Gembloux Agro-Bio Tech
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FUNGAL BIOFILM REACTOR IMPROVES THE QUALITY OF A FUSION PROTEIN GLA::GFP PRODUCED BY ASPERGILLUS ORYZAE

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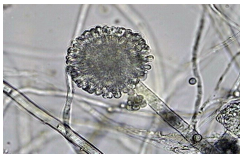
Context & Objectives

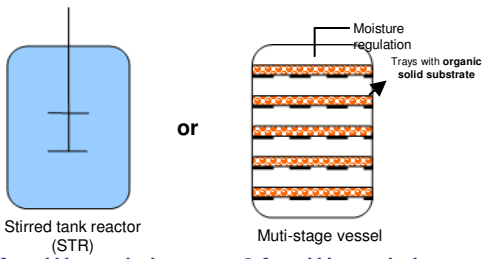
Fine chemical and pharmaceutical industry



Filamentous fungi such as *Aspergillus sp.* or *Trichoderma sp.*

- Fine chemicals (organic acids)
- Metabolites II (enzymes, antibiotics)
- **Recombinant protein**
  - ➔ high secretive power
  - ➔ post-translational modifications

Currents fermentation bioprocesses



➔ **fungal biomass looks like "balls of wool"**

(+) simple implementation

(-) high viscosity, shear stress

➔ **fungal biomass looks like a "wool carpet"**

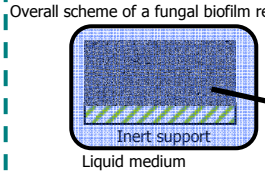
(++) enhancement of metabolites secretion and high productivity

(-) heat removal, downstream process operations

What? Weaknesses of these processes need to be improved!

Methodology

How? Design a Fungal biofilm bioreactor combining advantages from submerged and solid-state cultures!



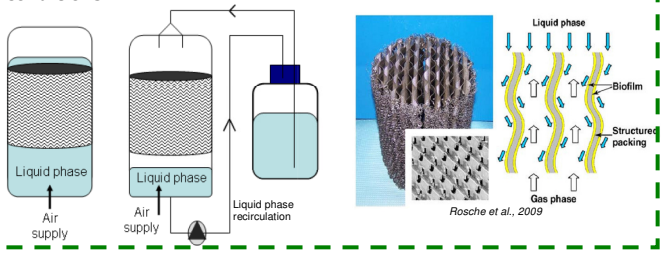
- biomass growth on inert support immerged in liquid medium

➔ enhances metabolites secretion

Fungal biofilm = structured wool carpet with high water content"

Task? Characterize secretion profile of two fungal biofilm reactors for the production of a recombinant protein

Scheme of fungal BfR designed for this work

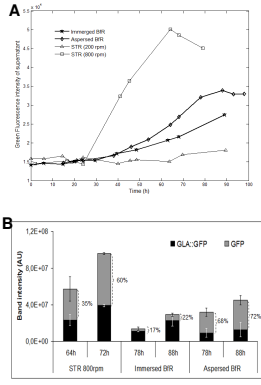


➔ GLA::GFP recombinant protein (RP) containing glaA sequence linked to the GFP sequence is under the control of the **glaB** promoter **only** induced in solid-state fermentation

➔ Secretion performances of the RP are compared between fungal BfR and submerged culture in STR. 2D-gel electrophoresis characterizes secretion profiles.

Results & Discussion

1. Production kinetic and detection of the RP



A shows production kinetic of RP in culture supernatant

Surprisingly, STR with intense agitation leads to highest RP production whereas low agitation leads to the lowest

➔ *Do high shear stress conditions activate pglaB?*

• Despite use of a specific promoter, we observe middle RP production in aspersed and immersed BfR

➔ *Does biofilm thickness influence diffusional mass transfer of RP?*

• For each sample of supernatant, immunodetection of the RP with an antibody anti-GFP detects two bands of different molecular weight :

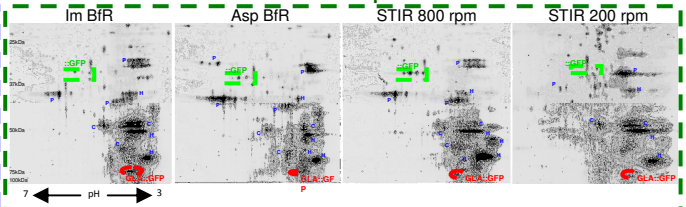
• 1st band at 70 kDa is the RP and 2nd band at 27 kDa is a proteolytically truncated GFP

B bar graph represents total amount of secreted RP for each culture condition. Fraction of truncated GFP is given in % of total amount

• Fraction of truncated GFP reaches less than 25% in immersed BfR whereas it reaches much than 50% in two other conditions

➔ *Do biofilm conditions alter secretion and quality of the RP?*

2. Secretion profile



C 2D-gel electrophoresis of extracellular proteom at the end of the culture

• Among **21 major spots** identified, 3 families of enzymes are mainly expressed at different levels in each culture condition : proteases (P) , polysaccharides hydrolases (H) and chitinases (C)

• The RP and several forms of ::GFP are identified in each gel. Proteolysis level of the RP supports results of 1D-gel electrophoresis

• Smeared spots and aligned spots of same MW would attest the presence of differently glycosylated or phosphorylated proteins

➔ *Culture conditions induce distinct secretion profiles. Presence of several protease families modify quality and recovery of the RP.*

Conclusion

• Productivity and quality of the recombinant product are influenced by culture conditions

➔ Surprisingly, glaB is broadly expressed in submerged culture at 800 rpm but is nearly absent in submerged culture at 200 rpm **(high shear stress effect?)**

➔ Aspersed BfR reaches middle RP productivity but immersed BfR leads to the best quality of the RP **(post-translational modifications effect?)**

• Secretion profile characterized by extracellular proteom is altered by culture conditions

➔ several proteins spots highlight different expression levels and different post-translational modifications : exoglucanase (exgA) and chitosanase (csnC)

Perspectives :

➔ implementation of the fungal BfR in a continuous process in order to improve productivity

➔ experiment cycles of aspersion/immersion in order to increase secretion and recovery of the RP

➔ construct and experiment a new transcriptional reporter with a promoter characterizing biofilm conditions

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