Annals of Microbiology, **57 (2)** 143-147 (2007)

Yeast strains from the endogenous microflora of the olive flies *Bactrocera oleae* larvae which could degrade the olive oil mill wastewaters polyphenols

Malika CHAKRI[1], Ahmed EL HAIDANI[1], Mohammed EL MZIBRI[2], Abdellatif HAGGOUD[1], Mohammed IRAQUI[1], Abdellah HOUARI[1], Saad IBNSOUDA KORAICHI[1]*

[1]Laboratoire de Biotechnologie Microbienne, Université Sidi Mohammed Ben Abdellah, Faculté des Sciences et Techniques de Fès, Route d'Immouzer, BP 2202, Fès; [2]Unité de Biologie et Recherche Médicale, Centre National de l'Energie, des Sciences et Techniques Nucléaires (CNESTEN), BP 1382 RP. 10001 Rabat, Maroc

Received 20 September 2006 / Accepted 2 March 2007

Abstract - Worldwide, wastewaters constitute a major environmental pollutant. They are very toxic against a wide range of plants and soil microorganisms. Their toxicity is due to the presence of compounds such as polyphenols. In this study, we have isolated yeast strains from the endogenous microflora of the olive flies *Bactrocera oleae* larvae that were capable of degrading the olive oil mill wastewater polyphenols. The results obtained showed the presence, in the digestive tract of the larvae, of yeast strains resisting to polyphenols. Two resistant strains were isolated and have shown variable capacity of polyphenols degradation that could reach up to 72%. The two isolated strains were identified by two methods: conventional technique and molecular method associating PCR amplification and DNA sequencing of the 5.8S ribosomal RNA gene. Both techniques showed that the two isolated strains corresponded to the *Candida diddensiae* specie. Related to its capacity to degrade polyphenols, this specie would be a potential candidate for wastewater treatment and environmental protection.

Key words: olive mill wastewater, aerobic treatment, *Bactrocera oleae*, yeast strains, polyphenols.

INTRODUCTION

Olive oil mill wastewater (OMW) is a major pollutant of both terrestrial and aquatic ecosystems in the Mediterranean region (Mendia *et al*., 1986; Servis, 1986; Moreno *et al*., 1990). OMW is a dark-coloured, mildly acidic liquid of high conductivity and whose composition varies depending on olive fruit maturity and oil extraction conditions (Ehaliotis *et al*., 1999). The organic fraction OMW is rich in polyphenols and contains sugars, nitrogenous compounds, volatile acids, alcohols, pectins and fats (Moreno *et al*., 1990; Fiestas Ros de Urisinos and Borja-Padilla, 1996). The elementary composition of the inorganic fraction shows the presence of chloride, sulphate, and phosphoric salts of potassium as well as calcium, iron, magnesium, sodium and copper, and other trace elements in various chemical forms. In the OMW, due to their low concentrations, inorganic constituents are reported to be not toxic, and may potentially serve as good source of plant nutrients (Piperidou *et al*., 2000).

OMW pollution is mainly due to the organic acids such as acetic and fumaric acids and to the phenolic compounds. These components display antibacterial properties, inhibit seed germination and are phytotoxic (Perez *et al*., 1986).

To minimise these pollution consequences, considerable efforts were carried out to find biological methods for the oil mill wastewater polyphenols treatment. Bioremediation techniques employed to treat oil mill wastewaters used various microorganisms such as *Phanerochaete chrysosporium*, *Aspergillus niger* and *Aspergillus terreus* which were shown to be able to remove 92%, 76% and 64% of total polyphenols, respectively (Garcia *et al*., 2000). On the other hand, the ability of *Phanerochaete chrysosporium* to decolourise OMW and the possible involvement of the lignin degrading system was investigated (Sayadi and Ellouz, 1992). Yesilada *et al.* (1998) have shown that there is an effective degradation of the olive oil mill wastewaters by white rot fungi while Kahraman and Yesilada (1999) found that *Phanerochaete chrysosporium* (in 20% of the medium with cotton stalk) reduced the COD and the coloration by 48% and 58% respectively. *Funalia trogii* (in 30% of the medium with cotton stalk) also reduced both parameters by 51% and 55% respectively.

Bactrocera oleae is the most important pest insect of the olive fruit. The development of the *Bactrocera oleae* larvae occurs while nourishing themselves on the olive pulp. Polyphenols are among the most components of the olive

* Corresponding author: Phone: 212 66038407;
Fax: 212 35608214; E-mail: Ibnsouda@hotmail.com

pulp. Also, endogenous microorganisms of these larvae would be resistant to polyphenols and perhaps degrade them.

In our study, we were interested in isolating yeast strains from the endogenous microflora of the olive flies *Bactrocera oleae* larvae able to grow on oil mill wastewaters and in evaluating their capacity to degrade the oil mill wastewater polyphenols.

MATERIAL AND METHODS

Olive oil mill wastewaters (OMW) used. The original wastewaters used in this study were obtained from an olive oil mill located in Sidi Brahim industrial area in Fès, Morocco, which uses a modern process of olive's oil extraction. The composition of OMW that was used in all experiments is shown in Table 1. The characteristics of the OMW were determined as described by Rodier (1996).

TABLE 1 - Composition of olive mill wastewater used in experiments

Parameters	Values
pH	4.7
Calcium (g/l)	0.2
Chlorure (g/l)	0.4
Nitrate (mg/l)	50
Sulphate (mg/l)	3550
Orthophosphate (mg/l)	60
Magnesium (g/l)	2.22
Total phenolic compounds (mg/l)	440
Total solids (g/l)	7.95
Mineral solids (g/l)	1.35

Yeast strains isolation. Three *Bactrocera oleae* larvae were treated by the method described by Cavados *et al*. (2001) to eliminate external microorganisms. The larvae were then crushed in 400 ml of YPG medium (1% yeast extract, 2% peptone and 2% glucose, pH 4.5). The so obtained suspension (100 µl) was spread over dishes containing the olive oil mill wastewaters supplemented with agar 1.5%. Various dilutions of the OMW in sterilised distilled water were used (25, 50, 75 and 100%). The dishes were then incubated at 30 °C. In this study, we have used only the yeast strains able to grow on crude OMW, so colonies with yeast's morphological aspect were re-isolated on agar medium with crude concentrated olive oil mill wastewater. These colonies were transferred once more on YPG medium containing three antibiotics (ampicilline, 100 µg/ml; kanamycine, 20 µg/ml and tetracycline, 20 µg/ml) to eliminate bacterial growth. Four yeast strains were therefore selected and preserved at 4 °C for frequent use and at –80 °C for long storage.

Numeration of the yeasts. The four yeast strains selected were inoculated into 50 ml of a crude olive oil mill wastewaters liquid medium and incubated at 30 °C under agitation. The strains growth was compared to the one obtained in YPG medium. For cell's numeration, a series of dilutions (10^{-1}-10^{-12}) from the cultures were done. A volume of 10 ml of each dilution was deposed on YPG-agar and incubated at 30 °C for 48 h. The number of cells by ml corresponds to the mean of the values obtained for the dilutions where the colonies were countables.

Dosage of the total phenolic compounds. A volume of 2 ml of a 48 h old culture in YPG of the tested strain was added to 50 ml of crude olive oil mill wastewaters and incubated at 30 °C under agitation for several days. To estimate the total phenolic compounds and thus, to determine their biological breakdown, 1 ml of filtrated OMW was added to 2.5 ml of Folin-Dennis reagent and 35 ml of distilled water. After homogenisation, 10 ml of a solution saturated with 20% sodium carbonate were added. The volume was brought to 50 ml with distilled water, then, the absorbance was determined at 725 nm. The calibration range was prepared with various concentrations of tannic acid solution (0.05, 0.03, 0.025, 0.015 and 0.01) (Maestro-Duran *et al*., 1991).

Identification of yeast strains. Yeast strains were first characterised by the conventional method (Kreger-Van Rij, 1987; Barnett *et al*., 2000), using the "Yeast identification PC program, version 5, May 2000" (Licence No. 50041, delivered to our laboratory on July 2005). In brief, the physiological tests included fermentation of D-glucose, assimilation of carbon compounds, assimilation of nitrogen compounds, determination of vitamin requirements, temperature tolerance tests, cycloheximide resistance test, growth on osmophilic media and urea hydrolysis. The morphological testing included mode of vegetative reproduction and formation of pseudohyphae, true mycelium, and arthroconidia.

The molecular identification was based on PCR amplification and DNA sequencing of a fragment of the ribosomal RNA 5.8S gene (Drik, 2000). The amplification reaction was performed in a final volume of 50 µl containing 50 pmol of each primer (ITS-1: 5'-TCCGTAGGTGAACCTGCGG-3' and ITS-4: 5'-TCCTCCGCTTATTGATATGC-3'), 200 µM each dNTP, 0.5 units *Taq* DNA polymerase and 3 µl of DNA sample in 1x *Taq* polymerase buffer. The mixture was first denatured at 94 °C for 7 min. Then, 35 cycles of PCR were performed by denaturation at 94 °C for 30 s, primers annealing at 55 °C for 45 s, and primer extension at 72 °C for 90 s. At the end of the last cycle, the mixture was incubated at 72 °C for 7 min. For each reaction, a negative control missing DNA template, and a positive control were included. Efficient amplification was confirmed by gel electrophoresis on 1.5% agarose gel.

PCR products were purified using the Magnesil yellow solution (Promega) to eliminate the primers used for PCR reactions, then the sequence reaction for each purified product was done in thermocycler. The sequencing reaction was performed in a final volume of 20 µl containing 20 pmol of one primer (ITS-1 or IST-4), 3 ml of Big Dye (version 1.1) and 2 µl of purified PCR product. Twenty-five cycles were performed: denaturation at 96 °C for 10 s, primer annealing at 55 °C for 10 s, and extension at 60 °C for 4 min. To eliminate the excess of labelled ddNTPs, sequencing reaction products were purified using the above mentioned Magnesil green solution.

TABLE 2 - Growth of the yeast strains on YPG and crude olive oil mill wastewaters after 15 days of incubation

Yeast strains	Culture on YPG (cells/ml) at days				Crude olive oil mill wastewaters (cells/ml) at days			
	0	5	10	15	0	5	10	15
YB37	1.2×10^3	2.5×10^6	7.6×10^7	8.4×10^{10}	2.2×10^6	1.5×10^5	1.3×10^7	9.9×10^8
YB40	9.7×10^4	2.1×10^7	9.8×10^8	2.5×10^{12}	1.8×10^5	8.9×10^4	7.8×10^7	8.8×10^9
YB39	1.9×10^4	9.2×10^5	3.2×10^9	2.3×10^{12}	0	0	0	0
YB42	2.3×10^4	2.4×10^7	9.6×10^9	9.3×10^{12}	0	0	0	0
YB9 (Control)	1.5×10^5	8.3×10^5	9.7×10^7	1.2×10^{13}	0	0	0	0

Direct sequencing of amplified PCR products for the ITS fragment of the ribosomal RNA 5.8S gene was performed on an ABI PRISM sequencing apparatus (ABI Prism 310 Genetic Analyser, Applied Biosystem) and data analysis was done by sequencing analysis software. The sequencing was carried out for both strands.

Control strain. The control yeast strain used in this study was isolated in our laboratory from the same larvae of *B. oleae* that showed no growth ability on solid or liquid medium containing the crude olive oil mill wastewaters. This strain was identified as *Pichia guilliermondii* (data not shown) and was used as control to estimate the rate of polyphenol degradation in comparison with the tested strains.

RESULTS AND DISCUSSION

Isolation of the yeast strains

The development of *B. oleae* larvae occurs inside the olive fruits, which are normally very rich on polyphenols. The polyphenols toxicity inhibits the growth of a great number of microorganisms. The internal microflora of *B. oleae* larvae would be resistant to the polyphenols present in the olive fruit and would probably be able to metabolise these compounds. In this work, and among all microorganisms from *B. oleae* larvae that were able to grow on a solid medium containing the crude olive oil mill wastewaters, we have randomly selected four yeast strains, YB37, YB40, YB39 and YB42.

Numeration of the viable cells in YPG and the olive mill wastewaters

The results (Table 2) showed, that as expected the control strain did not grow in oil mill wastewater medium. However, among the four strains isolated on solid medium with crude olive oil mill wastewaters, only two (YB37 and YB40) were able to grow on this medium in a liquid form. This could be explained by the fact that the two strains (YB39 and YB42) are less resistant to the crude olive oil mill wastewaters and are not able to adapt and grow in the liquid form of this medium. Indeed, we found that these two strains are able to grow on 3% of diluted olive oil mill wastewaters. Several studies showed that some strains like *Pleurotus ostreatus*, which are resistant to the olive oil mill wastewaters, are not able to grow on the crude state of this medium but can grow on the diluted medium. *Pleurotus ostreatus* is inhibited by concentrated olive oil mill wastewaters (> 20%) (Martirani *et al*., 1996). Aissam *et al.* (2002) showed that yeast strains which have a very effective secondary metabolism with respect to the degradation of the phenolic compounds were able to grow on crude olive oil mill wastewater only in conditions where an adaptation to increasing concentrations of OMW and that the same strains were enable to grow if they are directly exposed to high concentrations of these wastewaters without previous adaptation.

These results show that the endogenous flora of the *Bactrocera oleae* contains yeast strains with a relative resistance range to the olive oil mill wastewaters' polyphenols.

Degradation of the phenolic compounds

The percentage of the phenolic compounds degradation was estimated for the two oil mill wastewater liquid resistant strains YB37 and YB40. As shown in Table 3, the two strains S37 and YB40 were able to degrade the oil mill wastewater polyphenols. After 60 days of incubation, degradation of 72 and 54% of phenolic compounds was observed with strains YB37 and YB40, respectively.

It is very likely that these strains could metabolise phenolic compounds and use them for their own growth since these results were obtained with olive oil mill wastewaters without dilution or addition of any additional nutrients. Similar results were obtained by Fadil *et al*. (2003) who showed that the percentage of the phenolic compounds degradation by *Geotrichum* sp., *Aspergillus* sp. and *Candida tropicalis* was about 50%, 46% and 55% respectively, but in this case the olive oil mill wastewaters were diluted and $(NH_4)NO_3$ and K_2HPO_4 were added to have a ratio of the COD/N/P around 100/5/1 and $(NH_4)_2SO_4$ (1 g/l). Aissam (2003) obtained a reduction of the phenolic compounds around 75, 63, 48 and 40% by *Aspergillus niger*, *Penicillium* sp., *Geotrichum terrestre* and *Candida boidinii* respectively, after adaptation of these four indigenous strains to grow on various increasing concentrations (25, 50, 75 and 100%) of OMW; which lead to the resistance of the micro-organisms to the phenolic compounds toxicity. Yesilada *et al*. (1999) showed that the rate of abatement of the phenolic compounds by the fungus *Pleurotus sajor-caju* could reach 81% after three days of growth and they announced the importance in biotechnology of the bio-treatment of olive oil mill wastewaters without addition of any source of carbon or other energies. In

TABLE 3 - Degradation of phenolic compounds at different times of incubation

Sample	0 days	15 days		60 days	
	(mg/l)	(mg/l)	Degradation (%)	(mg/l)	Degradation (%)
YB37	460	405	12	130	72
YB40	465	420	10	215	54
YB9 (control)	450	430	4	420	7
Crude olive oil mill wastewaters (control)	440	415	6	410	7

our case, we have obtained a very important rate of degradation of the polyphenols (54-72%) without any nutriment addition. This rate could be improved by addition of some mineral elements. The choice of additional elements could be a decisive factor to use this biotechnology in the degradation of polyphenols and bio-treatment of OMW.

The results obtained show the presence in the digestive tract of the *B. oleae* larvae of yeasts resisting the polyphenols. The resistant strains have a variable capacity of degradation of polyphenols and can reach 72%.

Identification of the yeast strains

Yeast identification was carried out by the analysis of various characters, mainly physiological and metabolic. The results obtained were analysed by the data-processing program of Barnett *et al*. (2000). This enabled us to identify the two yeast strains YB37 and YB40 as *Candida diddensiae* with the following scores 0.883 and 0.899, respectively. These values give an indication of the reliability of the identification. In fact, YB37 and YB40 didn't show any difference with *Candida diddensiae*.

To confirm this result, the two strains were subject to a molecular identification based on PCR amplification of the ribosomal RNA 5,8S with specific primers of the preserved areas. The ITS1 and ITS4 primers amplified the region of 5.8S rRNA that is between the 18S rRNA and 28S rRNA. The two isolates gave PCR products with the same size (500 bp). The two PCR products were then sequenced on both strands. The sequences are reported in Fig. 1. The sequences were compared with available DNA sequence databases using BLAST program (Table 4).

After comparing the sequences to the GenBank database, the strains YB37 and YB40 were identified as *Candida diddensiae*. The 517 nucleotides sequenced of strain YB37 have shown 99% of homology with the ITS DNA sequences found in the GeneBank database that correspond to *Candida diddensiae* strain, whereas the 469 nucleotides sequenced of the strain YB40 have shown 98% of homology with the same ITS DNA sequences (Table 4 and Fig. 1).

Aissam *et al*. (2002) have already isolated the yeast strain *Candida diddensiae* from oil mill wastewaters in the region of Fès (Morocoo). They have shown that *Candida diddensiae* was able to grow on oil mill wastewaters. This is in agreement with our results, and we can postulate that the origin of this strain is the *B. oleae* microflora.

The olive grove is one of the principal richness of the region of Fès in Morocco. The economy of this area is mainly supported by the olive industry, which generates many wastes rich in polyphenols that have a harmful impact on the environment as well as on human health. This industry must be imperatively accompanied by bioremediation programs to reduce the impact of polyphenols on the ecosys-

A

```
  1 ACCAGAAATTTACACCTGATCCTTCAACGAGTTGGATAAACCTAATACATTGAATAATCA 60
 61 GAAAGCACTATCTAGCGCACTCATGCGCGATACATCTCAAGCAAACGCCTAGTCTGACTA 120
121 AGAGCTATCACTCAATACCAAACCCAAAGGTTTGAGAGAGAAATGACGCCTCAAACAGGC 180
181 ATGCCCCTTGGAATACCAAAGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACGAAAA 240
241 TCTGCAATTCATATTACTTATCGCATTTCGCCTGCGTTCTTCATCGATGCGAGAACCAAG 300
301 AGATCCGTTGTTGAAAGTTTTGAAGATTATTTTGAATTTAATCAAACAAATTGACAATAA 360
361 TTTAAAAAACAATTCAATAAAAAATTGAAGTTTATTCAAGTCTCTGGCCCAACCCGAAGG 420
421 CCAAGCCAAAGCAATAGTTCTTGTAATAACAAAAAACACAGTGTGTAAGGATTAGTTCCC 480
481 GCCGCGCAATTAAGCGCTGGCAAAGAATTAATAACTG 517
```

B

```
  1 CAATTGCCACCAGAAATTTACACCTGATCCTTCAACGAGTTGGATAAACCTAATACATTG 60
 61 AATAATCAGAAAGCACCTATCTAGCGCACCTCATGCCGATACATCTTCAAGCAAACGCCT 120
121 AGTCTGACTAAGAGTATCACCTCAATACCAAACCCAAAGGTCTTGAGAGAGAAATGACGC 180
181 TCAAACAGGCATGCCCCTTGGAATACCAAAGGGCGCAATGTGCGTTCAAAGATTCGATGA 240
241 TTCACGAAAATCTGCAATTCATATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCG 300
301 AGAACCAAGAGATCCGTTGTTGAAAGTTTTGAAGATTATTTTGAATTTAATCAAACAAAT 360
361 TGACAATAATTTAAAAAACAATTCAATAAAAAATTGAAGTTTATTCAAGTCTCTGGCCCA 420
421 ACCCGAAGGCCAAGCCAAAGCAATAGTTCTTGTAATAACAAAAAACACA 469
```

FIG. 1 - Sequence of the 5.8S ITS region of strain YB37 (A) and of strain YB40 (B).

TABLE 4 - Length of the 5.8S ITS sequenced regions of two yeast strains and their homology with *Candida diddensiae*

Candida diddensiae strain	Sequenced product	Percentage of homology	Ref
YB37	517	99	AY580135.1
YB40	469	98	AY580135.1

tem. Theses preliminary results suggest that the yeast strain *Candida diddensiae* could be used in the bioremediation program to degrade polyphenol in oil mill wastewaters and preserve the environment.

Acknowledgements
We thank Professor Khalid Sendid and Kawtar Fikri Benbrahim for their critical reading of the manuscript.

REFERENCES

Aissam H., Errachidi F., Merzouki M., Benlemlih M. (2002). Identification des levures isolées des margines et étude de leur activité catalase. Cahiers de l'Association Scientifique Européenne pour l'Eau et la Santé, 7 (1): 23-30.

Aissam H. (2003). Etude des Effluents des Huileries de la Ville de Fès (margines): Caractérisation, Biotraitement et Valorisation. Thèse pour l'obtention du diplôme de Doctorat National. Faculté des Sciences Dhar Elmahraz, Fes.

Barnett J.A., Payne R.W., Yarrow D. (2000). Yeasts: Characteristics and Identification, 3rd edn., Cambridge University Press, UK.

Cavados C.F.G., Fonseca R.N., Chaves J.Q., Rabinovitch L., Araujo C.J.P.C., Coutinho. (2001). Identification of entomopathogenic *Bacillus* isolated from *Simulium* (Diptera, Simuliidae) larvae and adults. Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. 96 (7): 1017-1021.

Drik R. (2000). Specific PCR primers to identify arbuscular mycorrhizal fungi within colonized roots. Mycorrhiza, 10: 73-80.

Ehaliotis C., Papadopoulou K., Kotsou M., Mari I., Balis C. (1999). Adaptation and population dynamics of *Azotobacter vinelandii* during aerobic biological treatment of olive-mill wastewater. FEMS Microbol. Ecol., 30: 301-311.

Fadil K., Chahlaoui A., Ouahbi A., Zaid A., Borja R. (2003). Aerobic biodegradation and detoxification of wastewaters from the olive oil industry. Int. Biodet. Biodegrad., 51: 37-41.

Fiestas Ros De Urisinos J.A., Borja-Pdilla R. (1996). Biomethanization. Int. Biodeterior. Biodegrad., 38: 145-153.

Garcia Garcia I., Jiménez Pena P.R., Bonilla Venceslada J.L., Martin Martin A., Martin Santos M.A., Ramos Gomez E. (2000). Removal of phenol compounds from olive mill wastewater using *Phanerochaete chrysosporium*, *Aspergillus niger*, *Aspergillus terreus* and *Geotrichum candidum*. Proc. Biochem., 35: 751-758.

Kahraman S., Yesilada O. (1999). Effect of spent cotton stalks on color removal and chemical oxygen demand lowering in olive oil mill wastewater by white rot fungi. Folia Microbiol., 44: 673-676.

Kreger-Van R.I.J. (1987). The Yeasts a Taxonomic Study, Third Revised and Enlarged Edition, Elsevier Science Publishers. B.V., Amsterdam, The Netherlands.

Maestro-Duran R., Borja R., Martin A., Fiestas Ros de Ursinos J.A., Alba Mendoza J. (1991). Biodegradation de los compuestos fenolicos presentes en el alpechin. Grasas Y Aceites, 42: 271-276.

Martirani L., Giardina P., Marzullo L., Sannia G. (1996). Reduction of phenol content and toxicity in olive mill wastewaters with the ligninolytic fungus *Pleurotus ostreatus*. Water Res., 1 (30): 1914-1918.

Mendia L., Carbone P., Dantonio G., Mendia M. (1986). Treatment of olive oil wastewaters. Water Sci. Technol., 18: 1254-1256.

Moreno E., Quevedo-Sarmiento J., Ramos-Cormenzana A. (1990). Antibacterial activity of wastewaters from olive mills. In: Cheremisinoff P.N., Ed., Hazardous Waste Containment and Treatment, Vol. IV, Gulf Publications, USA, pp. 731-757.

Perez J., Esteban E., Gomez M., Gallardolara F. (1986). Effects of wastewater from olive processing on seed germination and early plant growth of different vegetable species. J. Environ. Sci. Health, 21: 349-357.

Piperidou C.I., Chaidou C.I., Staliskas C.D., Soulti K., Pilidis G.A., Balis C. (2000). Bioremediation of olive oil mill wastewater: Chemical alterations induced by *Azotobacter vinelandii*. J. Agric. Food Chem., 48: 1941-1948.

Rodier J. (1996). L'Analyse de l'Eau: Eaux Naturelles, Eauxrésiduaires, Eaux de Mer, 8ème èdition. Dunod, Paris.

Sayadi S., Ellouz R. (1992). Decolorization of olive mill wastewaters by *Phanerochaete chrysosporium*, involvement of the lignin degrading system. Appl. Microbiol. Biotechnol., 37: 813-817.

Servis D. (1986). The soil as a receiving body of olive oil mill wastewaters (in Greek). M. Sc. Thesis, Agricultural University of Athens.

Yesilada O., Sik S., Sam M. (1998). Biodegradation of olive oil mill wastewater by *Coriolus versicolor* and *Funlia trogii*: effects of agitation, intial COD concentration, inoculum size and immobilization. World J. Microbiol. Biotechnol., 14: 37-42.

Yesilada O., Sik S., Sam M. (1999). Treatment of olive oil mill wastewater with fungi. Tr. J. Biology, 23: 231-240.