necessary to lower production costs. Considering this context, we herein investigated the potential of two newly yeast strains isolated from Brazilian Cerrado biome to produce this compound. Xylose conversion capacity by the new strains *Spathaspora*sp. JA1 and *Meyerozyma* sp. JA9 was evaluated and compared with control strains using xylose or sugarcane biomass hydrolysate in the fermentation medium. Among the tested strains, *Spathaspora* sp. JA1 was the best xylitol producer, reaching product yield as high as 0.74 g/g, which is a value similar to that obtained with the best xylitol producers described until now. Activities of enzymes related to the first steps of xylose metabolism and the hydrolysate detoxification process was determined in these yeasts. Moreover, the complete genome sequences of *Spathaspora* sp. JA1 and *Meyerozyma* sp. JA9 were obtained and annotated. Comparative genomic analysis was performed to clarify some aspects of yeast physiology.

IDENTIFICATION AND FUNCTIONAL ANALYSIS OF NOVEL ENDOGENOUS SIGNAL PEPTIDES FOR *KOMAGATAELLA PHAFFI*

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Pichia pastoris, reclassified as *Komagataella* sp, is considered an excellent platform for protein production. More than 600 proteins have been expressed using the *Komagataella* expression system. Most expression vectors use *Saccharomyces cerevisiae* α-factor pre-pro sequence as a secretion signal, which provides excellent secretion levels, but can be improperly processed in hyperexpression conditions. Using a native *Komagataella* sp secretion signal should overcome this problem, maintaining the right structure of the heterologous protein. *In silico* analysis identified thirteen secretion signals from the *Komagataella phaffii* secretome. These sequences and the putative α-factor of *K. phaffii* were cloned into pPIC9 carrying *Bacillus subtillis* α-amylase (*amyE*) as a reporter gene. All of them except PS9 showed a hydrolytic halo. Secretion levels were similar or superior to that of *S. cerevisiae* α-factor. All analyzed clones had only one copy of *amyE*. Amylases from four clones were purified for subsequent N-terminal sequencing. These new signals can be used as an alternative to the *S. cerevisiae* α-factor, mainly when more specific N-terminal processing is required or for better levels of secreted protein.

UTILIZATION OF METABOLIC FLUX ANALYSIS FOR METABOLOME DATA VALIDATION OF XYLOSE-FERMENTING YEASTS

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Metabolic flux analysis (MFA) is used to understand how fluxes are distributed in a metabolic network given a certain substrate. It can predict growth and product

distribution based on the stoichiometric reactions within a given network by addition of measured fluxes and production rates as constraints in the mathematical model. In this study, a stoichiometric model was developed using xylose fermentation data for the yeasts *Scheffersomyces stipitis*, *Spathaspora arborariae*, and S*pathaspora passalidarum*. Those models were utilized for the first time to validate the quantification of eleven intracellular metabolites within xylose and glucose catabolic pathways. Within the investigated metabolic network, eleven fluxes rates were calculated using the metabolomics data. Among them, 80% of total metabolites were validated with a correlation above 90% when compared to the stoichiometric model. Thus confirming that MFA can be utilized for metabolome data validation. Among the measured intracellular metabolites, fructose-6-phosphate, glucose-6-phosphate, malate, and ribulose-5-phosphate were validated in all studied yeasts. Nevertheless for the metabolites phosphoenolpyruvate and pyruvate the measured concentrations could not be correlated the predicted ones. Finally, it was possible to compare metabolism within the three different xylose-fermenting yeasts showing that xylose metabolism occurs at higher fluxes rate in *S. stipitis*than *S. passalidarum* and *S. arborariae*. The fluxes rate is divided similarly between pentose phosphate pathway and glycolysis. *S. arborariae* presents 3.0 times higher demand for NADPH regeneration than observed in *S. passalidarum*. The flux rate to glycerol formation in *S. passalidarum* is inactive and this yeast looks like occur a better NADH/NAD^+ balance, which permits efficient xylose fermentation.

BREWING WITH THE MOTHER OF THE LAGER YEAST IN PATAGONIA

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Saccharomyces eubayanus is a criotolerant yeast native to Andean Patagonia. It was formally described in 2011. Genetic studies confirmed that hybridization between this species and *S. cerevisiae* (Ale yeast) gave rise to *S. pastorianus* (Lager yeast, responsible for 96% of beer production worldwide). This discovery sparked interest in the scientific-technological sector due to the potential application of wild parental yeast as a starter culture in the brewing industry. From our laboratory we began to work to know its fermentative and organoleptic characteristics and to develop the starter cultures that could be transferred to the breweries. This labor forged a relationship between the scientific and the productive sector: prepare the industry to work with yeasts in liquid format (most of the craft breweries of Argentina only worked at that time with dry yeast), teach courses and instruct on the management and repitching of brewer´s yeasts, train on contaminant detection and perform controls in breweries. Experiments were started with brewers on a semi-pilot scale (20-50 Lts) with *S. eubayanus* to learn its behavior outside the laboratory. In May 2017, the first 100% Argentine beer was launched in Bariloche, where 5 breweries in the area presented their own styles made with the wild yeast. In conjunction with a local craft brewery, the low-alcohol beer “Wild Lager Sauvage” was presented at the Ironman 70.3 Bariloche. Currently, through an agreement with Bariloche´s local breweries association (ACAB), several beers are being made and commercialized with the Patagonian yeast at starting volumes ranging 150 to 1500 Lts. The “Proyecto Patagonia Salvaje” (Wild Patagonia Project) intends to create, in cooperation with local craft brewers, experimental beers employing *S. eubayanus* yeast, and local ingredients allowing to create new styles of