European Master in Biological and Chemical Engineering for a Sustainable Bioeconomy

Master thesis report

submitted to obtain the degrees of

Master in Biology AgroSciences (BAS) of the University of Reims Champagne-Ardenne

Master of Science in Engineering of Tallinn University of Technology

Master in Integrative Biology and Physiology (BIP) of the University Paris-Saclay, operated by AgroParisTech

Isolation and characterization of traditional tempeh starter culture *Rhizopus* sp.

Presented by: Terán Velástegui Martín Alejandro

Defended the: 06 /07 /2023

Supervised by

ANDRESON Maret, M.Sc.

PRIIDIK Reimo, M.Sc.

at: TFTAK - Center of Food and Fermentation Technologies.

Mäealuse 2/4,12618 Tallinn, Estonia

from 06/02/23 **to** 06/08/23

Bioceb supervisors: LANDAUD Sophie, Ph.D. AgroParisTech

SMOLANDER Olli-Pekka, Ph.D. TalTech

Confidentiality: Yes Privacy expiration date: 26/05/2028

SUMMARY (ENGLISH)

The development of alternative proteins is a key focus area on the transition to a sustainable bioeconomy. Alternative proteins have attracted commercial interest for their application in meat analogues. Between these different alternative sources of protein, mycoprotein has several examples of successful application for the production of meat analogues at industrial scale. In addition, fermented fungi products such as Indonesian tempeh traditionally have been used as meat replacement for generations.

The present study describes the isolation and characterization of different *Rhizopus* spp. from commercial tempeh starter cultures. The aim of the study was to obtain efficient *Rhizopus* sp. isolates and determine optimal growth conditions for biomass production for their future application in the formula of new meat analogues. Isolation was carried by cultivating on selective media. Isolates characterization was aided by a combination of ITS amplicon sequencing, morphological identification as well as study of growth kinetics in defined media during shake flask and microtiter plate-based fermentations. A 1 L bioreactor experiment was conducted for the most promising isolates. In addition, proteomic profiling of one isolate was performed to phenotype two different growth stages (before and after sporogenesis) and fermentation methods (submerged and solid state fermentation).

Eight pure *Rhizopus* sp. isolates were obtained from the starter cultures. Isolates screening was carried out in a mineral media with 0.2 g/L glucose and pH adjusted to 5.7 where pellet morphology, fastest growth and highest biomass yields were obtained. In these conditions, two clusters of isolates were observed differing in their lag period. Three of the most promising isolates were tested in a stirred tank reactor fermentation. However, cultivation in this configuration resulted in mycelium attached to sensors and stirrer mechanism, disturbing bioprocess control for any further studies.

Comparative proteomic profiling revealed unique proteins expressed in each growth stage. The biggest differences in protein signal expression were found between the two developmental stages. However, a large percentage of protein in each sample could not be assigned to any cellular localization or biological process. Insights gained in this study give the basis for future studies by setting approximate values for further optimization of stirred tank reactor fermentation, implement fermentation in airlift bioreactor or to serve as comparison for future experiments under different conditions.

SUMMARY (ESTONIAN)

Alternatiivsetest valkudest toodete arendamine on oluline samm jätkusuutliku biomajanduse suunas. Viimaste aastatega on muutunud alternatiivsete valkude kasutamine lihaanaloogide valmistamisel aina populaarsemaks. Võrreldes teiste alternatiivsete valkudega on mükoproteiini kasutamine tööstuslikus skaalas näidanud häid tulemusi lihaanaloogide tootmises. Näiteks indoneesia tempet, mis on fermenteeritud seente produkt, on kasutatud juba aastasadu liha asendajana.

Käes olev töö kirjeldab erinevate *Rhizopus* spp. isoleerimist ja karakteriseerimist kommertsiaalsetest tempe straterkultuuridest. Töö eesmärgiks oli saada effektiivsed *Rhizopus* spp. isolaadid ja leida nende optimaalsed kasvu tingimused biomassi tootmiseks, mida saaks edasi kasutada lihaanaloogide arendamises. Kultuuride isoleerimine viidi läbi selektiivsetel söötmetel. Lisaks eelnevale teostati ühe isolaadi proteoomi profiili analüüs kahe erineva kasufaasi (enne ja pärast sporuleerumist) ja fermentatsioonimeetodi (vedel- ja tahkekultuuri fermentatsioon) fenotüüpiliseks iseloomustamiseks.

Starterkultuuridest eraldati 8 *Rhizopus* spp. isolaati. Isolaatide skriinimine viidi läbi mineraalsöötmes, mille glükoosi sisaldus oli 0.2 g/L ja pH oli optimeeritud 5.7, kus saavutati pelleti kujuline morfoloogia, kiireim kasv ja kõrgeim biomass. Isolaadid jagunesid kahte klastrisse erinedes vaid lag-perioodi poolest nendel tingumustel. Kolme kõige paljulubavama isolaadiga teostati “stirred tank” bioreaktorites katse. Selgus, et sellises konfiguratsioonis bioreaktor ei sobi taolist tüüpi biomassi tootmiseks, kuna mütseel kattis sensorid ning kinnitus bioreaktori labade külge takistades bioprotsessi kontrollimist.

Võrdlev proteoomi analüüs näitas unikaalsete valkude ekspressiooni igas kasvufaasis. Suurim erinevus valgu signaali ekspressioonis oli kahe kasvufaasi vahel. Kõikides proovides esines palju valke, mille asukohta ega bioloogilist protsessi ei suudetud määrata. Selle töö raames kogutud informatsioon annab aluse edasisteks katseteks, et optimeerida “stirred tank” bioreaktoris fermenteerimist, kasutada “airlift” bioreaktorit seente kultiveerimisel või olla võrdluseks tulevaste katsete jaoks erinevates tingimustes.