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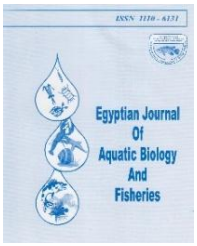

Production of biomass and exopolysaccharides obtained from *Pleurotus* hybrid strains under different pHs to remove heavy metals in aquaculture effluents.

Juan Valenzuela-Cobos[1]*, Fabricio Guevara-Viejó[1], Jesennia Cárdenas-Cobo[1]
Rafael Lazo[1], Delia Noriega[1], Ana Grijalva[2], and Fernanda Garcés[3]

[1]Facultad de Ciencias e Ingeniería, Universidad Estatal de Milagro (UNEMI) - Ecuador.
[2]Facultad de Ciencias Químicas, Universidad de Guayaquil – Ecuador
[3]Facultad de Ingeniería, Escuela Ingeniería Ambiental. Universidad Nacional de Chimborazo. Km 1 / vía a Guano s/n, Riobamba, 060150. Ecuador

***Corresponding Author:** juan_diegova@hotmail.com

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ABSTRACT

Hybrid strains of the genus *Pleurotus* (PO_5XPD_4 and PO_9XPD_5) were grown in glucose medium and yeast extract in air transport bioreactor (using different pHs 3.5, 4.5, 5.5, 6.5 and 7.5) for the production of mycelial biomass and exopolysaccharides (EPS). The highest biomass content (13.15 g L^{-1}) and the highest production of exopolysaccharides (1.95 g L^{-1}) were presented in the propagation of PO_5XPD_4 at pH 3.5. The liquid cultures under pH of 3.5 used in the cultivation of hybrid strains presented the highest production of exopolysaccharides that can be used to remove aquaculture effluents. The results showed that the production of biomass and exopolysaccharides (EPS) is directly related to the pH and the strain.

INTRODUCTION

Pleurotus is the second genus of edible fungi with the highest production in the world (**Adebayo *et al.*, 2018**), they are characterized by their nutritional value and are an important source of proteins, vitamins and minerals (**Manzi *et al.*, 2001; Reis *et al.* , 2012**). Studies have shown that some characteristics have been improved in the production of hybrid strains of these fungi such as: growth kinetics, morphological characteristics and nutritional composition (**Guadarrama-Mendoza *et al.*, 2014; Oropeza-Guerrero *et al.*, 2018**). These new characteristics are due to the separation of the nuclei during the formation of the monokaryons and their subsequent union during the formation of the dikaryon, causing phenotypic characteristics not present in the parental dikaryon to be expressed in the new hybrid strain (**Clark and Anderson, 2004**). The production of hybrid strains of edible fungi is a process that requires the mating of monokaryons previously obtained by chemical dedicariotization, a process that allows obtaining the two monokaryotic components of a dicarion using chemical substances such as: peptone and glucose (**Leal-Lara and Eger-Hummel , 1982; Aguilar-Doroteo *et al.*, 2018**).

Exopolysaccharides (EPS) have shown different applications in the food, pharmaceutical, cosmetic and other industries due to their properties such as: bioadhesives, probiotics, gelling, thickeners, bioadsorbents, emulsifiers and stabilizers (**Xu *et al.*, 2006; Öner *et al.*, 2016; Gil-Ramírez *et al.*, 2018**). Exopolysaccharides can be synthesized both intracellularly and extracellularly. If the first case occurs, they are excreted, but the synthesis route is unknown, and they also appear at different stages of the fungal life cycle and have different chemical structures; in turn, exopolysaccharides can remain attached to the cell surface or can be excreted in the culture medium (**Mahapatra and Banerjee, 2013**). The most common EPS producers are: bacteria and fungi, among the most important conditions to produce EPS are: pH between 3.0 and 6.5, temperature between 22 °C and 30 ° C (**Nguyen *et al.*, 2012; Mahapatra and Banerjee, 2013**). The use of industrial bioreactors allows obtaining large amounts of biomass in restricted spaces, having a uniform distribution in the substrate, reducing the cultivation periods without significant contamination problems (**Bae *et al.*, 2001; Kim *et al.*, 2002; García - Cruz *et al.*, 2019**). **Valenzuela-Cobos *et al.*, (2020)** presented that exopolysaccharides produced from the liquid culture of *Colletotrichum gloeosporioides* and *Rhizopus stolonifer* showed the adsorption of lead in in the sediment of aquaculture pool. However, there are no studies on obtaining biomass using hybrid strains of the genus *Pleurotus*, which will be used for the subsequent obtaining of exopolysaccharides that can be used to remove heavy metals in aquaculture effluents.

The objective of this research was to produce biomass and exopolysaccharides (EPS) of hybrid strains of the *Pleurotus* in an air transport bioreactor under different pHs (3.5, 4.5, 5.5, 6.5 and 7.5) .

MATERIALS AND METHODS

Biological material

In this research was used the hybrid strains of the *Pleurotus* (PO_5XPD_4 and PO_9XPD_5). The hybrid strain were obtained by mating compatible neohaplonts recovered by dedicariotization of parental strains of *Pleurotus djamor* and *Pleurotus ostreatus*.

Culture media

The culture medium was prepared by dissolving 39 g of PDA in 1 L of distilled water using an Erlenmeyer flask. The flask was sterilized in an autoclave at 15 psi (121 °C) for 15 min, subsequently, 10 mL of sterile medium was poured into Petri dishes. Plates with solidified medium were placed in plastic bags and incubated at 28 °C for 24 h to check sterility. Then, the Petri dishes without contamination were used for the propagation of the mycelium of the hybrid fungi (**Eger *et al.*, 1976; Coello-Loor *et al.*, 2017**).

Inoculation procedure

PDA agar mycelium discs (6 discs) (0.5 cm in diameter) were inoculated into 100 mL of glucose medium and yeast extract (5 g/L) for 4 days at 200 rpm on an orbital shaker. Subsequently, the 100 mL inoculated were added in a 1 L Erlenmeyer flask with 500 mL of fresh medium. Seven days later, it was used as inoculum for the 3.5 L bioreactor using 2.5 L of working volume (**Marquez-Rocha *et al.*, 1999**).

Bioreactor

A 3.5 L internal loop air transport bioreactor (with a 2.5 L working volume) of cylindrical geometry with an internal diameter of 14 cm and a height of 40 cm was used. The suction tubes are made of transparent acrylic plastic with a thickness of 0.5 cm. The draft tube has an inner diameter of 10 cm and a height of 16 cm. The upper and lower clearances were 17 cm and 3.5 cm, respectively. The ratio of the cross section of the down tube and the up tube areas (Ad/Ar) is approximately 1. Air was supplied through porous air diffusers at the bottom of the riser section. Inlet air was passed through a polytetrafluoroethylene (PTFE) membrane filter (0.1 µm pore size). Different pH levels were used (3.5, 4.5, 5.5, 6.5 and 7.5) using solutions of 2% sulfuric acid (H_2SO_4) and/or 2% sodium hydroxide (NaOH). The temperature was kept at 37 ^oC (**Nitayavardhana *et al*., 2013**).

Determination of biomass

The cultures were filtered through a 15 mm mesh Nitex cloth (B. & S. H. Thompson Co., Scarborough, Ontario, Canada). The mycelium (approximately 25 mL of broth) were washed with approximately 200 mL of deionized water and then placed on a previously weighed filter paper. The filter was placed in a drying oven at 90 ^oC overnight (**Nitayavardhana *et al*., 2013**).

Production of exopolysaccharides

The culture broth and water used to wash the biomass from the sieves were filtered through Whatman # 1 filter paper and evaporated to 50 mL under reduced pressure at 80 ^oC. In this reduced volume, 150 mL of ethanol was added to precipitate the polysaccharides. Finally, the precipitated exopolysaccharides (EPS) were filtered and dried at constant weight at 40 °C (**Wagner *et al*., 2004; Rasulov *et al*., 2013**).

Statistical analysis

In all analyzes, a completely randomized design and the results were studied using one-way analysis of variance (ANOVA) to determine the significance of individual differences at $p<0.05$ level, of the production of biomasses and exopolysaccharides, when statistical differences were found, the Duncan Test with $\alpha = 0.05$ was applied. The analyses were carried out using statistical software (Statgraphic ver. 16).

RESULTS

Production of biomass

Table 1 shows the biomass production from the two liquids submerged under different pHs (3.5, 4.5, 5.5, 6.5 and 7.5) used in the cultivation of PO_5XPD_4 and PO_9XPD_5. The liquid culture at different pHs (3.5, 4.5, 5.5, 6.5 and 7.5) used in the propagation of PO_5XPD_4 presented biomass contents ranged from 8.11 to 13.15 g L^{-1}. The results obtained using the liquid culture under pH 3.5 showed the highest biomass content (13.15 g L^{-1}), while the lowest biomass content (8.11 g L^{-1}) was presented at pH 7.5 in the propagation of this strain. Otherwise, the use of the liquid culture under different pH 3.5 in the cultivation of PO_9XPD_5 showed biomass contents between 7.41

and 12.58 g L^{-1}. The results obtained using the liquid culture under pH 3.5 showed the highest biomass production (13.15 g L^{-1}), while the lowest biomass content (7.41 g L^{-1}) at pH 7.5 in the propagation of this fungus.

Table 1. Biomass production obtained from hybrid strains of the genus *Pleurotus* grown in two different liquid culture media under different pHs.

Strains	pH	Biomasses (g L^{-1})
PO_5XPD_4	3.5	13.15±0.21[a]
	4.5	13.08±0.15[b]
	5.5	10.80±0.06[d]
	6.5	8.52±0.21[e]
	7.5	8.11±0.04[e]
PO_9XPD_5	3.5	12.58±0.12[c]
	4.5	12.05±0.42[c]
	5.5	10.42±0.09[d]
	6.5	9.84±0.24[e]
	7.5	7.41±0.05[f]

***All values are means ± standard deviation of ten replicates.**

Production of exopolysaccharides

Table 2 presents the exopolysaccharide production of the two liquid cultures under different initial pHs (3.5, 4.5, 5.5, 6.5 and 7.5) of hybrid strains (PO_5XPD_4 and PO_9XPD_5). The liquid cultures under different pHs used in the propagation of PO_5XPD_4 showed a production of exopolysaccharides since 0.52 to 1.95 g L^{-1}. The results obtained using the liquid culture at pH of 3.5 in the cultivation of PO_5XPD_4 showed the highest exopolysaccharide production (1.95 g L^{-1}), while using the liquid culture at 7.5 presented the lowest exopolysaccharide content (0.52 g L^{-1}). For otherwise, under different pHs in the cultivation of PO_9XPD_5 showed exopolysaccharide contents between 0.65 and 1.45 g L^{-1}. The results obtained using the liquid culture at pH of 3.5 in the cultivation of PO_5XPD_4 presented the highest content of exopolysaccharides (1.45 g L^{-1}).

Submerged cultivation represents an alternative form of rapid and efficient production of mycelial biomass and exopolysaccharides (**Confortin *et al.*, 2008**). The nitrogen source medium tends to be the most expensive. Peptones represent not only a source of organic nitrogen, but also a source of specific amino acids or peptides. They are defined as protein hydrolysates that are readily soluble in water and not precipitable by

heat, by alkalis, or by saturation with ammonium sulfate. The most commonly used peptones for microbiological studies are bactopeptone, tryptone peptone (TP), fish peptone (FP), meat peptone, neopeptone, and protease peptone (**Parrado *et al*., 1993; Dufosse *et al*., 1997; Taskin and Erdal, 2011; Vasileva-Tonkova *et al*., 2007**). The production of biomass and exopolysaccharides is modified with different pH values.

Table 2. Production of exopolysaccharides obtained from hybrid strains of the genus *Pleurotus* grown in two different liquid culture media under different pHs.

Strains	pHs	Exopolysaccharides (g L^{-1})
PO_5XPD_4	3.5	1.95 ± 0.01^{a}
	4.5	1.46 ± 0.02^{b}
	5.5	0.98 ± 0.00^{d}
	6.5	0.74 ± 0.12^{f}
	7.5	0.52 ± 0.00^{h}
PO_9XPD_5	3.5	1.45 ± 0.09^{b}
	4.5	1.21 ± 0.01^{c}
	5.5	0.85 ± 0.00^{e}
	6.5	0.74 ± 0.10^{f}
	7.5	0.68 ± 0.05^{g}

***All values are means ± standard deviation of ten replicates.**

CONCLUSION

The highest production of biomass was obtained in the internal loop air transport bioreactor working with a pH of 3.5 in the cultivation of hybrid strains of the genus *Pleurotus*. The liquid cultures under pH of 3.5 used in the cultivation of hybrid strains presented the highest production of exopolysaccharides that can be used to remove aquaculture effluents. The production of biomass and exopolysaccharides is modified with different pH values.

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