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A systems level approach for identification of molecular targets for antimicrobial intervention against *Pseudomonas aeruginosa*, while predicting biofilm formation

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Abstract

In this case study, we aimed at evaluating the suitability of genome-scale metabolic models to identify molecular targets that can potentially enhance antimicrobial effects of chemical preservatives against *P. aeruginosa*, while minimizing biofilm formation. For the case study, isothiazolinones were selected as a group of microbicides where their mechanism of action is well described in scientific literature. Target identification was carried out in several steps. First, we developed a computational model of *P. aeruginosa* metabolism under action of isothiazolinones. Action of sub-inhibitory concentrations of isothiazolinones was simulated based on extensive information on their mechanisms of action. Then, simulations of single and double gene deletion(s) were performed *in silico* to identify genes or combinations of genes that could be targeted to induce further reduction of bacterial growth rate. Finally, we assessed whether total or partial inhibition of these genes might activate biofilm formation.

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Keywords: Pseudomonas aeruginosa, genome-scale metabolic model, Flux Balance Analysis

1. Introduction

Genome-scale metabolic models (GSMMs) provide a biologically meaningful mechanistic basis for elucidating the genotype-phenotype relationship. GSMMs contain curated and systematized information about known metabolites

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and metabolic reactions of a given type of cell, based on its annotated genome, and on available data/information in experimental literature. The ability of GSMMs to predict effects of gene knockouts has enabled their application in metabolic engineering studies as well in the discovery of drug targets. However, little or no application of GSMMs has been reported to support the design of microbial control strategies based on chemical preservatives in consumer products. To evaluate this potential application, we have chosen isothiazolinones as a group of antimicrobial compounds with known mechanism of action reported in experimental literature. The mechanism by which isothiazolinones inhibit Gram-negative bacteria has been linked with disruption of the metabolic pathways involved in dehydrogenase, inhibition of oxygen consumption and energy generation (Williams, 2007). The aim of this work is to identify molecular targets that could enhance action of isothiazolinones against *P. aeruginosa* while minimizing biofilm formation.

A number of studies have shown that GSMMs could be used to accurately predict molecular targets essential for growth (e.g. Lee *et al*., 2009). The method consists in simulating gene(s) deletion using Flux Balance Analysis (FBA) and identifying a gene (or a group of genes) that could be targeted to prevent growth of the pathogen. These targets can then be mapped to existing compounds following e.g. the framework proposed by Chavali *et al.* (2012). More recently, GSMMs have also been used to predict metabolic rearrangements induced by gene inhibition. Of interest for this work, Xu *et al*. (2013) developed a framework to predict if exposure to low concentrations of antimicrobials can induce rearrangements in the metabolism of *P. aeruginosa* that activate biofilm pathways. In this work, target identification was carried out in several steps. First, we developed a computational model of *P. aeruginosa* metabolism under action of isothiazolinones. Then, simulations of gene deletions were performed *in silico* to identify genes and combinations of genes that could be targeted to potentiate the effects of isothiazolinones. Finally, we assessed whether partial inhibition of these genes might activate biofilm formation.

2. Materials and Methods

2.1. Computational model of P. aeruginosa in presence of sub-inhibitory concentrations of isothiazolinones

Metabolism of *P. aeruginosa* was modelled based the *iMO1086* reconstruction (Oberhardt *et al*., 2011). NADH dehydrogenases and reactions associated with PA1124 and PA3043 (two genes associated with biofilm formation) were implemented as in the previous *iMO1056* reconstruction (Oberhardt *et al*., 2008). For the purpose of this case study, uptakes of nutrients were defined to mimic water environments. In particular, carbon sources were assumed to be carboxylic acids (succinate, acetate, citrate and pyruvate). In absence of relevant gene expression data in public databases, effects of isothiazolinones on the cell metabolism of *P. aeruginosa* were simulated by reducing the bounds of fluxes in reactions known as being affected by isothiazolinones (pyruvate, lactate, succinate, oxogluterate and NADH dehydrogenases, ATP synthesis and aerobic respiration). First, the solution space corresponding to the maximum growth rate in absence of isothiazolones was sampled using the Artificial Centre Hit and Run (ACHR) sampling included in the COBRA Toolbox. For each metabolic reaction, it results in N sampled flux values which were analyzed to quantify the mean value and the probability density function of fluxes. Then, action of sub-inhibitory concentrations of isothiazolinones was mimicked by setting the upper bound of fluxes in reactions affected by these antimicrobials to 75% of the mean of the density function. For reversible reactions, the lower bound of fluxes were set to –75% of their mean value. Application of these constraints induced a reduction of 80% in the predicted growth rate.

2.2. Identification of targets that may enhance effects of isothiazolinones

Perturbation analysis was performed using FBA and the COBRA Toolbox on the metabolic model of *P. aeruginosa* in presence of isothiazolinones. We used maximization of biomass generation as an objective function for FBA, as it has proven to be effective in predicting redistribution of metabolic fluxes (Edwards *et al*., 2001; Brynildsen *et al*., 2013). Perturbation analysis was performed by simulating single and double gene deletion with FBA. For each single deletion, it was assessed if complete inhibition of gene(s) function results in a further reduction of the bacterial growth. Single and double genes deletions that result in further growth rate reduction are identified as potential targets and selected for further analysis.