

Understanding molecular weight control in bacterial fermentation: a systems biotechnology approach.

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Abstract

A system biotechnology approach was undertaken to understand molecular weight control of hyaluronic acid. The use of a genome scale model enables integration of other *omics* technologies.

Keywords: hyaluronic acid, *Streptococci zooepidemicus*, systems biotechnology, genome scale model

1 Introduction

Hyaluronic acid (HA) is a biopolymer with valuable applications in the pharmaceutical and cosmetic industries [1]. Many medical products require specific rheological properties (viscoelastic, pseudoplastic), which are dictated by the molecular weight (MW). Even when cross-linked HA has delivered good results for viscosupplementation, it has failed to provide satisfactory results in ophthalmic viscoelastic devices, where a linear polymer is required [2]. Currently, high MW HA is produced commercially mainly from animal tissues (i.e. rooster comb). Increased concerns over the contamination of animal derived products with infectious agents have made bacterial fermentation a more desirable production system to meet future demands.

Little is known about the mechanism of MW control of beta-polysaccharides such as HA, cellulose or chitin. Several research groups including ours have pursued various hypotheses for the past decade, but no hypothesis captures MW regulation observed in bioreactors [3]. *In vitro* studies [4] suggest that the MW is controlled by the hyaluronan synthase (HasA), but the overexpression of this particular enzyme in *Streptococcus zooepidemicus* (*S. zooepidemicus*) shows adverse results. A second hypothesis suggests that the supply of precursors in the synthesis pathway controls molecular weight.

Given previous failures of direct approaches, a systems approach was adopted. Using this approach, we aim to build models and quantitative simulations for a better understanding of the mechanism of MW control. Genome-scale metabolic maps (GSM) are useful tools to represent and analyze the metabolism of an organism, and the starting point for a systems approach.

2 Method and Results

Genes in the HA synthesis operon of *S. zooepidemicus* (*hasA*, *hasB*, *hasC*, *glmU* and *pgi*) were cloned and overexpressed using a nisin induced controlled expression vector. Overexpression of these genes changed the MW of HA. The greatest increase (72%) was observed in the recombinant strain overexpressing phosphoglucosomerase (*pgi*) (patent pending application no. EP 07115404.1.). There was also an increase in HA yield with overexpression of *glmU* and *pgi*, underlying the importance of the precursor UDP-N-acetylglucosamine in MW control. Results were confirmed by a targeted metabolomics approach using high pressure anion exchange chromatography.

S. zooepidemicus and the mutants (high MW producers) were characterised using differential gel electrophoresis (2D-DIGE) to evaluate changing levels of protein expression and to identify target proteins using mass spectrometry (LC/MS/MS and MALDI TOF/TOF). Seventy proteins were identified by MS and a reference map of the proteome was completed. However, integration of these *omics* technologies is necessary in order to fully understand the biological mechanism of MW control.

A metabolic network model for *S. zooepidemicus* has been reconstructed based on our genome annotation (Figure 1). Our GSM contains 450 reactions representing the carbon flux through the metabolic network. In the GSM, 342 metabolites, and 280 genes are represented. Our model successfully predicts *in vivo* growth and product formation.

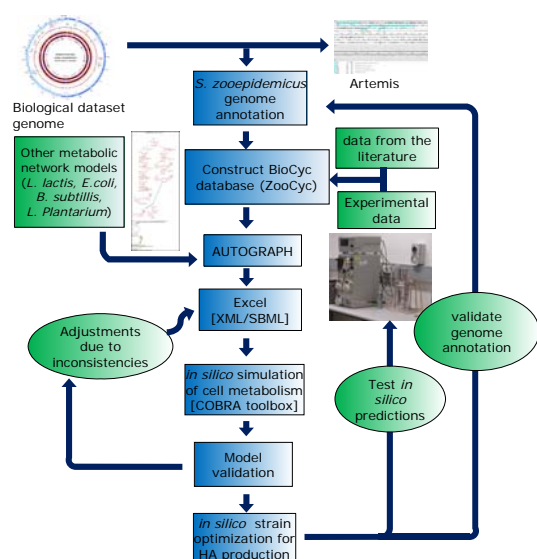


Figure 1. Methodology for generation of *S. zooepidemicus* GSM

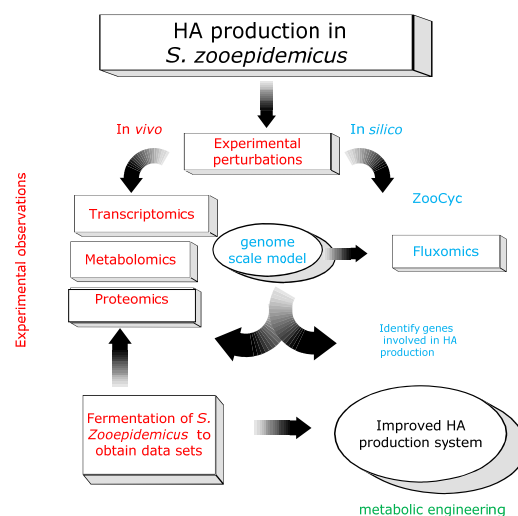


Figure 2. Genome scale model of *S. zooepidemicus* as a functional annotation and the first step towards a systems biotechnology approach.

3 Discussions

Genome scale models are useful tools to derive biological insight from complex systems and are essential tools to link *omics* technologies such as metabolomics and proteomics (Figure 2). Our GSM can predict bacterial growth and HA production in accordance with the experimental data. Furthermore, it is a potential platform to test hypothesis *in silico* and derive biological insights from the metabolism. It also represents a functional annotation and the first step towards a systems biotechnology approach for the better understanding of MW control in HA production.

The genome scale model can further be used to integrate other *omics* dataset such as proteomics and metabolomics. A proteomics approach was undertaken in order to understand the enzymatic interaction and the level of expression of the enzymes involved in HA MW control. Our proteomics experiment identified one enzyme, (UDP-N-Acetylglucosamide1-carboxyvinyl transferase) as a possible target for further improvement of the strain. Using our GSM, we have identified two copies of the enzyme *in silico*, suggesting that one of the copies can be knocked-out for further improvement of the strain.

A targeted metabolomics analysis of the intermediates involved in the HA pathway was also undertaken to identify possible bottle-necks. Manipulation of genes involved in the two HA precursors changed the MW, suggesting that the monomers should be balanced to achieve maximum MW. Using our GSM, we can test different strategies *in silico*, to balance the two monomers for increased MW production, which will lead us to our ultimate goal of strain optimization using metabolic engineering.

References

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