Insights on the metabolic response of *Papiliotrema laurentii* to acetic acid stress by RNA-seq and enzyme kinetic data integration into the genome-scale metabolic model

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Papiliotrema laurentii metabolizes lignocellulosic-derived sugars and can accumulate high lipid amounts. However, wild strains are sensitive to acetic acid, the main inhibitor of lignocellulosic hydrolysates. We recently selected the acetic acid tolerant strain, ATSI, by adaptive laboratory evolution (ALE). Herein, we integrated RNA-seq and enzyme kinetic data into the genome-scale metabolic model of *P. laurentii* (*papla*-GEM) to evaluate the endogenous and ALE-related metabolic responses to acetic acid stress. We cultivated ATSI (tolerant) and parental (sensitive) strains under chemostats with xylose in the absence and presence of acetic acid. We used the samples collected from these cultivations for total RNA extraction and sequencing and to adjust the biomass and growth parameters for metabolic modeling. After aligning, counting, and normalizing the RNA-seq data, we applied the Reaction Activity Score (RAS) approach to evaluate the expression of gene-associated reactions for each strain and condition in *papla*-GEM. We considered reactions with $|\log_2(\text{RAS fold change})| > 1.2$ and p-adjusted < 0.05 as differentially regulated. We used GECKO 3.1 to retrieve and integrate enzyme kinetic data (k_{cat}) and reconstruct the enzyme-constrained *papla*-GEM (*ecpapla*-GEM). Next, we performed a random sampling (RS) analysis with 10,000 observations to evaluate the fluxes toward ATP, NADPH, and NADH production. In the presence of acetic acid, ATSI upregulated reactions related to NADPH metabolism [two Fe(II):NADP+ oxidoreductases], consistent with the higher maximum theoretical yield predicted by the RS than the parental strain (17.96 times; 0.467 vs. 0.026), as well as higher ATP and NADH yield. Meanwhile, in the presence of acetic acid, the parental strain mainly downregulated reactions related to nitrogen and amino acid transport, while ATSI downregulated reactions related to fatty acid degradation and transport in the mitochondrion and citrate, cis-aconitate, isocitrate, and pantothenate metabolism. Without stress, ATSI upregulated asparaginase and cysteine transport but downregulated the transport of other amino acids, glutamate dehydrogenase, and ketone body metabolism versus the parental strain. In the presence of acetic acid versus the parental strain, ATSI upregulated methylglyoxal detoxification, water diffusion, organic acid transport, and aldehyde and formaldehyde dehydrogenases; in contrast, ammonia transport, xylose reductase, and reactions related to galactose, lipid, and nucleotide metabolism were downregulated. Therefore, the improved tolerance to acetic acid displayed by the ATSI might be related to higher ATP, NADH, and NADPH availability, the regulation of fatty acid metabolism, amino and organic acid transport and metabolism, water diffusion, and the removal of toxic compounds.

Keywords: Metabolic modeling; Transcriptome; Oleaginous yeasts.

***Insights* sobre a resposta metabólica de *Papiliotrema laurentii* ao estresse por ácido acético via integração de dados de RNA-seq e cinética enzimática ao modelo metabólico em escala genômica**

Papiliotrema laurentii metaboliza açúcares de biomassas lignocelulósicas e acumula lipídeos; contudo, é sensível ao ácido acético. Integramos RNA-seq e k_{cat} ao modelo metabólico para avaliar sua tolerância. Sob estresse, ATSI (tolerante) aumentou as reações e o rendimento de NADPH. A parental (sensível) reduziu transporte de compostos nitrogenados e aminoácidos, enquanto ATSI reduziu metabolismo de ácidos graxos e orgânicos. Sem estresse, ATSI aumentou asparaginase e transporte de cisteína, mas diminuiu glutamato desidrogenase e metabolismo de corpos cetônicos. A resposta ao estresse envolve disponibilidade de coenzimas, regulação do metabolismo de ácidos graxos, aminoácidos, ácidos orgânicos, difusão de H_2O, e remoção de toxinas.
Palavras-chave: Modelagem metabólica; Transcriptoma; Leveduras oleaginosas.

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