Detoxification of molasses and production of mycelial mass and polysaccharides from *Morchella conica* mushroom.

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Abstract The impact of molasses on the fungal mass, valuable secondary metabolites' production and quality characteristics of the edible wild ascomycetous *Morchella conica*, strain AMRL78 was examined in this study. *Morchella* mycelium were produced in submerged, static and agitated cultures and the C/N ratio of the medium was determined to ~20. Evaluation included determination of the dry mycelial mass (X, g/L), endopolysaccharides synthesized (IPS, g/L), the consumption of total sugars (TS, g/L) and molasses' phenolic-reduction and decolorization. The influence of agitation conditions was also evaluated. Results showed that *M. conica* appeared a significant production of dry mycelial mass (up to 16 g/L) and agitation had some impact upon X formation. A simultaneous high consumption of TS appeared both in agitated and static cultures. Maximum values of ~ 4.5 g/L IPS were recorded. *M. conica* reduced satisfactory phenolic compounds and the color of the molasse substrate (up to 46% and 20%, respectively). It is concluded that *M. conica* is a fungus of higher importance, amenable for simultaneous molasses detoxification and production of added-value compounds.

Keywords: liquid fermentation, mushrooms, *Morchella*, agro-industrial wastes, molasses, endopolysaccharides, detoxification

1. Introduction

Waste and by-product streams of food supply chain are generated from agricultural production to consumption. Cultivation of mushrooms in liquid fermentations is a biological treatment, which could be considered as a rapid alternative process to produce fungal biomass and secondary metabolites of high quality (Barros et al., 2007; Fazenda et al., 2008). In parallel, valorization of agro-industrial renewable resources as substrates could satisfy such needs along with positive environmental impact by achieving its detoxification (Diamantopoulou et al., 2021; Mukhopadhyay and Guha, 2015; Sarris et al., 2020). Liquid agro-industrial waste streams such as molasses, olive-mill wastes, bio-diesel derived glycerol etc. could be used as potential substrate for mycelial mass and valuable metabolites production (Diamantopoulou et al., 2021; Sarris et al., 2020). Molasses, the liquid agro-industrial waste streams contain the high molecular weight polymer melanoidin and its release into the environment without appropriate previous treatment, may lead to inhibition of seed germination and depletion of vegetation by reducing the soil alkalinity and manganese availability and when discharged to water may cause eutrophication phenomena (Kumar and Chandra, 2006).

Morels (*Morchella* spp., Pezizales, Ascomycota) are among the most known edible wild mushrooms. They have attracted research interest due to their commercial value, medicinal properties (antitumor, cardiovascular, anti-inflammatory and anti-allergenic) along with their unique taste and aroma which make them as an ideal source of nutrition, rich in minerals and vitamins (Barros et al., 2008; Wasser, 2002). The difficulties on the cultivation of morels in lignocellulosic wastes, like saprophytic mushroom species, have led some researchers on their growth in liquid cultures aiming at the production of edible mycelium and the synthesis of secondary metabolites like polysaccharides (Papadaki et al., 2019; Sarris et al., 2020). Aim of the current study was to achieve detoxification (phenolic content reduction and decolorization) of the molasses contained in the nutrient substrate of the liquid cultures and due to their high content in sugars, to examine their effect on the cell growth of the edible wild ascomycetous, the black morel, *Morchella conica*, along with the production of endopolysaccharides of high nutritional, medicinal and gastronomical value. The influence of agitated (or non-agitated) conditions was also assessed.

2. Materials and methods

Fungal species, medium and culture conditions: *Morchella conica*, AMRL78 was deposited at of the Edible Fungi Laboratory/ITAP culture collection. Beet molasses, yeast

Table 1. Data originated from maximum values on molasses substrates in static-flask and agitated (120 ± 5 rpm) cultures of *Morchella conica* grown at 26 ±0.5 °C.

Parameters	Static cultures	Agitated cultures
X_{max} (g/L)	16.0 ± 0.3	18.2 ± 0.1
TS_{con} (%)	96.9 ± 0.6	97.4 ± 0.8
IPS (g/L)	4.6 ± 0.1	4.8 ± 0.1
Phenol reduction (%)	46.8 ± 0.6	56.5 ± 0.5
Color removal (%)	26.7 ± 0.5	20.3 ± 0.4

extract and peptone were mixed and the final C/N ratio determined to ~20 (TS_0 = 22.0 g/L). Either static or agitated (120 ± 5 rpm) cultures were performed in 100 mL Erlenmeyer flasks containing 30 ± 1 mL of growth medium. Flasks were autoclaved for 20 min at 121 ± 1 °C, allowed to cool and inoculated with 9 mm agar plugs cut from a growing colony in a PDA Petri dish. Incubation took place at 26±0.5 °C, in the dark, until total consumption of carbon sources.

Analytical methods: Dry weight (X, g/L) was gravimetrically determined. Total consumed sugars (TS_{con}) were determined according to Roukas (1996) and expressed as glucose equivalents according to 3.5-dinitrosalicylic acid (DNS) assay (Miller, 1959). Total endopolysaccharides (IPS, g/L) were determined according to Diamantopoulou et al. (2014) and were expressed as glucose equivalents. The phenolic content reduction in the culture medium was determined according to Folin - Ciocalteau method (Slinkard and Singleton, 1977), measured at 750 nm and expressed as gallic acid equivalent. The reduction of medium color was performed according to Sayadi and Ellouz (1992) protocol. Within experiments, three non-agitated and three agitated flasks were used to generate each data point.

3. Results and discussion

M. conica produced satisfactory amount of dry mycelial mass, both on agitated (18.2 g/L) and static (16.0 g/L) cultures and in parallel, up to 96% of total sugars were consumed at the end of fermentation period (19th day) (**Table 1**). Papadaki et al. (2019) reported lower biomass production (9.4 g/L) by the black morel *M. conica* grown on a glucose-based medium (14th day), whereas more than 90% of the initial sugars were consumed. Also, black morel, *Morchella elata* produced lower biomass (9.8 g/L) when was cultivated on molasses substrate and up to 80% of initial sugars were consumed (Sarris et al., 2020). As it turned out, agitation enhanced biomass formation and sugars assimilation in agreement with the perception that aeration favors mycelial growth in liquid media (Diamantopoulou et al., 2014). Total IPS in absolute values (g/L) was quite remarkable (4.5–4.8 g/L). However, agitation seem not to favor biosynthesis of IPS to a great degree, since in agitated (4.8 g/L) and static flask (4.6 g/L) cultures, IPS_{max} quantities were synthesized in a similar amount. The kinetic profile of IPS showed that there was a notable difference between agitated and static cultures, as IPS constantly increased as a function of the fermentation time on agitated flasks, whereas on static cultures values declined after the 14th day (**Figure 1**). This fact showed that agitation contributed to the adequate supply of nutrients to cells and facilitated the removal of gases and other by-products of catabolism from the microenvironment of cells until the end of the fermentation (Oh et al., 2007). Lower IPS values achieved when *Morchella esculenta* (yellow morel) was examined in liquid media with glucose as substrate, in static (3.9 g/L) and agitated (3.8 g/L) cultures by Diamantopoulou et al. (2014). Furthermore, a reduction in the IPS (g/L) values was detected towards the end of *M. esculenta* and were not significantly affected by agitation. Concerning the detoxification of molasses substrate, *M. conica* reduced satisfactory its phenolic compounds (46.8%; static, 56.5%; agitated cultures) especially on agitated cultures and sufficiently decolorization values were achieved (26.7%; static, 20.3%; agitated; **Figure 2**). This lower percentage of color removal suggests the occurrence of other type of compounds, different from the phenols that are not eliminated by the fungi assayed (Jiménez et al., 2003).

Concluding, the results of the present study are very interesting as there are not many studies in which edible wild ascomycetes are examined and finally achieve biological detoxification of liquid wastes' pollutant load (reduction of phenolic compounds and decolorization) and in particular of molasses that was used without further processing. Apart from ecological waste treatment, *Morchella* mushroom took advantage of the high sugars content of molasses and produced a significant amount of mycelial mass with simultaneous biosynthesis of endopolysaccharides that are important metabolites with great nutritional and commercial value used in both food and pharmaceutical industry.

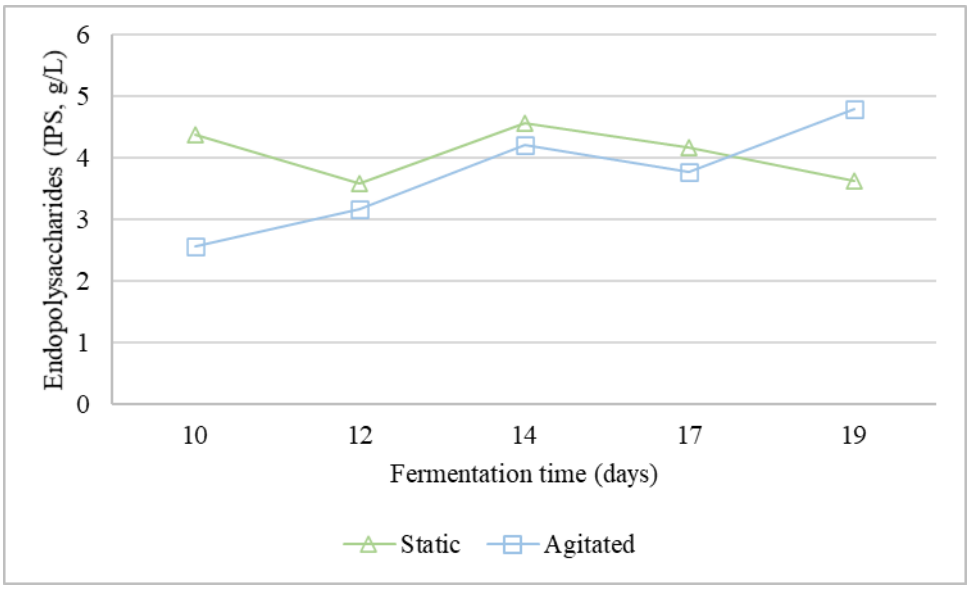

Figure 1. Produced endopolysaccharides (IPS, g/L) during the cultivation of *Morchella conica* in static and agitated (120 ± 5 rpm) cultures at 26±0.5 °C on molasses substrate (C/N =20).

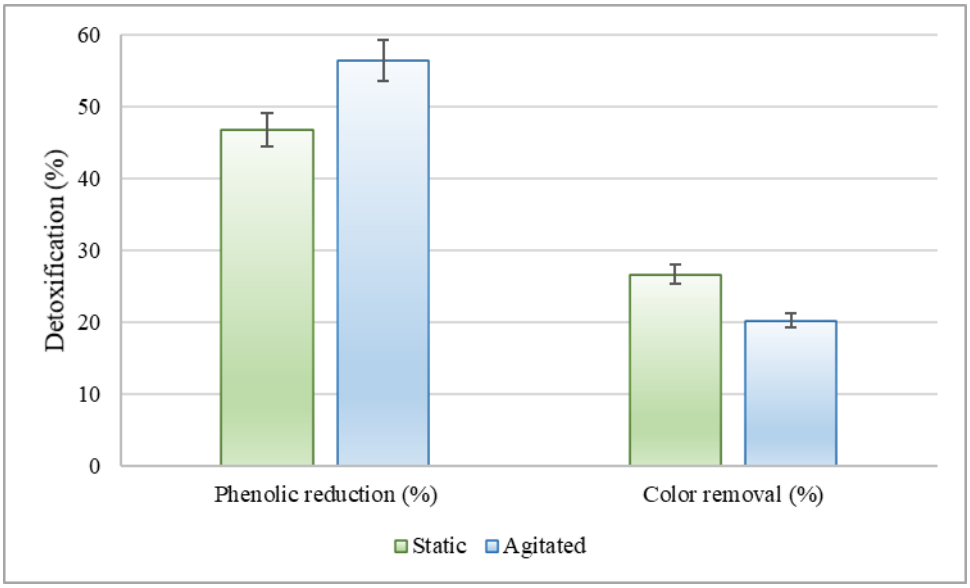

Figure 2. Phenolic reduction (%) and color removal of molasses substrates (C/N ~20) during the cultivation of *Morchella conica* mushroom in static and agitated (120 ± 5 rpm) cultures at 26±0.5 °C.

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