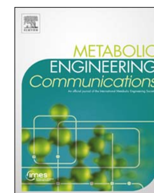
Metabolic Engineering Communications 6 (2018) 1–12

Contents lists available at ScienceDirect

Metabolic Engineering Communications

journal homepage: www.elsevier.com/locate/mec



Genome-scale model guided design of *Propionibacterium* for enhanced propionic acid production

Laura Navone[a,1,2], Tim McCubbin[a,1], Ricardo A. Gonzalez-Garcia[a], Lars K. Nielsen[a], Esteban Marcellin[a,b,*]

[a] *Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Australia*
[b] *Queensland Node of Metabolomics Australia, The University of Queensland, Australia*

ARTICLE INFO

Keywords:
Propionic acid
Propionibacteria
Metabolic engineering
Genome-scale modelling
Pentose phosphate pathway
Phosphoenolpyruvate carboxykinase overexpression

ABSTRACT

Production of propionic acid by fermentation of propionibacteria has gained increasing attention in the past few years. However, biomanufacturing of propionic acid cannot compete with the current oxo-petrochemical synthesis process due to its well-established infrastructure, low oil prices and the high downstream purification costs of microbial production. Strain improvement to increase propionic acid yield is the best alternative to reduce downstream purification costs. The recent generation of genome-scale models for a number of *Propionibacterium* species facilitates the rational design of metabolic engineering strategies and provides a new opportunity to explore the metabolic potential of the Wood-Werkman cycle. Previous strategies for strain improvement have individually targeted acid tolerance, rate of propionate production or minimisation of by-products. Here we used the *P. freudenreichii* subsp. *shermanii* and the pan-*Propionibacterium* genome-scale metabolic models (GEMs) to simultaneously target these combined issues. This was achieved by focussing on strategies which yield higher energies and directly suppress acetate formation. Using *P. freudenreichii* subsp. *shermanii*, two strategies were assessed. The first tested the ability to manipulate the redox balance to favour propionate production by over-expressing the first two enzymes of the pentose-phosphate pathway (PPP), Zwf (glucose-6-phosphate 1-dehydrogenase) and Pgl (6-phosphogluconolactonase). Results showed a 4-fold increase in propionate to acetate ratio during the exponential growth phase. Secondly, the ability to enhance the energy yield from propionate production by over-expressing an ATP-dependent phosphoenolpyruvate carboxykinase (PEPCK) and sodium-pumping methylmalonyl-CoA decarboxylase (MMD) was tested, which extended the exponential growth phase. Together, these strategies demonstrate that *in silico* design strategies are predictive and can be used to reduce by-product formation in *Propionibacterium*. We also describe the benefit of carbon dioxide to propionibacteria growth, substrate conversion and propionate yield.

1. Introduction

Propionic acid is a three-carbon compound with a broad range of applications in the food, pharmaceutical and chemical industries. In addition to its use as an intermediate for the synthesis of cellulose fibres, herbicides, perfumes and pharmaceuticals, propionic acid is a strong antimicrobial agent used in animal feed and as a food preservative (Guan et al., 2015b). Propionic acid is mainly produced by petrochemical processes. However, the demand for green chemicals has renewed attention to fermenting propionibacteria for propionic acid production. Propionibacteria are gram-positive, rod-shaped, facultative anaerobes. Dairy propionibacteria are important industrial GRAS (generally recognized as safe) strains used in the food and cheese industry (Meile et al., 2008). Propionibacteria have long been employed as starter and ripening cultures, responsible for flavour development and participating in the formation of cheese eyes (Britz and Riedel, 1994; Thierry and Maillard, 2002). Dairy strains are also used industrially to produce trehalose and vitamin B12 (Kośmider et al., 2012; Ruhal and Choudhury, 2012).

The bioprocess for propionate production still suffers from low productivity, low yield, and expensive downstream purification costs; acetic and succinic acids are produced alongside propionic acid making

* Correspondence to: Australian Institute for Bioengineering and Nanotechnology (AIBN), The University of Queensland, Corner College and Cooper Rds (Bldg 75), Brisbane, Qld 4072, Australia.

E-mail address: e.marcellin@uq.edu.au (E. Marcellin).

[1] Authors contributed equally to this work.

[2] Present address: Science and Engineering Faculty, Queensland University of Technology, Australia.

https://doi.org/10.1016/j.meteno.2017.11.001
Received 7 June 2017; Received in revised form 12 October 2017; Accepted 20 November 2017
Available online 24 November 2017
2214-0301/ © 2017 The Authors. Published by Elsevier B.V. on behalf of International Metabolic Engineering Society. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/BY-NC-ND/4.0/).

the downstream separation costly (Liu et al., 2012; Rodriguez et al., 2014). Metabolic engineering strategies aiming to reduce by-products are thus needed. Improved yields can theoretically be achieved by deleting by-product genes, but metabolic manipulation of propionibacteria has developed slowly, and only a few examples exist in the literature engineering the best propionic acid producer, *P. acidipropionici.* Metabolic engineering strategies have focussed on enhancing flux through the Wood-Werkman cycle, either by directing carbon into the cycle through over-expression of pyruvate and phosphoenolpyruvate carboxylases or the pyruvate:methylmalonyl-CoA carboxyltransferase (Ammar et al., 2014; Liu et al., 2016; Wang et al., 2015b). Other strategies that have been tested include the over-expression of components of the Wood-Werkman cycle itself (Liu et al., 2015; Wang et al., 2015a) or the targeting of acid tolerance associated processes such as trehalose synthesis (Jiang et al., 2015) and the glutamate decarboxylase or arginine deiminase pathways (Guan et al., 2015a). While *P. acidipropionici* remains resistant to engineering, unique metabolic features of this species were over-expressed in lower producers (Wang et al., 2015b). Few studies have targeted by-product production, and these have ultimately had limited success (Liu et al., 2016; Suwannakham et al., 2006). Ultimately, the limited phenotypic improvements in current studies and limitations in genetic engineering tools necessitate a genome-scale model to further aid design (Wang et al., 2015b).

Here, we utilise a recently developed genome-scale model for *P. freudenreichii* subsp. *shermanii* and the pan-*Propionibacterium* GEM to design genetic modification strategies. Because the production of propionic acid is already growth-coupled, classic growth-coupling strain design algorithms such as OptKnock (Burgard et al., 2003) favour suboptimal strategies that limit energy generation, leaving cells more susceptible to acid stress. Given the depletion of the pH gradient is regarded as a major mechanism by which weak acids exert their toxicity on cells, we explored strategies favouring propionate production while maintaining high levels of energy generation or even exceeding those of wild-type strains. Strategies probed pathways consistent with the maximum production of energy and propionate in the native metabolism. By simultaneously trying to improve both energy output and propionate production, strains are expected to be readily evolvable to a higher producing phenotype while also improving acid tolerance. Such approaches facilitate the production of high producing mutants with minimum genetic perturbations; a requirement enforced by the few genetic modification tools available. Given the limited success of reducing acetate production in previous studies (Liu et al., 2016; Suwannakham et al., 2006), we over-expressed the PPP to test *in silico* simulation predictions and show that such a strategy results in lower acetate production without sacrificing growth. We then tested an alternative strategy consisting of over-expressing an ATP-PEPCK and MMD to enhance energy available to the cell and analyse how this affects the phenotype. We observed an extended exponential growth phase in the mutant strain. Because this strategy was reliant on the addition of CO_2, the influence of CO_2 on the *Propionibacterium* fermentation was also assessed, revealing undescribed biomass and propionate stimulating effects in propionibacteria.

2. Materials and methods

2.1. Model preparation

Simulations using the pan-*Propionibacterium* and *P. freudenreichii* subsp. *shermanii* models (manuscript in preparation) were performed in MATLAB using the COBRA toolbox (Hyduke et al., 2011). The original excel models were first converted to SBML format and boundary metabolite exchanges were added to allow accumulation using an in-house tool, as previously described (Quek and Nielsen, 2008). Glucose was allowed to be consumed as a carbon source at a rate of 1 mmol/gDW/h, while all other carbon-containing exchange metabolites were set to production only. No external terminal electron acceptors were available, including oxygen, sulphate and nitrate. Ammonium, phosphate, water and hydrogen sulphide fluxes were unconstrained. When required, additional reactions were added from the modelSEED database (Overbeek et al., 2005). Reactions referenced herein have been matched to their unique modelSEED reaction identifiers or by the identifiers given within the original model (Supplementary material Table S1).

2.2. Identification of pathways of interest

Pathways enabling maximum theoretical yield of propionate were hierarchically screened with respect to ATP yield in *P. freudenreichii* subsp. *shermanii*. This was performed by changing the objective function to maximise for the propionate boundary exchange reaction with a small weighting for the ATP drain reaction. Subsequent minimisation of total flux was utilised to assist in the identification of pathways that maximise yield. A key reaction in the pathway was then constrained to carry no flux so that alternative pathways could be determined. The procedure was repeated until the maintenance fell below 75% of the wild-type value. The same analysis was also performed on the pan-model. Pathways that could increase propionate production while maintaining a constant maximum energy yield were similarly calculated by maximising for the ATP drain reaction with a small bias for the propionate exchange flux. OptKnock simulations were implemented through the COBRA toolbox (Burgard et al., 2003).

2.3. Pathway yield calculation

Pathway yields were calculated by allowing free flux through central carbon metabolism. Flux through pathways that were identified to enhance propionate production were constrained as appropriate for the relevant scenario by constraining key enzymatic steps. These included citramalate synthase for the citramalate pathway, citrate synthase for the TCA cycle, and formate-tetrahydrofolate ligase for the glycine cleavage pathway. For each strategy, the expected yield of propionate with energy optimisation was calculated by maximising and minimising the propionate yield after maximising for the ATP yield. Additionally, the maximum energy yield associated with maximum propionate production was calculated by maximising the ATP yield with propionate constrained at the maximum theoretical yield of 1.714 moles/mole glucose. In all strategies, the methylmalonyl-CoA carboxyltransferase was set to irreversible (lower bound 0) to prevent infinite energy cycling when the sodium-pumping MMD was added. The relevant constraints for each strategy are detailed as follows: 1. GAPDH replacement of PGK and *Bifidobacterium* shunt knockout (constrained to 0 flux: phosphoglycerate kinase, phosphoketolase; added to model: GAPDH); 2. Acetate knock-out (constrained to 0 flux: citrate synthase, acetate kinase, cystathionine gamma-synthase, formate-tetrahydrofolate ligase); 3. PPP over-expression (constrained to 0 flux: citrate synthase, citramalate synthase, formate-tetrahydrofolate ligase); 4. Citramalate pathway over-expression (constrained to 0 flux: citrate synthase); 5. TCA cycle over-expression (constrained to 0 flux: citramalate synthase); 6. Glycine cleavage pathway over-expression (added to model: formate dehydrogenase); 7. Linear propionate pathway (added to model: PEPCK, MMD); 8. Combination of strategies 6 and 7 (added to model: formate dehydrogenase, PEPCK, MMD).

2.4. Bacterial strains and growth conditions

E. coli DH5α was grown aerobically in Luria-Bertani medium at 37 °C and supplemented with 50 µg/mL apramycin (Am), 100 µg/mL ampicillin (Ap) or 50 µg/mL kanamycin (Km) when needed. *Propionibacterium* strains were grown anaerobically at 32 °C on complex medium containing yeast extract 10 g/L, trypticase soy broth 5 g/L, K_2HPO_4 0.25 g/L, $MnSO_4$ 0.05 g/L and glucose 80 g/L to mimic

Download English Version:

https://daneshyari.com/en/article/6964990

Download Persian Version:

https://daneshyari.com/article/6964990

Daneshyari.com