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Detoxification of olive mill wastewaters by Moroccan yeast isolates

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Abstract A total of 105 yeast strains were isolated from Moroccan olive oil production plants and evaluated for their ability to grow in olive oil mill wastewaters (OMW). The 9 isolates that grew best on OMW were selected for further study to evaluate their effect on removal of organic pollutants and OMW phytotoxicity (barley seed germination test). The results showed that at least four yeast isolates effectively lowered the toxicity of this effluent in addition to providing very useful materials in terms of both yeast biomass (6 g/l DW) and an irrigation fluid. This group of yeast isolates significantly reduced the concentration of total phenols (44% removal) and Chemical Oxygen Demand, COD (63% removal). The best germination rate of 80% for undiluted OMW was obtained for strain *Candida holstii* that also increased the pH from 4.76 to 6.75. Principal component analysis of the results obtained for the best yeast strains confirmed the importance of COD and total phenol reduction along with increase of organic nitrogen and final pH for the improvement of germination rates and phytotoxic reduction. This study has highlighted the potential of indigenous yeasts in detoxification of olive mill wastewaters.

Keywords Carbohydrate assimilation · COD · Detoxification · Germination assays · Olive oil mill wastewaters · Total phenols · Yeasts

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Introduction

In the last 20 years there has been increasing interest in the treatment and disposal of the Olive oil Mill wastewater (OMW) which represents a major threat to the environment in the Mediterranean region (Assas et al. 2002).

Physical and chemical treatments aimed at reducing OMW toxicity such as ozonation and advanced oxidation processes can effectively detoxify OMW (Monteagudo et al. 2005). Nevertheless, most of these treatments are costly and technically unsuitable for developing countries such as Morocco. In such countries, with severe water deficit, treatments aimed

at reducing toxicity for water reuse for irrigation seem to be more suitable. Indeed, OMW can be a source of water especially in the arid zones of southern Morocco. In addition, OMW contains large amounts of minerals and organic matter that can be beneficial to plant growth (Ramos-Cormenzana et al. 1995). Nevertheless, because of its phytotoxic and antimicrobial effects related mainly to the presence of phenolic compounds, organic volatile acids, acidic pH and the high level of salts such as potassium (Paredes et al. 1999), raw OMW can not be used without prior treatment.

Biological treatments of OMW using anaerobic digestion to produce biogas and to reduce OMW phytotoxicity have been attempted with limited success (Ramos-Cormenzana et al. 1995). Aerobic treatment using fungi such as *Phanerochaete flavido-alba* (Perez et al. 1998), *Lentinula edodes* (D'Annibale et al. 2004), *Pleuroteus ostreatus* (Aggelis et al. 2003) as well as bacteria such as *Ralstonia* sp. and *Pseudomonas putida* (Di Gioia et al. 2001) were shown to reduce toxicity and colour in OMW. Recently, investigations have evaluated the ability of yeasts for the treatment and detoxification of OMW along with the production of enzymes and yeast biomass (Lanciotti et al. 2005). In fact, yeasts are more adapted to the growth in OMW as they can resist the high concentrations of phenols (Shivarova et al. 1999; Yan et al. 2005) and low pH. Moreover, they seem to be the dominant microorganisms in this wastewater compared to bacteria and moulds (Ben Sassi et al. 2005). Collection yeasts such as *Yarrowia lipolytica* (Lanciotti et al. 2005) as well as isolates from the olive wastes such as *Candida tropicalis* (Ettayebi et al. 2003) and *Geotrichum candidum* (Assas et al. 2002) led to significant reduction of polyphenols and COD in OMW.

These investigations have shown encouraging results for organic pollutants removal from OMW using yeasts. The present work brings additional data by comparing the effect of many natural yeast isolates on the reduction of pollutants and phytotoxicity of OMW. A collection of 105 yeast strains was isolated and purified from different olive oil production processes in Morocco (Ben Sassi et al. 2005). The ability of these strains to grow on this wastewater was carried out using OMW agar solidified and raw liquid OMW. The best yeast strains were selected for the evaluation of total phenols removal, organic matter degradation and barley seed germination in OMW from the olive oil extraction continuous process.

Material and methods

Biological material

In Morocco, the olive oil is predominantly extracted using three processes. The discontinuous press process where olives are crushed using stone mills. The olive mash is then filled in bags made of date palm leaves. The bags are subsequently put in mechanical presses to extract the oil. This process is either traditional in small units powered by animals or semi-modern in bigger electrically powered units. The third process called continuous process uses rotating hammers to ground up the olives. The oil is separated from the rest of the olive parts using industrial centrifuges. The samples of OMW used in this study originated from factories in the area of Marrakech that use the three extraction systems (continuous, semi-modern and traditional). The effluent used in the aerobic treatment with yeasts originated from the continuous process. The general characteristics of this effluent are: pH: 4.76 ± 0.08; total solids (g/l): 3.07 ± 0.02; COD (g/l): 176.32 ± 0.04; total phenols (g/l): 1.43 ± 0.07; Reducing sugars (g/l): 14.3 ± 0.02; Glucose (g/l) 4.6, Fructose (g/l) 3.8; total nitrogen (g/l): 0.29 ± 0.04 and colour A_{395}: 31.56 ± 0.03. All samples were immediately analyzed for pH, prior to centrifugation and freezing at −20°C.

Ability of yeasts to grow on OMW

The isolates (105) were purified and maintained on slants of Yeast Malt (YM) agar at 4°C. The isolates were grown in YM broth at 30°C for 48 h, until a final concentration of 10^8CFU/ml was reached. Yeasts were then harvested by centrifugation (5,000 g/10 mn), washed twice with sterile physiological water and diluted in 1 ml physiological water (the yeast suspension).

Isolates were tested for their ability to grow on OMW agar by inoculating a loop of the yeast suspension on plates containing sterile OMW from each process solidified with 1.5% agar. Plates were then incubated for 48 h at 30°C.

Eighty five isolates were tested for their ability to grow in OMW from the continuous process and 30 isolates were tested for growth ability on OMW from semi-modern and traditional processes as follow: a volume of 0.5 ml of the yeast suspension was inoculated into flasks containing 5 ml of sterile OMW (121°C/10 mn) and incubated at 30°C under agitation (200 rpm) for 48 h. Yeast growth was determined on YM agar at $t = 0$ h (N0h) and after 48 h of incubation (N48h). The results were given as the growth magnitude (N48h/N0h).

Carbohydrates assimilation

Thirty selected isolates were tested for their ability to utilize some sugars and especially those that are the most abundant in OMW: D-glucose, D-xylose, fructose and D-sucrose (Salvemini 1985). Sugar assimilation was assayed according to Middelhoven (2002): to a solution of 0.67% of Yeast Nitrogen Base (Difco), 0.5% of the sugars to analyze are added. After sterilization, a fresh culture of yeast is inoculated and the growth is estimated after 4 to 5 days of incubation at 30°C.

Yeast identification

Nine yeast isolates were identified according to molecular techniques. DNA extraction was performed as described by Cocolin et al. (2000). Cell broth was centrifuged at 16,000 × *g* for 10 mn at 4°C and the cell pellet (approximately 100 µl volume) was suspended in 200 µl of breaking buffer (2% Triton X-100, 1% SDS, 200 mM NaCl, 10 mM Tris pH 8, 1 mM EDTA pH 8). The cells were homogenized with 0.3 g of glass beads (0.5 mm in diameter) in the presence of 200 µl chloroform. The mixture was centrifuged at 16,000 × *g* at 4°C to collect the aqueous phase.

DNA was precipitated with 2.5 volume of 100% ethanol in the presence of 100 mM de NaCl at −20°C for 30 mn and centrifuged at 16,000 × *g* at 4°C for 10 mn. The pellet washed twice with 70% ethanol, centrifuged at 16,000 × *g* at 4°C for 5 mn, dried at 30°C and suspended in 20 µl sterile filtered water and stored at −20°C.

Universal fungal primers ITS1 and ITS4, directed to the conserved regions of ribosomal DNA, were used to amplify DNA. PCR was performed in a final volume of 20 µl containing 2 µl of *Taq*-DNA polymerase buffer (Promega), 1.5 mM $MgCl_2$, 0.2 mM each dATP, dCTP, dGTP and dTTP (Promega), 0.2 mM of the primers ITS1 and ITS4 (Promega), 1.25 IU *Taq*-DNA polymerase (Promega) and 2 µl of the extracted DNA (approximately 10 ng). The reactions were run for 35 cycles: denaturising at 95°C for 30 s, annealing at 55°C for 45 s and extension at 72°C for 90 s. An initial 5 mn denaturisation at 95°C and a final 7 mn extension at 72°C were used. The PCR products were sequenced using an ABI Prism 310 Applied Biosystems. Identification was carried out by comparing the sequences obtained to those of the Pub Med database.

Treatment of OMW and cell growth

The nine isolates were grown in a 2l reactor (New Brunswick—Bioflo III). About 5 log CFU/ml of the yeast suspension was inoculated into the reactor containing 1200 ml of sterilized OMW (121°C/10 mn). Temperature was maintained at 30°C, agitation at 150 rpm and aeration at a flow rate of 1 ml/s.

Analytical methods

Cell dry weight was estimated on samples centrifuged 15 mn at 5000 g and dried at 105°C until constant weight.

Chemical Oxygen Demand (COD) and total nitrogen of culture supernatants were analyzed according to Standards methods (APHA; AWWA; WEF, 1998). Protein content was determined by the Kjeldahl method, using a factor of 6.25 to convert nitrogen to protein content. Total phenols were determined according to the method of Garcia Garcia et al. (2000) using the Folin-Ciocalteau reagent and cafeic acid as standard. Colour was measured spectrophotometrically at 395 nm on OMW samples adjusted to pH 7 (Flouri et al. 1996). Total sugars were determined by the dinitrosalicylic acid method (Miller 1959). All analyses were performed in triplicates.

Gas chromatography analysis

Glucose and fructose content was measured on raw and treated samples of OMW by gas chromatography

(GC). GC was performed in an Agilent Technologies 6800 gas chromatograph. The column used is a SGE BPX5 (25 m x 0.32 mm I.D., 0.25 μm film thickness). Temperature was: 60°C for 2 mn and then 150°C at a rate of 20°C/mn and then 260° at a rate of 60°C/mn. The flame ionization detector (FID) was at 280°C. A volume of 1 μl of the sample was injected.

The oxime-trimethylsilyl (TMS) formation was done as follow: 0.5 ml of each sample was freeze dried. Then, 100 μl of 2% hydroxylamine in pyridine was added to the sample and the temperature was kept at 80°C for 30 mn. The reaction was completed with 900 μl of HMDS and 100 μl of trifluoracetic acid at a temperature of 100°C for 1 h. Finally 1 μl of the sample was injected and analysed.

Germination assay

A local variety of barley (*Ordeum vulgare.* Var. Tissa) was used for germination assays. Three replicates of 50 barley seeds per Petri dish were used. The temperature was kept constant at 22°C in the dark. The experiments were performed both on treated and raw undiluted and 1/2-diluted OMW. Controls irrigated with distilled water were run in parallel. The number of seeds germinated was counted each day and the rate of germination calculated as percent of number of seeds germinated in comparison with the control (100% germinated seeds).

Statistical analysis

Principal component analysis was performed on raw data of Tables 2 and 3 using Statistica Program (version 5, 1997).

Results and discussion

Growth of isolates on OMW

Results of the growth of the 105 yeast isolates on OMW agar from the three processes (continuous, semi-modern and traditional) showed that almost all the isolates were able to grow on solidified OMW from the continuous process (about 94%). About 78% were able to grow on OMW from the semi-modern process while only 19% grew on the traditional OMW agar. These results seem to confirm that the traditional OMW is the most toxic effluent and that OMW from the continuous process is the less toxic as already established using germination tests (Ben Sassi et al. 2005). Among the 105 strains, the 20 isolates that grew in the traditional OMW agar also grew in OMW agar from the two other processes (continuous and semi-modern). These 20 isolates were selected for the rest of this investigation. To increase the number of selected strains to 30, 10 additional isolates that gave the best growth magnitude on liquid OMW from the continuous process, were also investigated.

The growth results (Table 1) confirmed the earlier results with OMW from the traditional process being the most toxic and OMW from the continuous process the least toxic. Almost all strains were able to assimilate D-glucose and fructose. Among the 30 isolates, 10 were able to degrade all the sugars analyzed.

These 10 isolates that gave the best growth results on liquid OMWs and the isolate ymc83 that assimilated all the tested sugars (the 11 isolates with numbers in bold in 1) were kept for further study. Upon further culture transfers, the isolates ymc86 and ymt102 (preserved in glycerol under −20°C) could not be subsequently cultured. Hence, only the other nine strains were used in the next sections of the work.

Yeast identification

The nine selected isolates were identified by means of molecular techniques (Cocolin et al. 2000) and the isolates were identified as the yeasts:

Isolat ymc51, Isolat ymc80, Isolat ymc85 : *Pichia guilliermondii.*
Isolat ymc83, Isolat ymt95 : *Candida ernobii.*
Isolat ymc78, Isolat ymc98 : *Candida diddensiae.*
Isolat ymc73 : Candida holstii.
Isolat ymt103 : *Pichia sp.*

Yeast treatment of OMW

The aerobic treatment of OMW in a batch reactor with the 9 selected isolates showed that some of the isolates adapted well to this effluent as expressed by dry weight (DW) biomass production (Table 2). The

Table 1 Growth magnitude (cfu at 48 h / cfu at 0h) on different OMW and sugar utilization by the 30 selected yeast strains

Strains	Continuous	Semi-modern	Traditional	D-Glucose	Fructose	D-Sucrose	D-Xylose
Candida diddensiae ymc98	80503	4615	0	+	+	+	+
Candida diddensiae ymc78	41714	48232	0	+	+	−	+
Candida ernobii ymt95	31594	74	0	+	+	+	+
Candida holstii ymc73	29250	350	0	+	+	+	+
ymc86	9479	1631	0	+	+	+	+
Pichia guilliermondii ymc85	9048	0	0	+	+	+	+
Pichia guilliermondii ymc80	2804	0	0	+	+	+	+
ymt102	2538	333	1	+	+	+	+
Pichia sp. ymt103	1582	31	0	+	+	+	+
Pichia guilliermondii ymc51	1469	206	0	+	+	+	+
Candida ernobii ymc83	0	228	0	+	+	+	+
ymc100	7718	406	0	+	+	−	+
yms53	1123	58	0	+	+	+	−
ymc101	920	51	0	+	+	+	−
ymc38	820	354	0	+	+	+	−
ymc74	486	349	5	+	+	+	−
ymc48	343	0	0	+	+	+	−
ymc54	167	95	7	+	+	+	−
ymc55	123	194	5	+	+	+	−
ymt69	97	3204	20	+	+	+	−
ymt34	91	2219	22	+	+	+	−
ymt82	53	0	15	+	+	+	−
ymt57	26	272	0	+	+	+	−
ymc87	21	64	1	+	+	+	−
ymt42	19	1712	6	+	+	+	−
ymt35	15	2889	14	+	+	+	−
ymt56	12	12	0	+	+	+	−
ymt36	11	321	43	+	+	+	−
ymc84	0	1556	10	+	+	+	−
ymt91	0	0	0	+	+	+	−

lowest values were around 1.52 g/l (DW) obtained for *Pichia sp*. ymt103 while the highest value reached 6.18 g/l for the yeast *C. diddensiae* ymc98. Hence, there seems to be good growth of almost all selected yeasts even if no external nutrients were added to the effluent as recommended by some authors (Fadil et al. 2003). Sugar consumption was detected by GC analyses following the growth of *C. diddensiae* ymc98 and no glucose was detected and only 4.13% of original fructose detected after 48 h of incubation. The yeasts *P. guilliermondii* ymc51, *C. diddensiae* ymc78 and *Candida ernobii* ymc83 presented a good growth level and used almost all the glucose present in OMW after the growth. For the other yeasts, different results were obtained. For example, *C. holstii* ymc73 exhibited slight growth despite more than 90% consumption of glucose and fructose and *C. ernobii* ymt95 exhibited poor growth with more than 93% of glucose consumed. *P. guilliermondii* ymc80 and ymc85 grew well but didn't consume high concentrations of glucose and fructose. These results suggest that yeast growth is not related to the consumption of the sugars analysed. These results were confirmed by the results of the yeast *Pichia sp*.ymt103 which consumed 100% of the two sugars but grew poorly.

Yeast growth was also followed by a decrease in COD and total phenols (Table 3). The best removal

Table 2 Growth parameters of the 12 best performing yeast isolates (average of three replicates ±95% confidence interval) and percent of glucose and fructose remaining in percentage of the initial concentration of each sugar

	Dry biomass (g/l)	Proteins (mg/l)	Sugars	
			Glucose	Fructose
P. guilliermondiiIsolate ymc51	3.04 ± 0.00	25.09 ± 0.39	0.00	100.00
C. holstii ymc73	2.63 ± 0.00	35.21 ± 0.00	7.74	3.75
C. diddensiae ymc78	4.18 ± 0.00	37.00 ± 0.49	9.71	77.47
P. guilliermondii ymc80	3.04 ± 0.00	14.25 ± 1.27	76.65	100.00
C. ernobii ymc83	3.29 ± 0.00	14.60 ± 0.05	8.85	91.29
P. guilliermondii ymc85	3.50 ± 0.00	27.69 ± 0.59	13.77	22.78
C. ernobii ymt95	1.83 ± 0.00	22.94 ± 0.00	6.19	94.38
C. diddensiae ymc98	6.18 ± 0.00	64.54 ± 0.10	4.13	0.00
Pichia sp. ymt103	1.52 ± 0.00	22.19 ± 0.10	0.00	0.00

Table 3 Growth and Reduction of OMW organic parameters after treatment by the 9 isolates (average of three replicates ± 95% confidence interval)

Isolate	% COD reduction	% phenols reduction	% from initial colour	Final pH
P. guilliermondii ymc51	51.57 ± 0.00	31.89 ± 0.01	92.84 ± 0.00	4.87 ± 0.07
Candida holstii ymc73	57.93 ± 0.02	39.00 ± 0.03	137.68 ± 0.03	6.75 ± 0.03
C. diddensiae ymc78	64.84 ± 0.01	32.14 ± 0.03	96.67 ± 0.01	5.33 ± 0.07
P. guilliermondii ymc80	34.47 ± 0.00	33.52 ± 0.03	92.84 ± 0.00	4.87 ± 0.07
C. ernobii ymc83	51.85 ± 0.00	35.23 ± 0.04	108.92 ± 0.00	4.64 ± 0.01
P. guilliermondii ymc85	53.21 ± 0.02	25.09 ± 0.02	93.62 ± 0.01	5.28 ± 0.07
C. ernobii ymt95	62.65 ± 0.00	34.09 ± 0.00	144.18 ± 0.00	4.61 ± 0.02
C. diddensiae ymc98	55.40 ± 0.00	43.56 ± 0.01	97.97 ± 0.01	4.64 ± 0.01
Pichia sp. ymt103	41.04 ± 0.04	40.00 ± 0.02	112.70 ± 0.02	4.87 ± 0.01

of 65% COD was obtained for the yeast *C. diddensiae* ymc78. Lanciotti et al. (2005), using *Yarrowia lipolytica*, obtained 41% COD reduction on undiluted OMW. Ettayebi et al. (2003) have reported similar results with 70% COD reduction although they used diluted OMW supplemented with nutrients. The highest COD reduction on undiluted OMW was obtained by Scioli and Vollaro (1997) who obtained 80% COD reduction on OMW supplemented with ammonium sulphate and yeast extract using the culture collection yeast *Yarrowia lipolytica* ATTC 20255. Total phenol removal was also comparable to COD reduction with around 40% for the yeasts *C. diddensiae* ymc98, *Pichia sp.*ymt103 and *C. holstii* ymc73. The reduction of total phenols concentration by yeasts may be ascribed mainly to the degradation by the yeasts of the phenolic compounds as previously reported by many authors. For example, Shivarova et al. (1999) reported that *Trichosporon cutaneum* was capable of metabolizing high concentrations of phenols, up to 1 g/l. Similarly, the yeast *Candida tropicalis* showed high phenol degradation potential, either in mineral salt medium, where the phenol degradation reached values up to 2 g/l (Yan et al. 2005), or in OMW under metabolic induction, where it reduced the initial total phenols by 36.5% (Ettayebi et al. 2003). The reduction of total phenol levels may also be attributed, in part, to the adsorption of phenols to yeast cells through the formation of protein-polyphenol complexes (Papadopoulou and Frazier 2004).

The reduction of total phenol content did not appear to be correlated with colour removal (Table 3). In fact, the black colour in OMW is attributed to the polymerized fraction composed of the highly recalcitrant but less toxic high molecular-mass polyphenols such as

anthocyanins and catechins (Hamdi et al. 1992). Most of the yeast isolates, in this study, did not significantly decrease colour levels. The ability to degrade the recalcitrant molecules responsible of colour in OMW has been traditionally reported for filamentous white-rot fungi such as *Phanerochaete chrysosporium*, *Lentinula edodes* and *Pleurotus ostreatus* which possess enzymes (manganese and lignin peroxidases and laccases) capable of oxidizing lignin-like molecules (Perez et al., 1998). Yeasts are not known for this ability but recently, the filamentous yeast *Geotrichum candidum* has been reported to produce peroxidase (Kim and Shoda 1999) and reduce high and low molecular weight phenolics including those in OMW (Assas et al. 2002). None of the yeast strains isolated in this work were filamentous and the reduction of colour did not exceed around 7% for yeasts *P. guilliermondii* ymc51 and *P. guilliermondii* ymc80. On the contrary, a few strains increased the colour level. The increase in colour may be explained by the polymerization of polyphenols that occur with high levels of oxygen transfer due to the agitation and aeration (D'Annibale et al. 1998).

Most of the yeast strains did not dramatically change pH (Table 3). However, one isolate, *C. holstii* ymc73**,** changed the pH of OMW from 4.76 to 6.75. Such an increase in pH during the aerobic treatment of OMW has been reported for *Candida tropicalis* (Fadil et al., 2003) and may be attributed either to ammonia produced by yeasts following the deamination of amino acids or to the consumption of organic acids present in OMW.

Toxicity of yeast-treated OMW toward barley seed germination

Whereas most of undiluted yeast-treated OMW (Fig. 1A) almost completely blocked seed germination, pre-treatment with isolate *C. holstii* ymc73 showed a marked increase in germination (80%), compared to the control. Isolates *P. guilliermondii* ymc85 and *C. diddensiae* ymc98 induced a slight increase in germination around 30% and 12% respectively. All other yeast isolates tested induced germination lower than 10% (only germination higher than 10% is represented in Fig. 2 for increased clarity). Even after dilution of the treated OMW to 1/2, most isolates induced germination rates lower than 50% or even led to a germination percentage lower

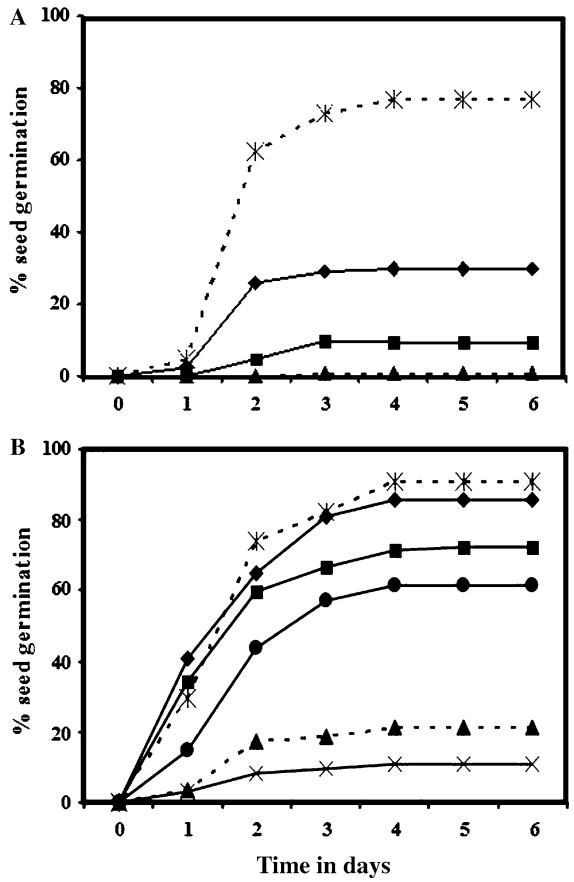

Fig. 1 Percentage of germinated barley seeds irrigated with (**A**) Undiluted and (**B**) 1/2 diluted yeast-treated OMW (Only five of the nine isolates and the raw OMW are represented in the figures)

than the control (raw OMW) for isolate *P. guilliermondii* ymc80 (Fig. 1B). Nevertheless, four isolates led to higher germination rates with 90% for isolate *C. holstii* ymc73, 80% for *P. guilliermondii* ymc85 and around 60% for isolates *C. diddensiae* ymc98 and *Candida ernobii* ymt95.

These results do not correlate with the other parameters presented in Tables 2 and 3. A multifactorial principal component analysis of data in these tables (Fig. 2) showed that the OMW treated with these four isolates (***C****. holstii* ymc73, *P. guilliermondii* ymc85, *C. ernobii* ymt95 and *C. diddensiae*

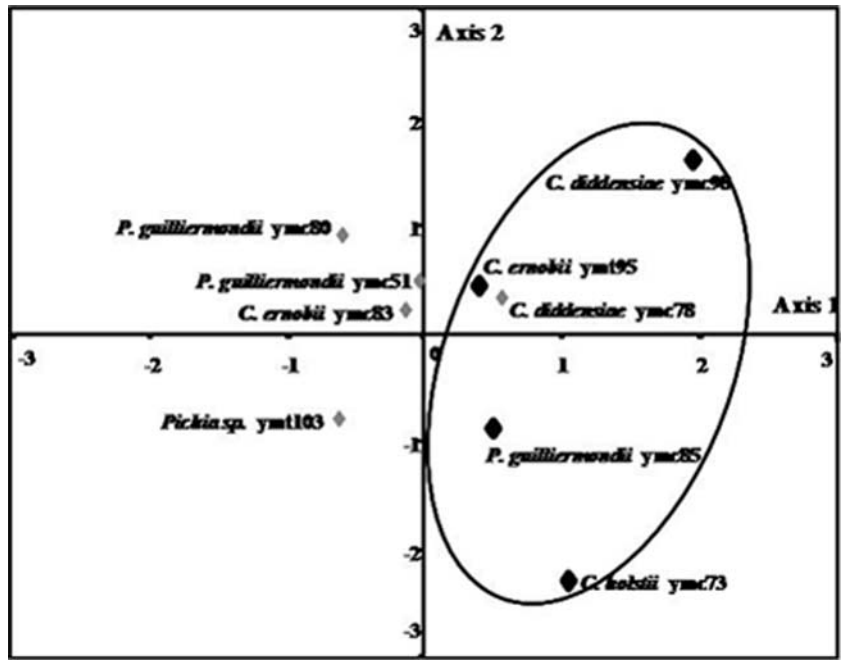

Fig. 2 Plot of the OMW yeast treated samples in the first (47 % of total variance) and second (19% of total variance) axes of the principal component analysis using raw data of tables 2 and 3 (markers in bold indicate the best germination rates)

ymc98) form a group at the right side of the first axis. This axis, explaining 46.57 % of the total variance, is defined by four variables with protein concentration (factorial weight of 0.83), COD removal (factorial weight of 0.76), germination percentage (factorial weight of 0.74) and total phenol removal (factorial weight of 0.71). The second axis, explaining 18% of the total variance, is mainly defined by the final pH (factorial weight of 0.78). Hence, it seems that the yeast strains that gave the best toxicity reduction (best germination rates) significantly reduced total phenols and COD along with good growth as expressed by organic nitrogen production. This result seems to confirm other reports claiming that the reduction of both COD and phenolic compounds improves germination of durum wheat in OMW treated with aerobic treatment (D'annibale et al. 2004). Nevertheless, decrease of phenolics (65%) with minimal change in COD (only 5.3% reduction) increased germination of durum wheat after an enzymatic treatment (Casa et al. 2003). In contrast, Cereti et al. (2004) have reported that the reduction of COD alone increased the germination of durum wheat using *Aspergillus niger* on OMW despite minimal reduction of total phenol.

In addition to total phenols and COD, the acidic pH is also thought to contribute to the phytotoxic effect of OMW (Paredes et al. 1999). The results in the present work showed that the best germination percentage was obtained for strain *C. holstii* ymc73 that also increased the pH to 6.7 along with total phenol and COD reduction. Marques (2001) reported similar results for the anaerobic treatment of OMW that led to a neutralization of the pH and the use of this treated fluid in soil irrigation. Nevertheless, OMW treated with *C. diddensiae* ymc98 which reduced total phenols and COD but almost no pH change still induced high germination percentage (70% for OMW).

These observations imply that parameters such as COD, total phenols and pH contribute dependently as a group to the phytotoxic effect of OMW. Depending on the yeast strains and the type of OMW treatment one of these parameters may appear as the key factor in lowering phytotoxic effect. It is likely that other factors such as the increase of organic nitrogen and growth factors, as well as parameters not measured in this work, also contribute to the observed changes in seed germination rates. This claim is supported by the results obtained for strain *C. diddensiae* ymc78 that led to good COD and total phenol removal: 65% and 32%, respectively and increased the pH to 5.33. However, the germination rate was low reaching only 28% for OMW treated with this strain.

Conclusion

The yeast strains isolated from local factories of olive oil extraction were grown in samples of raw OMW with no nutritional supplements. Some of the isolates (*C. holstii* ymc73, *C. diddensiae* ymc78, *P. guilliermondii* ymc85, *C. ernobii* ymt95, *C. diddensiae* ymc98 and *Pichia* sp. ymt103) grew well and were able to significantly reduce COD and total phenol along with the production of yeast biomass. The results showed that yeast pre-treatment reduced OMW phytotoxicity as determined by barley seed germination. Isolate *C. holstii* ymc73 induced germination higher than 70% for the undiluted OMW. Based on multifactorial principal component analysis, the isolates that increased the germination percentages have also increased organic nitrogen content along with a significant reduction of COD and total phenols. Neutralization of the pH seems to be a contributing factor to the improvement of germination rates by certain yeast strains.

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