Development of biotechnological tools for modulating an industrial microbiome

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Development of biotechnological tools for modulating an industrial microbiome

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Abstract: Industrial scale bioprocesses are poised to suffer from continuous contaminations. It reduces process efficiency and increases the demand for raw materials. In order to advance towards a biobased economy we need to address these bottlenecks. In this project we plan to understand the microbial consortia that resides in a well stablished industrial bioprocess – sugarcane ethanol fermentation – and devise solutions to rationally modulate it.

***Main Objective*: Improve an industrial bioprocess performance by modulating its intrinsic microbiome.**

Introduction: Ethanol is the most important commodity produced via biotechnology today. Most of it is produced via starch (corn) or sucrose (sugarcane) based fermentations. For several socio-economical reasons, sugarcane is the preferred carbon source for bulk biochemical production.
Having harvested over 600 million tons of sugarcane in 2015, resulting in more than 28 billion litres of ethanol in the same year, Brazil is the world leader sugarcane ethanol producer.
The Brazilian ethanol production process makes use of very high cell density, non-aseptic, fed-batch fermentations (10% w/v), achieved via cell recycling. In this setup, yeast slurry is chronically treated with sulfuric acid – acid wash – in order to control contamination (**Figure 1**).
Still, contamination outbreaks emerge continuously. Mills add antibiotics in order to control these contaminant strains, with limited success. After some time, resistant contaminants will overtake the process, leading to several issues like: yield loss, operational costs increase, flocculation, foaming, yeast viability drop, etc. (**Figure 2**).

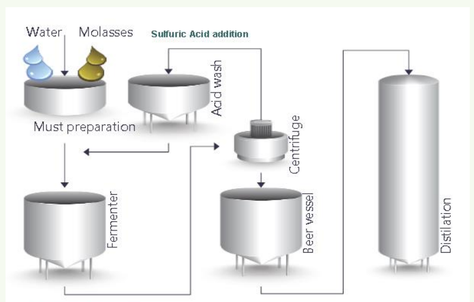

Figure 1: The Brazilian ethanol production process, and its several unitary steps…

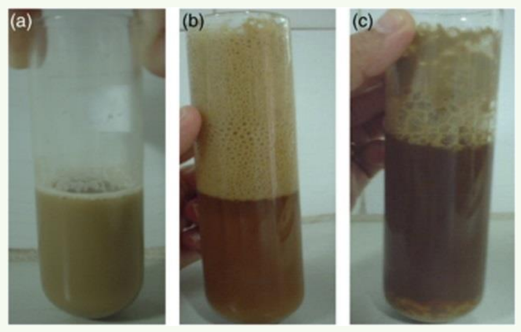

Figure 2: … and some of its contamination issues. Comparison between a selected industrial yeast strain (2a); a strain presenting foaming phenotype (2b) and one presenting flocculation phenotype (2c).

Strategy: Actual samples from Brazilian ethanol production process will be used to draw the profile of the industrial microbiome, via 16S/18S amplicon analysis and metagenomics. These samples will also be the source of microbial isolates for further lab studies (**Figure 3a**).
After, microbial isolates will be co-cultivated with an industrial selected yeast strain, each one separately, in a high throughput manner. By barcoding the strain of interest, we will be able to determine the real impact on growth that each contaminant strains has. Further co-cultivating these contaminant strains with each other will allow us to determine the ecological interactions between these contaminants. By doing so we expect to pin point the most detrimental strains for the fermentation, and also potential antagonists for these strains (**Figure 3b**).
Based on the knowledge gained in step 3a, we will simulate the industrial ethanol production process at lab scale. By comparing the performance of fermentations with and without the most detrimental contaminants – found in 3b – we will demonstrate their real impact to the process. Finally, by treating contaminated fermentations with isolated antagonists for the worst contaminants – also from 3b – we will treat infection in a more specific, environmentally friendly and effective manner (**Figure 3c**).
Finally, we will compare physiological parameters and metagenomics data of chemostat fermentations, with a co-cultivation of most impacting contaminant strains with industrial selected yeast strains and reference laboratory yeast strains. With this, we expect to identify which set of genes and metabolic pathways are necessary for a species to remain in an industrial process, putting also the ecological interactions under perspective of an active selection pressure (**Figure 3d**). This knowledge can help to tailor the next generation of industrial workhorses.

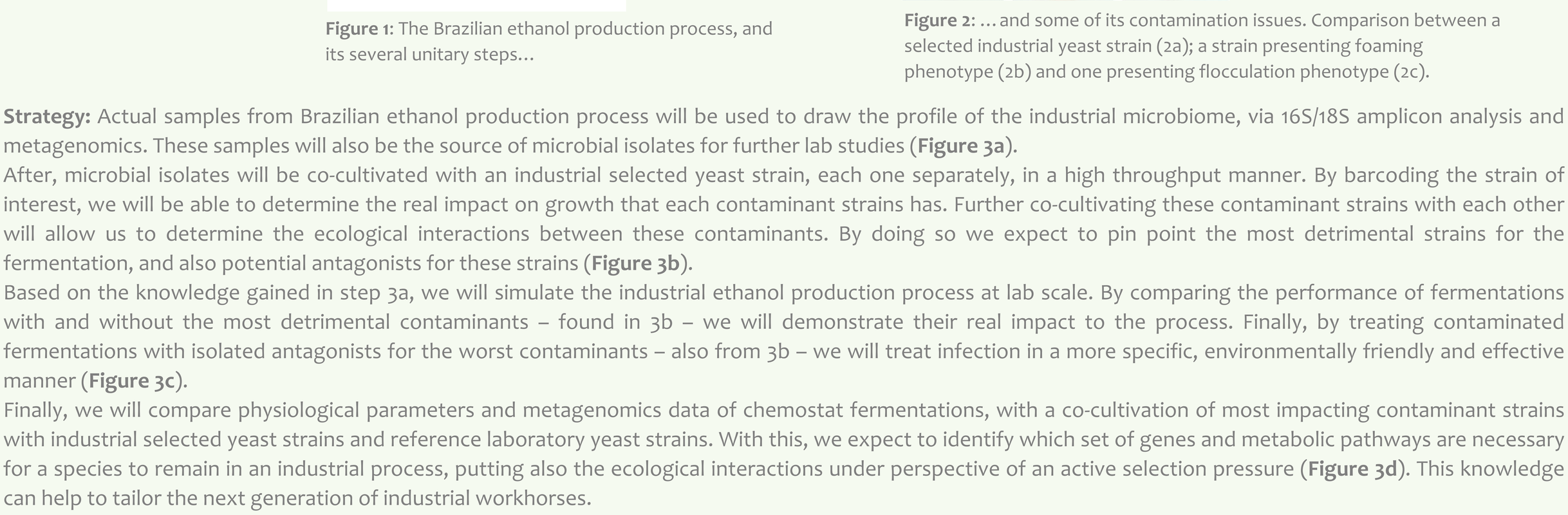

Figure 3: Workflow of projects proposed for this study. Microbiome analysis of the sugarcane ethanol industry via 16S/18S amplicons and metagenomics, and further isolation of contaminants (3a). High throughput screening of ecological interactions of the species present in the industrial process. Barcoding will allow to follow through fitness parameters of important strains (3b). Laboratory simulation of the ethanol production process, demonstrating the concept of microbiome modulation (3c). Fitness studies using chemostat cultivations of a population of contaminants with either selected industrial yeast strains or laboratory yeast strains (3d).

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