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Lipase production by a Tunisian *Fusarium solani* strain cultivated on olive oil wastewater-based media and a biotreatment assay

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ABSTRACT

The ability of a lipolytic fungus, *Fusarium solani*, to grow on olive mill wastewater (OMW)-based medium and to produce a high value compound, while degrading this waste was tested. OMW with high values of chemical oxygen demand (COD) was supplemented with yeast extract to ensure the growth of this fungus. In shake flask batch cultures, OMW led to a lipase activity of about 14 U/ml. *F. solani* growth on OMW showed high oil and COD removal efficiencies of 100 and 24.1%, respectively. A maximum phenolic compounds removal, about 46% of the initial content, was achieved and a decrease in 68% of total sugars was registered at the second day of culture.

Keywords: Fusarium solani; Olive mill wastewaters; Phenolic compounds; Lipase activity

1. Introduction

The olive mill industry is a traditional agricultural industry in Mediterranean countries. These countries produce almost all the olive oil sold worldwide. Large amount of liquid waste named olive mill wastewater (OMW) is generated. The organic fraction of OMW includes sugar, polyphenols, pectins, and lipids, which results in high values of chemical oxygen demand (COD) [1]. These components found in OMW are difficult to degrade and their disposal presents a critical environmental problem [2].

Different approaches have been developed for OMW biological treatment, such as anaerobic digestion [3], natural biodegradation in evaporation ponds [4], using bacteria, such as *Ralstonia* sp. and *Pseudomonas* [5] and using fungi [6]. Biological treatment of OMW by aerobic microorganisms (fungi and yeasts) has been shown to reduce OMW toxicity and to improve the biodegradability in anaerobic digestion [7], but no valorization of the OMW is made [8]. Yeast species, such as *Yarrowia lipolytica*, *Candida rugosa*, and *Candida cylindracea*, can grow well in OMW media, consume the organic material and, at the same time, produce biomass and other valuable products, like enzymes (such as lipases) and organic acids (such as citric acid) [1,9]. Other reports describe the reduction of the COD and the phenolic fraction of an OMW using immobilized *Geotricum candidum* cells [10]. Fungi were also used to degrade phenolics in OMW. Several filamentous fungi have revealed interesting capacities for the removal of problematic OMW compounds [11]. A variety of white-rot fungi have been used including *Pleurotus ostreatus*. This fungus grown in bioreactor batch cultures in a model phenolic wastewater (OMW), caused significant phenolic removal which

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resulted in a decrease of the OMW toxicity against the seeds of *Lepidium sativum* [12].

The aim of the present investigation is to study: the ability of a filamentous fungus—*Fusarium solani*—to grow on OMW, the valorization of this waste by producing lipase and to test the potential of this lipolytic fungus for OMW bioremediation.

2. Materials and methods

2.1. Olive mill waste water

The composition of OMW obtained from local olive oil manufacture (Sfax-Tunisia) and used in this study is shown in Table 1. It was centrifuged for 15 min at 4,000*g* to eliminate solids and materials and stored at −20°C until further use.

2.2. Microorganism and inoculum preparation

F. solani was isolated and identified in our laboratory [13]. The culture medium, in which the inoculum was prepared, contained 15 g/l casein peptone, 5 g/l yeast extract, 1.75 g/l KH_2PO_4, and 0.5 g/l $MgSO_4$ at pH 6. Preculture was incubated aerobically for 24 h at 30°C and 160 r/min.

Table 1
Composition and features of the OMW used

Parameter	Olive-mill wastewater used
pH	5.46 ± 0.04
Total sugars (g/L)	40.11 ± 0.29
Total phenols (as gallic acid) (g/L)	12.24 ± 0.15
COD (g/L)	80.59 ± 0.25
Total nitrogen (g/L)	ND
Lipids (g/L)	2.4 ± 0.36

Note: ND: Not Detected.

2.3. Growth of F. solani in OMW

Cultures of *F. solani* were carried out in 250-ml Erlenmeyer flasks containing 50 ml sterilized OMW. In order to counteract the lack of nitrogen, the OMW was enriched with different source of nitrogen at a 5 g/l concentration (Table 2) and pH was adjusted to 6. After inoculation with 1% (v/v) of *F. solani* suspension, cultures were incubated aerobically for 5 d on a rotary shaker set at 160 r/min and 30°C. A control flask (without inoculation with *F. solani* suspension) was run at the same time of inoculated flasks.

2.4. Analytical methods

Mycelium was filtered on preweighed Whatman discs (diameter, 47 mm) and the harvested biomass was washed with distilled water. The filter was dried at 100°C until constant weight, cooled in desiccators and weighed. Determination of total sugars concentration was conducted according to Dubois method [14]. Total phenols were assessed by the Folin–Ciocalteu method using gallic acid as a standard. Lipids were determined gravimetrically after chloroform/methanol (2/1) (v/v) extraction. Oil-degradation rate (%) was defined as the amount of oil degraded vs. the amount of initial oil. COD and nitrogen were determined according to standard methods [15].

2.5. Lipase activity measurement

Lipase activity was assayed potentiometrically by automatic titration of free fatty acids released from mechanically stirred triacylglycerol emulsions using 0.1 N NaOH and a pH-stat. Assay was performed at 37°C in a thermostated vessel using olive oil emulsion as substrate containing 3 mM $CaCl_2$ and 2 mM NaTDC.

One unit of lipase activity corresponds to 1 μmol of fatty acid released per minute under the assay conditions used.

Table 2
Effect of different nitrogen source on cell-growth and lipase production by *F. solani*

	Nitrogen source				
OMW	$(NH_4)_2SO_4$	Yeast extract	Soja peptone	Biomasse	Lipase activity
	−	−	−	−	−
	+	−	−	+	−
	−	+	−	++	++
	−	−	+	++	+

Notes: (−) not added and (+) added.

3. Results and discussions

3.1. Physicochemical characterization of the OMW

The OMW was characterized for total sugars, polyphenol content, COD, lipids, and total nitrogen. Mean results are presented in Table 1. This wastewater has a very high organic load (COD about 80.59 g/l). It contains also a high levels of phenolics and long-chain fatty acids (12.24 and 2.4 g/l, respectively) judged as phytotoxic and microbially inhibitory compounds. OMW used in this study was very poor in nitrogen, which can inhibit the growth of *F. solani*.

3.2. Effect of nitrogen source supplemented to OMW on growth and lipase activity of F. solani

To counteract the lack of nitrogen, we have added different nitrogen source ($(NH_4)_2SO_4$, yeast extract and soy peptone) to OMW. The effect of different nitrogen source on the growth of *F. solani* on OMW and on lipase production was shown in Table 2.

F. solani was able to grow on OMW used without dilution (data not shown). Table 2 shows that yeast extract supplemented to OMW was the best nitrogen source since it provide high lipase activity in the culture medium. The optimal yeast extract concentration added to OMW was about 5 g/L.

Fig. 1(a) shows the time courses of cell-growth and pH variation of the culture broth. As can be seen, the maximum dry weight reached 15.37 g/L after five days of culture. Since no significant change in pH value was occurred, cultures were conducted without pH correction.

Lipase production by yeast and fungi grown on OMW-based medium was previously studied in previous work. These studies reported that a lipase activity of 2.2 U/mL and 1.041 U/mL were produced by *C. Rugosa* and *Y. Lipolytica*, respectively [9]. Moreover, *C. cylindracea* [6] and *G. candidum* [16] produce a lipase activity about 16.8 U/L and 30 U/mL, respectively, on OMW-based medium. In the herein presented work, an important lipase activity of 14 U/mL, which as high as that measured using synthetic medium by the same strain, was reached [13].

Results show that OMW might be upgraded as a basis of a growth medium for *F. solani* producing an enzyme of commercial interest such as lipase.

Fig. 1(b) shows that the oil degradation rate increases with the time extension of the culture. The efficiency of removal of oil was 100% after five days of culture. The degradation of wastewater containing high concentrations of oil by *F. solani* was in accordance with other publications describing the removal of oil from industrial wastewater by lipolytic strains. Wu et al. [17] reported that *Y. Lipolytica* remove 93.3% of oil from salad oil wastewater.

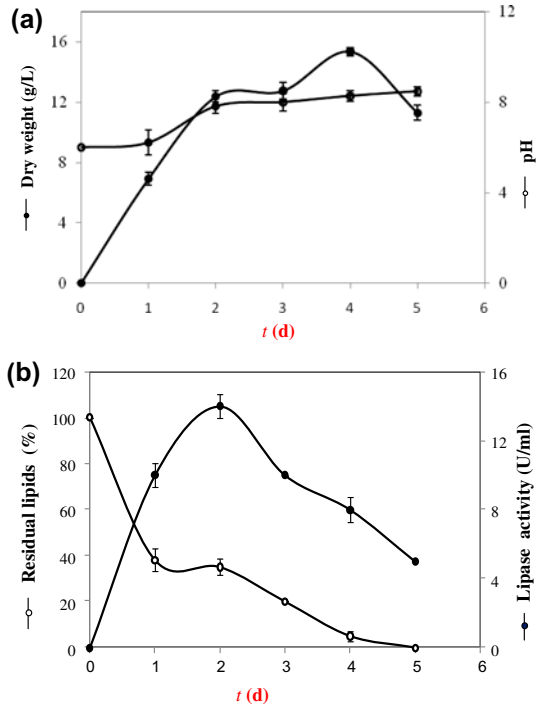

Fig. 1. (a) Time course of biomass (●) and pH (○) during the growth of *F. solani* on OMW. (b) Time course of extracellular lipase activity (●) and residual lipids (○) during the growth of *F.solani* on OMW. Values shown are mean ± SD.

3.3. Detoxification of OMW by F. solani

Pretreatment of OMW by the culture of filamentous fungi has been shown to reduce OMW toxicity [7]. In this study, we have used *F. solani* to evaluate the reduction of total phenol content of OMW operated by this strain. In fact, several species belonging to the genus *Fusarium* have been isolated from both soils and industrial effluents characterized by the presence of either phenols or aromatic hydrocarbons and oils [18,19]. For this reason, these fungi appear to be good putative candidates to perform the detoxification of an industrial effluent such as OMW.

For that, cultures were incubated at 30°C and 160 rpm for 5 d. As can be seen in Fig. 2, the total phenol removal was rapid during the first 3 d and decreased slowly thereafter. In fact, the maximum phenolic removal was about 46% of the initial content. The removal of phenolic compounds was attributed to the fungal activity not to the agitation since there is

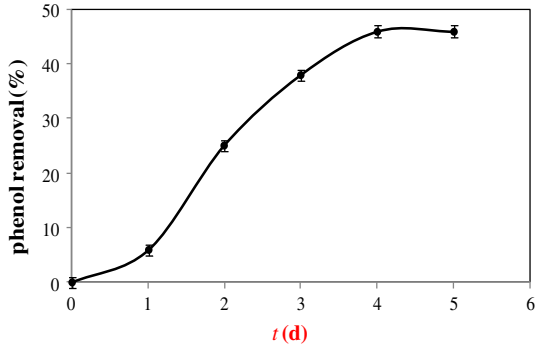

Fig. 2. Time course of phenol removal during the growth *F. solani* on OMW. Values are the averages of two independent experiments ± SD.

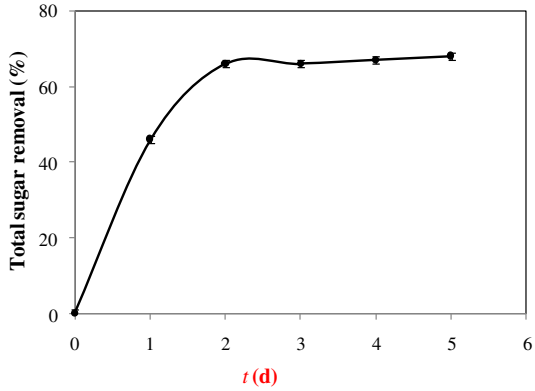

Fig. 3. Time course of total sugars consumption during the growth of *F. solani* on OMW. Values are means ± SD.

not a change in phenolic compound concentration observed in control experiment (agitation of OMW without a cultivation of *F. solani*). In fact, several investigations have been carried out using microorganisms able to grow on OMW in order to reduce its phenolic content. A variety of white rot fungi have been used for the remediation of OMW, such as *Corolus Versicolor* and *F. trogii* [20]. These fungi appear quite effective by removing phenolic compounds at high rate. The detoxification capability was also tried with other fungi. The use of *Aspergillus tereus* on OMW bioremediation reduces 60% of phenol concentration [21]. Moreover, *Y. Lipolytica* and *C. Rugosa* [9] were demonstrated to remove 31.3 and 27% of the initial content phenol, respectively. *F. solani* described in this study decreased efficiently the rate of phenol content without reducing color levels of treated OMW. Similar behavior was described by Ben Sassi et al. using a Marrocan yeast to detoxify OMW [22]. Besides the reduction of phenol concentration, the potential of removal of organic matter from this residue by *F. solani* was evaluated. Measure of COD was done before and after inoculation of OMW with *F. solani* (data not shown). Reduction of the COD was scored at the fifth day of culture and it was 24.1%. Similar results were observed by the use of the filamentous yeast *G. candidum* which achieve 25% of COD reduction [23]. However, it was reported that *Y. Lipolytica* and *C. Rugosa* reduce COD at an important rate of 51.3 and 70.2%, respectively [9,24].

The OMW used in this study contains an important concentration of sugars. The kinetic reduction of sugars during the culture of *F. solani* on OMW was described (Fig. 3). A high reduction value of sugars was obtained in this study; a rate of 68% was achieved at the 2nd day of culture (Fig. 3).

The *F. solani* strain used in this study has proved to grow well in undiluted OMW. Results of this study confirmed the potential application of *F. solani* for OMW valorization, by its use as culture medium for biomass and enzymes production. Moreover, the reduction of COD and phenolic compounds of OMW by this fungus underline the promising use of *F. solani* as a biotechnological tool for the removal of organic pollutants from OMW. Thus, the utilization of OMW as a resource to be valorized is of greater interest, since it could be used to produce high-value products while is being degraded.

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