Enzyme activity screening methods for fungal solid-state fermentation

Tuisku Immonen[1], Ph.D. Outi Mäkinen[2] and M.Sc. Anniina Valtonen[2]

[1]Department of Life Technologies, University of Turku, [2]Biomush Ltd, Espoo

BIOMOLECULAR PRODUCTION (TECH.)

@biomush

Introduction

Solid-state fermentation is a fermentation technology that takes place in a moist particle without being submerged into a liquid (Fig. 1).[2]

Enzymes can be easier to produce in higher quantities in solid-state fermentation because of a more natural-like environment. Enzymes are commercially used in many processes. They have value in food industry applications among others.[2]

Glutaminase and protease are both used in the food industry.[3] Glutaminase affects flavor compounds in food and beverage solid-state fermentation applications.[3] Protease productivity is also a desired characteristic in food and beverage solid-state fermentation applications.[2]

Aim of the Study

The aim of the study is to establish screening methods to differentiate enzyme activity and form a big picture of how enzyme activities change and develop in a fungal solid-state fermentation.

Screening is done with four different strains of filamentous fungi. The aim is to be able to also establish differences between the four strains and their effects on enzyme activity.

Solid-state fermentation

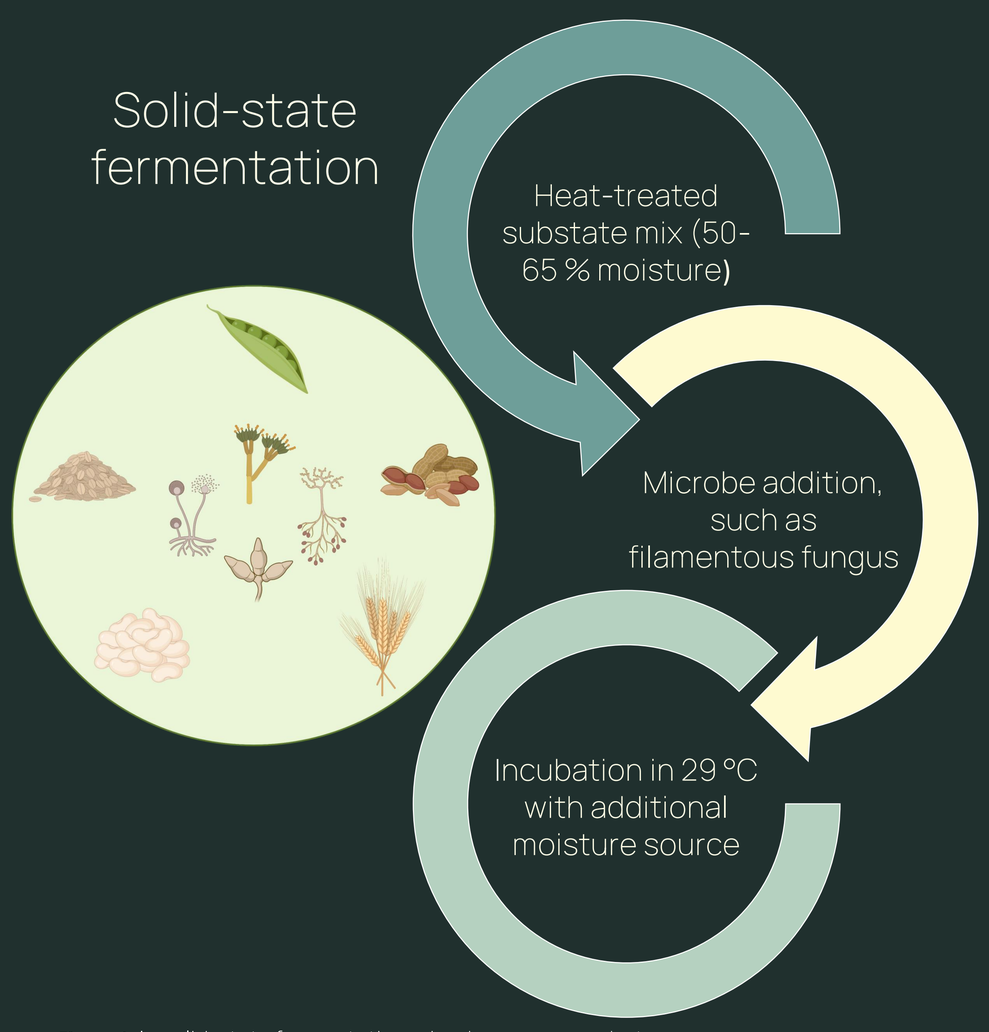


Figure 1. In solid-state fermentation microbes grow on a heterogenous surface. In this study the solid material consist of food industry residues.

Methods

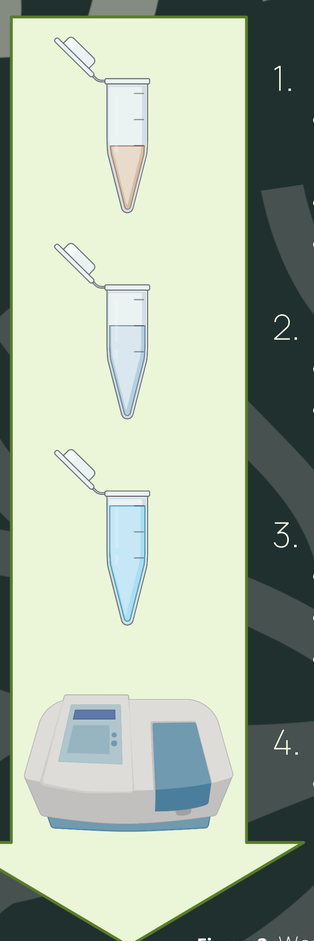
Solid-state fermentations are done with two different substrate mixes with four different strains.

General differences in enzyme activities in different strains are determined with on/off enzyme assay. Specific enzyme activities are analyzed with spectrophotometric methods as a function of time.

The general idea is that the activity of a sample is determined by the rate of substrate hydrolysis that is detected by a specific kit (Fig. 2).

Methods are first modified in practice. Thereafter, these methods are used to continue the comprehensive activity screening.

Enzyme activity assay



1. Extraction
   - 1 g solid sample per 10 ml water
   - 30 min extraction
   - 10 min 4 000 g
2. Hydrolysis
   - Substrate addition
   - Incubation in 37 °C 30 min
3. End of hydrolysis
   - TCA addition
   - Ice bath
   - 10 min 10 000 g
4. Analysis
   - Spectrophotometric analysis with specific kit

Figure 2. Workflow of the spectrophotometric enzyme activity assay methods include four steps.[1]

Results

Protease activity screening has required development. Casein as a substrate is suspected to cause interference and a new substrate is to be tested.

Glutaminase method has been proven to function. Some differences in the glutaminase activity between strains have been established already. Glutaminase activity levels differ between strains. All strains follow a similar pattern with glutaminase progression (Fig 3). The highest activities are detected in the final parts of the cultivation. Also, different growth habits are shown to reflect on the glutaminase activity.

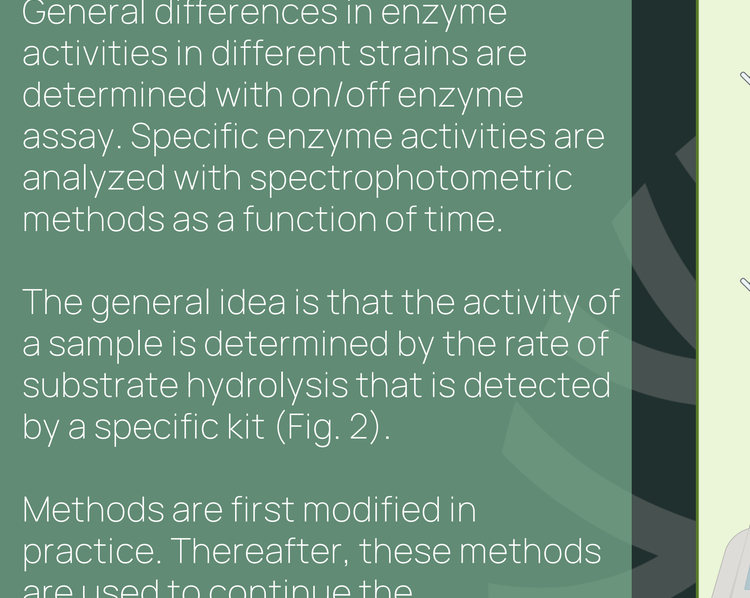


Figure 3. Glutaminase activity of the four strains (1, 5, L and SS) and pH of the corresponding substrate as a function of time.

Conclusions

Problems regarding casein as a substrate were important to be found early. Still, absolute conclusions can't be made yet.

The revealed differences between strains shows promise. The results will aid future research undoubtedly. Such as, strain L's constant glutaminase progression should be investigated further.

References

[1]Created with BioRender.com
[2]Chen, J., & Zhu, Y. (Eds.). (2013). Solid state fermentation for foods and beverages. Taylor & Francis Group.
[3]Matsuyama, A. (2021). Koji Molds for Japanese Soy Sauce Brewing: Characteristics and Key Enzymes. Journal of Fungi, 7(8), 658.

Chutmanop, J., Chuichulcherm, S., Chisti, Y. and Srinophakun, P. (2008), Protease production by Aspergillus oryzae in solid-state fermentation using agroindustrial substrates. J. Chem. Technol. Biotechnol., 83: 1012-1018.
Ito, K., Hanya, Y., & Koyama, Y. (2013). Purification and characterization of a glutaminase enzyme accounting for the majority of glutaminase activity in Aspergillus sojae under solid-state culture. Applied Microbiology and Biotechnology, 97(19), 8581-90.
Kumar Chandrawanshi, N., Koreti, D., Kosre, A., & Kumar, A. (2022). Proteolytic Enzymes Derived from a Macro Fungus and Their Industrial Application. IntechOpen.