IN SILICO ANALYSIS OF XYLOSE CONVERSION INTO BIOPRODUCTS BY *Burkholderia sacchari*

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ABSTRACT

Burkholderia sacchari has been proposed as a chassis to convert agroindustrial waste into bioproducts. It efficiently consumes xylose, a sugar abundant in agricultural residues, yielding bioproducts such as xylonic acid, xylitol and polyhydroxyalkanoates (PHA). A search in the *B. sacchari* genome detected the full catabolic xylose isomerase pathway and the partial Weimberg pathway, where the *xylX* gene is missing. Thus *xylX* expression could lead to a full functional Weimberg pathway. No evidence of Dahms, xylitol dehydrogenase or xylose reductase pathways was detected, but a gene putatively encoding an aldo-keto reductase was found that could catalyze the same reaction as xylose reductase. An Elementary Mode analysis (EMA) of *B. sacchari* metabolic network, considering the expression of *xylX*, suggested that simultaneous expression of the xylose isomerase and the Weimberg pathways could improve xylose catabolism and cell growth, though with less PHA and xylonic acid production.

Keywords: Xylose. Xylonic Acid. Xylitol. Polyhydroxyalkanoates. *Burkholderia sacchari*.

1 INTRODUCTION

Burkholderia sacchari was isolated from Brazilian sugarcane soil and is a chassis to generate bioproducts such as polyhydroxyalkanoates (PHA), xylonic acid and xylitol. Due to its ability to efficiently consume a great array of carbon sources, such as sucrose, fructose and xylose, it can be utilized in second generation ethanol biorefineries, allowing the co-generation of these bioproducts.[1]

PHA is a family of biocompatible and biodegradable thermoplastic polymers, produced by various bacteria. Plastics derived from these biopolymers can have a great diversity of physical properties, due to the existence of more than 150 types of monomers already detected as constituents of different PHAs, which may form copolymers or homopolymers. Different mutants and recombinants strains of *B. sacchari* LFM101 were built, allowing modulation of the PHA composition.[1]

Raposo and coworkers demonstrated the production of xylonic acid and xylitol by *B. sacchari* LFM101.[2] Xylonic acid was listed, by the US National Renewable Energy Laboratory, as one of the top 30 chemical compounds derived from biomass with application in industry, while xylitol was ranked as one of the top 12. Xylonic acid can be used as a substitute for gluconic acid (due to its similar structure) and used as an additive in foods and drinks, granting a refreshing flavor. It can also be used as an additive in cement, granting better resistance. Furthermore, it has excellent chelating capacity in alkaline conditions, some studies cite its use as a green solvent and as a material for synthesis of 1,2,4-butanetriol and other compounds. Xylitol is widely applied as a healthier "artificial" sweetener than glucose, valuable for low calorie diets and for patients with diabetes. Moreover, xylitol has antibacterial and anti-inflammatory properties, being used to reduce dental caries.[3]

In this work, considering the recently re-sequenced *B. sacchari* genome, we searched for genes related to the catabolism of xylose. Additionally, an elementary modes analysis (EMA) was done to give insights into the metabolic effects of *xylX* gene expression, establishing a functional Weimberg pathway.

2 MATERIAL & METHODS

Reference sequences of genes and proteins were obtained at Uniprot (https://www.uniprot.org/) (Uniprot Consortium, 2023) and NCBI (https://www.ncbi.nlm.nih.gov/) platforms. The search for xylose catabolism genes in the *B. sacchari* genome was done using the standard protein BLAST (Basic Local Alignment Search Tool). The reference genes and protein sequences utilized for the Weimberg pathway (*xylB*, *xylC*, *xylD*, *xylX* and *xylA*) were from *Burkholderia xenovorans*. Xylose isomerase (*xylA* and *xylB*) were from *Escherichia coli*. Xylose reductase and xylitol dehydrogenase (*GRE3* and *XYL2*) were from *Saccharomyces cerevisiae* and Dahms aldolase (*yjhH*) was from *E. coli* (strain K12).

The elementary modes were obtained with the Metatool software. The metabolic network utilized was adapted from previous works of elementary mode analysis of *B. sacchari* and genomic data. It contains the following pathways: Xylose isomerase pathway, Weimberg pathway, Entner-Doudoroff pathway (linear and cyclic form), Pentose-Phosphate pathway, Krebs Cycle, Glyoxylate Cycle, the reaction catalyzed by the Pyruvate Dehydrogenase Complex, the Biomass formation reaction and anaplerotic reactions associated with the oxidation-reduction of coenzymes. The analysis considered xylose as the only carbon source and poly-3-hydroxybutyrate [P(3HB)], xylonic acid and biomass as bioproducts. Figure 1 shows this metabolic network.[4]

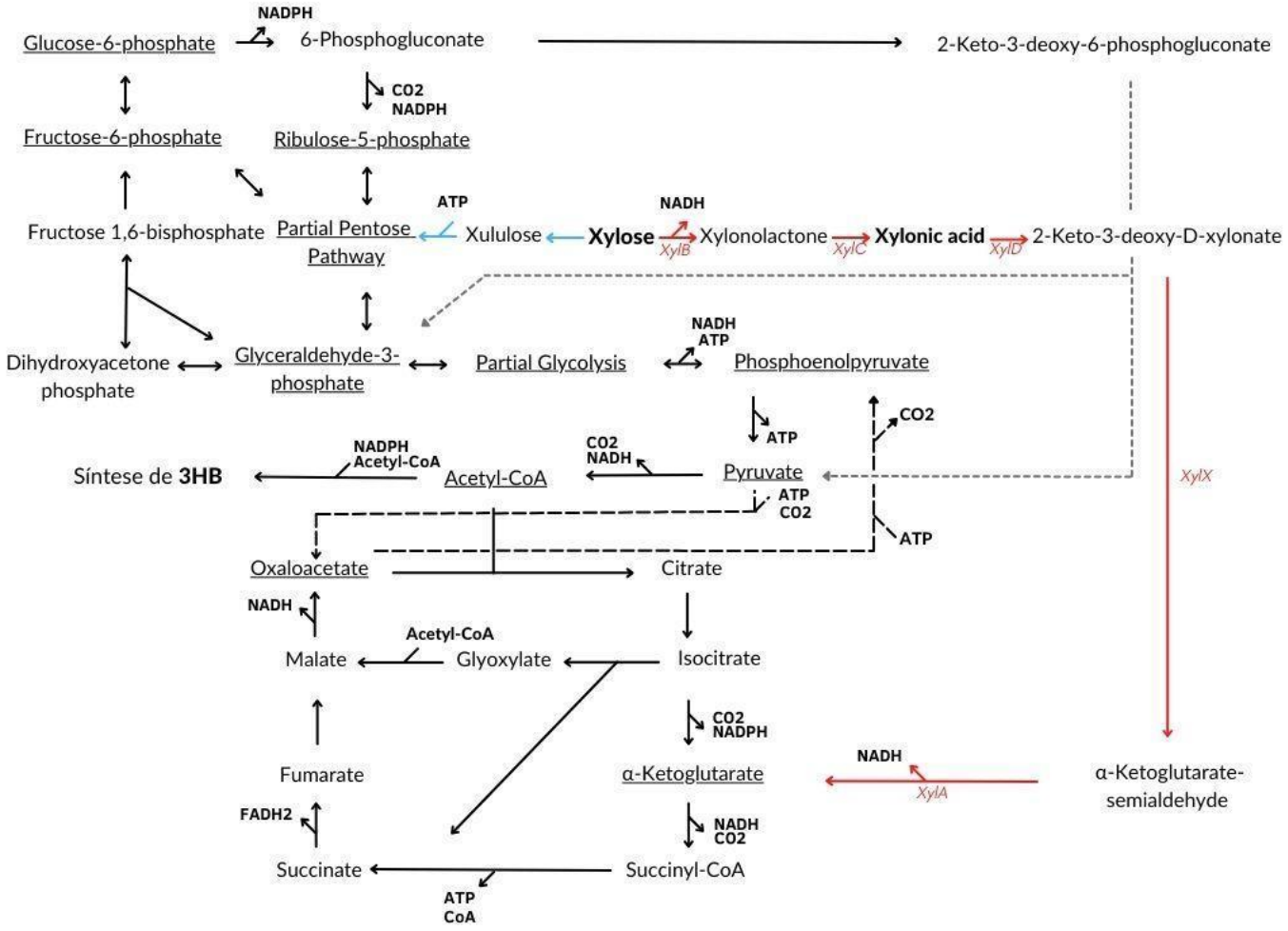

Figure 1 *B. sacchari* metabolic network. The reactions in red comprehend the Weimberg pathway and in blue the summarized xylose isomerase pathway. The underlined metabolites represent the compounds that are essential to form biomass.

3 RESULTS & DISCUSSION

Xylose is the second most abundant sugar in nature, being the main compound derived from hemicellulose and can be catabolized through three main pathways: the xylose isomerase, oxidoreductase and non-phosphorylant pathways. The xylose isomerase pathway is the most common among bacteria, whilst the oxidoreductase (xylose reductase-xylitol dehydrogenase) pathway is more predominant in fungi, the latter also contains xylitol as an intermediate. The non-phosphorylant Weimberg and Dahms pathways are less common, both have xylonic acid (D-xylonate) as an intermediate.

Cherix already searched for xylose catabolism genes in the *B. sacchari* genome. However, there are new genome sequences and there is the reference sequence of the Dahms aldolase enzyme, that was previously unknown. Like the previous analysis, the pathway for xylose catabolism found in *B. sacchari* is the xylose isomerase pathway. The Weimberg pathway seems to be almost complete. The *xylX* gene was only found in the *B. sacchari Suichang626* strain and seems absent in the wild type strain. The best alignment of the *xylC* gene show low score and identity (177 and 37.67% respectively), however, the reaction mediated by the protein coded by the *xylC* gene can occur spontaneously. Cherix observed similar results and hypothesized that expressing the *xylX* gene could be enough to have a full functional Weimberg pathway in *B. sacchari*. Finally, there were no significant results regarding the presence of aldolase from the Dahms pathway, xylose reductase and xylitol dehydrogenase. However, the corresponding alignment of xylose reductase is annotated as an aldo-keto reductase, the family of enzymes of which xylose reductase is a part, so this alignment could correspond with a gene that codes for a new undescribed xylose reductase or another enzyme from the same family that can catalyze this reaction nonspecifically.[5]

Raposo[2] and Bondar and coworkers[3] showed that *B. sacchari* produces xylonic acid, and argued that this production occurs through the accumulation of D-xylonate, due to the absence of the *xylX* gene. They also verified the production of xylitol, despite

the absence of the xylose reductase. This corroborates the hypothesis that expression of the *xylX* gene could lead to the complete functioning of the Weimberg pathway. This also corroborates the hypothesis that the alignment found, which was annotated as a gene coding for an aldo-keto reductase enzyme, could function as or be an undescribed xylose reductase. Considering that the Dahms pathway shares the first three reactions of the Weimberg pathway (*xylB*, *xylC* and *xylD*), it is possible that expressing *yjhH*, which codes for the aldolase that converts 2-keto-3-deoxy-D-xylonate into pyruvate and glycolaldehyde, could also lead to complete functioning of the Dahms pathway.

The elementary modes obtained from metatool were organized and normalized accordingly with xylose. Then they were classified according to the highest and lowest biomass and PHB production. The elementary mode with the highest biomass production was: 1 Xylose + 2.61 ATP + 0.18 O_2 → 2.61 ADP + 0.22 CO_2 + 1.12 Biomass. The metabolic flux of the catabolism of xylose was 0.88 for the xylose isomerase pathway and 0.12 for the Weimberg pathway. The elementary mode with the highest biomass production when the xylose was catabolized only by the xylose isomerase ptahway (flux of 1.0) was: 1 Xylose + 1.6 ATP + 0.41 O_2 → 1.6 ADP + 0.33 CO_2 + 1.09 Biomass. This could indicate that the simultaneous expression of the xylose isomerase pathway and Weimberg pathway could lead to a more efficient cell growth.

When it comes to PHB production, the elementary mode with the highest production was: 1 Xylose + 7.5 ADP + 2.5 O_2 → 7.5 ATP + 1.66 CO_2 + 0.83 3HB. Its metabolic flux only used the xylose isomerase pathway (flux of 1.0). The elementary mode with the highest production that utilizes the Weimberg pathway was: 1 Xylose + 13.12 ADP + 4.37 O_2 → 13.12 ATP + 2.5 CO_2 + 0.62 3HB. Also, the metabolic flux was 0.37 for the xylose isomerase pathway and 0.63 for the Weimberg pathway. These results indicate that although the expression of the *xylX* gene could avoid the accumulation of xylonic acid, focusing the carbon to the production of PHB and biomass, it could also lead to a decrease of the production of PHB. Further experiments are required to predict the best condition.

The results of the elementary modes analysis suggests that the expression of *xylX* could benefit cell growth at the cost of less accumulation of PHA and xylonic acid. This could occur because xylonic acid as an intermediate of the Weimberg pathway would be consumed and PHB has NADPH and acetyl-CoA as precursors. Hence, as the Weimberg pathway is included in the central metabolism as α-ketoglutarate, in the Krebs cycle, the metabolic routes that lead to the production of NADPH and acetyl-CoA are associated with a great loss of carbon as carbon dioxide (CO_2).

4 CONCLUSION

The genome of *B. sacchari* contains the genes of the xylose isomerase pathway and all the genes of the Weimberg pathway, except the *xylX* gene (which is only found in the *B. sacchari Suichang626* strain). The *yjhH* gene of the Dahms pathway and genes of the xylose reductase and xylitol dehydrogenase were not detected. The production of xylitol could be attributed to a novel or nonspecific aldo-keto reductase. It is hypothesized that the expression of *xylX* in *B. sacchari* could induce a full functional Weimberg pathway while the expression of *yjhH* could induce a full functional Dahms pathway. It is also hypothesized that expressing a full functional Weimberg pathway can lead to more efficient xylose consumption and cell growth, at the expense of less PHB and xylonic acid accumulation.

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