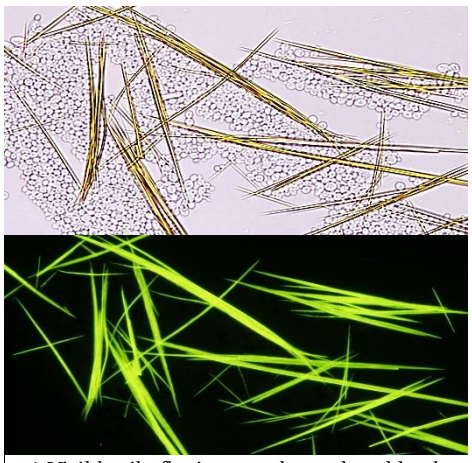
General public abstract

Riboflavin, also known as vitamin B_2, is essential for the life and proper functioning of animals and humans, so we must provide it with food. There are many studies showing the health-promoting and anti-disease effects of riboflavin, mainly the anti-inflammatory effect. Riboflavin is involved in the production of red blood cells, is successfully used in the treatment of iron deficiency (anemia), and helps reduce the negative effects of eye diseases such as cataracts. Taking riboflavin supplements is also a cure for migraines. Riboflavin is obtained biotechnologically using mainly modified *Bacillus subtilis* and *Ashbya gossypii* strains, however, due to several advantages, flavinogenic yeast *Candida famata* can still compete with them. Over the years, thanks to the use of modern techniques of genetic engineering, many strains that overproduce riboflavin on have been obtained, as well as on various wastes including lignocellulosic hydrolysates and cheese whey. It is necessary to focus on the genes involved in the biosynthesis pathway and to understand the regulatory mechanisms.



1.Visible riboflavin crystals produced by the yeast C. famata and fluorescence of the crystals under UV light.

In recent years, the tendency to protect the environment has been intensifying and concerns mainly the reduction of pollution and, comsidering the fact that someday natural energy sources will run out, the search for alternative energy sources and the reuse of waste (recycling). One of the most important in biotechnology is plant waste, which after appropriate pre-treatment (hydrolysis) can be reused as a source of carbon, for example bagasse. In addition, widespread hunger in the world makes it unethical to use raw materials rich in sugar, i.e. cereals or sugar cane, in the industry, while lignocellulosic waste, the largest source of renewable biomass, is treated as inedible waste, which is an additional asset. Currently, energy obtained from biomass already accounts for over 14% of global energy consumption, but the question should be asked, can we use these raw materials more efficiently?

So far, hydrolysates have been successfully used in industry for the production of compounds useful by microorganisms, e.g., biodiesel, biohydrogen, lipids, carotenoids or even polymers. As we have shown, our recombinant *C. famata* strains are able to grow and produce riboflavin on lignocellulosic hydrolysates, and we have already taken steps to make this process more efficient.

Lignocellulosic waste must be processed through pre-treatment and hydrolysis, unfortunately, the big problem is the inhibitors generated during these processes, mainly acids, furans and phenols, strongly affect cells, damaging proteins, lipids and DNA, consequently leading to cells death, and from a biotechnological point of view, it stops the whole production process. At present, however, little is known about genes that could simultaneously counteract the toxic effects of inhibitors and increase the efficiency of *C. famata's* production of riboflavin. A very important aspect is also increasing the utilization capacity of glucose and xylose sugars, the two main ones present in the hydrolysate. Various genes have been identified in many yeast species that have a real impact on improving production from lignocellulosic biomass. **Therefore, our main objective of this proposal is an attempt to analyze genes that could significantly improve the ability to produce riboflavin in flavinogenic yeast *C. famata* (*Candida flareri*, teleomorph, *Debaryomyces subglobosus*) on lignocellulosic hydrolysates.**

In the first place, we want to improve the use of the main sugars in the hydrolysate, i.e., glucose and xylose, by overexpressing the genes responsible for the pathways of these sugars utilization. Inspired by similar studies in *S. cerevisiae* and *O. polymorpha*, we want to overexpress genes from the HGT and XYL groups. Preliminary studies have confirmed that overexpression of XYL1 increases xylose utilization relative to the parental strain on mineral medium. Effective detoxification of toxic biochemicals in fermentation media remains a challenge. To counteract this, we intend to carry out evolutionary mutagenesis in a bioreactor, preceded by UV mutagenesis in order to increase the frequency of mutations. Next, we also planned total genome sequencing of the most resistant mutants, which will allow us to compare sequences and changes in genes. After sequencing, genes that could significantly contribute to improved production will be analyzed and then amplified by PCR and inserted into the cell with the help of a plasmid. The successful implementation of these plans will translate into a deeper insight into the mechanisms regulating the synthesis of riboflavin on lignocellulosic hydrolysates.