Biochemical Production of Endophytic Yeast

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Biofuel: what and why
In the U.S., the debate about biofuel - an alternative transportation fuel coming from currently living organisms - is still continued because the farms are growing corns (first generation) for fuel production instead of for human and animal consumptions. The future of biofuel relies on lignocellulose from fast growing plants native to the sites and able to tolerate harsher conditions. Lignocellulose is the major component of plant cell wall - a carbohydrate with different sorts of sugar types. Cellulose (30-50%) is made up by glucose, while hemicellulose (20-40%) commonly comprises of glucose (C6), xylose (C5), arabinose (C5), and mannose (C6).

Biochemical Production of Endophytic Yeast

The two graphs above show the various concentration of sugar-alcohol during fermentation processes. They are averaged based on the three trials done, each conducted in triplicates. The following pictures are from different yeast strains out of 15 screened. Yeast strains were subjected to three different treatments to test hypothesis: rich media with agitation at 30°C, rich media without agitation at 4°C, nitrogen limited media with agitation at 30°C. The upper parts of each photographs set were taken under a reflective light microscope, showing what the real yeast morphology while the bottom parts were taken under the fluorescence where the green parts show lipid accumulation. Shown below are of WP1 and AD1 respectively.

Fermentation by endophytic yeast
I focused on the endophytic yeast strains which are able to ferment sugar into various biochemicals. Endophytes are microbes that live inside the plant tissues without causing diseases, but rather help the plants thrive in various environments. The Doty Lab favorite is poplar rocks, which happen to be the biofuel crop chosen for the Pacific Northwest. The strains that I used are: Candida lignohabitans strain AR1, Rhodotorula mucilaginosa strain PTD3 and Rhodotorula graminis strain WP1. The first was isolated from a wild red alder, the second was from a hybrid poplar grown in Boardman, OR as biofuel crop feedstock, while the last was from a wild poplar by Skykomish river, WA.

Since they are so used to digesting different sugar types, they are able to utilize the various sugar types found in plants: glucose, xylose and arabinose. Baker’s yeast on the other hand is not able to ferment the latter two due to its lack of appropriate enzymes, namely: Xylose reductase (XR) and Xylitol Dehydrogenase (XDH). XR will digest xylitol into ethanol, while XDH will digest xylitol into ethanol. PTD3 was previously screened for such genes and found to have them although only the former seems to be expressed.

Methods
The yeast strains would be grown on rich media for two days to reach a high cell density before starting fermentation process. During fermentation itself, the cells are switched into a media that mimics the plant hydrolysate. Depending upon the given sugar, it takes 1-5 days to obtain the chemical of interests: bioethanol, xylitol and triglyceride. Fermentation typically has much more sugar concentration than the growing media as the high C:N ratio triggers switch of metabolic activities in the yeast. While bioethanol and xylitol use C:N of 20:1 in the absence of oxygen or very limited oxygen level, triglyceride accumulation is more effective at 40:1 with limited oxygen level.

Quantitative measurement was done for the first two products while for the accumulation of lipid a qualitative method was used instead. The first utilized a high performance liquid chromatography (HPLC) instrument where sugar-alcohol concentrations were read off collected samples. The latter used the aid of fluorescent microscopy after staining the cells with a green fluorescence dye that emits the wavelength at 505 nm, while absorbing at 515 nm.

Results
The yeast strains would be grown on rich media for two days to reach a high cell density before starting fermentation process. During fermentation itself, the cells are switched into a media that mimics the plant hydrolysate. Depending upon the given sugar, it takes 1-5 days to obtain the chemical of interests: bioethanol, xylitol and triglyceride. Fermentation typically has much more sugar concentration than the growing media as the high C:N ratio triggers switch of metabolic activities in the yeast. While bioethanol and xylitol use C:N of 20:1 in the absence of oxygen or very limited oxygen level, triglyceride accumulation is more effective at 40:1 with limited oxygen level.

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