Project brief

Thünen Institute of Agricultural Technology 2023/30a

Replacement of Contentious Inputs in Organic Farming Systems (RELACS)

Anja Kuenz[1], Sarah Bromann

- **Many of the vitamins available on the market, such as vitamin B2 (riboflavin), are produced using genetically modified organisms (GMOs) and therefore cannot be added to feed on organic farms.**
- **A screening of non-GMO wild-type microorganisms that overproduce riboflavin was conducted. The wild-type yeast *Meyerozyma guilliermondii* produced the highest riboflavin concentration in the screening procedure.**
- **Using an adapted fermentation strategy, 30 mg riboflavin per g dry cell weight was achieved on a laboratory scale with a GMO-free yeast strain.**

Background and Objectives

In contrast to many bacteria and yeasts higher eukaryotes cannot synthesize riboflavin (Vitamin B2) and therefore this vitamin is a dietary necessity. The supply of riboflavin in poultry diets is essential as it is required for many metabolic processes. Riboflavin is currently produced biotechnologically, based on genetically modified organisms (GMOs). Due to legislation, organically raised animals must be fed GMO-free feed, which also applies to the manufacturing processes of vitamin supplements in feed. To ensure a continuous supply of riboflavin to organic livestock, the isolation and optimization of a new GMO-free yeast strain for vitamin B2 was carried out and this project focused on:

- Screening for new riboflavin producers from specific habitats
- Identification of new previously unknown riboflavin producers based on the phylogenetic tree of known natural overproducers
- Optimization of the fermentation process of the new potent riboflavin-producing yeast strains
- Fermentation parameters such as media, inocula, pH, temperature, stirring and aeration rates were under investigation

Procedure

Screening non-GMO wild-type yeast strains that overproduce riboflavin is the first step in developing GMO-free riboflavin production lines.

Samples were cultured from special habitats such as compost, tree bark, rotting fruit, homemade sauerkraut and sourdough, homemade beer from grapes, soils at oil production pumps and gas stations, and from rumen fluid on various media (Figure 1). From these approaches, 37 different yeast strains could be isolated. Furthermore, 14 phylogenetically close relatives of known naturally overproducing vitamin B2 strains were ordered from culture collections.

Figure 1: Overview of sampling and isolation of new yeast strains

Quelle: Thünen-Institut/Anja Kuenz(2022).

Results

Of these 51 tested strains, the wild-type yeast strain *Meyerozyma guilliermondii* DSM 11947 produced the highest riboflavin concentration in the screening procedure. *M. guilliermondii* is a yeast species that is widely distributed in the environment. It is classified as Risk Group 1, making it a low individual and community risk organism, meaning it is very unlikely to cause disease. This natural overproducer was selected for further process optimization.

The nutrient requirements of the wild-type strain and scale-up from shake flasks to a 1-liter bioreactor were investigated.

To make the process economical, it is important to eliminate expensive components during the yeast cultivation process. Besides the substrate, nutrient sources such as yeast extract cause the highest production costs. Therefore, the focus here was primarily on yeast extract, which accounts for an enormous

share of the media costs, but on the other hand serves as an important source of nitrogen and nutrients such as vitamins, trace elements and others.

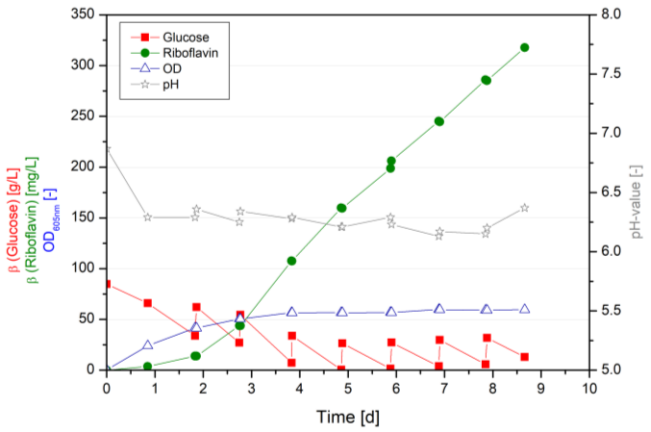
Therefore, the yeast extract concentration was reduced. In the variants with lower yeast extract concentration, glucose utilization took longer and the maximum productivity of riboflavin formation was significantly lower. With urea the production medium already contains another nitrogen source, that is much cheaper compared to yeast extract. Therefore, the complete replacement of yeast extract with urea was tested. Complete omission of yeast extract resulted in slower riboflavin formation regardless of the used urea concentration. Maximum productivity was lower, but after 14 days of cultivation the same titer of riboflavin was produced. So, yeast extract could be replaced by urea.

After varying the nitrogen source, the influence of the vitamin and amino acid content of the yeast extract was studied. The addition of B vitamins is not necessary for overproduction of riboflavin with *M. guilliermondii*, because the strain was able to produce riboflavin even without the addition of vitamins. The situation is different with the influence of amino acids, here further studies are necessary to get more information about the requirement.

After that, the riboflavin production was successfully transferred to the 1-liter bioreactor scale (Figure 2).

Here, the most critical parameter seems to be the supply of sufficient oxygen in combination with the right speed range. The highest titer achieved so far, 317.6 mg/L riboflavin, was reached during fed-batch cultivation after 9 days. A total yield of 30 mg riboflavin per g dry mass of yeast cells was achieved.

Figure 2: Fed-batch cultivation of *M. guilliermondii* DSM 11947 in a 1 L-bioreactor fermentation, agitation speed 500 - 800 rpm, aeration rate of 9 sL/h, dissolved oxygen setpoint of 30%



Quelle: Thünen-Institut/Anja Kuenz(2022).

This demonstrated that it is possible to achieve higher riboflavin contents with the wild-type strain *M. guilliermondii* using bioprocessing technology and not genetic modification. The selected strain has the potential for much higher riboflavin concentration than currently shown. In the nearer future, this should increase the availability of non-GM vitamin B2 for organic feed production.

Further information

Contact

[1] Thünen-Institute of Agricultural Technology
Anja.Kuenz@thuenen.de

www.thuenen.de/at
Project website: www.relacs-project.eu
Social media: Facebook (RELACSeu) & Twitter (@RELACSeu)

DOI:10.3220/PB1687162447000

Project period

5.2018 - 4.2022

Project-ID

2023

Publications

Kuenz, A., Tölle, M. & Bromann, S. Investigations on riboflavin production by wild-type yeast strain for supplementation of organic feed. Org. Agr. (2023). https://doi.org/10.1007/s13165-023-00435-4

Financed by

'Replacement of Contentious Inputs in Organic Farming Systems' (RELACS) has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 773431.