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SHORT COMMUNICATION

Survival of *Candida parapsilosis* yeast in olive oil

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Abstract *Candida parapsilosis* is a human opportunistic pathogen yeast isolated from different habitats like animals, man, pickled cucumber, fruit juices, and water. Recent studies have demonstrated that *C. parapsilosis* can survive in olive oil for very long periods even exceeding 24 months. The survival of two strains of *C. parapsilosis* named DAPES 1890 and 1892, previously isolated from extra virgin olive oil, was influenced by the state of hydration of the cells and the polyphenols concentration of olive oil. When the cells of the two strains of *C. parapsilosis* were inoculated under a liophilized form into olive oil containing 45–312 mg/kg of total polyphenols, their survival in some olive oil samples reached approximately 18 months. However, if the above-mentioned inoculum was rehydrated with 1 % of distilled water, then the survival of both yeast strains in some samples of oil exceeded 24 months. The two yeast strains, recovered from the olive oil samples after 24 months of storage, showed, under SEM, spherical shapes with and without buds according to whether the inoculum was made up of rehydrated or lyophilized cells. The survival of all the *C. parapsilosis* strains was also negatively influenced by the polyphenols concentration of the olive oil samples inoculated both with lyophilized and rehydrated yeast cells. In the oily habitat, the polyphenols sorption to the *C. parapsilosis* yeast surface was observed, and during storage the polyphenols reacted with the yeast cell walls according to their concentration in the inoculated olive oil.

Keywords *Candida parapsilosis* · Dimorphic yeast · Olive oil · Yeast survival

Introduction

Candida parapsilosis is a ubiquitary dimorphic yeast isolated from different habitats such as pickled cucumbers, olives, animals, man, soft drinks, fruit juices, and water where they seem to be capable of surviving for very long periods (Barnett et al. 2000). In man, it has been quite frequently isolated from hospitalized patients where it behaves as an opportunistic pathogen responsible for pericarditis and urinary infections (Wong et al. 2000). As far as food of vegetable origin is concerned, it has been found by various authors in the brine of table olives during the debittering process (Mourad and Nour-Eddine 2006). Nevertheless, it is not certain whether the yeast which develops in the brine of table olives comes solely from the carposphere of the fruits or also from other sources of contamination, since in some studies it was found in the brine but not on the fruits used for processing (Hernández et al. 2007). Olive oil constitutes a very particular habitat where *C. parapsilosis* can be found. In fact, some recent studies by Zullo and Ciafardini (2008a) have, for the first time, highlighted the presence of these yeasts in three commercial extra virgin olive oils on the market, while Koidis et al. (2008) reports the presence of this yeast in cloudy olive oils produced by some olive varieties in Greece. However, considering the food safety aspect, since this yeast species has only recently been found in oil, from the scientific data already available it is not possible to understand if *C. parapsilosis* strains are able to survive for a long time and reproduce in olive oil or if it is just of short endurance. This investigation reports a study on the survival of two oil-borne *C. parapsilosis* strains inoculated in lyophilized and rehydrated forms in an olive oil characterized by different polyphenols concentrations.

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Materials and methods

Long-term *C. parapsilosis* survival in olive oil and oil polyphenols profile

The *C. parapsilosis* cultures used in the microbiological trials were represented by two oil-borne strains named, respectively, DAPES 1890 and DAPES 1892 isolated in our laboratory from different commercial olive oils, and characterized previously

according to their carbohydrate fermentation patterns and genetic characteristics (Zullo et al. 2010). The polyphenols profile of the different olive oil samples was evaluated separately according to Mateos et al. (2001). The studies made on the long-term survival of the DAPES 1890 and DAPES 1892 *C. parapsilosis* strains were accomplished using an olive oil containing three different concentrations of polyphenols. The yeast cultures were prepared by growing the two strains separately in 1-L Pyrex flasks containing 800 mL of MYGP (malt extract, yeast extract, glucose, peptone) (Kurtzman and Fell 1998) broth medium, and after 3 days of incubation at 30 °C, the yeasts were collected by centrifugation at 5,000 *g* for 10 min. Finally, the two yeast biomasses were dried by lyophilization and used within 48 h for the tests described below. At the same time, 3 L of extra virgin olive oil originating from the "Leccino" olive variety were used for the preparation of three fractions containing, respectively, total polyphenols concentrations of 45, 178, and 312 mg Kg^{-1} olive oil, evaluated as previously reported by Zullo and Ciafardini (2008b) and expressed as gallic acid. The olive oil fraction containing a total polyphenols concentration equal to 45 mg of gallic acid kg^{-1} was obtained from an extra virgin olive oil originally containing total polyphenols equal to 312 mg of gallic acid per kg of oil. An amount of 1,500 mL of the original mass of extra virgin olive oil were taken and transferred into 4-L flasks fitted with screw caps, into which an equal volume of a solution of methanol/water (80:20 v/v) was added in order to extract the polyphenols. After 30 min of magnetic stirrer agitation, the mass was centrifuged for 5 min at 4,000 *g* and the oily fraction was taken and stored in a 2-L screw-top flask. After two extractions, the oily mass was treated as above with an equal volume of distilled water to remove traces of methanol. A fraction of olive oil containing the intermediate concentrations of total polyphenols equal to 178 mg of gallic acid per kg was also prepared by mixing one volume of oil with a polyphenols content equal to 45 mg of gallic acid per kg reported above with the same volume of the original extra virgin olive oil containing 312 mg of gallic acid per kg. Finally, the stocks of the olive oil with the three different polyphenols concentration reported above were filter-sterilized (Minisart NML-Sartorius, Göttingen, Germany), transferred to 2-L Pyrex flasks with screw caps which had been previously sterilized in an autoclave at 121 °C for 20 min and used for the inoculation trials as follows. An amount of 200 mL of each olive oil fraction was transferred in sterile Pyrex flasks with screw caps and inoculated separately with the DAPES 1890 and DAPES 1892 strains of *C. parapsilosis* using, respectively, 40 mg of yeast lyophilized biomass or 40.2 mg of rehydrated biomass reconstructed at the moment of inoculation by adding 1 % (wt/wt) of sterile distilled water to the lyophilized biomass. For each strain of *C. parapsilosis* and for each type of oil, two repetitions were carried out. The inoculated samples were mixed with a vortex and stored in a dark room for 24 months at 15–17 °C. At the beginning of the incubation, 15 mL of olive oil aseptically sampled were used for the preliminary scanning electron microscopy (SEM) observation and microbiological analysis in order to evaluate the presence of injured cells and their viability after drying processes. The survival of the two yeast strains along the 24 months of incubation was evaluated through the microbiological analysis carried out after each 6 months of storage. At the end of the incubation, the yeast cells recovered from the inoculated olive oil samples were used for SEM observation and chemical analysis as described below.

Microbiological analysis and SEM observation

The microbiological analysis was accomplished by serial dilution of 10 mL of olive oil with sterile distilled water. After mixing, 0.2 mL of oil–water emulsion were inoculated in Petri dishes containing the above-mentioned MYGP agar medium using five repetitions for each sample. The inoculated plates were incubated at 28 °C, and after 3 days the yeast colonies obtained were recorded and, for each olive oil sample, 20 colonies were randomly chosen for the preparation of a master accomplished in Petri dishes containing MYGP agar and used for the biochemical analysis reported by Zullo et al. (2010). Finally, the conformity between the biochemical characteristics of the two *C. parapsilosis* strains used in the trials and those of the inoculated olive oil yeast colonies was checked. The SEM observations were made considering lyophilized and rehydrated yeast cells used as inoculum at the beginning of the trials and the yeast cells recovered from olive oil after 24 months of storage. Volumes of olive oil equal to 10 mL, taken at zero time and after 24 months of storage, respectively, from the olive oil samples characterised by a phenolic content equal to 312 mg of gallic acid per kg and containing lyophilized or rehydrated yeast cells of *C. parapsilosis* yeast strains were used for the SEM observation as described by Zullo et al. (2010) using an SEM Zeiss DSM 940 (Zeiss, Rome, Italy).

Evaluation of olive oil polyphenol absorption by yeast cells

The olive oil phenolic compounds adsorption by yeast cells during storage was evaluated after 24 months of incubation through the polyphenols extraction from the yeast cell biomass according to the procedure reported by Ciafardini and Zullo (2003). In short, the yeast biomass was recovered after 24 months of storage using 80 mL of each inoculated olive oil sample. After 20 min of centrifugation at 12,000 *g*, the yeast biomass was resuspended in 2 mL of sterile distilled water and, after a brief stir, the sample was centrifuged as reported above and the liquid fraction also containing oil traces was discarded. The phenolic compounds entrapped in the yeast biomass were extracted with ethyl acetate and evaluated as reported by Ciafardini and Zullo (2003). The initial lyophilized cells inoculum of both DAPES 1890 and 1892 *C. parapsilosis* strains stored for 24 months at –20 °C were used as a control.

Results

Candida parapsilosis DAPES 1890 and 1892 strains when inoculated in the different olive oil samples survived a long time according to the yeast cells hydration and the chemical composition of the oil. The microbiological analysis and the SEM observation accomplished at the beginning of the trials, considering the cells of the two strains of *C. parapsilosis* before and after undergoing lyophilization, demonstrated a low presence of injured cells (data not shown) and a high level of vitality of both the lyophilized and the rehydrated yeast biomass used as inoculum. In fact, at the beginning of the long-term survival experiments (zero time), in the inoculated olive oil samples more than 10^6 CFU (colony forming units) per mL of both the *C. parapsilosis* strains were recorded (Figs. 1, 2). The microbiological analyses carried out regularly every 6 months for the period of incubation of 24 months demonstrated that these yeast strains can survive in olive oil for a long time, and that the degree of survival is linked to the concentration of total polyphenols present in the oil and to the degree of hydration of the yeast cells inoculated in the oil samples. In fact, the DAPES 1890 of *C. parapsilosis* strain inoculated into the oil under a lyophilized form survived for no more than 18 months, whereas the rehydrated yeast cells containing 1 % of added water survived for more than 24 months in the presence of low polyphenols content (Fig. 1). Nevertheless, for both types of inoculum, the survival of the DAPES 1890 strain was negatively influenced by the high concentration of total polyphenols present in the olive oil especially for the lyophilized form (Fig. 1). The DAPES 1892 strain of *C. parapsilosis*, compared to the previous strain, showed a similar behavior. In fact, also in this case, the survival of the yeast inoculated under a lyophilized form did not exceed 18 months,

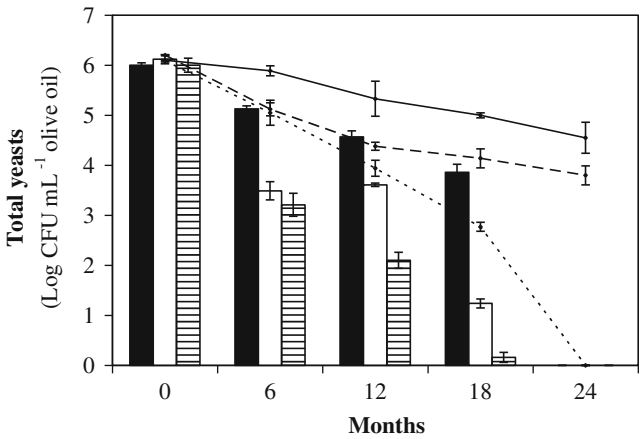

Fig. 1 Survival of *C. parapsilosis* 1890 DAPES strain in olive oil. ■ lyophilized cells in olive oil with 45 mg gallic acid kg^{-1}; □ lyophilized cells in olive oil with 178 mg gallic acid kg^{-1}; ▤ lyophilized cells in olive oil with 312 mg gallic acid kg^{-1}; ___ rehydrated cells in olive oil with 45 mg gallic acid kg^{-1}; _ _ _ rehydrated cells in olive oil with 178 mg gallic acid kg^{-1}; ..... rehydrated cells in olive oil with 312 mg gallic acid kg^{-1}. All the values are the mean of two determinations ± SD

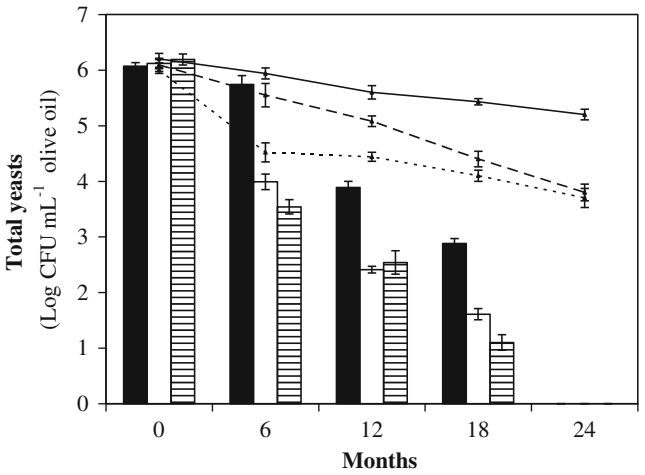

Fig. 2 Survival of *C. parapsilosis* 1892 DAPES strain in olive oil. ■ lyophilized cells in olive oil with 45 mg gallic acid kg^{-1}; □ lyophilized cells in olive oil with 178 mg gallic acid kg^{-1}; ▤ lyophilized cells in olive oil with 312 mg gallic acid kg^{-1}; ___ rehydrated cells in olive oil with 45 mg gallic acid kg^{-1}; _ _ _ rehydrated cells in olive oil with 178 mg gallic acid kg^{-1}; ..... rehydrated cells in olive oil with 312 mg gallic acid kg^{-1}. All the values are the mean of two determinations ± SD

whereas the survival of the rehydrated cells exceeded 24 months (Fig. 2). However, compared to the previous strain, the DAPES 1892 *C. parapsilosis* strain showed a better olive oil polyphenols tolerance especially when rehydrated cells were used as inoculum (Fig. 2). The ultrastructural characteristics of both *C. parapsilosis* strains differed according to the inoculum type. The SEM observations of the DAPES 1890 and 1892 *C. parapsilosis* strains, both inoculated into the oil under a lyophilized form and recovered from the same samples after 24 months of storage, showed spherical shapes without buds and some cells with a

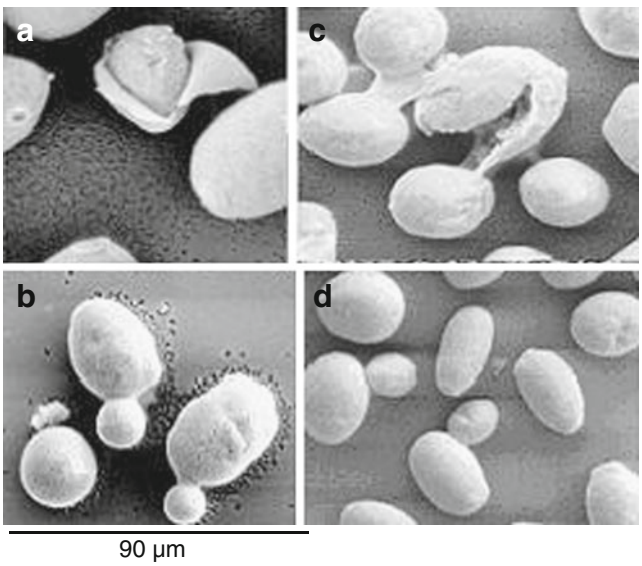

Fig. 3 SEM observation of *C. parapsilosis* recovered after 24 months of storage from original olive oil inoculated with lyophilized (**a**) or rehydrated (**b**) 1890 DAPES yeast strain and lyophilized (**c**) or rehydrated (**d**) 1892 DAPES yeast strain

damaged wall (Fig. 3a, c), whereas the yeast strains inoculated in the olive oil in a hydrated form showed mainly cells characterized by the presence of single buds (Fig. 3b, d). The chemical analysis carried out on the biomass of *C. parapsilosis* strains recovered from the oil samples after 24 months storage indicated that the olive oil polar phenolic compounds attached themselves steadily to the yeast cells. The concentration of the total polyphenols extracted from the biomass of the two *C. parapsilosis* strains positively correlated with the total polyphenols concentration of the inoculated olive oil samples, whereas no differences were found between the lyophilized and the rehydrated biomass of both the yeast strains (data not shown).

Discussion

The microbiological analyses and the SEM observations showed that the vitality of the two strains of *C. parapsilosis* is mainly conditioned by two parameters represented by the state of hydration of the yeast cells used as inoculum and the concentration of total polyphenols present in the oil. Considering the first parameter, the data reported in Figs. 1 and 2 indicate that both the *C. parapsilosis* strains survive better in the olive oil when, other than the free water normally present in the olive oil, other external water is added. The presence of buds observed with the SEM confirmed the positive role played by the rehydration of the yeast cells used as inoculum; however, comparing the results of the microbiological analyses reported in Figs. 1 and 2 with the observations made on the SEM (Fig. 3b, d), it can be hypothesized that even the yeast cells inoculated in the oil in a lyophilized form can survive a long time and also partly multiply by using the watery fraction dissolved in the olive oil. As is well known, all olive oils contain water which is partly found linked and partly free and available for chemical and enzymatic reactions (Tsimidou et al. 2005; Koidis et al. 2008). The olive oil polyphenols negatively influence the vitality of *C. parapsilosis*, but such action takes place very slowly, so that the death of all of the cells was only found in the olive oil fractions characterized by high concentrations of total polyphenols and not before 18 months of preservation (Figs. 1, 2). Comparing these results to the findings reported by other authors, it seems that yeast vitality is not inhibited to a large extent by olive phenolics (Medina et al. 2006; Zullo et al. 2010). In fact, the survival rates shown in Figs. 1 and 2 seems to be related to the concentration of polyphenols in the oil samples and consequently to their sorption to the yeast cell walls. The slow inhibiting effect demonstrated by the polyphenols in regard to the *C. parapsilosis* has important practical implications, since if we consider that the average *shelf life* of an extra virgin olive oil is approximately 12–18 months, during which it is used as an aliment by consumers, it is possible that, in the case of a high level of contamination of the newly-produced oil, this opportunistic pathogen yeast remains viable in the oil for several months before being completely devitalized by the same oil components. However, even though for analytical reasons (the recovery from the oil samples of enough cell biomass), these experiments were carried out through the inoculation of the olive oil with a very high number of yeast cells, scientific data currently available indicated a low number of *C. parapsilosis* in the commercial extra virgin olive oil (Koidis et al. 2008; Zullo et al. 2010). For this reason, there is not enough insight to demonstrate that the actual yeast cell density in olive oils can represent an effective threat to the consumer health.

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