Potential use of vine shoots as support to carry solid-state culture

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Keywords: Solid-state fermentation, *Aspergillus niger*, vine shoots, naphtho-gamma-pyrones, conidia**.**

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Abstract

In a context of growing awareness regarding environmental protection, biomass valorization is gaining a lot of attention. The byproducts volumes generated by agro-industry are massive and, left to decay, can constitute environmental pollutions. In France, the intensive production of wine produces tons of vine shoots every year and some of these byproducts enter in a primary valorization with polyphenols extraction. This high value-added molecules recovery does not reduce the volume of the vine shoots but generates materials exhausted in chemically active compounds. In this study, we evaluate the use of exhausted vine shoots as a support for fungal growth for conidia and secondary metabolites productions using solid state fermentation. The carbon/nitrogen ratio of the obtained fermented material is also measured in order to propose an ultimate way of valorization through composting.

Introduction

Solid state fermentation **(**SSF) is a very ancient process, initially used for human alimentation [1]. Numerous studies have shown advantages of SSF over its liquid homologous; advantages related to quantitative and qualitative aspects in the productions of enzymes and secondary metabolites by filamentous fungi [2]. However, one of the main driving forces in the gain of popularity SSF is gaining over the last decades is the growing concern about sustainability in bioprocess development linked to the possibility to use agro-industrial wastes, as cheap culture media to perform fermentations [3, 4]. Even so, to be fully both economically competitive and eco-friendly, the SSF must, to a larger extent, be carried on local byproducts. In this study, we investigate the potential use of Exhausted Vine Shoots (EVS) – *Vitis vinifera* – as support to carry SSF. EVS result from the ethanolic extraction of polyphenols present in the vine shoots. Indeed, wine production, as a major agricultural activity in France produces large amounts of waste including important volume of vine shoots [5]. Even so, many studies have shown that a diversity of polyphenol exists in the vine wood and, thus after extraction; it is possible to collect molecules with interesting properties [6,7]. This valorization process however does not reduce significantly the volume of the byproducts, which remains as a lignocellulosic substrate, the EVS [8]. In the present work, this by-product should not be considered only as a source of nutrients but mainly as a support providing suitable physical textures for the conidial anchorage and for mycelial growth of *Aspergillus niger* for the production of naphtho-gamma-pyrones (NγPs) which are secondary metabolites with interesting biological activities, including antioxidant properties. The potential use of EVS as a medium component in SSF process is evaluated in view of the metabolic performances of the fungus and compared to usual solid supports such as sugarcane bagasse and pine sawdust.

Material and methods

Microorganism and inoculum

A. niger G131 a non-ochratoxicogenic strain, provided by École nationale supérieure agronomique de Toulouse, France, was used for its ability to produce antioxidant NγPs. The fungal conidia were stored at 4°C in a 5 ml bottle on potato dextrose agar culture medium.

Solid-state fermentation

SSF was performed in 250 ml bottles containing 5 g of dry matter (DM), itself composed by 2.5 g of lignocellulosic support (sugarcane bagasse, EVS or pine sawdust) and 2.5 g of wheat bran. Each mixture was sieved to a 2-3 mm particle size. The humidity was set to 50% before sterilization. All the culture media were then autoclaved at 121°C during 1 hour. Each mixture was then inoculated with 2.10^7 conidia/g(DM), the volume of this added conidial suspension sets the initial humidity to

66%. The cultures were performed during 7 days in a laboratory incubator at 25°C. Each condition has been performed in triplicate.

Conidia and secondary metabolites analysis

All the results being expressed in by gram of dry matter, the water content of each sample was measured as followed: after 7 days, 1 g of fermented material was introduced into a lab oven at 105°C to analyze the relative humidity of the sample.
For the NγPs analysis, 1 g of fermented material was mixed to 10 ml of analytical ethanol during 1 hour. The ethanolic extract was then filtered with a 0.2 µm millipore filter into vials and then analyzed using HPLC system equipped with a DAD (Agilent Technologies©, United States of America). NγPs were detected using an analytical reversed-phase column C18 RP, Zorbax Eclipse XDB (Agilent Technologies©), 150 mm, 4.6 mm, 5 µm particle size. The initial elution conditions were 70% A (water with 2% of acetic acid) and 30% of B (acetonitrile) following an increasing gradient up to 100% of B during 50 min. The NγPs peaks were detecting using their UV absorption specters on ChemStation (Agilent Technologies©) and their concentrations were expressed using a commercial standard, the rubrofusarin (Bioviotica Naturstoffe GmbH©, Germany).
Conidia suspension was prepared introducing 1 g of fermented material in 10 ml of distilled water with 0.01% Tween 80. The conidia were subsequently counted using a traditional hemacytometer counting as described by Roussos et al. [9].

Elemental analysis

Elemental analysis have been performed on culture medium prior and after a 7 days fermentation. The medium was composed of 50% of EVS and 50% of wheat bran. Total carbon (% C), total nitrogen (% N) and C/N ratio were determined with an elemental analyser FlashEA 1112 (Thermo Fisher Scientific©, United States of America) from homogenized oven-dried samples.

Results and Discussion

The NγPs and conidia productions show no differences depending on the support used (Fig. 1). This means that no elements linked to the physical structure or the chemical composition of the EVS hindered the growth and the metabolism of *A. niger* cultivated in SSF.

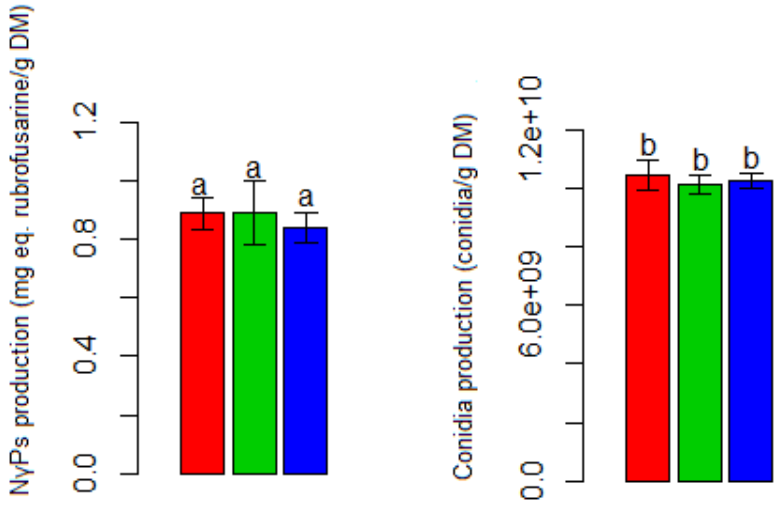

Figure. 1. Fungal production on solid medium using various supports.
On the left, the NγPs production and on the right the conidia production. In red the sugarcane bagasse, in green the EVS and in blue the pine sawdust. A t-test showed no significant difference between the production according to the support used at a risk α = 5%.

In SSF, the medium has to be considered following two aspects: as substrate, it has to efficiently provide microbial nutritional needs, and as support of culture, it has to possess favorable physical properties having consequence on water availability and allowing initial conidial anchorage, mycelial elongation in space and mass and heat transfers to occur over time [3]. In this case, the focus is made on the support aspect of the medium. Indeed, ligneous byproducts like EVS or pine sawdust are rich in biopolymers recalcitrant to the enzymatic degradation by *A. niger*. Each of the tested supports is the final byproduct of a previous agro-industrial process: sugar extraction for the sugarcane bagasse, residual woodchips from sawmills for pine sawdust and polyphenols extraction for the vine shoots.

The lack of significant difference in the NγPs and conidia productions between the medium containing EVS and the other two suggests that the vine shoots have been correctly exhausted in polyphenols that may otherwise inhibit the fungal growth. Polyphenols like stilbenes have indeed antifungal properties [10, 11]. Sugarcane bagasse and pine sawdust being considered neutral in terms of polyphenols composition, the EVS are therefore suitable for fungal culture.
The choice of industrially proceeded byproducts is also important for SSF processes because it decreases the batch-to-batch variations. Indeed, because of their complex nature, natural byproducts may increase the experimental variability between replicates leading to variations in the process performance. As an example, polyphenol composition varies a lot in numerous plants, depending on the year and climatic events that occur during it. Therefore, using byproducts from the same industrial origin that share the same standardized pretreatments reduces the heterogeneity that may exist between two stocks of the same byproduct [12].
The EVS, despite similar levels of polymer composition, is less water absorbent than the other two substrates [13, 14]. This difference, linked to the flexibility of the structures may however be an advantage in case of agitated cultures. Indeed, the resistance to compaction phenomenon occurring in agitated bioreactors may allow the medium to keep its favorable structural properties towards the fungal growth. Encouraging results (data not shown) seem to validate this hypothesis, as good conidia and secondary metabolites production were obtained when *A. niger* G131 was grown on EVS in a pilot-scaled agitated bioreactor.
Composting is a possible ultimate way of valorization that could follow the previous high value-added molecules or conidia production. The C/N ratio is a marker to determine if the obtained fermented material is a good candidate to composting process. The Fig. 2 shows the evolution of C/N ratio of a solid medium – containing 50% of EVS and 50% of wheat bran – prior inoculation and after 7 days of fermentation. The fermentation process has no effect on the global elemental composition of the solid material.

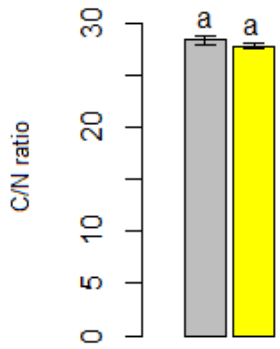

Figure 2. Evolution of C/N ratio of a solid medium after a 7 days fermentation.
In grey the C/N ratio of the medium prior inoculation and in yellow the C/N ratio of the medium after 7 days of fermentation. A t-test showed no significant difference between the ratio used at a risk α = 5%.

The initial carbon to nitrogen (C/N) ratio is one of the most important factors influencing compost quality). In general, initial C/N ratio of 25–30 are considered ideal for composting [15]. With a C/N ratio below 30, the fermented material may be composted, therefore completing in a final step the valorization of vine shoots.

Conclusion

Cost and availability are the main factors to consider for the selection of suitable medium component in SSF. An abundant and low-cost agricultural solid byproduct such as EVS which is generated by the annual pruning of vineyards, has been successfully used as solid support in the preparation of SSF with a view to develop a new process for fungal conidia and secondary metabolites production. The opportunity for local agro-industrial byproducts of low commercial value to be used in a biotechnological process, positions this process in an important valorization approach.

Acknowledgements

Support from Vinovalie (CIFRE ANRT N° 2015/0027) is gratefully acknowledged.

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