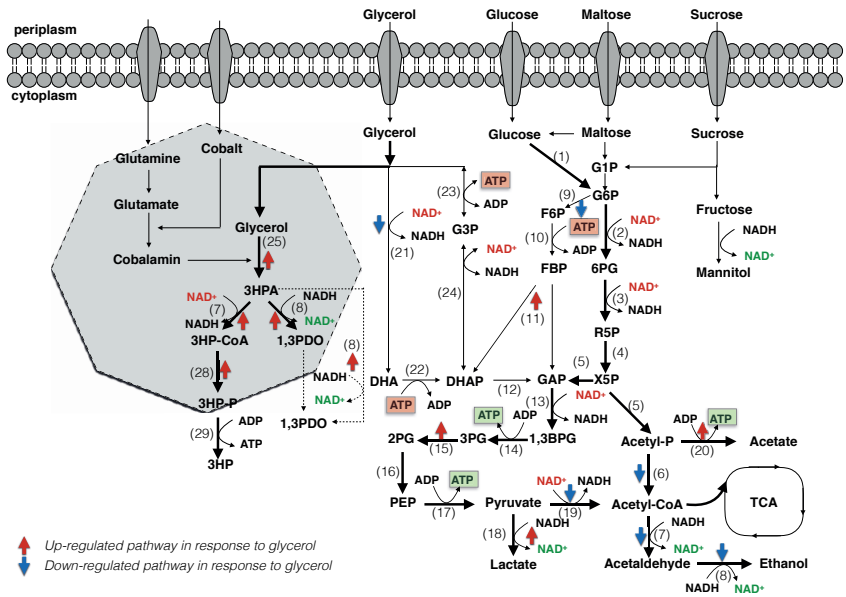


Figure 17 – Pathways of glucose and glycerol metabolism in *L. reuteri* DSM20016. A) The proposed routes for glucose metabolism using Embden-Meyerhof (EMP) and -phosphoketolase (PKP) pathways, and model of the Pdu microcompartment and -pathway for glycerol transformation. G1P, glucose-1-phosphate; 6PG, 6-phosphogluconate; X5P, xylulose-5-phosphate; Acetyl-P, acetyl phosphate; FBP, fructose-1,6-bisphosphate; GAP, glyceraldehyde-3-phosphate; 3PG, 3-phosphoglycerate; G6P, glucose-6-phosphate; 2PG, 2-phosphoglycerate; R5P, ribulose-5-phosphate; PEP, Phosphoenolpyruvate; G3P, glycerol-3-phosphate; 1,3BPG, 1,3-biphosphoglycerate; F6P, fructose-6-phosphate; DHAP, dihydroxyacetone phosphate; Adh, alcohol dehydrogenase; Aldh, aldehyde dehydrogenase; Ldh, lactate dehydrogenase; Gld, glycerol-2-dehydrogenase; G6pd, glucose-6-phosphate dehydrogenase; Gpd, glycerol-3-phosphate dehydrogenase; Gapdh, glyceraldehyde phosphate dehydrogenase; Pgdh, 6-phosphogluconate dehydrogenase; PDC, pyruvate dehydrogenase complex. Genes that are differentially expressed in response to glycerol are indicated as red (up-regulated) and blue (down-regulated). (Data derived from Santos FB et al. Vitamin B12 synthesis in *Lactobacillus reuteri*. 2008).

7.1 Redox Homeostasis

The biosynthetic pathways of most alcohols are linked to intracellular redox homeostasis, which is crucial for life. This crucial balance is primarily controlled by the generation of reducing equivalents, as well as the (reduction)-oxidation metabolic cycle and the thiol redox homeostasis system. As a main oxidation pathway of reducing equivalents, the biosynthesis of most alcohols includes redox reactions, which are dependent on cofactors such as NADH or NADPH(Fig. 18).

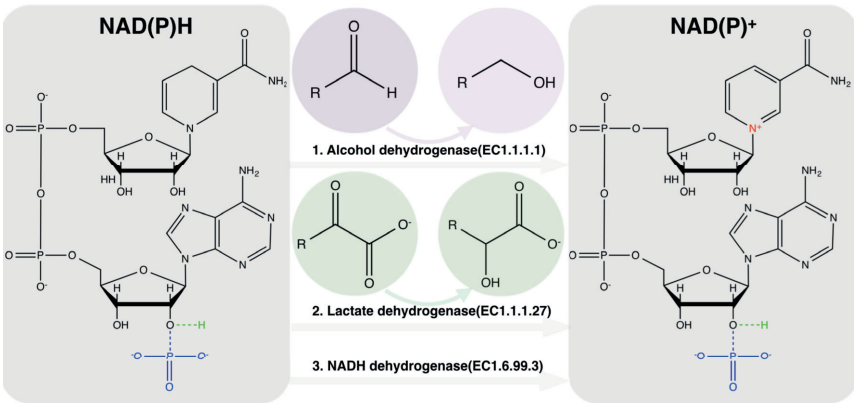


Figure 18 – NAD(P)$^+$ regeneration catalyzed mainly by three enzymes in *L. reuteri* (Alcohol dehydrogenase (EC1.1.1.1), Lactate dehydrogenase (EC1.1.1.27) and NADH dehydrogenase (EC1.6.99.3). ADHs catalyse the NAD(P)$^+$-dependent interconversion of aldehydes or ketones and enantiomeric alcoholic compounds. The complete structure of NAD$^+$/NADH is indicated by a green H-atom, while the additional phosphate group of NADP$^+$/NADPH is indicated in blue.

Metabolic flux analysis has shown that *L. reuteri* uses both Embden-Meyerhof pathway (EMP) and phosphoketolase pathway (PKP) for glucose metabolism, and the main flux is through the PKP while the EMP is used as a mere shunt [213][3]. The growth rate and biomass yield obtained in the presence of non-limiting concentration of glucose are however quite low (maximum specific growth rate of 0.45/h). It has been reported by Årsköld et al. [3] that the choice of carbon source can influence the growth performance of *L. reuteri*. The organism does not grow on glycerol, but its addition to the cultivation medium alleviates the growth limitation, which is ascribed to alleviation of redox imbalance (**Paper III**). Furthermore, glycerol induces the expression of genes in the propanediol-utilization (Pdu) operon encoding enzymes and structural proteins needed for metabolism of glycerol (or the other diols) and use as electron acceptor [3].

8 Kinetic Characterization and Molecular Modeling of PduQ (Paper IV)

Alcohol dehydrogenases (ADHs) are highly diverse enzymes catalysing the interconversion of alcohols and aldehydes or ketones. Several categories of ADHs can be distinguished based on their cofactor specificity, these being: (i) NAD or NADP, (ii) the pyrroloquinoline quinone, haem or cofactor F420, and (iii) FAD. The NAD(P)-dependent ADHs are further sub-divided into zinc-dependent (group I), short-chain (group II), and iron-activated (group III) ADHs. Most of the known ADHs from *Lactobacillus* sp. are NAD(P)-dependent, which indicates that they share a common catalytic mechanism. Among the group III NAD(P)-dependent bacterial ADHs that have been described and characterised so far are lactaldehyde dehydrogenases, butanol dehydrogenases, 1,3-PDO dehydrogenases while there are several more remaining to be characterized. In general, the group III enzymes have been found to preferentially catalyse reduction of aldehydes.

Paper IV has a focus on the iron containing PduQ, a 1,3-propanediol oxidoreductase of *L. reuteri*. The gene (1122 base pairs) encoding the enzyme, identified through sequence analysis of *L. reuteri* DSM20016 genome, shared 100% nucleotide sequence with the PduQ of *L. reuteri* JCM 1112. Homology modeling for the prediction of tertiary structure resulted in a model based on the crystal structure of chain A of the probable alcohol dehydrogenase of *Geobacillus thermoglucosidasius* [71]. PduQ is composed of two structural domains. The N-terminal domain has a Rossmann-fold architecture, which is a typical protein structure motif that binds nucleotide cofactor such as FAD, NAD or NADP. The C-terminal domain is alpha-helical, with an up-down bundle architecture known as a dehydroquinate synthase-like alpha-domain. The active site is probably located between the Rossmann fold and the alpha-helical domain. According to the model, the binding of NADH to Gly91, Ser92, Asp95, Gly260, His180 of PduQ via hydrogen bonding is proposed.

The PduQ gene of *L. reuteri* with His6 coding gene was cloned and expressed in *Escherichia coli* BL21(DE3) and the recombinant enzyme was purified to homogeneity by metal ion affinity chromatography. Kinetic tests for aldehyde reduction and alcohol oxidation using the pure enzyme confirmed significantly higher activity (K_{cat}/K_m) for aldehyde reduction than the oxidation of the corresponding alcohol due to lower K_m as well as higher V_{max} with the aldehyde and NADH. The PduQ showed higher catalytic efficiency towards propionaldehyde than 3-HPA in terms of K_{cat}/K_m value (3.02 vs 0.64 $S^{-1}\cdot mM^{-1}$). The enzyme exhibited optimal activity for propionaldehyde reduction at pH 7.0 (15.0± 0.6 U/mg), and for 3-HPA reduction at pH 8.0 (25.1± 2.3 U/mg) at 30 °C. The recombinant *L. reuteri* PduQ-His6 used both NADH and NADPH as cofactors, but the activity with the former was 10-fold higher. Maximum specific activity of PduQ-His6 with NADH as cofactor (12.6± 0.7 U/mg) was about

three-fold higher than that obtained with NAD^+ (3.9± 0.2 U/mg).

The enzyme was highly sensitive to the exposure to air, resulting in significant loss of its iron content. The loss of iron as well as enzyme activity could be overcome by the addition of ascorbic acid, an antioxidant. This would suggest that the loss of enzyme activity occurs through metal catalyzed oxidation [214]. The proposed metal binding amino acid residues (Asp176, His180, His245, His 249, His259) on the PduQ were confirmed by their mutations to alanine by site directed mutagenesis. All the mutants except H249A were inactive; H249A exhibited 1.5-fold higher K_m and approximately 2-fold lower K_{cat}/K_m.

9 Concluding Remarks

Glycerol has emerged as a versatile feedstock for the production of a variety of chemicals, polymers and fuels [174]. With increasing awareness of GHG emissions and consequent impact on climate change coupled to fossil based production, biorefineries based on renewable feedstocks including glycerol will increase in number.

Over the past 10 years, various pathways for biological 1,3-PDO, 3-HPA and 3-HP production have been examined [128]. Production of 1,3-PDO has indeed become a commercial reality but the production is based on glucose as feedstock. Two routes for 3-HP production, one from glucose via malonyl-CoA pathway and the other from glycerol by CoA-independent pathway using engineered microorganisms, are most promising [36][128]. *Lactobacillus reuteri* is an interesting, safe microbial host for the production of 1,3-PDO, 3-HPA and 3-HP from glycerol through the Pdu pathway. The main requirements for the pathway are the availability of Vitamin B12, coenzyme A and the cofactor NADH/NAD^+. *L. reuteri* itself is en efficient producer of Vitamin B12, but if the Pdu pathway is to be engineered in another microorganism, B12 synthesis route would also need to be engineered. The vitamin itself could be a valuable co-product in a glycerol biorefinery. The need for CoA, which is a requirement in the oxidative branch of the pathway, can be eliminated by substitution of PduP, L and W by an aldehyde dehydrogenase, an enzyme available also in *L. reuteri* and several other microorganisms.

The study presented in this thesis confirmed the existence of the Pdu pathway inside microcompartments that are enveloped by a proteinaceous shell, which regulates the passage of substrates, intermediates and products in and out of the microcompartment. Bacterial microcompartments are present in many ecologically diverse and important bacteria and have a number of potential biotechnology applications [133]. Metabolic benefits gained from the microcompartment system in enhancing production of valuable chemicals via enhancing pathway flux and establishing concentration gradient, can be exploited as a way of improving biotransformation [104]. It was shown in this thesis that the microcompartment shell limited the entry of longer chain substrates through its pores, which coincided also with the susbstrate selectivity of glycerol dehydratase. The substrate scope of such a system could however be increased by altering the pore structures in the major shell proteins through mutating the amino acid residues lining the pores and also be rational mutagenesis of glycerol dehydratase.

The thesis also shows that the PduQ enzyme inside the microcompartment is active in resting cells and helps to establish redox homeostasis with coproduction of 3-HP from glycerol. It was also shown that a cytoplasmic alcohol dehydrogenase ADH7 participates in 1,3-PDO production in growing cells, which is logical for enabling

cofactor recycling for the central glucose metabolism in the cytoplasm. While PduQ was found to be a highly sensitive enzyme, ADH7 needs further characterization to determine its potential in glycerol transformation to 1,3-PDO.

10 References

[1] MD Altaf, M Venkateshwar, M Srijana, and G Reddy. An economic approach for L-(+) lactic acid fermentation by *Lactobacillus amylophilus* GV6 using inexpensive carbon and nitrogen sources. *Journal of Applied Microbiology*, 103(2):372–380, 2007.

[2] M Anitha, SK Kamarudin, and NT Kofli. The potential of glycerol as a value-added commodity. *Chemical Engineering Journal*, 295:119–130, 2016.

[3] E Årsköld, E Lohmeier-Vogel, R Cao, S Roos, P Rådström, and EW van Niel. Phosphoketolase pathway dominates in *Lactobacillus reuteri* ATCC 55730 containing dual pathways for glycolysis. *Journal of Bacteriology*, 190(1):206–212, 2008.

[4] MA Asadollahi, J Maury, KR Patil, M Schalk, A Clark, and J Nielsen. Enhancing sesquiterpene production in *Saccharomyces cerevisiae* through *in silico* driven metabolic engineering. *Metabolic Engineering*, 11(6):328–334, 2009.

[5] L Asveld, R Van Est, and D Stemerding. Getting to the core of the bioeconomy. A perspective on the sustainable promise of biomass. 2011.

[6] S Atsumi, T Hanai, and JC Liao. Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels. *Nature*, 451(7174):86, 2008.

[7] TW Aukrust, MB Brurberg, and IF Nes. Transformation of *Lactobacillus* by electroporation. In *Electroporation Protocols for Microorganisms*, pages 201–208. Springer, 1995.

[8] L Axelsson, G Lindstad, and K Naterstad. Development of an inducible gene expression system for *Lactobacillus sakei*. *Letters in Applied Microbiology*, 37(2):115–120, 2003.

[9] RH Baltz. Genetic manipulation of secondary metabolite biosynthesis for improved production in *Streptomyces* and other actinomycetes. *Journal of Industrial Microbiology & Biotechnology*, 43(2-3):343–370, 2016.

[10] K Båth. *Factors important for persistence of Lactobacillus reuteri in the gastrointestinal tract*, volume 2007. 2007.

[11] D Baum. *Extreme mindstorm: an advanced guide to LEGO mindstorms*. Apress, 2000.

[12] J Becker, R Schäfer, M Kohlstedt, B Harder, NS Borchert, N Stöveken, E Bremer, and C Wittmann. Systems metabolic engineering of *Corynebacterium glutamicum* for production of the chemical chaperone ectoine. *Microbial Cell Factories*, 12(1):110, 2013.

[13] J Becker and C Wittmann. Bio-based production of chemicals, materials and fuels–*Corynebacterium glutamicum* as versatile cell factory. *Current Opinion in Biotechnology*, 23(4):631–640, 2012.

[14] J Bell, L Paula, T Dodd, S Nemeth, C Nanou, V Mega, and P Campos. EU ambition to build the world's leading bioeconomy–uncertain times demand innovative and sustainable solutions. *New Biotechnology*, 2017.

[15] OV Berezina, NV Zakharova, A Brandt, SV Yarotsky, WH Schwarz, and VV Zverlov. Reconstructing the *Clostridial n*-butanol metabolic pathway in *Lactobacillus brevis*. *Applied Microbiology and Biotechnology*, 87(2):635–646, 2010.

[16] M Berlanga and R Guerrero. Living together in biofilms: the microbial cell factory and its biotechnological implications. *Microbial Cell Factories*, 15(1):165, 2016.

[17] L Bervoets, K Van Hoorenbeeck, I Kortleven, C Van Noten, N Hens, C Vael, H Goossens, KN Desager, and V Vankerckhoven. Differences in gut microbiota composition between obese and lean children: a cross-sectional study. *Gut Pathogens*, 5(1):10, 2013.

[18] H Biebl, K Menzel, AP Zeng, and WD Deckwer. Microbial production of 1, 3-propanediol. *Applied Microbiology and Biotechnology*, 52(3):289–297, 1999.

[19] RH Bischof, J Ramoni, and B Seiboth. Cellulases and beyond: the first 70 years of the enzyme producer *Trichoderma reesei*. *Microbial Cell Factories*, 15(1):106, 2016.

[20] TA Bobik, GD Havemann, RJ Busch, DS Williams, and HC Aldrich. The propanediol utilization (*pdu*) operon of *Salmonella enterica* serovar typhimurium LT2 includes genes necessary for formation of polyhedral organelles involved in coenzyme B12-dependent 1, 2-propanediol degradation. *Journal of Bacteriology*, 181(19):5967–5975, 1999.

[21] RS Bongers, MH Hoefnagel, and M Kleerebezem. High-level acetaldehyde production in *Lactococcus lactis* by metabolic engineering. *Applied and Environmental Microbiology*, 71(2):1109–1113, 2005.

[22] I Borodina, J Siebring, J Zhang, CP Smith, G van Keulen, L Dijkhuizen, and J Nielsen. Antibiotic overproduction in *Streptomyces coelicolor* A3(2) mediated by phosphofructokinase deletion. *Journal of Biological Chemistry*, 283(37):25186–25199, 2008.

[23] EF Bosma, J Forster, and AT Nielsen. *Lactobacilli* and *pediococci* as versatile cell factories–evaluation of strain properties and genetic tools. *Biotechnology Advances*, 35(4):419–442, 2017.

[24] EF Bosma, AH van de Weijer, MJ Daas, J van der Oost, WM de Vos, and R van Kranenburg. Isolation and screening of *thermophilic bacilli* from compost for electrotransformation and fermentation: characterization of *Bacillus smithii* et 138 as a new biocatalyst. *Applied and Environmental Microbiology*, pages AEM–03640, 2015.

[25] JJ Bozell and GR Petersen. Technology development for the production of biobased products from biorefinery carbohydrates–the US Department of Energy's "Top 10" revisited. *Green Chemistry*, 12(4):539–554, 2010.

[26] JR Broadbent, TS Oberg, JE Hughes, RE Ward, C Brighton, DL Welker, and JL Steele. Influence of polysorbate 80 and cyclopropane fatty acid synthase activity on lactic acid production by *Lactobacillus casei* ATCC 334 at low pH. *Journal of Industrial Microbiology & Biotechnology*, 41(3):545–553, 2014.

[27] SA Buffington, GV Di Prisco, TA Auchtung, NJ Ajami, JF Petrosino, and M Costa-Mattioli. Microbial reconstitution reverses maternal diet-induced social and synaptic deficits in offspring. *Cell*, 165(7):1762–1775, 2016.

[28] G Cambray, JC Guimaraes, VK Mutalik, C Lam, QA Mai, T Thimmaiah, JM Carothers, AP Arkin, and D Endy. Measurement and modeling of intrinsic transcription terminators. *Nucleic Acids Research*, 41(9):5139–5148, 2013.

[29] N Campo, ML Daveran-Mingot, K Leenhouts, P Ritzenthaler, and P Le Bourgeois. *Cre-loxP* recombination system for large genome rearrangements in *Lactococcus lactis*. *Applied and Environmental Microbiology*, 68(5):2359–2367, 2002.

[30] G Cavallaro, L Decaria, and A Rosato. Genome-based analysis of heme biosynthesis and uptake in prokaryotic systems. *Journal of Proteome Research*, 7(11):4946–4954, 2008.

[31] GQ Chen. New challenges and opportunities for industrial biotechnology. *Microbial Cell Factories*, 11(1):111, 2012.

[32] L Chen, PD Bromberger, G Nieuwenhuiys, and R Hatti-Kaul. Redox balance in *Lactobacillus reuteri* DSM20016: roles of iron-dependent alcohol dehydrogenases in glucose/glycerol metabolism. *PloS One*, 11(12):e0168107, 2016.

[33] L Chen and R Hatti-Kaul. Exploring *Lactobacillus reuteri* dsm20016 as a biocatalyst for transformation of longer chain 1, 2-diols: limits with microcompartment. *PloS One*, 12(9):e0185734, 2017.

[34] XL Chen, XX Dong, YC Wang, ZH Zhao, and LM Liu. Mitochondrial engineering of the TCA cycle for fumarate production. *Metabolic Engineering*, 31:62–73, 2015.

[35] SQ Cheng, S Sinha, CG Fan, Y Liu, and TA Bobik. Genetic analysis of the protein shell of the microcompartments involved in coenzyme B12-dependent 1, 2-propanediol degradation by *Salmonella*. *Journal of Bacteriology*, 2011.

[36] Z Cheng, JQ Jiang, H Wu, ZM Li, and Q Ye. Enhanced production of 3-hydroxypropionic acid from glucose via malonyl-coa pathway by engineered *Escherichia coli*. *Bioresource Technology*, 200:897–904, 2016.

[37] F Cherubini, G Jungmeier, M Wellisch, T Willke, I Skiadas, R Van Ree, and Ed de Jong. Toward a common classification approach for biorefinery systems. *Biofuels, Bioproducts and Biorefining*, 3(5):534–546, 2009.

[38] HY Cheung, KT Lau, TP Lu, and D Hui. A critical review on polymer-based bio-engineered materials for scaffold development. *Composites Part B: Engineering*, 38(3):291–300, 2007.

[39] C Chowdhury, S Chun, A Pang, MR Sawaya, S Sinha, TO Yeates, and TA Bobik. Selective molecular transport through the protein shell of a bacterial microcompartment organelle. *Proceedings of the National Academy of Sciences*, page 201423672, 2015.

[40] C Chowdhury, S Sinha, S Chun, TO Yeates, and TA Bobik. Diverse bacterial microcompartment organelles. *Microbiology and Molecular Biology Reviews*, 78(3):438–468, 2014.

[41] VL Colin, A Rodríguez, and HA Cristóbal. The role of synthetic biology in the design of microbial cell factories for biofuel production. *BioMed Research International*, 2011, 2011.

[42] JL Corchero, B Gasser, D Resina, W Smith, E Parrilli, F Vázquez, I Abasolo, M Giuliani, J Jäntti, and P Ferrer. Unconventional microbial systems for the cost-efficient production of high-quality protein therapeutics. *Biotechnology Advances*, 31(2):140–153, 2013.

[43] RI Corona-Hernandez, E Álvarez-Parrilla, J Lizardi-Mendoza, AR Islas-Rubio, LA de la Rosa, and A Wall-Medrano. Structural stability and viability of microencapsulated probiotic bacteria: a review. *Comprehensive Reviews in Food Science and Food Safety*, 12(6):614–628, 2013.

[44] CS Crowley, D Cascio, MR Sawaya, JS Kopstein, TA Bobik, and TO Yeates. Structural insights into the mechanisms of transport across the *Salmonella en-*

terica Pdu microcompartment shell. *Journal of Biological Chemistry*, pages jbc–M110, 2010.

[45] ME Csete and JC Doyle. Reverse engineering of biological complexity. *Science*, 295(5560):1664–1669, 2002.

[46] GP Da Silva, M Mack, and J Contiero. Glycerol: a promising and abundant carbon source for industrial microbiology. *Biotechnology Advances*, 27(1):30–39, 2009.

[47] LM Damasceno, CJ Huang, and CA Batt. Protein secretion in *Pichia pastoris* and advances in protein production. *Applied Microbiology and Biotechnology*, 93(1):31–39, 2012.

[48] R Davis, K Schooley, B Rasmussen, J Thomas, and P Reddy. Effect of PDI overexpression on recombinant protein secretion in CHO cells. *Biotechnology Progress*, 16(5):736–743, 2000.

[49] AM Davy, HF Kildegaard, and MR Andersen. Cell factory engineering. *Cell Systems*, 4(3):262–275, 2017.

[50] M De Angelis and M Gobbetti. *Lactobacillus* spp.: general characteristics. 2015.

[51] V de Lorenzo and M Schmidt. Biological standards for the knowledge-based bioeconomy: what is at stake. *New Biotechnology*, 2017.

[52] MM de Nadra. Nitrogen metabolism in lactic acid bacteria from fruits: a review. *Communicating Current Research and Educational Topics and Trends in Applied Microbiology*, pages 500–510, 2007.

[53] GLV de Oliveira, AZ Leite, BS Higuchi, MI Gonzaga, and VS Mariano. Intestinal dysbiosis and probiotic applications in autoimmune diseases. *Immunology*, 152(1):1–12, 2017.

[54] WM de Vos. Systems solutions by lactic acid bacteria: from paradigms to practice. In *Microbial Cell Factories*, volume 10, page S2. BioMed Central, 2011.

[55] C Della Pina, E Falletta, and M Rossi. Oxidation of allyl alcohol in the presence of a gold catalyst: A route to 3-hydroxypropionic acid. *ChemSusChem: Chemistry & Sustainability Energy & Materials*, 2(1):57–58, 2009.

[56] PM Derkx, T Janzen, KI Sørensen, JE Christensen, B Stuer-Lauridsen, and E Johansen. The art of strain improvement of industrial lactic acid bacteria without the use of recombinant DNA technology. In *Microbial Cell Factories*, volume 13, page S5. BioMed Central, 2014.

[57] H Dhamankar and KL Prather. Microbial chemical factories: recent advances in pathway engineering for synthesis of value added chemicals. *Current Opinion in Structural Biology*, 21(4):488–494, 2011.

[58] M Diaz, V Ladero, B del Rio, B Redruello, M Fernández, MC Martin, and MA Alvarez. Biofilm-forming capacity in biogenic amine-producing bacteria isolated from dairy products. *Frontiers in Microbiology*, 7:591, 2016.

[59] T Dishisha. *Microbial production of bio-based chemicals*. PhD thesis, Lurid: Lund University, 2013.

[60] T Dishisha, R Hatti-Kaul, RR Sardari, B Grund, and O Thum. 3-hydroxypropionaldehyde detection and extraction, August 21 2018. US Patent App. 10/053,411.

[61] T Dishisha, LP Pereyra, SH Pyo, RA Britton, and R Hatti-Kaul. Flux analysis of the *Lactobacillus reuteri* propanediol utilization pathway for production of 3-hydroxypropionaldehyde, 3-hydroxypropionic acid and 1, 3-propanediol from glycerol. *Microbial Cell Factories*, 13(1):76, 2014.

[62] WJ Dobrogosz and SE Lindgren. Antibiotic reuterin, May 9 1995. US Patent 5,413,960.

[63] FP Douillard and WM De Vos. Functional genomics of lactic acid bacteria: from food to health. In *Microbial Cell Factories*, volume 13, page S8. BioMed Central, 2014.

[64] FP Douillard, J Mahony, V Campanacci, C Cambillau, and D van Sinderen. Construction of two *Lactococcus lactis* expression vectors combining the Gateway and the NIsin Controlled Expression systems. *Plasmid*, 66(3):129–135, 2011.

[65] J Du, ZY Shao, and HM Zhao. Engineering microbial factories for synthesis of value-added products. *Journal of Industrial Microbiology & Biotechnology*, 38(8):873–890, 2011.

[66] A Dudnik. Development of tools for precise genome engineering in lactic acid bacteria. In *Cell Factories and Biosustainability 2015*, 2015.

[67] JE Dueber, GC Wu, GR Malmirchegini, TS Moon, CJ Petzold, AV Ullal, KL Prather, and JD Keasling. Synthetic protein scaffolds provide modular control over metabolic flux. *Nature Biotechnology*, 27(8):753, 2009.

[68] MJ Dunlop, ZY Dossani, HL Szmidt, HC Chu, TS Lee, JD Keasling, MZ Hadi, and A Mukhopadhyay. Engineering microbial biofuel tolerance and export using efflux pumps. *Molecular Systems Biology*, 7(1):487, 2011.

[69] SO Elferink, J Krooneman, JC Gottschal, SF Spoelstra, F Faber, and F Driehuis. Anaerobic conversion of lactic acid to acetic acid and 1, 2-propanediol by *Lactobacillus buchneri*. *Applied and Environmental Microbiology*, 67(1):125–132, 2001.

[70] M Emptage, SL Haynie, LA Laffend, JP Pucci, and G Whited. Process for the biological production of 1, 3-propanediol with high titer, February 4 2003. US Patent 6,514,733.

[71] J Extance, SJ Crennell, K Eley, R Cripps, DW Hough, and MJ Danson. Structure of a bifunctional alcohol dehydrogenase involved in bioethanol generation in *Geobacillus thermoglucosidasius*. *Acta Crystallographica Section D: Biological Crystallography*, 69(10):2104–2115, 2013.

[72] SH Fang, YJ Lai, and CC Chou. The susceptibility of *Streptococcus thermophilus* 14085 to organic acid, simulated gastric juice, bile salt and disinfectant as influenced by cold shock treatment. *Food Microbiology*, 33(1):55–60, 2013.

[73] C Ferndahl, N Bonander, C Logez, R Wagner, L Gustafsson, C Larsson, K Hedfalk, RA Darby, and RM Bill. Increasing cell biomass in *Saccharomyces cerevisiae* increases recombinant protein yield: the use of a respiratory strain as a microbial cell factory. *Microbial Cell Factories*, 9(1):47, 2010.

[74] N Ferrer-Miralles, J Domingo-Espín, JL Corchero, E Vázquez, and A Villaverde. Microbial factories for recombinant pharmaceuticals. *Microbial Cell Factories*, 8(1):17, 2009.

[75] AK Fisher, BG Freedman, DR Bevan, and RS Senger. A review of metabolic and enzymatic engineering strategies for designing and optimizing performance of microbial cell factories. *Computational and Structural Biotechnology Journal*, 11(18):91–99, 2014.

[76] A Fleißner and P Dersch. Expression and export: recombinant protein production systems for *Aspergillus*. *Applied Microbiology and Biotechnology*, 87(4):1255–1270, 2010.

[77] S Frank, AD Lawrence, MB Prentice, and MJ Warren. Bacterial microcompartments moving into a synthetic biological world. *Journal of Biotechnology*, 163(2):273–279, 2013.

[78] AC Franken, BC Lokman, AF Ram, PJ Punt, CA van den Hondel, and S de Weert. Heme biosynthesis and its regulation: towards understanding and improvement of heme biosynthesis in filamentous fungi. *Applied Microbiology and Biotechnology*, 91(3):447–460, 2011.

[79] SA Frese, AK Benson, GW Tannock, DM Loach, J Kim, M Zhang, PL Oh, NC Heng, PB Patil, and N Juge. The evolution of host specialization in the vertebrate gut symbiont *Lactobacillus reuteri*. *PLoS Genetics*, 7(2):e1001314, 2011.

[80] MG Gaenzle. Lactic metabolism revisited: metabolism of lactic acid bacteria in food fermentations and food spoilage. *Current Opinion in Food Science*, 2:106–117, 2015.

[81] JD Galley, MC Nelson, ZT Yu, SE Dowd, J Walter, PS Kumar, M Lyte, and MT Bailey. Exposure to a social stressor disrupts the community structure of the colonic mucosa-associated microbiota. *BMC Microbiology*, 14(1):189, 2014.

[82] C Gandini, L Tarraran, D Kalemasi, E Pessione, and R Mazzoli. Recombinant *Lactococcus lactis* for efficient conversion of cellodextrins into L-lactic acid. *Biotechnology and Bioengineering*, 114(12):2807–2817, 2017.

[83] P Gaspar, AL Carvalho, S Vinga, H Santos, and AR Neves. From physiology to systems metabolic engineering for the production of biochemicals by lactic acid bacteria. *Biotechnology Advances*, 31(6):764–788, 2013.

[84] P Gaspar, AR Neves, MJ Gasson, CA Shearman, and H Santos. High yields of 2, 3-butanediol and mannitol in *Lactococcus lactis* through engineering NAD+ cofactor recycling. *Applied and Environmental Microbiology*, pages AEM–05544, 2011.

[85] B Gasser, M Saloheimo, U Rinas, M Dragosits, E Rodríguez-Carmona, K Baumann, M Giuliani, E Parrilli, P Branduardi, and C Lang. Protein folding and conformational stress in microbial cells producing recombinant proteins: a host comparative overview. *Microbial Cell Factories*, 7(1):11, 2008.

[86] M Gavrilescu. Biorefinery systems: an overview. In *Bioenergy Research: Advances and Applications*, pages 219–241. Elsevier, 2014.

[87] DG Gibson, GA Benders, C Andrews-Pfannkoch, EA Denisova, H Baden-Tillson, J Zaveri, TB Stockwell, A Brownley, DW Thomas, and MA Algire. Complete chemical synthesis, assembly, and cloning of a *Mycoplasma genitalium* genome. *Science*, 319(5867):1215–1220, 2008.

[88] DG Gibson, JI Glass, C Lartigue, VN Noskov, RY Chuang, MA Algire, GA Benders, MG Montague, L Ma, and MM Moodie. Creation of a bacterial cell controlled by a chemically synthesized genome. *Science*, page 1190719, 2010.

[89] YJ Goh, MA Azcárate-Peril, S O'Flaherty, E Durmaz, F Valence, J Jardin, S Lortal, and TR Klaenhammer. Development and application of a *upp*-based counterselective gene replacement system for the study of the S-layer protein

SlpX of *Lactobacillus acidophilus* NCFM. *Applied and Environmental Microbiology*, 75(10):3093–3105, 2009.

[90] RR Gokarn, OV Selifonova, HJ Jessen, SJ Gort, T Selmer, and W Buckel. 3-hydroxypropionic acid and other organic compounds, March 6 2007. US Patent 7,186,541.

[91] YM Gong, HH Hu, Y Gao, XD Xu, and H Gao. Microalgae as platforms for production of recombinant proteins and valuable compounds: progress and prospects. *Journal of Industrial Microbiology & Biotechnology*, 38(12):1879–1890, 2011.

[92] G Greifová, H Májeková, G Greif, P Body, M Greifová, and M Dubničková. Analysis of antimicrobial and immunomodulatory substances produced by heterofermentative *Lactobacillus reuteri*. *Folia Microbiologica*, 62(6):515–524, 2017.

[93] Y Gu, XH Xu, YK Wu, TF Niu, YF Liu, JH Li, GC Du, and L Liu. Advances and prospects of *Bacillus subtilis* cellular factories: from rational design to industrial applications. *Metabolic Engineering*, 2018.

[94] YX Guo, DD Pan, H Li, YY Sun, XQ Zeng, and BX Yan. Antioxidant and immunomodulatory activity of selenium exopolysaccharide produced by *Lactococcus lactis* subsp. *lactis*. *Food Chemistry*, 138(1):84–89, 2013.

[95] T Hagi, M Kobayashi, S Kawamoto, J Shima, and M Nomura. Expression of novel carotenoid biosynthesis genes from *Enterococcus gilvus* improves the multistress tolerance of *Lactococcus lactis*. *Journal of Applied Microbiology*, 114(6):1763–1771, 2013.

[96] T Hamann and L Lange. Discovery, cloning and heterologous expression of secreted potato proteins reveal erroneous pre-mRNA splicing in *Aspergillus oryzae*. *Journal of Biotechnology*, 126(3):265–276, 2006.

[97] J Hasegawa, M Ogura, S Tsuda, SI Maemoto, H Kutsuki, and T Ohashi. High-yield production of optically active 1, 2-diols from the corresponding racemates by microbial stereoinversion. *Agricultural and Biological Chemistry*, 54(7):1819–1827, 1990.

[98] R Hatti-Kaul, U Törnvall, L Gustafsson, and P Börjesson. Industrial biotechnology for the production of bio-based chemicals–a cradle-to-grave perspective. *Trends in Biotechnology*, 25(3):119–124, 2007.

[99] GD Havemann and TA Bobik. Protein content of polyhedral organelles involved in coenzyme B12-dependent degradation of 1, 2-propanediol in *Salmonella enterica* serovar Typhimurium LT2. *Journal of Bacteriology*, 185(17):5086–5095, 2003.

[100] BK He, TK Hoang, T Wang, M Ferris, CM Taylor, XJ Tian, M Luo, DQ Tran, JI Zhou, and N Tatevian. Resetting microbiota by *Lactobacillus reuteri* inhibits T reg deficiency–induced autoimmunity via adenosine A2A receptors. *Journal of Experimental Medicine*, 214(1):107–123, 2017.

[101] SA Heider and VF Wendisch. Engineering microbial cell factories: metabolic engineering of *Corynebacterium glutamicum* with a focus on non-natural products. *Biotechnology Journal*, 10(8):1170–1184, 2015.

[102] S Heiss, A Hörmann, C Tauer, M Sonnleitner, E Egger, R Grabherr, and S Heinl. Evaluation of novel inducible promoter/repressor systems for recombinant protein expression in *Lactobacillus plantarum*. *Microbial Cell Factories*, 15(1):50, 2016.

[103] F Hempel, AS Bozarth, N Lindenkamp, A Klingl, S Zauner, U Linne, A Steinbüchel, and UG Maier. Microalgae as bioreactors for bioplastic production. *Microbial Cell Factories*, 10(1):81, 2011.

[104] TI Herring, TN Harris, C Chowdhury, S Mohanty, and TA Bobik. A bacterial microcompartment is used for choline fermentation by *Escherichia coli* 536. *Journal of Bacteriology*, pages JB–00764, 2018.

[105] C Hidalgo-Cantabrana, S O'Flaherty, and R Barrangou. CRISPR-based engineering of next-generation lactic acid bacteria. *Current Opinion in Microbiology*, 37:79–87, 2017.

[106] JE Holladay, JF White, JJ Bozell, and D Johnson. Top value-added chemicals from biomass. Volume II-Results of screening for potential candidates from biorefinery lignin. Technical report, Pacific Northwest National Lab.(PNNL), Richland, WA (United States), 2007.

[107] H Holo and IF Nes. High-frequency transformation, by electroporation, of *Lactococcus lactis* subsp. *cremoris* grown with glycine in osmotically stabilized media. *Applied and Environmental Microbiology*, 55(12):3119–3123, 1989.

[108] P Hols, M Kleerebezem, A Schanck, T Ferain, J Hugenholtz, J Delcour, and WM de Vos. Conversion of *Lactococcus lactis* from homolactic to homoalanine fermentation through metabolic engineering. *Nature Biotechnology*, 17(6):588, 1999.

[109] WH Holzapfel and BJ Wood. *Lactic acid bacteria: biodiversity and taxonomy*. John Wiley & Sons, 2014.

[110] J Hou, K Tyo, ZH Liu, D Petranovic, and J Nielsen. Engineering of vesicle trafficking improves heterologous protein secretion in *Saccharomyces cerevisiae*. *Metabolic Engineering*, 14(2):120–127, 2012.

[111] J Hugenholtz. Citrate metabolism in lactic acid bacteria. *FEMS Microbiology Reviews*, 12(1-3):165–178, 1993.

[112] J Hugenholtz, M Kleerebezem, M Starrenburg, J Delcour, W de Vos, and P Hols. *Lactococcus lactis* as a cell factory for high-level diacetyl production. *Applied and Environmental Microbiology*, 66(9):4112–4114, 2000.

[113] SA Ibrahim. Lactic acid bacteria: *Lactobacillus* spp.: other species. 2015.

[114] PR Jensen and K Hammer. The sequence of spacers between the consensus sequences modulates the strength of prokaryotic promoters. *Applied and Environmental Microbiology*, 64(1):82–87, 1998.

[115] XL Jiang, X Meng, and M Xian. Biosynthetic pathways for 3-hydroxypropionic acid production. *Applied Microbiology and Biotechnology*, 82(6):995–1003, 2009.

[116] B Kamm and M Kamm. Biorefinery systems. *Chemical and Biochemical Engineering Quarterly*, 18(1):1–7, 2004.

[117] S Karimi, D Ahl, E Vågesjö, L Holm, M Phillipson, H Jonsson, and S Roos. *In vivo* and *in vitro* detection of luminescent and fluorescent *Lactobacillus reuteri* and application of red fluorescent mCherry for assessing plasmid persistence. *PloS One*, 11(3):e0151969, 2016.

[118] CA Kerfeld, S Heinhorst, and GC Cannon. Bacterial microcompartments. *Annual Review of Microbiology*, 64, 2010.

[119] C Kiel, E Yus, and L Serrano. Engineering signal transduction pathways. *Cell*, 140(1):33–47, 2010.

[120] JE Kim, HJ Eom, YJ Kim, JE Ahn, JH Kim, and NS Han. Enhancing acid tolerance of *Leuconostoc mesenteroides* with glutathione. *Biotechnology Letters*, 34(4):683–687, 2012.

[121] JE Kim, HJ Eom, L Li, KS Yoo, and NS Han. Induction of the acid tolerance response in *Leuconostoc mesenteroides* ATCC 8293 by pre-adaptation in acidic condition. *Food Science and Biotechnology*, 23(1):221–224, 2014.

[122] S Kind, WK Jeong, H Schröder, and C Wittmann. Systems-wide metabolic pathway engineering in *Corynebacterium glutamicum* for bio-based production of diaminopentane. *Metabolic Engineering*, 12(4):341–351, 2010.

[123] T Klaenhammer, E Altermann, F Arigoni, A Bolotin, F Breidt, J Broadbent, R Cano, S Chaillou, J Deutscher, and M Gasson. Discovering lactic acid bacteria by genomics. In *Lactic Acid Bacteria: Genetics, Metabolism and Applications*, pages 29–58. Springer, 2002.

[124] W Kong, VS Kapuganti, and T Lu. A gene network engineering platform for lactic acid bacteria. *Nucleic Acids Research*, 44(4):e37–e37, 2015.

[125] AA Koutinas, A Vlysidis, D Pleissner, N Kopsahelis, IL Garcia, IK Kookos, S Papanikolaou, TH Kwan, and CSK Lin. Valorization of industrial waste and by-product streams via fermentation for the production of chemicals and biopolymers. *Chemical Society Reviews*, 43(8):2587–2627, 2014.

[126] H Krauter, T Willke, and KD Vorlop. Production of high amounts of 3-hydroxypropionaldehyde from glycerol by *Lactobacillus reuteri* with strongly increased biocatalyst lifetime and productivity. *New Biotechnology*, 29(2):211–217, 2012.

[127] OP Kuipers, MM Beerthuyzen, PG de Ruyter, EJ Luesink, and WM de Vos. Autoregulation of nisin biosynthesis in *Lactococcus lactis* by signal transduction. *Journal of Biological Chemistry*, 270(45):27299–27304, 1995.

[128] V Kumar, S Ashok, and S Park. Recent advances in biological production of 3-hydroxypropionic acid. *Biotechnology Advances*, 31(6):945–961, 2013.

[129] V Kumar, S Ashok, and S Park. Microbial production of 3-hydroxypropionic acid from renewable sources: A green approach as an alternative to conventional chemistry. *Bioprocessing of Renewable Resources to Commodity Bioproducts*, pages 381–407, 2014.

[130] JM Lambert, RS Bongers, and M Kleerebezem. *Cre-lox*-based system for multiple gene deletions and selectable-marker removal in *Lactobacillus plantarum*. *Applied and Environmental Microbiology*, 73(4):1126–1135, 2007.

[131] GM Lari, G Pastore, M Haus, YY Ding, S Papadokonstantakis, C Mondelli, and J Pérez-Ramírez. Environmental and economical perspectives of a glycerol biorefinery. *Energy & Environmental Science*, 11(5):1012–1029, 2018.

[132] J Law, G Buist, A Haandrikman, J Kok, G Venema, and K Leenhouts. A system to generate chromosomal mutations in *Lactococcus lactis* which allows fast analysis of targeted genes. *Journal of Bacteriology*, 177(24):7011–7018, 1995.

[133] AD Lawrence, S Frank, S Newnham, MJ Lee, IR Brown, WF Xue, ML Rowe, DP Mulvihill, MB Prentice, and MJ Howard. Solution structure of a bacterial microcompartment targeting peptide and its application in the construction of an ethanol bioreactor. *ACS Synthetic Biology*, 3(7):454–465, 2014.

[134] JH Lee, DE Lee, BU Lee, and HS Kim. Global analyses of transcriptomes and proteomes of a parent strain and an L-threonine-overproducing mutant strain. *Journal of Bacteriology*, 185(18):5442–5451, 2003.

[135] JW Lee, D Na, JM Park, J Lee, S Choi, and SY Lee. Systems metabolic engineering of microorganisms for natural and non-natural chemicals. *Nature Chemical Biology*, 8(6):536, 2012.

[136] KH Lee, JH Park, TY Kim, HU Kim, and SY Lee. Systems metabolic engineering of *Escherichia coli* for L-threonine production. *Molecular Systems Biology*, 3(1):149, 2007.

[137] LG Lee and GM Whitesides. Preparation of optically active 1, 2-diols and .alpha.-hydroxy ketones using glycerol dehydrogenase as catalyst. limits to enzyme-catalyzed synthesis due to noncompetitive and mixed inhibition by product. *The Journal of Organic Chemistry*, 51(1):25–36, 1986.

[138] SY Lee and HU Kim. Systems strategies for developing industrial microbial strains. *Nature Biotechnology*, 33(10):1061, 2015.

[139] SY Lee, D Mattanovich, and A Villaverde. Systems metabolic engineering, industrial biotechnology and microbial cell factories, 2012.

[140] SY Lee, J Nielsen, and G Stephanopoulos. *Industrial biotechnology: products and processes*. John Wiley & Sons, 2016.

[141] K Leenhouts, G Buist, A Bolhuis, A Ten Berge, J Kiel, I Mierau, M Dabrowska, G Venema, and J Kok. A general system for generating unlabelled gene replacements in bacterial chromosomes. *Molecular and General Genetics MGG*, 253(1-2):217–224, 1996.

[142] A Li, N Pfelzer, R Zuijderwijk, A Brickwedde, C van Zeijl, and P Punt. Reduced by-product formation and modified oxygen availability improve itaconic acid production in *Aspergillus niger*. *Applied Microbiology and Biotechnology*, 97(9):3901–3911, 2013.

[143] HJ Li, N Sethuraman, TA Stadheim, DX Zha, B Prinz, N Ballew, P Bobrowicz, BK Choi, WJ Cook, and M Cukan. Optimization of humanized IgGs in glycoengineered *Pichia pastoris*. *Nature Biotechnology*, 24(2):210, 2006.

[144] Z Li, JX Yan, JK Sun, P Xu, CQ Ma, and C Gao. Production of value-added chemicals from glycerol using *in vitro* enzymatic cascades. *Communications Chemistry*, 1(1):71, 2018.

[145] JA Linares-Pastén, R Sabet-Azad, L Pessina, RR Sardari, MH Ibrahim, and R Hatti-Kaul. Efficient poly (3-hydroxypropionate) production from glycerol using *Lactobacillus reuteri* and recombinant *Escherichia coli* harboring *L. reuteri* propionaldehyde dehydrogenase and *Chromobacterium* sp. PHA synthase genes. *Bioresource Technology*, 180:172–176, 2015.

[146] DR Mack. D(-)-lactic acid producing probiotics, d(-)-lactic acidosis and infants. *Canadian Journal of Gastroenterology and Hepatology*, 18(11):671–675, 2004.

[147] JM Macklaim, JC Clemente, R Knight, GB Gloor, and G Reid. Changes in vaginal microbiota following antimicrobial and probiotic therapy. *Microbial Ecology in Health and Disease*, 26(1):27799, 2015.

[148] G Mathiesen, A Sveen, JC Piard, L Axelsson, and VGH Eijsink. Heterologous protein secretion by *Lactobacillus plantarum* using homologous signal peptides. *Journal of Applied Microbiology*, 105(1):215–226, 2008.

[149] D Mattanovich, P Branduardi, L Dato, B Gasser, M Sauer, and D Porro. Recombinant protein production in yeasts. In *Recombinant Gene Expression*, pages 329–358. Springer, 2012.

[150] R Mazzoli, F Bosco, I Mizrahi, EA Bayer, and E Pessione. Towards lactic acid bacteria-based biorefineries. *Biotechnology Advances*, 32(7):1216–1236, 2014.

[151] P Mengal, M Wubbolts, E Zika, A Ruiz, D Brigitta, A Pieniadz, and S Black. Bio-based industries joint undertaking: the catalyst for sustainable bio-based economic growth in Europe. *New Biotechnology*, 2017.

[152] V Meyer, B Wu, and AF Ram. *Aspergillus* as a multi-purpose cell factory: current status and perspectives. *Biotechnology Letters*, 33(3):469–476, 2011.

[153] GL Mi, L Zhao, DD Qiao, WQ Kang, MQ Tang, and JK Xu. Effectiveness of /textitLactobacillus reuteri in infantile colic and colicky induced maternal depression: a prospective single blind randomized trial. *Antonie Van Leeuwenhoek*, 107(6):1547–1553, 2015.

[154] LM Miller and DW Keith. Climatic impacts of wind power. *Joule*, 2018.

[155] T Min, CE Ergenekan, MK Eidsness, T Ichiye, and C Kang. Leucine 41 is a gate for water entry in the reduction of *Clostridium pasteurianum* rubredoxin. *Protein Science*, 10(3):613–621, 2001.

[156] J Mohseni, M Fazeli, and AS Lavasani. Effect of various parameters of carbon and nitrogen sources and environmental conditions on the growth of *Lactobacillus Casei* in the production of lactic acid. *Global Journal of Medical Research*, 2017.

[157] MR Monteiro, CL Kugelmeier, RS Pinheiro, MO Batalha, and A da Silva César. Glycerol from biodiesel production: technological paths for sustainability. *Renewable and Sustainable Energy Reviews*, 88:109–122, 2018.

[158] C Moon, JH Ahn, SW Kim, BI Sang, and Y Um. Effect of biodiesel-derived raw glycerol on 1, 3-propanediol production by different microorganisms. *Applied Biochemistry and Biotechnology*, 161(1-8):502–510, 2010.

[159] F Mozzi and GM Vignolo. *Biotechnology of lactic acid bacteria: novel applications*. John Wiley & Sons, 2010.

[160] QH Mu, J Kirby, CM Reilly, and XM Luo. Leaky gut as a danger signal for autoimmune diseases. *Frontiers in Immunology*, 8:598, 2017.

[161] QH Mu, V Tavella, and XM Luo. Role of *Lactobacillus reuteri* in human health and diseases. *Frontiers in Microbiology*, 9:757, 2018.

[162] Y Mu, H Teng, DJ Zhang, W Wang, and ZL Xiu. Microbial production of 1, 3-propanediol by *Klebsiella pneumoniae* using crude glycerol from biodiesel preparations. *Biotechnology Letters*, 28(21):1755–1759, 2006.

[163] CE Nakamura and GM Whited. Metabolic engineering for the microbial production of 1, 3-propanediol. *Current Opinion in Biotechnology*, 14(5):454–459, 2003.

[164] AR Neves, WA Pool, R Castro, A Mingote, F Santos, J Kok, OP Kuipers, and H Santos. The α-phosphoglucomutase of *Lactococcus lactis* is unrelated to the α-D-phosphohexomutase superfamily and is encoded by the essential gene *pgmH*. *Journal of Biological Chemistry*, 281(48):36864–36873, 2006.

[165] HM Nguyen, G Mathiesen, EM Stelzer, ML Pham, K Kuczkowska, A Mackenzie, JW Agger, VG Eijsink, M Yamabhai, and CK Peterbauer. Display of a β-mannanase and a chitosanase on the cell surface of *Lactobacillus plantarum* towards the development of whole-cell biocatalysts. *Microbial Cell Factories*, 15(1):169, 2016.

[166] TT Nguyen, TH Nguyen, T Maischberger, P Schmelzer, G Mathiesen, VG Eijsink, D Haltrich, and CK Peterbauer. Quantitative transcript analysis of the inducible expression system pSIP: comparison of the overexpression of *Lactobacillus* spp. β-galactosidases in *Lactobacillus plantarum*. *Microbial Cell Factories*, 10(1):46, 2011.

[167] J Nielsen. Synthetic biology for engineering acetyl coenzyme A metabolism in yeast. *MBio*, 5(6):e02153–14, 2014.

[168] J Nielsen. Yeast cell factories on the horizon. *Science*, 349(6252):1050–1051, 2015.

[169] J Nielsen, C Larsson, A van Maris, and J Pronk. Metabolic engineering of yeast for production of fuels and chemicals. *Current Opinion in Biotechnology*, 24(3):398–404, 2013.

[170] L Nissen, G Pérez-Martínez, and MJ Yebra. Sorbitol synthesis by an engineered *Lactobacillus casei* strain expressing a sorbitol-6-phosphate dehydrogenase gene within the lactose operon. *FEMS Microbiology Letters*, 249(1):177–183, 2005.

[171] JH Oh and JP van Pijkeren. CRISPR-Cas9-assisted recombineering in *Lactobacillus reuteri*. *Nucleic Acids Research*, 42(17):e131–e131, 2014.

[172] PH Opgenorth, TP Korman, and JU Bowie. A synthetic biochemistry molecular purge valve module that maintains redox balance. *Nature Communications*, 5:4113, 2014.

[173] S Ostergaard, L Olsson, M Johnston, and J Nielsen. Increasing galactose consumption by *Saccharomyces cerevisiae* through metabolic engineering of the *GAL* gene regulatory network. *Nature Biotechnology*, 18(12):1283, 2000.

[174] M Pagliaro, R Ciriminna, H Kimura, M Rossi, and C Della Pina. From glycerol to value-added products. *Angewandte Chemie International Edition*, 46(24):4434–4440, 2007.

[175] M Pagliaro and M Rossi. *The future of glycerol*. 2008.

[176] A Pang, MZ Liang, MB Prentice, and RW Pickersgill. Substrate channels revealed in the trimeric *Lactobacillus reuteri* bacterial microcompartment shell protein pdub. *Acta Crystallographica Section D: Biological Crystallography*, 68(12):1642–1652, 2012.

[177] K Papadimitriou, Á Alegría, PA Bron, M De Angelis, M Gobbetti, M Kleerebezem, JA Lemos, DM Linares, P Ross, and C Stanton. Stress physiology of lactic acid bacteria. *Microbiology and Molecular Biology Reviews*, 80(3):837–890, 2016.

[178] M Papagianni and M Legiša. Increased mannitol production in *Lactobacillus reuteri* atcc 55730 production strain with a modified 6-phosphofructo-1-kinase. *Journal of Biotechnology*, 181:20–26, 2014.

[179] JH Park, JE Oh, KH Lee, JY Kim, and SY Lee. Rational design of *Escherichia coli* for L-isoleucine production. *ACS Synthetic Biology*, 1(11):532–540, 2012.

[180] JB Parsons, SD Dinesh, E Deery, HK Leech, AA Brindley, D Heldt, S Frank, CM Smales, H Lünsdorf, and A Rambach. Biochemical and structural insights into bacterial organelle form and biogenesis. *Journal of Biological Chemistry*, 283(21):14366–14375, 2008.

[181] R Patnaik, S Louie, V Gavrilovic, K Perry, WP Stemmer, CM Ryan, and S del Cardayré. Genome shuffling of *Lactobacillus* for improved acid tolerance. *Nature Biotechnology*, 20(7):707, 2002.

[182] P Pawelzik, M Carus, J Hotchkiss, R Narayan, S Selke, M Wellisch, M Weiss, B Wicke, and MK Patel. Critical aspects in the life cycle assessment (LCA) of bio-based materials–reviewing methodologies and deriving recommendations. *Resources, Conservation and Recycling*, 73:211–228, 2013.

[183] K Petersen, PV Nielsen, G Bertelsen, M Lawther, MB Olsen, NH Nilsson, and G Mortensen. Potential of biobased materials for food packaging. *Trends in Food Science & Technology*, 10(2):52–68, 1999.

[184] BA Pfeifer and C Khosla. Biosynthesis of polyketides in heterologous hosts. *Microbiology and Molecular Biology Reviews*, 65(1):106–118, 2001.

[185] LB Pickens, Y Tang, and YH Chooi. Metabolic engineering for the production of natural products. *Annual Review of Chemical and Biomolecular Engineering*, 2:211–236, 2011.

[186] S Piotrowski, M Carus, and D Carrez. European bioeconomy in figures. *Industrial Biotechnology*, 12(2):78–82, 2016.

[187] K Qi, JJ Zhong, and XX Xia. Triggering respiro-fermentative metabolism in a Crabtree-negative yeast *Pichia guilliermondii* by disrupting the *CAT8* gene. *Applied and Environmental Microbiology*, pages AEM–00854, 2014.

[188] K Ramana, J Xavier, and R Sharma. Recent trends in pharmaceutical biotechnology. *Pharm Biotechnol Curr Res*, 1:1–10, 2017.

[189] AL Rodrigues, N Trachtmann, J Becker, AF Lohanatha, J Blotenberg, CJ Bolten, C Korneli, AO de Souza Lima, LM Porto, and GA Sprenger. Systems metabolic engineering of *Escherichia coli* for production of the antitumor drugs violacein and deoxyviolacein. *Metabolic Engineering*, 20:29–41, 2013.

[190] MC Rodríguez, MT Alegre, and JM Mesas. Optimization of technical conditions for the transformation of *Pediococcus acidilactici* p60 by electroporation. *Plasmid*, 58(1):44–50, 2007.

[191] MR Rondon and JC Escalante-Semerena. The *poc* locus is required for 1, 2-propanediol-dependent transcription of the cobalamin biosynthetic (*cob*) and propanediol utilization (*pdu*) genes of *Salmonella typhimurium*. *Journal of Bacteriology*, 174(7):2267–2272, 1992.

[192] I Rud, PR Jensen, K Naterstad, and L Axelsson. A synthetic promoter library for constitutive gene expression in *Lactobacillus plantarum*. *Microbiology*, 152(4):1011–1019, 2006.

[193] WM Russell and TR Klaenhammer. Efficient system for directed integration into the *Lactobacillus acidophilus* and *Lactobacillus gasseri* chromosomes

via homologous recombination. *Applied and Environmental Microbiology*, 67(9):4361–4364, 2001.

[194] R Sabet-Azad, JA Linares-Pastén, L Torkelson, RR Sardari, and R Hatti-Kaul. Coenzyme a-acylating propionaldehyde dehydrogenase (pdup) from *Lactobacillus reuteri*: kinetic characterization and molecular modeling. *Enzyme and Microbial Technology*, 53(4):235–242, 2013.

[195] R Sabet-Azad, RR Sardari, JA Linares-Pastén, and R Hatti-Kaul. Production of 3-hydroxypropionic acid from 3-hydroxypropionaldehyde by recombinant *Escherichia coli* co-expressing *Lactobacillus reuteri* propanediol utilization enzymes. *Bioresource Technology*, 180:214–221, 2015.

[196] MJ Salas-Jara, A Ilabaca, M Vega, and A García. Biofilm forming *Lactobacillus*: new challenges for the development of probiotics. *Microorganisms*, 4(3):35, 2016.

[197] M Sankaranarayanan, A Somasundar, E Seol, AS Chauhan, S Kwon, GY Jung, and S Park. Production of 3-hydroxypropionic acid by balancing the pathway enzymes using synthetic cassette architecture. *Journal of Biotechnology*, 259:140–147, 2017.

[198] Y Sanno, TH Wilson, and ECC Lin. Control of permeation to glycerol in cells of *Escherichia coli*. *Biochemical and Biophysical Research Communications*, 32(2):344–349, 1968.

[199] RR Sardari, T Dishisha, SH Pyo, and R Hatti-Kaul. Biotransformation of glycerol to 3-hydroxypropionaldehyde: improved production by *in situ* complexation with bisulfite in a fed-batch mode and separation on anion exchanger. *Journal of Biotechnology*, 168(4):534–542, 2013.

[200] RR Sardari, T Dishisha, SH Pyo, and R Hatti-Kaul. Improved production of 3-hydroxypropionaldehyde by complex formation with bisulfite during biotransformation of glycerol. *Biotechnology and Bioengineering*, 110(4):1243–1248, 2013.

[201] M Sauer, H Russmayer, R Grabherr, CK Peterbauer, and H Marx. The efficient clade: lactic acid bacteria for industrial chemical production. *Trends in Biotechnology*, 35(8):756–769, 2017.

[202] RK Saxena, P Anand, S Saran, and J Isar. Microbial production of 1, 3-propanediol: recent developments and emerging opportunities. *Biotechnology Advances*, 27(6):895–913, 2009.

[203] M Schröder. Engineering eukaryotic protein factories. *Biotechnology Letters*, 30(2):187–196, 2008.

[204] J Schroeter and T Klaenhammer. Genomics of lactic acid bacteria. *FEMS Microbiology Letters*, 292(1):1–6, 2009.

[205] D Segre, D Vitkup, and GM Church. Analysis of optimality in natural and perturbed metabolic networks. *Proceedings of the National Academy of Sciences*, 99(23):15112–15117, 2002.

[206] MS Siddiqui, K Thodey, I Trenchard, and CD Smolke. Advancing secondary metabolite biosynthesis in yeast with synthetic biology tools. *FEMS Yeast Research*, 12(2):144–170, 2012.

[207] RS Singh. Industrial biotechnology: an overview.

[208] S Sinha, SQ Cheng, CG Fan, and TA Bobik. The PduM protein is a structural component of the microcompartments involved in coenzyme B12-dependent 1, 2-propanediol degradation by *Salmonella*. *Journal of Bacteriology*, pages JB–06529, 2012.

[209] MF Slininger Lee, CM Jakobson, and D Tullman-Ercek. Evidence for improved encapsulated pathway behavior in a bacterial microcompartment through shell protein engineering. *ACS Synthetic Biology*, 6(10):1880–1891, 2017.

[210] HH Smits, A Engering, D van der Kleij, EC de Jong, K Schipper, TM van Capel, BA Zaat, M Yazdanbakhsh, EA Wierenga, and Y van Kooyk. Selective probiotic bacteria induce IL-10-producing regulatory T cells *in vitro* by modulating dendritic cell function through dendritic cell–specific intercellular adhesion molecule 3–grabbing nonintegrin. *Journal of Allergy and Clinical Immunology*, 115(6):1260–1267, 2005.

[211] C Solem, T Dehli, and PR Jensen. Rewiring *Lactococcus lactis* for ethanol production. *Applied and Environmental Microbiology*, pages AEM–03623, 2013.

[212] L Song, HY Cui, L Tang, XY Qiao, M Liu, YP Jiang, W Cui, and YJ Li. Construction of *upp* deletion mutant strains of *Lactobacillus casei* and *Lactococcus lactis* based on counterselective system using temperature-sensitive plasmid. *Journal of Microbiological Methods*, 102:37–44, 2014.

[213] DD Sriramulu, MZ Liang, D Hernandez-Romero, E Raux-Deery, H Lünsdorf, JB Parsons, MJ Warren, and MB Prentice. *Lactobacillus reuteri* DSM 20016 produces cobalamin-dependent diol dehydratase in metabolosomes and metabolizes 1, 2-propanediol by disproportionation. *Journal of Bacteriology*, 190(13):4559–4567, 2008.

[214] ER Stadtman. Metal ion-catalyzed oxidation of proteins: biochemical mechanism and biological consequences. *Free Radical Biology and Medicine*, 9(4):315–325, 1990.

[215] L Staffas, M Gustavsson, and K McCormick. Strategies and policies for the bioeconomy and bio-based economy: an analysis of official national approaches. *Sustainability*, 5(6):2751–2769, 2013.

[216] ZH Sun, HM Harris, A McCann, CY Guo, S Argimón, WY Zhang, XW Yang, IB Jeffery, JC Cooney, and TF Kagawa. Expanding the biotechnology potential of *lactobacilli* through comparative genomics of 213 strains and associated genera. *Nature Communications*, 6:8322, 2015.

[217] PF Suthers and DC Cameron. Production of 3-hydroxypropionic acid in recombinant organisms, February 8 2005. US Patent 6,852,517.

[218] G Sweet, Ch Gandor, R Voegele, N Wittekindt, J Beuerle, V Truniger, EC Lin, and W Boos. Glycerol facilitator of *Escherichia coli*: cloning of *glpF* and identification of the *glpF* product. *Journal of Bacteriology*, 172(1):424–430, 1990.

[219] HW Tan, AA Aziz, and MK Aroua. Glycerol production and its applications as a raw material: a review. *Renewable and Sustainable Energy Reviews*, 27:118–127, 2013.

[220] MP Taranto, JL Vera, J Hugenholtz, GF De Valdez, and F Sesma. *Lactobacillus reuteri* CRL1098 produces cobalamin. *Journal of Bacteriology*, 185(18):5643–5647, 2003.

[221] L Tarraran and R Mazzoli. Alternative strategies for lignocellulose fermentation through lactic acid bacteria: state-of-the-art and perspectives. *FEMS Microbiology Letters*, 2018.

[222] OK Tawfik and S Dan. Enzyme promiscuity: a mechanistic and evolutionary perspective. *Annual Review of Biochemistry*, 79:471–505, 2010.

[223] F Theil. Diols as substrates in lipase-catalyzed enantioselective acylations-a brief review. *Catalysis Today*, 22(3):517–536, 1994.

[224] H Trip, NL Mulder, and JS Lolkema. Improved acid stress survival of *Lactococcus lactis* expressing the histidine decarboxylation pathway of *Streptococcus thermophilus* CHCC1524. *Journal of Biological Chemistry*, 287(14):11195–11204, 2012.

[225] M Urbańska, D Gieruszczak-Białek, and H Szajewska. Systematic review with meta-analysis: *Lactobacillus reuteri* dsm 17938 for diarrhoeal diseases in children. *Alimentary Pharmacology & Therapeutics*, 43(10):1025–1034, 2016.

[226] H van Bokhorst-van de Veen, T Abee, M Tempelaars, PA Bron, M Kleerebezem, and ML Marco. Short- and long-term adaptation to ethanol stress and its cross-protective consequences in *Lactobacillus plantarum*. *Applied and Environmental Microbiology*, pages AEM–00515, 2011.

[227] MJ van Hoek and RM Merks. Redox balance is key to explaining full *vs.* partial switching to low-yield metabolism. *BMC Systems Biology*, 6(1):22, 2012.

[228] JP van Pijkeren and RA Britton. High efficiency recombineering in lactic acid bacteria. *Nucleic Acids Research*, 40(10):e76–e76, 2012.

[229] JP van Pijkeren, KM Neoh, D Sirias, AS Findley, and RA Britton. Exploring optimization parameters to increase ssDNA recombineering in *Lactococcus lactis* and *Lactobacillus reuteri*. *Bioengineered*, 3(4):209–217, 2012.

[230] S Veluturla, N Archna, D Subba Rao, N Hezil, IS Indraja, and S Spoorthi. Catalytic valorization of raw glycerol derived from biodiesel: a review. *Biofuels*, 9(3):305–314, 2018.

[231] GJM Versteegh, HJ Bosch, and JW De Leeuw. Potential palaeoenvironmental information of C24 to C36 mid-chain diols, keto-ols and mid-chain hydroxy fatty acids-a critical review. *Organic Geochemistry*, 27(1-2):1–13, 1997.

[232] NR Vestman, T Chen, PL Holgerson, C Öhman, and I Johansson. Oral microbiota shift after 12-week supplementation with *Lactobacillus reuteri* DSM 17938 and PTA 5289: a randomized control trial. *PloS One*, 10(5):e0125812, 2015.

[233] H Waegeman and W Soetaert. Increasing recombinant protein production in *Escherichia coli* through metabolic and genetic engineering. *Journal of Industrial Microbiology & Biotechnology*, 38(12):1891–1910, 2011.

[234] T Walther and JM François. Microbial production of propanol. *Biotechnology Advances*, 34(5):984–996, 2016.

[235] JY Wang, W Liu, WT Ding, GC Zhang, and JJ Liu. Increasing ethanol titer and yield in a *gpd1*Δ *gpd2*Δ strain by simultaneous overexpression of *GLT1* and *STL1* in *Saccharomyces cerevisiae*. *Biotechnology Letters*, 35(11):1859–1864, 2013.

[236] P Wang, Y Li, H Xiao, YH Shi, GW Le, and J Sun. Isolation of *Lactobacillus reuteri* from Peyer's patches and their effects on sIgA production and gut microbiota diversity. *Molecular Nutrition & Food Research*, 60(9):2020–2030, 2016.

[237] YC Wang and O Yu. Synthetic scaffolds increased resveratrol biosynthesis in engineered yeast cells. *Journal of Biotechnology*, 157(1):258–260, 2012.

[238] OP Ward. Production of recombinant proteins by filamentous fungi. *Biotechnology Advances*, 30(5):1119–1139, 2012.

[239] T Werpy, G Petersen, A Aden, J Bozell, J Holladay, J White, A Manheim, D Eliot, L Lasure, and S Jones. Top value added chemicals from biomass. Volume I-Results of screening for potential candidates from sugars and synthesis gas. Technical report, Department of Energy Washington DC, 2004.

[240] L Westers, H Westers, and WJ Quax. *Bacillus subtilis* as cell factory for pharmaceutical proteins: a biotechnological approach to optimize the host organism. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, 1694(1-3):299–310, 2004.

[241] V Wigneswaran. *Pseudomonas putida as a microbial cell factory*. PhD thesis, Department of Systems Biology, Technical University of Denmark, 2016.

[242] HW Wisselink, RA Weusthuis, G Eggink, J Hugenholtz, and GJ Grobben. Mannitol production by lactic acid bacteria: a review. *International Dairy Journal*, 12(2-3):151–161, 2002.

[243] CD Wu, J Huang, and RQ Zhou. Progress in engineering acid stress resistance of lactic acid bacteria. *Applied Microbiology and Biotechnology*, 98(3):1055–1063, 2014.

[244] CD Wu, J Huang, and RQ Zhou. Genomics of lactic acid bacteria: current status and potential applications. *Critical Reviews in Microbiology*, 43(4):393–404, 2017.

[245] CD Wu, J Zhang, GC Du, and J Chen. Heterologous expression of *Lactobacillus casei* RecO improved the multiple-stress tolerance and lactic acid production in *Lactococcus lactis* NZ9000 during salt stress. *Bioresource Technology*, 143:238–241, 2013.

[246] CD Wu, J Zhang, M Wang, GC Du, and J Chen. *Lactobacillus casei* combats acid stress by maintaining cell membrane functionality. *Journal of Industrial Microbiology & Biotechnology*, 39(7):1031–1039, 2012.

[247] JJ Wu, GC Du, JW Zhou, and J Chen. Systems metabolic engineering of microorganisms to achieve large-scale production of flavonoid scaffolds. *Journal of Biotechnology*, 188:72–80, 2014.

[248] M Xian. *Sustainable production of bulk chemicals: Integration of bio-, chemo-resources and processes*. Springer, 2015.

[249] JW Xu, YN Xu, and JJ Zhong. Enhancement of ganoderic acid accumulation by overexpression of an N-terminal truncated 3-hydroxy-3-methylglutaryl

CoA reductase gene in basidiomycete *Ganoderma lucidum*. *Applied and Environmental Microbiology*, pages AEM–01263, 2012.

[250] Y Yamauchi, T Hirasawa, M Nishii, C Furusawa, and H Shimizu. Enhanced acetic acid and succinic acid production under microaerobic conditions by *Corynebacterium glutamicum* harboring *Escherichia coli* transhydrogenase gene *pntAB*. *The Journal of General and Applied Microbiology*, 60(3):112–118, 2014.

[251] LD Ye, H Zhao, Z Li, and JC Wu. Improved acid tolerance of *Lactobacillus pentosus* by error-prone whole genome amplification. *Bioresource Technology*, 135:459–463, 2013.

[252] TO Yeates, CS Crowley, and S Tanaka. Bacterial microcompartment organelles: protein shell structure and evolution. *Annual Review of Biophysics*, 39:185–205, 2010.

[253] TO Yeates, CA Kerfeld, S Heinhorst, GC Cannon, and JM Shively. Protein-based organelles in bacteria: carboxysomes and related microcompartments. *Nature Reviews Microbiology*, 6(9):681, 2008.

[254] TO Yeates, MC Thompson, and TA Bobik. The protein shells of bacterial microcompartment organelles. *Current Opinion in Structural Biology*, 21(2):223–231, 2011.

[255] BB Yoo and SK Mazmanian. The enteric network: interactions between the immune and nervous systems of the gut. *Immunity*, 46(6):910–926, 2017.

[256] D Zabala, AF Braña, AB Flórez, JA Salas, and C Méndez. Engineering precursor metabolite pools for increasing production of antitumor mithramycins in *Streptomyces argillaceus*. *Metabolic Engineering*, 20:187–197, 2013.

[257] AP Zeng and H Biebl. Bulk chemicals from biotechnology: the case of 1, 3-propanediol production and the new trends. In *Tools and Applications of Biochemical Engineering Science*, pages 239–259. Springer, 2002.

[258] T Zeuthen and DA Klaerke. Transport of water and glycerol in aquaporin 3 is gated by h+. *Journal of Biological Chemistry*, 274(31):21631–21636, 1999.

[259] J Zhang, CD Wu, GC Du, and J Chen. Enhanced acid tolerance in *Lactobacillus casei* by adaptive evolution and compared stress response during acid stress. *Biotechnology and Bioprocess Engineering*, 17(2):283–289, 2012.

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