Popular Science Summary

Characterization and optimization of *Rhodosporidium toruloides* for production of lipids

This thesis was based on *Rhodosporidium toruloides* a super pink, super oily yet super useful species of yeasts! This thesis resulted in the yeasts storing about 40% of the cell mass as oils also known as lipids. The storage capability of these yeasts is what resulted in them being called oleaginous yeasts. How do they accumulate lipids you ask? Simple, they channel all the extra sugar one feeds them when you starve them of an essential nutrient such as nitrogen. The research here focuses on finding which sugar was the best at which temperature and at what pH do these cells grow the best. Once those conditions were selected, the process was then scaled up from shake flask to reactors. The main end goal was to see how well these pink yeasts store lipids.

Rhodosporidium toruloides are yeasts that can store lipids hence are termed as oleaginous. For a microorganism to be termed as "oleaginous", they should be able to store at least 20% of their cell mass as lipids. As mentioned in the introduction, these yeasts need to be starved of an essential nutrient namely nitrogen, sulfur, or phosphate; in this research nitrogen was the preferred nutrient. Before the cells were starved, the best growth conditions were decided. The first condition was to check which sugar was taken up readily amongst glucose, glycerol, and xylose. As it turns out this yeast can grow in all of them, but it is faster in glucose and xylose compared to glycerol. This can be interesting as if this process were to be industrialized it is important for the price of the sugar to be affordable, and glucose is a pretty expensive sugar! So, in situations like this, a source like glycerol which is a by-product of many other industrial processes such as manufacturing of biodiesel could lead to a beneficial process economy. The second condition was temperature, previous research suggested we use 30°C as the growth temperature. So, to verify that claim the research checked the growth of the yeast in 27, 33, and 36°C. The results once again showed that our pink yeast is quite strong and is capable of growing at all those temperatures just at different speeds. The cells preferred 27°C as the most comfortable condition, thereby reassuring the above-mentioned claim. The third and final condition was checking the best-suited pH, which is a measure of whether the yeasts prefer an acidic, basic, or neutral growth media environment. Previous research once again suggested a pH of 5.5 to check whether that was true for our yeasts, we grew it in pH 4.0, 4.5, and 6.5. The change in pH can lead to the cells experiencing stress. The yeasts once again showed that it is very strong and can grow in all conditions of stress, but it is the most comfortable in pH 6.5. So, the final conditions to grow the yeasts were set at glucose (the sugar source), 30°C (temperature source), and 5.5 (pH source).

Once the yeasts grew well the starvation process to produce lipids were tested, but to produce lipid, one must decide what ratio of sugar to nitrogen they want to use? That ratio is called C:N ratio, where C:N stands for carbon (the sugar) and nitrogen ratio that is put in the growth medium. The ratios tested were 20, 40,80, and 100. The yeasts showed no significant storage at C:N of 20, showed about 20% storage at C:N 40, around 35% storage at C:N 80, and 40% storage at C:N 100. The C:N experiments were initially performed in 300 mL shake flasks to check which ratio gives more lipids, and then scaled up to check if the cells could store more lipids in a more controlled environment which a reactor can provide. A C:N ratio of 80 was chosen as it did not put the cells under too much stress. The reactor experiments were separated in two categories: (i) Batch- this means throwing in everything in the reactor at once and just waiting for the cells to become pink and consume all of the sugar; (ii) Fed-Batch- in this category the cells are given a little sugar so they can grow well until the nitrogen gets completely used up, once the nitrogen is used up they are fed sugar little by little until they store as much lipids as they can. The batch experiments resulted in the cells storing 35% of their cell mass as lipids, but the experiment can only run for 72 hours as the cells completely consume the sugar. The fed-batch experiments resulted in ~37% of their cell mass as lipids but they continued to store lipids for 192 hours. Now, one would start to think how is that the experiment runs longer yet the amount lipids has not increased much? Well, there were more lipids, but they were no longer in the yeasts. The lipids were in the growth medium, how you ask? The pink yeasts burst! Yes, burst because they stored more lipids than they could handle. Another observation we made was that the cells grew in size as the experiment progressed, this is possible because the nitrogen starvation doesn’t allow them to divide but the excess sugar that is getting stored as lipids increase the size.

To conclude, *Rhodosporidium toruloides* have a bright (pink) future in this area of research! Who knows, maybe some time from now they would be able to store plastics?