

Optimization of 6-pentyl-alpha-pyrone, lytic enzymes, and spores production by Trichoderma harzianum in solid state culture

Rayhane Hamrouni, Josiane Molinet, Nathalie Dupuy, Ahmed Masmoudi, Sevastianos Roussos, Magali Claeys-Bruno

▶ To cite this version:

Rayhane Hamrouni, Josiane Molinet, Nathalie Dupuy, Ahmed Masmoudi, Sevastianos Roussos, et al.. Optimization of 6-pentyl-alpha-pyrone, lytic enzymes, and spores production by Trichoderma harzianum in solid state culture. Chimiométrie 2018, Jan 2018, Paris, France. hal-01782461

HAL Id: hal-01782461

https://hal.science/hal-01782461

Submitted on 2 May 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



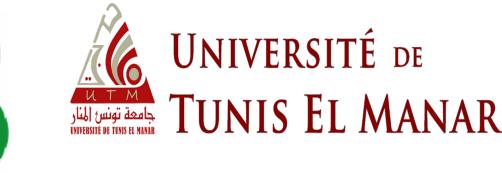












Optimization of 6-pentyl-alpha-pyrone, lytic enzymes, and spores production by Trichoderma harzianum in solid state culture

HAMROUNI R.^{1, 2}, MOLINET J. ¹, DUPUY N. ¹, MASMOUDI A ², ROUSSOS S.¹, CLAEYS-BRUNO M.¹

1. Aix Marseille Univ, Avignon Université, CNRS, IRD, IMBE, Marseille, France.

2. Univ. Manouba, ISBST, BVBGR-LR11ES31, Biotechpole Sidi Thabet, 2020, Ariana, Tunisia.

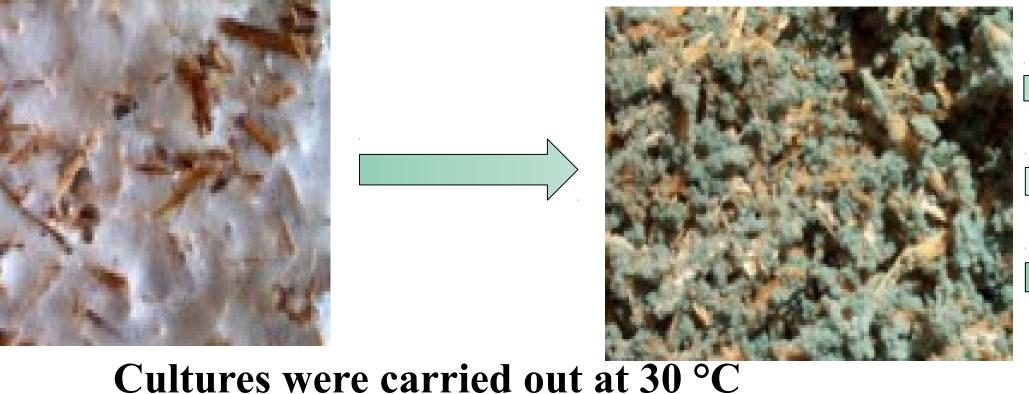
Introduction

Commonly the pests causing problems on food crops are treated with chemical pesticides, but the wrong and excessive application are causing severe damages to human health, to ecology by non-target organisms eliminated and the creation of pests resistant to pesticide. In recent years biopesticides have placed as a viable alternative to control pests and as a possible substitute for the traditional chemical used. Trichoderma is a filamentous fungus considered a biocontrol agent. It has been a very important model for study because of its mechanisms of action; nutrients and space competence, antibiotics production and lytic enzymes production systems using solid substrates have shown excellent yields for the production of filamentous fungal spores. Solid state culture (SSC) allows the valorization of agroindustrial wastes having an impact on the worldwide ecology. This production system has the potential to produce value-added products such as antibiotics, pigments, aromas like 6-pentyl-alpha-pyrones (6-PP) and enzymes like cellulases, chitinases, amylases, etc.. In this investigation, a 6-PP, lytic enzymes and spores producing strain of two *Trichoderma sp.*, were subjected to optimization of media and cultivation parameters for metabolites production.

SSC Production

was conducted in 250ml flasks containing 10g of mixed substrates were sterilized at 121 °C for 30 min. After cooling the medium was inoculated with a concentrated spore suspension of 2x107 spores/g dry matter (DM). Initial pH and moisture contents were 5.6 and 55 %, respectively.

6-PP production by *Trichoderma* species using SSC



6-PP (GC analysis) Spores (hemocytometer) Lytic enzymes (dilution suspension)

Precursors

Structure of 6-PP

Optimization of medium components for 6-PP, enzymes and spores production by two Trichoderma harzianum G7 and G18

Experimental design

The SSC allows the valorization of agroindustrial wastes generating positive impact on the worldwide ecology. Sugarcane bagasse and vine shoots are the major agroindustrial waste generated globally. They are an inexpensive material and could be used as carbon source. Today vine shoot is more accessible in Tunisia than sugarcane bagasse. Potatoes flour and wheat bran are used as starch source (20%). Due to there low concentration of ashes, Jaropha and Moringa are an excellent substrate for bioconversion process offering great approaches instead of wheat bran. They could be used as amino acid source. For oil source two sources are tested: argan and olive pomace because they are agroindustrial waste in Maghreb. Olive oil and chitin could be used as precursors enzymatic.

Factors Experience Amino acid source **Support Starch source** Oil source 30% **20%** 20% vine shoots potatoes flour Jatropha

enzymatic **20%** 10% olive oil olive pomace Chitin potatoes flour sugarcane bagasse Jatropha Argan wheat bran sugarcane bagasse olive oil Jatropha Argan vine shoots wheat bran Moringa olive oil Argan sugarcane bagasse potatoes flour Moringa olive oil Olive pomace vine shoots wheat bran Chitin Jatropha Olive pomace vine shoots wheat bran Jatropha Chitin Olive pomace vine shoots wheat bran Chitin Jatropha Olive pomace vine shoots potatoes flour Moringa Chitin Argan sugarcane bagasse wheat bran Moringa Chitin Olive pomace

A Hadamard design was used to study the effects of 5 variables in 8 experiments. The experiment 6 has been made in triplicate. The responses variables were; 6-PP, spores production and lytic enzymes.

Extraction and analysis of 6-PP

10 g of the fermented matter

Soxhlet extraction: at 60°C with 100ml heptane

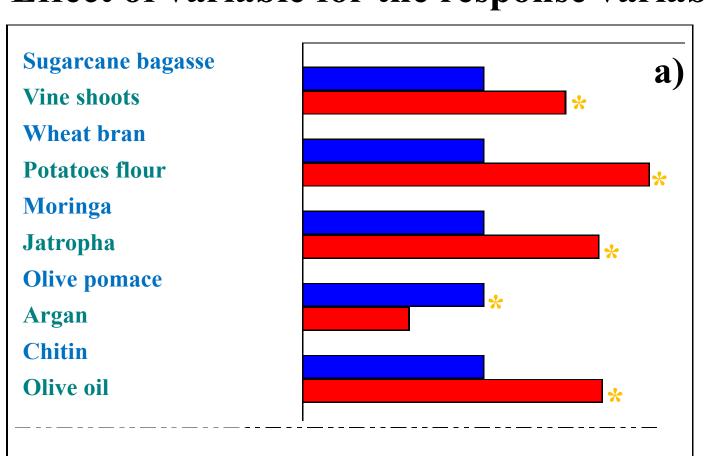
GC analysis: Supelcowax capillary column (\emptyset : 0.25mm, L: $60m, e_f : 0.25\mu m$), the carrier gas :H₂, 1,2ml.min; Split ratio: 2:1

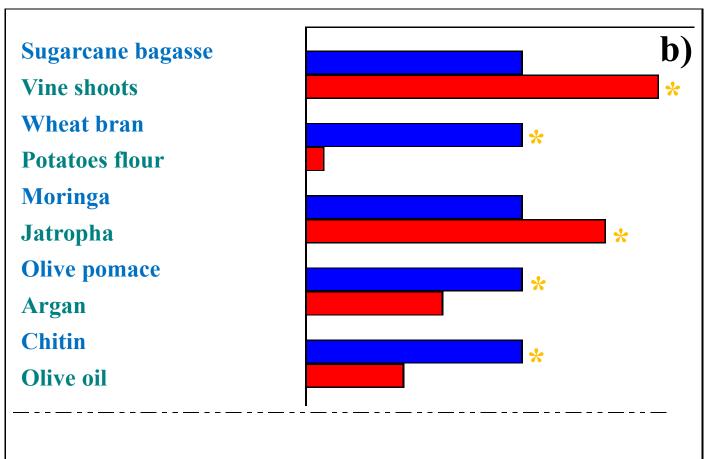
Quantitative analysis Internal calibration: γ-undecalactone

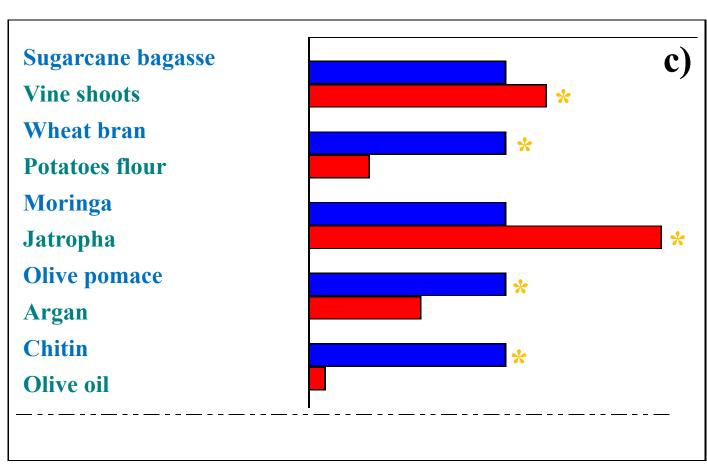
Results and interpretation of Hadamard design

From the results, we can calculate the estimation of the model coefficients using multilinear regression. In order to check the significance of these coefficients, a student test has been performed.

Effect of variable for the response variable 6-PP (a), cellulase enzyme (b) and spores (c) by and growth of *T,harzianum* G7







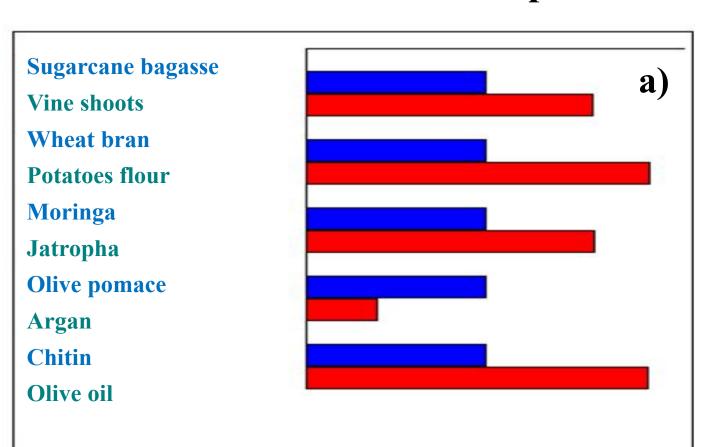
The data obtained showed a range of positive main effect values indicating that the presence of vine shoots, Jatropha and olive pomace in the growth medium positively affects 6-PP, spores and cellulases production by *T,harzianum* G7.

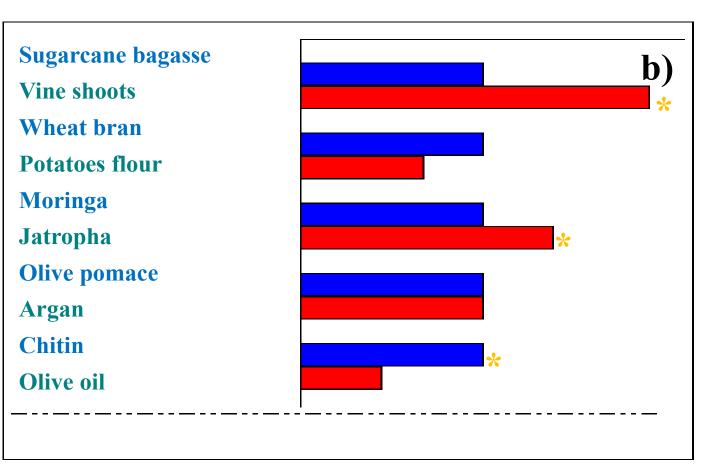
On the other hand, the presence of olive oil and potatoes flour result in low spores and cellulases activity and an increase in 6-PP production.

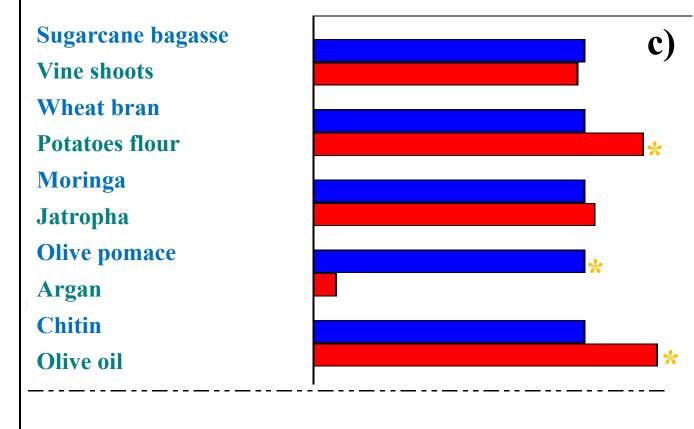
Olive oil was chosen to the optimized medium to stimulate *T.harzianum* G7 6-PP, even if it was not the best conditions for cellulases and spores production,

*: Significant variation of the response

Effect of variable for the response variable 6-PP (a), cellulase enzyme (b) and spores (c) by and growth of *T,harzianum* G18







The Hadamard design showed that cellulase production is more efficient when Jatropha is present in the medium. Vine shoot, wheat bran and chitine are better levels for cellulase production by *T,harzianum G 18*.

Olive pomace, olive oil and potatoes flour are more interesting for the spore production.

Conclusion and perspectives

- The Hadamard design showed that for *T,harzianum* G7 and *T,harzianum* G18 the optimized culture medium are different.
- As a result a medium of the following formula was predicted to be near the optimum, for producing metabolites in the culture filtrate of Trichoderma harzianum G7: Vine shoots, potatoes flour, jatropha, olive pomace, and olive oil (recuperation of 1mg/g substrate of 6-PP).
- For *T,harzianum* G18, Jatropha is replace by Moringa and olive oil by chitin.