

Context & Objectives

Fine chemical and pharmaceutical industry

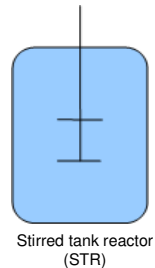


Filamentous fungi such as *Aspergillus sp.* or *Trichoderma sp.*

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

Currents fermentation bioprocesses

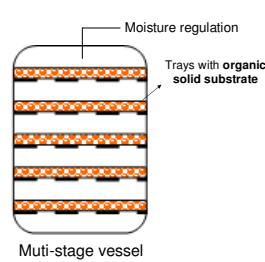
Submerged culture



→ fungal biomass looks like "balls of wool"

- (+) simple implementation
- (-) high viscosity, shear stress

Solid-state culture



→ fungal biomass looks like a "wool carpet"

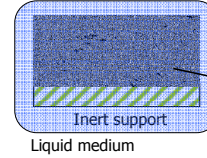
- (++) enhancement of metabolites secretion and high productivity
- (-) heat removal, downstream process operations

What? Weaknesses of these processes need to be improved!

Methodology

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

Overall scheme of a fungal biofilm reactor

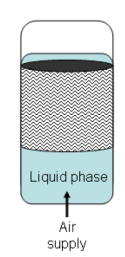


- biomass growth on inert support immersed in liquid medium
- enhances metabolites secretion!
- Fungal biofilm = "structured wool carpet with high water content"

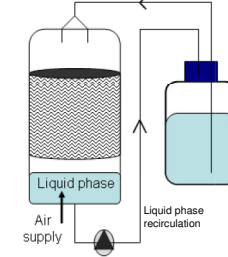
Task? Characterize secretion profile of two fungal biofilm reactors for the production of a recombinant protein

Scheme of fungal BfR designed for this work

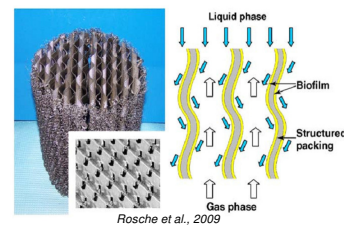
Immersed conditions



Aspersed conditions



Support = Metal structured packing with high specific area (750 m²/m³)

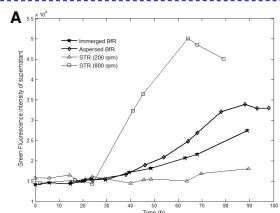


→ GLA::GFP recombinant protein (RP) containing glaA sequence linked to the GFP sequence is under the control of the **glaB** promoter **only** induced in **solid-state fermentation**

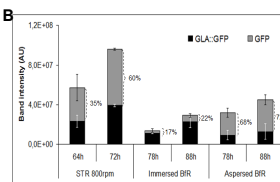
→ Secretion performances of the RP are compared between fungal BfR and submerged culture in STR. 2D-gel electrophoresis characterizes secretion profiles.

Results & Discussion

1. Production kinetic and detection of the RP

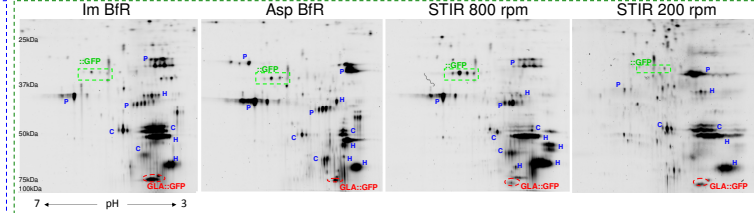


A shows production kinetic of RP in culture supernatant
 Surprisingly, STR with intense agitation leads to highest RP production whereas low agitation leads to the lowest
 → **Do high shear stress conditions activate *glaB*?**
 • Despite use of a specific promoter, we observe middle RP production in aspersed and immersed BfR
 → **Does biofilm thickness influence diffusional mass transfer of RP?**
 • a fraction of RP has been extracted from the biofilm matrix (results not shown)



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
- Smear 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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