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# Diversity of lipase-producing yeasts from marine environments and oil hydrolysis by their crude enzymes

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**Abstract** - Total 427 yeast strains from seawater, sediments, mud of salterns, guts of the marine fish and marine algae were obtained. After lipase activity of the yeast cultures was estimated, we found that nine yeast strains obtained in this study grown in the medium with olive oil could produce lipase. The results of routine identification and molecular methods show that they belonged to *Candida intermedia* YA01a, *Pichia guilliermondii* N12c, *Candida parapsilosis* 3eA2, *Lodderomyces elongisporus* YF12c, *Candida quercitrusa* JHSb, *Candia rugosa* wl8, *Yarrowia lipolytica* N9a, *Rhodotorula mucilaginosa* L10-2 and *Aureobasidium pullulans* HN2.3, respectively. The optimal pHs and temperatures of lipases produced by them were between 6.0 and 8.5 and between 35 and 40 ºC, respectively. Majority of lipases from the yeast strains were cell-bound and only lipase from *A. pullulans* HN2.3 was extracellular. Some lipases from the yeast strains could actively hydrolyse different oils, indicating that they may have potential applications in industry.

**Key words:** lipase, marine-derived yeasts, diversity, marine environments, lipid hydrolysis.

## INTRODUCTION

Lipases are a class of hydrolyases that are primarily responsible for the hydrolysis and the synthesis of esters formed from glycerol and long-chain fatty acids *in vitro* and *in vivo*. They catalyse a wide range of reactions, including hydrolysis, inter-esterification, alcoholysis, acidolysis, esterification and aminolysis. Therefore, lipases, especially microbial lipases have many industrial applications in the detergent, food, flavour industry, biocatalytic resolution of pharmaceuticals, esters and amino acid derivatives, making of fine chemicals, agrochemicals, use as biosensor, bioremediation and cosmetics and perfumery (Hasan *et al.,* 2006). Following proteases and carbohydrases, lipases are considered to be the third largest group based on total sales volume. The commercial use of lipases is a billion-dollar business that comprises a wide variety of different applications. Usually, extracellular lipase is produced by submerged liquid fermentation, solid state fermentation and immobilised cell culture (Sharma *et al*., 2001). Numerous species of bacteria, yeasts and molds were found to produce lipases. Among the terrestrial yeasts, several *Candida* spp., *Yarrowia lipolytica*, several *Rhodotorula* spp., some *Pichia* spp., *Saccharomycopsis crataegensis*, *Torulospora globosa* and *Trichosporon asteroides* have been found to be able to produce lipase (Vakhlu and Kour, 2006; Sharma *et al.,* 2001). Because most of yeast strains are considered as non-pathogenic, the processes for lipase production based on yeasts have been classified as GRAS (generally regarded as safe). Extracellular lipases from several yeasts have been purified and characterised and the genes encoding lipase in *Candida*, *Geotrichum, Trichosporon* and *Y. lipolytica* have been cloned and overexpressed (Vakhlu and Kour, 2006; Sharma *et al.,* 2001). It has been found that most of lipases are serine hydrolyases according to their biochemical properties (Vakhlu and Kour, 2006). Although lipases from *Candida rugosa* and *Candida antarctica* have been extensively used in flavour industry, synthesis of lipophillic antioxidants, detergent industry, lipid hydrolysis, biosensors and clinical purpose, very few studies exist on the lipase produced by the yeasts obtained from marine environments (Chi *et al.,* 2006).

After we screened over 400 yeast strains from different marine environments, we found that some yeast strains from different marine environments could produce lipase. The main purpose of the present study was to analyse diversity of lipase-producing yeasts from different marine environments. We also carried out the hydrolysis of various oils by the crude lipases or cell-bound lipases produced by the lipase-producing yeasts. To our knowledge, this is the first report on the lipase-producing yeasts derived from the marine environments.

## MATERIALS AND METHODS

**Sampling.** Different samples of seawater and sediments in China South Sea, China East Sea, Indian Ocean and the Pacific Ocean were collected during the Antarctic exploration in 2004 and hypersaline sea water, sediments of the salterns, different species of marine animals and algae along the coast of Qingdao, China were also collected.

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**Isolation of marine yeasts.** Two millilitres of the seawater or 2 g of the sediments or 2 ml of homogenised guts of marine animals or homogenised marine algae were suspended in 20 ml of YPD medium containing 2% (w/v) glucose, 2% (w/v) polypeptone and 1% (w/v) yeast extract and supplemented with 0.05% (w/v) chloramphenicol immediately after sampling and cultivated at natural temperature on the ship for five days. After suitable dilution of the cell cultures, the dilute was plated on YPD plates with 0.05% chloramphenicol and the plates were incubated at 20-25 °C for five days. Different colonies from the plates were transferred to the YPD slants, respectively.

**Lipase production**. One loop of the cells of the yeast strains was transferred to 50 ml of YPD medium prepared with distilled water in 250-ml flask and aerobically cultivated for 24 h. The cell culture (0.2 ml, $OD_{600nm}$ = 20.0) was transferred to 50 ml of the production medium which contained 3% (w/v) olive oil, 1% (w/v) ammonium sulphate, 0.2% (w/v) $K_2HPO_4$, 0.03% (w/v) $MgSO_4 \cdot 7H_2O$, 1.6% (w/v) NaCl, 0.1% (w/v) yeast extract, pH 7.0 and grown by shaking at 170 rpm and 25 °C for 4 days. The culture was centrifuged at 5000 rpm and 4 °C and the supernatant obtained was used as the crude lipase. For preparation of cell-bound lipase, the culture was washed three times by centrifugation at 5000 rpm and 4 °C and the cell pellets obtained were resuspended in 100 mM potassium phosphate buffer (pH 7.0).

**Determination of lipase activity.** The substrate emulsions were prepared by dropwise addition of 0.2 ml solution A (40 mg of *ρ*-nitrophenyl-laurate was dissolved in 12 ml isopropanol) into 3 ml solution B (0.4 g Triton X-100 and 0.1 g gum arabic were dissolved in 90 ml of 100 mM potassium phosphate buffer, pH 6.0-8.5) under intense vortexing. These emulsions were stable for 1 h at room temperature. The crude lipase (0.1 ml) or the cell-bound lipase (0.1 ml) was added to 3.2 ml of the substrate emulsion and the mixture was incubated for 20 min in a shaking water bath at 35-40 °C. Then, the mixture was put into ice and OD value at 410 nm in the mixture was read by using spectrophotometer. The same mixture to which the same amount of the inactivated crude lipase or the inactivated cell-bound lipase (heated at 100 ºC for 10 min) was added before the reaction was used as the control. One unit (U) of enzyme activity was defined as the amount of enzyme required for the liberation of 1.0 μM *ρ*-nitrophenol per minute under the assay conditions. The specific lipase activity was units per mg of protein or per g of cell dry weight. Protein concentration was measured by the method of Bradford, and bovine serum albumin served as standard (Bradford, 1976). Cell dry weight of the yeast culture was measured according to the methods described by Chi *et al.* (2001).

**DNA extraction and PCR.** The total genomic DNA of the yeast strains was isolated and purified by using the methods as described by Sambrook *et al.* (1989). Amplification and sequencing of 18S rDNA and ITS from the yeasts were performed according to the methods described by Chi *et al.* (2007). The common primers for amplification of D1/D2 26S rDNA in yeasts were used, the forward primer was NL-1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and the reverse primer was NL-4 (5′-GGTCCGTGTTTCAAGACGG-3′) (Sugita *et al.,* 2003). The reaction system (25 μl) was composed of 10x buffer 2.5 μl, dNTP 0.8 μM, $MgCl_2$ 1.5 mM, NL-1 0.5 μM, NL-4 0.5 μM, Taq DNA polymerase 1.25 U, template DNA 1.0 μl and $H_2O$ 16.6 μl. The conditions for the PCR amplification were as follows: initial denaturation at 94 °C for 10 min, denaturation at 94 °C for 1 min, annealing temperature at 53 °C for 1 min, extension at 72 °C for 2 min, final extension at 72 °C for 10 min. PCR was run for 32 cycles and PCR cycler was GeneAmp PCR System 2400 made by Perkin-Elmer. PCR products were separated by agarose gel electrophoresis and recovered by using UNIQ-column DNA gel recovery kits (BIOASIA, Shanghai). The recovered PCR products were ligated into pGEM-T easy vector and transformed into the competent cells of *Escherichia coli* JM109. The transformants were selected on plates with ampicillin. The plasmids in the transformant cells were extracted by using the methods as described by Sambrook *et al.* (1989). The D1/D2 26S rDNA fragments inserted on the vector were sequenced by Shanghai Sangon Company.

**Phylogenetic analysis and identification of the yeasts.** The sequences obtained above were aligned by using BLAST analysis (http://www.ncbi.nlm.nih.gov/BLAST). For comparison with currently available sequences, several sequences were retrieved with over 98% similarity belonging to different genera from NCBI (http://www.ncbi.nlm.nih.gov) and multiple alignment was performed by using ClustalX 1.83 and phylogenetic trees were constructed by PHYLIP 3.56. The routine identification of the yeasts was performed by using the methods as described by Kurtzman and Fell (2000).

**Effects of pH and temperature on lipase activity.** The effects of pH on the enzyme activity were determined by incubating the culture supernatant or the cell-bound lipase at different pH between 4.0 and 9.0 using the standard assay conditions described above. The buffers used were 0.1 M citric-sodium dihydrogen phosphate buffer (pH 3.0), 0.1 mM acetate buffer (pH 4.0-5.0), and 0.1 M phosphate buffer (pH 6.0-9.0). The optimal temperature for activity of the enzyme was determined at 30, 35, 40, 45, 50, 55, 60 and 65 °C in the same buffer as described above.

**Determination of hydrolytic activity of the lipases.** Five millilitres of 20 mM phosphate buffer (pH 6.0-8.5) and 4.0 ml of 50% oil emulsion were added to 100-ml flasks and the mixture was kept at 35-40 °C for 5 min. The crude lipase (1 ml) or the cell-bound lipase (1 ml) was added to the mixture in one of the flasks. Another flask without addition of the crude lipase or the cell-bound lipase was used as the blank. The flasks were shaken at 35-40 °C in the water bath for 30 min. Ten millilitres of 95% ethanol solution were added to the mixture immediately to cease the reaction and 1.0 ml of the crude lipase or the cell-bound lipase was added to the blank. Liberated free fatty acids were titrated with 0.05 M NaOH using phenolphthalein as indicator (Wu *et al.,* 1996). One unit of hydrolytic activity of the lipase was defined as the amount of enzyme which catalyses the release of one μM of free fatty acids per min under the above conditions. The specific lipase activity was units per mg of protein or per g of cell dry weight.

TABLE 1 - Sources of the lipase-producing marine yeasts

| Strains | Sources |
|---|---|
| YA01a | Surface of *Sargassum pallidum* collected from seawater at Changdao Island, China |
| N12c | Seawater at China South Sea |
| 3eA2 | Gut of *Apostichopus japonicus* from seawater in Srilank |
| YF12c | Gut of *Pseudosciaena crocea* collected at the coastline in Qingdao |
| JHSb | Salterns in Qingdao |
| HN2.3 | Salterns in Qingdao |
| N9a | Deep seawater in South pole |
| L10-2 | Surface of *Laminaria japonica* collected from seawater at Changdao Island, China |
| Wl8 | Gut of *Nemipterus virgatus* collected from seawater at China East Sea |

## RESULTS AND DISCUSSION

### Screening of the lipase-producing yeasts

Some of terrestrial yeasts have been confirmed to have the capacity to produce over 150 U/ml of lipase (Lotti *et al*., 1998). Therefore, we want to know if such yeasts exist in marine environments. After over 400 yeast strains from seawater, sediments, guts of the marine fish and marine algae were screened, we found that nine yeast strains from different marine environments grown in the medium with olive oil could produce lipase (data not shown). As shown in Table 1, the nine yeast strains were isolated from surface of *Sargassum pallidum* collected at Changdao Island, China, seawater at China South Sea, gut of *Apostichopus japonicus* from seawater in Srilanka, gut of *Pseudosciaena crocea* collected at coastline in Qingdao, salterns in Qingdao, deep seawater in South pole, surface of *Laminaria japonica* collected from seawater at Changdao Island, China and gut of *Nemipterus virgatus* collected at China East Sea, respectively. In recent years, we have found that diversity of marine yeasts is very rich. However, to our knowledge, the yeast from marine environments is still an untouched bioresource for lipase production (Chi *et al.,* 2006). It is very interesting to observe from the results in Table 1 that lipase-producing yeasts were distributed in many marine environments and many species of lipase-producing yeasts occurred in the marine environments.

### Physiological and biochemical characterisation

Based on the fermentation spectra and carbon source assimilation spectra of the yeasts isolated in this study and those of the type strains listed in The Yeast, A Taxonomic Study (Kurtzman and Fell, 2000), we found that strains YA01a, N12c, 3eA2, YF12c, JHSb, HN2.3, N9a, L10-2 and w18 looked similar to *Candida intermedia, Pichia guilliermondii*, *Candida parapsilosis*, *Lodderomyces elongisporus*, *Candida quercitrusa, Aureobasidium pullulans*, *Yarrowia lipolytica*, *Rhodotorula mucilaginosa* and *Candida rugosa*, respectively (Table 2).

TABLE 2 - The fermentation and assimilation of different carbohydrates by the marine yeast strains

| | | Marine yeast strains | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | YA01a | N12c | 3eA2 | YF12c | JHSb | HN2.3 | N9a | L10-2 | wl8 |
| Fermentation | Glucose | + | + | + | + | – | – | – | – | – |
| | Maltose | – | – | – | – | – | – | – | – | – |
| | Galactose | + | – | + | – | + | – | – | – | – |
| | Sucrose | + | – | – | – | – | – | – | – | + |
| | Lactose | – | – | – | – | – | – | – | – | – |
| | Raffinose | – | – | – | – | – | – | – | – | + |
| | Melibiose | – | – | – | – | – | – | – | – | – |
| Assimilation | Glucose | + | + | + | + | + | + | + | + | + |
| | Maltose | + | + | + | + | + | + | – | + | + |
| | Galactose | + | + | + | + | + | + | + | + | + |
| | Sucrose | + | + | + | + | + | + | – | + | + |
| | Lactose | + | – | W | – | – | – | – | – | – |
| | Raffinose | + | W | W | – | + | + | – | + | + |
| | Melibiose | – | – | – | + | + | + | – | – | – |
| | Amidulin | W | – | W | – | W | + | – | + | + |
| | Trehalose | + | – | + | + | + | + | – | + | + |
| | Cellobiose | + | W | W | W | + | + | – | – | – |
| | D–Arabinose | W | – | – | – | – | + | + | + | + |
| | Xylose | + | + | + | + | + | + | W | + | + |
| | L–Arabinose | W | – | + | + | + | + | – | + | + |

+: positive; –: negative; W: weak.
Fermentation and assimilation were performed at 25 °C in the media with 0.5% sugars at natural pH (Kutzman and Fell, 2000).

**Phylogenetic analysis of partial sequences of the 18S rRNA genes, D1/D2 26S rDNA and ITS**

According to Kurtzman and Fell (2000), traditional and routine identification methods which depend on phenotype, are usually leading to uncertain and inaccurate interpretations of species interaction. Sequence analysis of phylogeny for microbial taxonomy, is a more accurate method for determining inter- and intra-specific relationships. Therefore, 18S rDNA partial sequences, D1/D2 26S rDNA sequences and ITS of the yeast strains were determined and aligned by using BLAST analysis (http://www.ncbi.nlm.nih.gov/BLAST). Phylogenetic trees were constructed by using PHYLIP software package version 3.56 (Felsenstein, 1995). Distance matrices were generated by the DNADIST program, based on Kimura's two-parameter model (Kimura, 1980). Neighbour-joining analysis of the data sets was carried out with the program Neighbour of the PHYLIP package. *Cafeteria roenbergensis* (heterotrophic flagellates), *Ricciocarpos natans* and *Gianda intestinalis* were used as out-groups during the construction of consensus trees of the isolates based on 18S rRNA gene sequences, D1/D2 26S rDNA sequences and ITS, respectively. The search for the similarity between 18S rDNA sequences, D1/D2 26S rDNA sequences and ITS of the isolates and those in the NCBI database shows that many phylogenetically related yeast species were similar to the yeast strains obtained in this study. Phylogenetic relationships of 18S rDNA sequences, D1/D2 26S rDNA sequences and ITS of the yeast strains isolated in this study were shown in Figs. 1, 2 and 3 and their GenBank accession numbers and the closest relatives are shown in Table 3. The topology of the phylogram in Figs.1, 2 and 3 confirms that the strain YA01a was closely related *Candida intermedia,* whereas the strain N12C could be in close relationship to *Pichia guilliermondii*. The strain 3eA2 was assigned to *Candida parapsilosis*. D1/D2 26S rDNA sequences and ITS of yeast strain YF12C were identical to those of *Lodderomyces elongisporus*, respectively. Strain JHSb was identified to be a strain of *Candida quercitrusa*. Strain HN2.3 was found to be a strain of *Aureobasidium pullulans* while strains N9a, L10-2 and w18 were similar to *Yarrowia lipolytica*, *Rhodotorula mucilaginosa* and *Candida rugosa*, respectively. The results were identical to those from fermentation spectra and carbon source assimilation spectra of the yeasts obtained in this study (Table 2).

To date, only *Candida rugosa*, *Candida tropicalis*, *Candida antarctica*, *Candida cylindracea*, *Candida parapsilosis*, *Candida deformans*, *Candida curvata*, *Candida valida*, *Yarrowia lipolytica*, *Rhodotorula glutinis*, *Rhodotorula pilimanae*, *Pichia bispora, Pichia mexicana*, *Pichia silvicola*, *Pichia xylosa*, *Pichia burtonii*, *Saccharomycopsis lipolytica*, *Saccharomycopsis crataegenesis*, *Torulospora globosa* and *Trichosporon asteroides* have been found to be able to produce lipase (Sharma *et al.,* 2001; Vakhlu and Kour, 2006) among the terrestrial yeasts. Therefore, the yeast strains

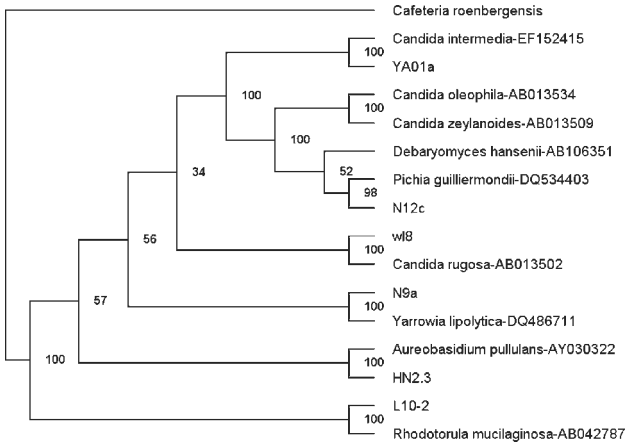


FIG. 1 - Consensus tree of the isolates based on 6 18S rRNA gene sequences obtained in this study and 9 previously published sequences obtained from GenBank. The outgroop we used was *Cafeteria roenbergensis*. The numbers above the branches are bootstrap, only values 98% are shown.

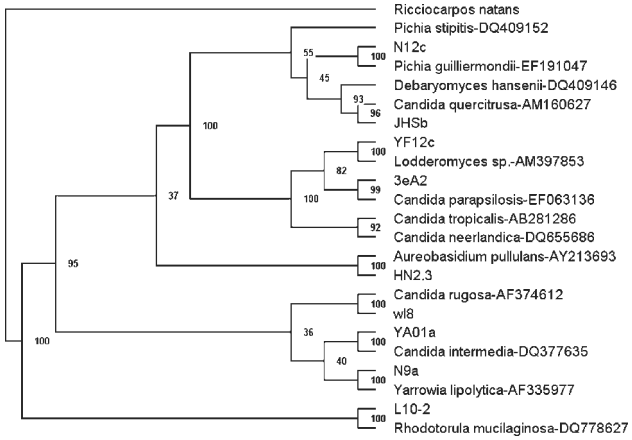


FIG. 2 - Consensus tree of the isolates based on 9 D1/D2 26S rDNAs obtained in this study and 13 previously published sequences obtained from GenBank. The outgroop we used was *Ricciocarpos natans*. The numbers above the branches are bootstrap, only values 96% are shown.

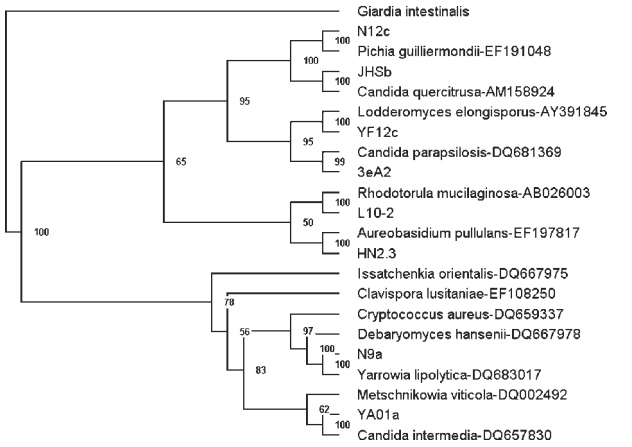


FIG. 3 - Consensus tree of the isolates based on 8 ITS sequences obtained in this study and 13 previously published sequences obtained from GenBank. The outgroop we used was *Gianda intestinalis*. The numbers above the branches are bootstrap, only values > 99% are shown.

TABLE 3 - The accession numbers of ITS, 18S rDNA and D1/D2 26S rDNA from the nine lipase-producing marine yeasts

| Strains | The closest relatives (% similarity) | The accession numbers of 18S rRNA gene | The accession numbers of ITS | The accession numbers D1/D2 26S rRNA gene |
|---|---|---|---|---|
| YA01a | *Candida intermedia* (99% ) | EF408189 | DQ680837 | EF362751 |
| N12c | *Pichia guilliermondii* (99% ) | DQ438179 | EF375704 | EF375700 |
| 3eA2 | *Candida parapsilosis* (over 99% ) | EF208925 | DQ681358 | EF362748 |
| YF12c | *Lodderomyces elongisporus* (100% ) | DQ515959 | EF394941 | EF394940 |
| JHSb | *Candida quercitrusa* (99% ) | EF152413 | DQ665264 | EF375703 |
| HN2.3 | *Aureobasidium pullulans* (over 99% ) | EF125668 | DQ680685 | EF375702 |
| N9a | *Yarrowia lipolitica* (100% ) | EF190312 | DQ683016 | EF362750 |
| L10-2 | *Rhodotorula mucilaginosa* (over 99% ) | EF218987 | DQ681372 | EF362749 |
| wl8 | *Candida rugosa* (over 99% ) | EF371020 | EF198009 | EF375701 |

*C. intermedia* YA01a, *L. elongisporus* YF12c, *P. guilliermondii* N12c, *C. quercitrusa* JHSb, *R. mucilaginosa* L10-2 and *A. pullulans* HN2.3 obtained in this study were the new producers of lipase.

**Effects of different temperature and pH on activity of the crude lipases**

The results in Table 4 show that the optimal pHs for the crude lipases produced by the yeasts *C. intermedia* YA01a, *P. guilliermondii* N12c, *C. parapsilosis* 3eA2 and *L. elongisporus* YF12c were 7.5 while the optimal pHs for the crude lipases produced by the yeasts *C. quercitrusa* JHSb and *C. rugosa* wl8 were 7.0. It also can be seen from Table 4 that the optimal pHs for the crude lipases from *A. pullulans* HN2.3, *Y. lipolytica* N9a and *R. mucilaginosa* L10-2 were 8.5, 8.0 and 6.0, respectively. It can be observed from the results in Table 4 that the optimal temperatures of the crude lipases produced by the yeasts *C. intermedia* YA01a, *C. parapsilosis* 3eA2, *L. elongisporus* YF12c and *Y. lipolytica* N9a were 40 ºC while the optimal temperatures of the crude lipases produced by other yeast strains were 35 ºC, respectively.

Usually, the optimal pH and temperature for lipase from terrestrial yeasts are between 5.0 and 8.0 and between 30 and 50 ºC (Vakhlu and Kour, 2006). For example, the optimal pH and temperature for activity of the crude lipase produced by *C. rugosa* were 30 °C and pH 7.2, respectively (Lotti *et al.,* 1998). This means that the optimal pH and temperature of the crude lipase from the yeasts obtained in this study were in agreement with those from terrestrial yeasts.

**Lipase activity and cellular location of lipases from the yeasts**

It is very interesting to note from data in Table 5 that *C. intermedia* YA01a, *P. guilliermondii* N12c, *C. parapsilosis* 3eA2, *L. elongisporus* YF12c, *C. quercitrusa* JHSb, *C. rugosa* wl8, *Y. lipolytica* N9a and *R. mucilaginosa* L10-2 produced cell-bound lipase whereas *A. pullulans* HN2.3 secreted lipase into the medium. Table 5 also shows that the yeast strains *C. intermedia* YA01a and *L. elongisporus* YF12c had higher cell-bound lipase activity than any other yeast strains tested in this study and *A. pullulans* HN2.3 had the highest extracellular lipase activity.

Our results (Table 5) show that majority of lipases from the yeast strains used in this study were cell-bound and only *A. pullulans* HN2.3 secret a large amount of lipase into the medium. However, it has been confirmed that most of lipases from terrestrial yeast are extracellular, but lipase from terrestrial yeasts *C. parapsilosis* CBS 604 and some lipase from *Y. lipolytica* are also cell-bound (Vakhlu and Kour, 2006).

TABLE 4 - The optimal temperature and pHs of the crude lipases produced by the marine yeast strains

| Strains | Optimal temperature (ºC) | Optimal pH |
|---|---|---|
| *C. intermedia* YA01a | 40 | 7.5 |
| *P. guilliermondii* N12c | 35 | 7.5 |
| *C. parapsilosis* 3eA2 | 40 | 7.5 |
| *L. elongisporus* YF12c | 40 | 7.5 |
| *C. quercitrusa* JHSb | 35 | 7.0 |
| *A. pullulans* HN2.3 | 35 | 8.5 |
| *Y. lipolytica* N9a | 40 | 8.0 |
| *R. mucilaginosa* L10-2 | 35 | 6.0 |
| *C. rugosa* wl8 | 35 | 7.0 |

TABLE 5 - The lipase activities produced by the marine yeast strains and cellular location of lipases

| Strains | Lipase activity (U $g^{-1}$) | Cellular location | Fermentation period (days) |
|---|---|---|---|
| *C. intermedia* YA01a | 42.0 ± 1.2 | Cell-bound | 3 |
| *P. guilliermondii* N12c | 43.6 ± 1.8 | Cell-bound | 2 |
| *C. parapsilosis* 3eA2 | 10.4 ± 0.12 | Cell bound | 3 |
| *L. elongisporus* YF12c | 16.6 ± 0.9 | Cell bound | 2 |
| *C. quercitrusa* JHSb | 9.6 ± 0.3 | Cell bound | 4 |
| *A. pullulans* HN2.3 | 8.2 ± 0.12 | Extracellular | 2 |
| *Y. lipolytica* N9a | 5.1 ± 0.73 | Cell-bound | 3 |
| *R. mucilaginosa* L10-2 | 4.0 ± 0.23 | Cell-bound | 3 |
| *C. rugosa* w18 | 26.9 ± 1.2 | Cell-bound | 2 |

Values are given as mean ± SD, n = 3.

TABLE 6 - Oil hydrolysis by the crude lipases

| Strains | Olive oil | Lard | Peanut oil | Soybean oil |
|---|---|---|---|---|
| *C. intermedia* YA01a | 200.0 ± 18.1[a] | 16.6 ± 2.4[a] | 160.0 ± 3.2[a] | 13.2 ± 0.5[a] |
| *P. guilliermondii* N12c | 21.0 ± 0.9[a] | 196.5 ± 2.7[a] | 119.5 ± 0.9[a] | 14.2 ± 1.1[a] |
| *C. parapsilosis* 3eA2 | 94.1 ± 2.7[a] | 8.8 ± 0.5[a] | 10.1 ± 0.4[a] | 86.3 ± 3.5[a] |
| *L. elongisporus* YF12c | 74.4 ± 2.8[a] | 12.6 ± 0.7[a] | 93.0 ± 1.2[a] | 54.6 ± 0.5[a] |
| *C. quercitrusa* JHSb | 30.7 ± 3.2[a] | 4.3 ± 0.8[a] | 12.0 ± 0.1[a] | 8.3 ± 0.5[a] |
| *C. rugosa* wl8 | 14.3 ± 0.6[a] | 6.8 ± 1.2[a] | 11.9 ± 1.5[a] | 9.1 ± 0.7[a] |
| *Y. lipolytica* N9a | 8.8 ± 0.4[a] | 3.3 ± 0.2[a] | 10.3 ± 0.8[a] | 13.5 ± 0.4[a] |
| *R. mucilaginosa* L10-2 | 28.2 ± 1.7[a] | 7.4 ± 0.3[a] | 18.3 ± 0.9[a] | 16.7 ± 1.5[a] |
| *A. pullulans* HN2.3 | 35.2 ± 1.3[b] | 33.2 ± 0.9[b] | 37.3 ± 1.9[b] | 29.6 ± 0.9[b] |

[a]: units per g of cell dry weight; [b]: units per mg of protein. Values are given as mean ± SD, n = 3.

**Hydrolytic activity of the crude lipases**

The hydrolytic reaction catalysed by lipase generally takes place at the oil-water interface (Sharma *et al*., 2001). The hydrolytic activity is the basic characteristic of lipase. It is required that lipase is non-specific, hydrolysing different kinds of lipids from different sources when it is applied to lipase biosensor, detergent industry and digestion of lipids in the food and medicine (Wu *et al.,* 1996). Therefore, the crude lipase hydrolytic activity by the marine yeasts obtained in this study was assayed towards olive oil, peanut oil, soybean oil and lard. The results in Table 6 indicate that the crude lipase produced by the yeast strain HN2.3 which could produce extracellular lipase had high hydrolytic activity towards all the oils, especially peanut oil while the cell-bound lipase from strain YA01a had high hydrolytic activity towards olive oil and peanut oil and cell-bound lipase from strain N12c had high hydrolytic activity towards lard and peanut oil, suggesting that the extracellular lipase from strain HN2.3 and cell-bound lipases from strains YA01a and N12c had highly potential application in digestion of lipids. However, cell-bound lipases from other yeast strains had very low hydrolytic activity towards the oils tested in this study (Table 6). The results in Table 6 also show cell-bound lipases from strain YA01a had very low hydrolytic activity towards lard and soybean oil and cell-bound lipases from strain N12c had very low hydrolytic activity towards olive oil and soybean oil. Although lipase from terrestrial yeast *C. rugosa* has been extensively used in industrial and clinical purpose (Vakhlu and Kour, 2006), lipase activity produced by the yeast *C. rugosa* wl8 obtained in this study was lower than that produced by *C. intermedia* YA01a and *L. elongisporus* YF12c tested in this study.

In China, a large amount of peanut oil and lard is discharged from the restaurants and homes each day and causes heavy pollution in fresh water and seawater. Therefore, the lipase with high hydrolytic activity towards peanut oil and lard may have highly potential applications in degradation of oil in fresh water and seawater and reuse of the wasted peanut oil. It should be stressed that the cell-bound lipase from the marine yeasts had many advantages over the extracellular lipase when it is applied to industries because it is very easy to collect and concentrate the cell-bound lipase by centrifugation of yeast culture and yeast cell flocculation.

In recent years, many results have also shown that lipase in the intestine of marine animals can help digest lipid in their feed, inferring that lipase activity may be implicated in regulating the use of dietary components and possibly influencing the stages of development in marine animals (Fu *et al*., 2005). Therefore, marine yeasts with high lipase activity also can be used in mariculture. We also think that lipase-producing marine yeasts are new bioresouces and gene resources (Chi *et al*., 2006).

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