Economic laccase production from halotolerant fungi

1, Zeyneb BOUCHERIT; 2, Raouf ZEHIOUA ; 3, Aicha MECHAKRA; 3, Souad AMEDDAH

1: Laboratoire d'Obtention des Substances Thérapeutiques, UFMC1, 25000, Constantine, Algeria

2: Laboratoire de l'ingénierie des procédés de l'environnement. Université UC3SB. 25000, Constantine, Algeria

3: Laboratoire de Biologie et Environnement, UFMC1, 25000, Constantine, Algeria

INTRODUCTION and OBJECTIVE

Laccases are a multi-copper enzymes capable of oxidising poly phenols, non phenolic compounds , lignin and its derived compounds by reducing molecular oxygen as co-substrate to water, with notable stability to environmental conditions. Which makes them highly interesting for a wide variety of processes like paper and pulp industry, food processing, textile, dye degradation and bioremediation. All these applications involve the production of large scale of enzymes. Which is challenge in the case of laccase because it requires various carbon and nitrogen sources as well as expansive inducers like 2,5-xylidine, ferulic acid, guaiacol, aliphatic alcohols and some polysaccharides. Therefore, the use of the agro-industrial wastes for fungi growth could make the whole process much more economical. Consequently, the aim of this work is the production of laccases on the Olive-Oil Wastes (Olive Pomace and Olive Mill Wastewater) as unique substrate under SSF and SmF respectively by a halotolerant isolated fungi

METHODS

Microorganisms

A total of 23 strains were isolated on 20 % NaCl PDA from Algerian saline soil.

Screening for laccase activity

Each pure culture was inoculated on PDA supplemented with of Guaiacol then Petri dishes were incubated at 25° C until formation of a brown halo around colonies (Qasemian et al. 2002)

Screening for OMWW-decolourisation activity

Strains which presented laccase activity were cultured on diluted OMWW 2% Agar medium (Daassi et al 2014). The incubation was carried out at 25° C until discolored halos appeared.

Identification of the efficient fungus

Macroscopic aspects was evaluated By Pitt et Hocking (2009) method while microscopic identification was carried using optic microscope after coloration with blue cotton.

Salinity essay

The selected fungus was incubated in presence of increasing concentrations of NaCl (from 0 to 21%) then incubated under static conditions .

Laccase production on OMWW under SmF

The efficient strain was cultured on 50ml of OMWW liquid medium diluted to 10 and 20% with distilled water. the flasks were incubated at 25°C for 10 days under static conditions.

Laccase production on OP under SSF

10g of grinded and un-grinded OP with no nutrients supplementation were prepared separtelly in 250 ml flasks then inoculated and incubated up to 7 days at 25 °C.

RESULTS

Screening for laccase activity and *decolourisation of OMWW.*

Among the 23 halotolerant fungi isolated form the Algerian saline soil, three strains exibited laccase activity on Gauacol agar plate (brown halo around colonie) (Fig.1a). While on OMWW-agar plate method, one strain (GS15) showed the capacity of growth and decolourisation of the medium (Fig 1 b, c)

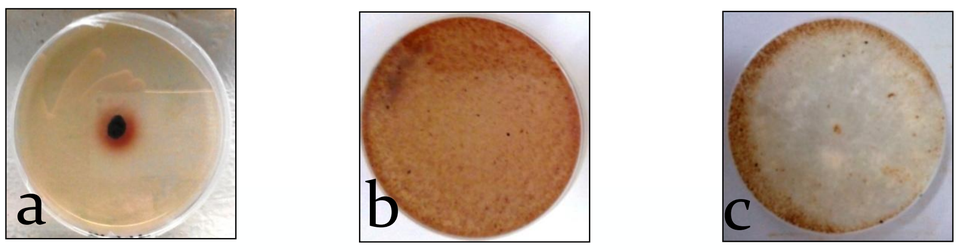


Fig 1. Laccase and decoulorization activity of GS15.(a): Guaiacol gar plate; (b): OMWW agar; (c): OMWW agar with GS15.

Identification of the efficient fungus

The strain GS15 was identified phenotypically as Penicillium sp according to Pitt et Hocking (2009) (fig.2 a,b,c)

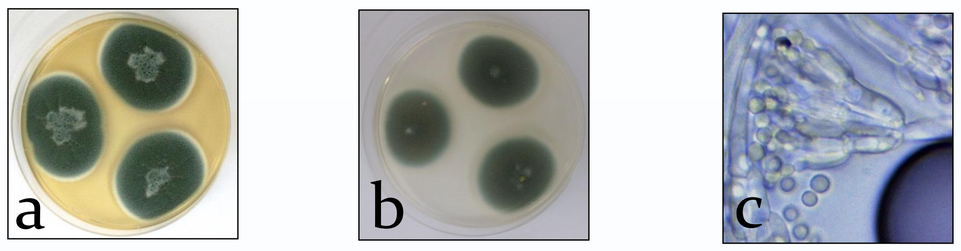


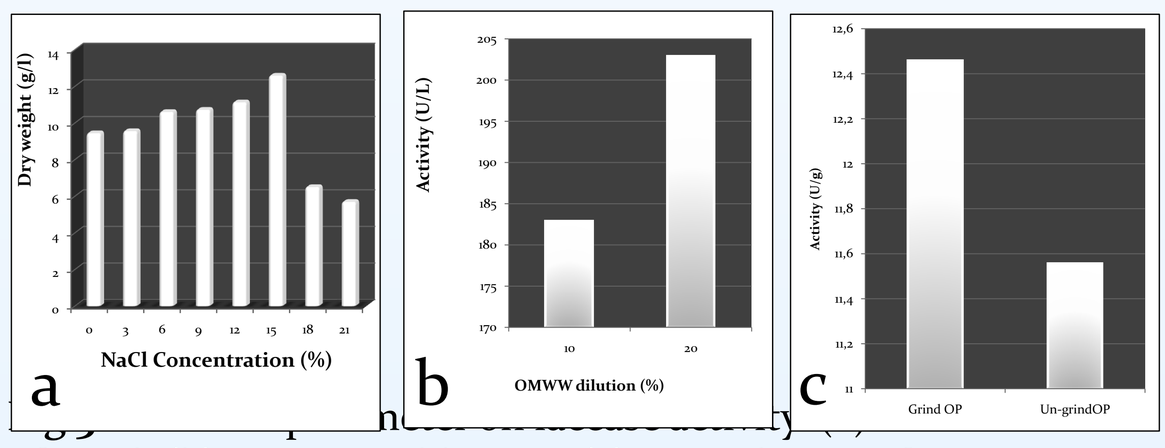
Fig 2: Different aspects of *Penicillium GS15*. (a): MEA, 25°C ; (b): CYA, 25°C; (c): microscope x100.

Salinity assay

P. Chrysogenum GS15 shows the ability to grow in the absence and in the presence of NaCl up to 21% (w/v) with the best growth at 15 % of liquid medium (Fig. 3a).

Laccase production on olive wastes

The enzymatic activities obtained on OMW's submerged culture were 183 U/L and for 10% dilution and 203 U/L for 20% dilution (Fig. 3b) whereas the solid culture on humidified OP shows 12,46 and 11,56 U/g for respectively un-grinded and grinded OP (Fig. 3c).



of NaCl; (b): OMWW dilution ; (c): Particle size of OP

CONCLUSION

Promoted laccase activity was determined on OMW and OP cultures media with halotolerant *P. chrysogenum* under low-coast conditions. Neither pretreatment except for sterilization nor supplementation excluding distilled water was utilised on SSF experiments. Where for SmF trials, none treatment or nutrient addition was carried out on OMW used for fungal growth. Which suggest the possible use of the halophilic strain for the economic production of laccases using phenolic agro-industrial wastes.

REFERENCES

- Pitt, J.I., Hocking, A.D., 2009. Fungi and Food Spoilage. Springer US, Boston, MA.

-Qasemian, L., Billette, C., Guiral, D., Alazard, E., Moinard, M., Farnet, A.-M., 2012. Halotolerant laccases from Chaetomium sp., Xylogone sphaerospora, and Coprinopsis sp. isolated from a Mediterranean coastal area. Fungal Biology 116, 1090–1098.

-- Daâssi, D.,Lozano-Sánchez,J.,Borrás-Linares, I., Belbahri, L., Woodward, S., Zouari-Mechichi, H., Mechichi, T., Nasri, M., Segura-Carretero, A., 2014. Olive oil mill wastewaters: Phenolic content characterization during degradation by Coriolopsis gallica. Chemosphere 113, 62–70. https://doi.org/10.1016/j.chemosphere.2014.04.053