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Taxonomic Characterization and Potential Biotechnological Applications of *Yarrowia lipolytica* Isolated From Meat and Meat Products

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ABSTRACT

Background: Some species of yeast such as *Yarrowia lipolytica* produce citric acid, lipases, single-cell oil, etc. *Y. lipolytica* can degrade renewable, low-cost substrates to produce organic acids like citric acid, more efficiently than *Aspergillus niger*, and result in higher product yield and lesser waste production and toxicity.
Objectives: The aim of this study was to isolate yeast strains with potential for use in biotechnological applications such as production of citric acid and lipase.
Materials and Methods: For yeast strain screening, we isolated 179 yeast strains from meat and meat products that were prepared at the RAK and Pegah factories in Isfahan, Iran. Different media were used for screening of yeast colonies and for analyses of citric acid and lipase production; the production of these metabolites was assayed over time.
Results: One of the yeast strains isolated from poultry produced 55.5 g/L of citric acid and 12.3 U/mL of lipase. Biochemical and molecular tests showed that this strain belonged to the species *Y. lipolytica*. Molecular identification was confirmed by DNA sequencing, and the strain was named *Y. lipolytica* M7 (GenBank accession number, HM011048).
Conclusions: The results of this study suggest that meat and its products, especially poultry products, are suitable sources for isolation of yeast strains that produce two biotechnologically valuable products—citric acid and lipase. The yeast strain *Y. lipolytica* M7 can be used for citric acid production in bioreactor.

Implication for health policy/practice/research/medical education:
This study introduced *Y. lipolytica* M7 as suitable wild strain with high potential for citric acid production and acceptable amount of lipase activity.

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1. Background

Yeasts are commonly used microorganisms in the production of various foods and beverages. They are also involved in the spoilage of food products either due to an impaired fermentation process or post-process contamination. Thus far, yeast isolation has been mostly performed to determine food spoilage. Encinas *et al.* investigated the yeast populations present on Spanish fermented sausages and reported that *Debaryomyces hansenii* was the dominant strain in their samples (1). Deak *et al.* and Sanz *et al.* reported that *Yarrowia lipolytica* was the predominant strain isolated from meat products (2, 3). Citric acid is widely used in many industrial applications, manufacture of cosmetics, and buffering and chelating (4-6). All developed countries follow a conventional procedure involving the use of *Aspergillus niger* (producer) and molasses (substrate) for citric acid production.

Use of yeasts such as *Y. lipolytica* has several advantages

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over the use of Aspergillus spp, since yeast strains show lower sensitivity to low dissolved oxygen concentrations and heavy metals, and give higher product yields. Wild-type strains of *Y. lipolytica* can use a wide spectrum of carbon sources as substrates for production of organic acids, except for sucrose, a cheap and commonly used source of carbon for citric acid production with *A. niger*. Therefore, yeasts are better candidates for citric acid production than *A. niger* (4-6). Levinson *et al.* screened yeasts isolated from different geographic areas and concluded that *Y. lipolytica* was the best strain for citric acid production (range, 1.4–21.6 g/L) (7). Anastassiadis *et al.* x investigated the citric acid levels produced by some *Candida* strains and found the yield range to be 2.4–50.1 g/L (7). In literature, different *Y. lipolytica* strains have been compared (9-11), and the effects of different compositions of growth culture media and other parameters have been studied simultaneously (10, 12).

Lipases (EC 3.1.1.3) are among the most important classes of industrial enzymes and have many interesting applications in lipid biotechnology as well as synthesis of biosurfactants, pharmaceuticals, optically pure compounds, and flavor compounds (13, 14). Some yeasts can produce citric acid and lipase from a wider range of carbon sources than can fungi and bacteria, including glucose, molasses, fatty acids, alcohols, acetate, n-alkanes, and plant oils. Among these sources, glucose has gained considerable interest after the oil crisis of 1973, which is a very plausible situation in the present scenario (15, 16).

2. Objectives

The aim of this study was to isolate yeast strains with potential for use in biotechnological applications, especially citric acid and lipase productions. Therefore, we screened the yeast strains obtained from meat and studied their potential for producing citric acid and lipase, and identified the best strain by performing biochemical and molecular tests.

3. Materials and Methods

3.1. Isolation and Screening Procedures

We used routine microbiological procedures and selective nutrition media for isolation of yeasts and obtained 37 samples from different meat and meat products from the food factories in Isfahan, Iran. The samples were serially diluted, and 100 µl from each dilution was cultivated on yeast extract glucose chloramphenicol (YGC) agar (Merck, Germany) and incubated at 29°C for 2 days. The yeast strains were conserved at 4°C on yeast extract peptone dextrose (YPD) medium (Merck, Germany) (17).

The medium used for screening citric acid production was composed of 20 g glucose, 0.7 g $MgSO_4$, 0.4 g $Ca(NO_3)_2$, 0.5 g NaCl, 1 g KH_2PO_4, 0.1 g K_2HPO_4, 8 g yeast autolysate, and 20 g/L agar supplemented with trace elements in the solution (18). Bromocresol green indicator (0.4%) was used for qualitative screening of citric acid (19). Qualitative screening of lipase from isolated yeasts was performed on tributyrin agar and rhodamine B agar (Sigma, USA). After cultivation, the media were incubated at 29°C for 3–10 days. Strains producing citric acid and lipase were identified on the basis of the hydrogen-to-carbon (H/C) index for each isolated yeast strain. The H/C index was determined by the ratio of the diameters of the yellow halo on the citric acid screening medium and hydrolysis halo or the orange halo on the tributyrin agar or rhodamine B agar (H), respectively, relative to cell colony size (C). An H/C ratio of 1 meant no hydrolysis, and thus no production of citric acid and lipase, whereas a higher H/C ratio indicated production of these metabolites (20-22). *Y. lipolytica* DSM 3286 strain was obtained from the culture collection of DSM, Germany. This strain could produce citric acid and lipase; therefore, it was used as the standard strain to compare the production of citric acid and lipase in the other isolated yeast strains (8).

Table 1. Yeast Strains Isolated From Meat and Meat Products

	Poultry meat	Beef	Sausages	Lamb	Hamburgers	Red meat
Strains	M1–53	M54–67	M68–102	M103–114	M115–142	M143–179

Table 2. Comparison of Assimilation Test Results Between Two Yeast Strains (*Y. lipolytica* DSM 3286 and Isolated *Y. lipolytica* M7)

	Citrate	Gelatin	Acetyl-D-glucosamin	Ethanol	Erythritol	Glycerol	Melibiose	Starch	Rhamnose	Galactitol	Ribitol	D-glucosamin	Lactose	Galactose	Maltose	Trehalose	Raffinose	Inulin	Xylose	Ribose	Mannitol	Arabinose	Cellobiose	Glucose	Sucrose
M7	+	+	+	+	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	+/-	+	-
DSM 3286	+	+	+/-	+	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+/-	-	+	+	-

3.2. Production Media and Analysis Procedures

The fermentation media for citric acid and lipase production contained glucose and olive oil as carbon sources, respectively (23). Glucose concentration was determined using a glucose kit (Darvash, Iran). Initial pH of the media was adjusted to 7. For cultivation, the strains were grown at 29°C with constant agitation at 200 rpm in 250-ml flasks. Citric acid assay was performed using a K-CITR enzymatic test kit (Megazyme, Ireland) according to manufacturer's instructions. Extracellular lipase production was determined spectrophotometrically by using ρ-nitrophenyl laurate (ρ-NPL) as the substrate (24). A titrimetric method described by Darvishi *et al.* was used to confirm the results (25). For biomass determination, the cells were harvested by centrifugation at 4600 × g for 6 min and then washed with hexane. The dry weight of the centrifuged biomass was determined after drying at 80°C for 24 h (25, 26).

3.3. Identification of Yeast Strains

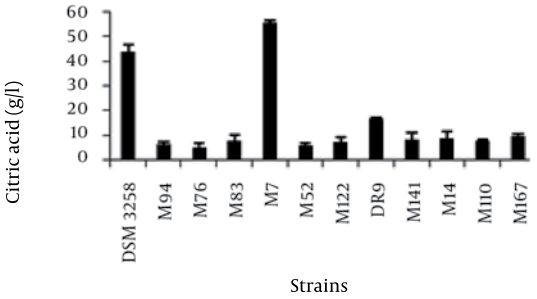
The isolated yeast strains were identified by assimilation and fermentation tests according to previously described methods (27). The results of the biochemical tests were further confirmed by molecular testing. Isolation and purification of DNA were performed according to the modified method put forth by Vasdinyei and Deak (28). Internal transcribed spacer-polymerase chain reaction (ITS-PCR) was performed according to the method described by Dlauchy *et al.* (29). The following primer sequences were used as described by White *et al.*: ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (30). Twenty-five cycles of PCR were carried out using the following program: denaturation at 95°C for 30 s, followed by annealing at 55°C for 30 s, and extension at 72°C for 1 min. DNA was sequenced on an automated sequencer (3730xl DNA Analyzer, Applied Biosystems) by using synthetic primers and the dye-terminator procedure. These processes were outsourced to Macrogen, South Korea.

4. Results

About 179 yeast strains were isolated from poultry, beef, and lamb, and meat products like hamburgers and sausages depending on the morphological and physiological characteristics of the yeast strains (*Table 1*).

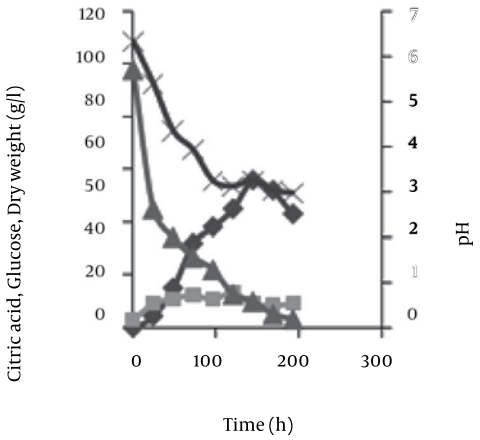
Eleven different yeast strains were selected on citric acid and lipase screening media on the basis of zone-formation around the colonies and H/C ratio. Isolated strains were cultured on citric acid fermentation medium for 8 days and quantitative assay for citric acid was performed every 24 h. Our results showed that the maximum amount of citric acid was produced 144 h after inoculation. One of the strains isolated from poultry (M7) produced the maximum amount of citric acid, 55.5 g/L, which was more than that produced by the standard strain *Y. lipolytica* DSM 3286 (*Figure 1*). Changes in citric acid production, glucose consumption, dry weight, and pH associated with the M7 strain on the fermentation medium during the 192 h are shown in *Figure 2*. The M7 strain was cultured on the basal lipase production medium for the quantitative lipase production assay. Our results showed that maximum lipase production occurred at 24 h after inoculation, and the M7 strain produced 12.3 U/mL of lipase (*Figure 3*). In the biochemical

Figure 1. Comparison of Citric Acid Production by Isolated Yeasts on Citric Acid Fermentation Medium at 144 h After Inoculation



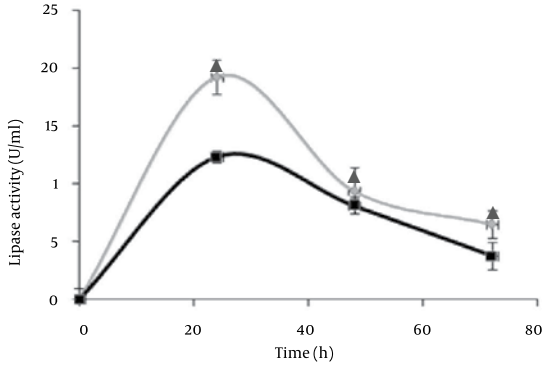
DSM 3286: *Yarrowia lipolytica* DSM 3286. Error bars represent the standard error associated with the findings from at least 3 experiments

Figure 2. Changes Associated With Citric Acid Production, Glucose Concentration, Dry Weight, and pH in the *Y lipolytica* M7 Strain on Citric Acid Fermentation Medium (Incubation Time, 192 h)



(♦) citric acid, (▲) glucose, (■) dry weight, (×) pH

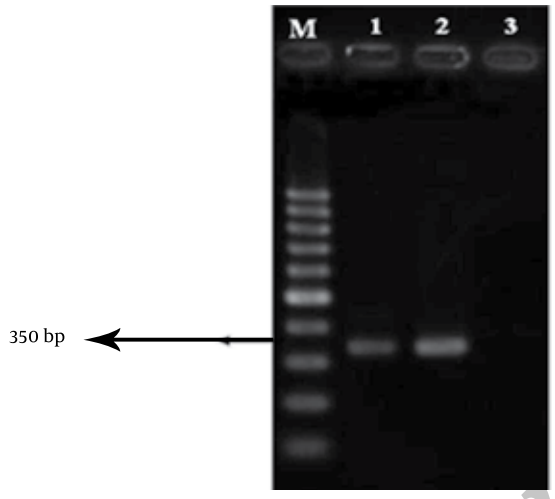
Figure 3. Comparison of Lipase Production Between *Yarrowia lipolytica* strains M7 and DSM 3286 on the Basal Lipase Production Medium



Error bars represent the standard error associated with the findings from at least 3 experiments (▲) DSM 3286, (■) M7

test performed for M7 strain identification, negative results were obtained for fermentation tests of sugars, including glucose, galactose, sucrose, maltose, lactose, raffinos, trehalose and positive results were obtained for assimilation of glucose, cellobiose, mannitol, galactose, glycerol, D-acetyl glucosamine, citrate, gelatin, and ethanol; the results of other assimilation tests were negative (Table 2). To confirm the results of the biochemical tests, ITS-PCR was performed (results have been shown in Figure 4). The results of the biochemical tests, PCR, and DNA sequencing indicated that the distinct M7 strain belonged to the *Y. lipolytica* species. Therefore, the M7 strain was named *Y. lipolytica* M7. The nucleotide sequence of *Y. lipolytica* M7 was registered with GenBank (accession number, HM011048). A dendrogram was obtained using the MEGA4 software (Center for Evolutionary Medicine and Informatics, USA) (*Figure 5*).

Figure 4. ITS-PCR Results Obtained for *Yarrowia lipolytica* Strains M7 and DSM 3286 by Using ITS1 and ITS4 Primers



M: 100-bp DNA ladder; 1: *Y. lipolytica* DSM 3286 [positive control]; 2: M7 strain; 3: negative control
ITS-PCR: Internal transcribed spacer-polymerase chain reaction

5. Discussion

This study focused on the isolation of citric acid-producing yeasts such as *Y. lipolytica* or Candida oleophila that were previously described as the best citric acid producers (8, 16, 24). *Y. lipolytica* strains can be easily isolated from dairy products and salads containing meat or shrimps (8). Deak *et al.* (2) reported that 39% of the yeast isolates from poultry meat belonged to the *Y. lipolytica* strain. Yeast strains are usually considered unimportant in meat spoilage because their low initial numbers and slow growth rates at refrigeration temperatures prevent them from competing effectively with psychrotrophic bacteria (31). However, yeasts strains are more resistant than bacteria to several food-related stresses, such as low water activity, low pH, high salinity, and chemical preservatives. Therefore, these yeast strains may become important opportunistic spoilage agents in meat products when bacterial growth gets retarded due to the inhibitory effects of the above stress factors (31, 32).

Contamination and spoilage of raw and processed poultry meat with bacteria are well documented. However, not much is known about the presence and role of yeasts in spoilage of poultry meat, although yeasts are a stable part of the microbiota on raw red-meat, poultry, and fish (32, 33). In this study, we focused on the isolation of yeasts from meat and meat products. Some of the strains were

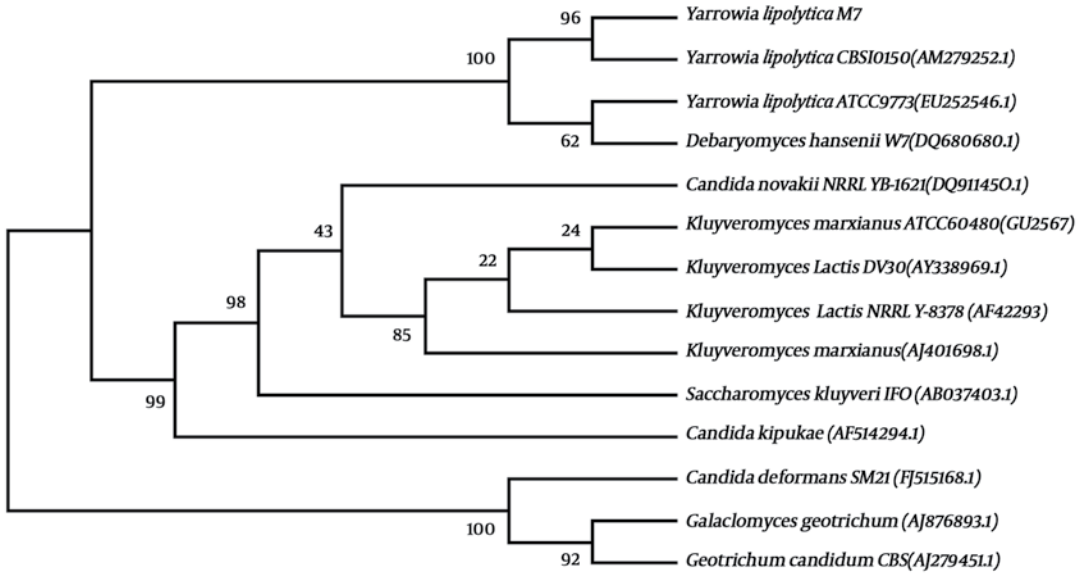


Figure 5. Cluster Analysis of the Patterns of the Relationship of *Y. lipolytica* with the Source of Isolation. (Dendrogram was Obtained Using MEGA4 Software)

isolated for their ability to produce citric acid. Anastassiadis *et al.* isolated a strain of *C. oleophila* with citric acid yields of 50 g/L citric acid (in shake-flask fermentation) and 80 g/L (in fed-batch fermentation) with 1.5 and 3 g/L NH_4Cl, respectively, under non-optimized conditions (8). Papanikolaou *et al.* changed the initial glucose concentration from 34 to 149.5 and reported 10.5–42.9 g/L of citric acid production by *Y. lipolytica* (34). Forster *et al.* used sucrose as the carbon source and recombinant *Y. lipolytica* and reported a citric acid yield of only 78 g/L after 191 h (35, 36). Our results showed that the wild-type *Y. lipolytica* M7 strain produced high levels of citric acid (55.5 g/L) on a medium containing glucose as the sole carbon source in shake-flask fermentation. Therefore, large amounts of citric acid can be produced by optimization of growth conditions of this wild-type *Y. lipolytica* by fed-batch fermentation or by maintaining chemostat conditions.

Lipase production by yeast strains present in some plant and vegetable oils and animal fats has been studied (3, 9, 33, 37). Kamzolova *et al.* screened a huge number of *Y. lipolytica* strains and reported the lipase yield ranging from 1.8 to 45.5 U/mL (13). The results of lipase production for other wild-type *Candida* spp were similar to those obtained in our study. The isolated M7 strain could produce an acceptable amount of lipase (12.3 U/mL). These data are similar to the data reported by Corzo *et al.* and Pereira-Meirelles et al (37, 38). Reports have shown that *Y. lipolytica* can be used to decrease the biochemical and chemical oxygen demands of waste water from oil factories, and this wastewater can be treated using the wild-type strain that has adapted to the ecological conditions of the particular area (39, 40). Our findings indicate that *Y. lipolytica* M7 is a suitable wild-type strain with high potential for citric acid production and acceptable amounts of lipase activity. Meat and meat products, especially poultry meat, are good sources for isolation of yeast that produce citric acid and lipase, two valuable biotechnological products.

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References

1. Encinas JP, López-Díaz TM, García-López ML, Otero A, Moreno B. Yeast populations on Spanish fermented sausages. *Meat Sci*. 2000;**54**(3):203-8.
2. Deak T, Chen J, Beuchat LR. Molecular characterization of *Yarrowia lipolytica* and *Candida zeylanoides* isolated from poultry. *Appl Environ Microbiol*. 2000;**66**(10):4340-4.
3. Sanz A, Martin R, Mayoral MB, Hernandez PE, Gonzalez I, Lacarra TG. Development of a PCR-culture technique for rapid detection of yeast species in vacuum packed ham. *Meat science*. 2005;**71**(2):230-7.
4. Antonucci S, Bravi M, Bubbico R, Di Michele A, Verdone N. Selectivity in citric acid production by *Yarrowia lipolytica*. *Enzyme Microb Technol*. 2001;**28**(2-3):189-95.
5. Soccol CR, Vandenberghe LPS, Rodrigues C, Pandey A. New perspectives for citric acid production and application. *Food Technol Biotech*. 2006;**44**(2):141.
6. Vandenberghe LPS, Soccol CR, Pandey A, Lebeault JM. Microbial production of citric acid. *Braz Arch Biol Technol*. 1999;**42**(3):263-76.
7. Levinson WE, Kurtzman CP, Kuo TM. Characterization of *Yarrowia lipolytica* and related species for citric acid production from glycerol. *Enzyme Microb Tech*. 2007;**41**(3):292-5.
8. Anastassiadis S, Aivasidis A, Wandrey C. Citric acid production by Candida strains under intracellular nitrogen limitation. *Appl Microbiol Biotechnol*. 2002;**60**(1-2):81-7.
9. Moresi M. Effect of glucose concentration on citric acid production by *Yarrowia lipolytica*. *J Chem Tech Biot*. 1994;**60**(4):387-95.
10. Rane KD, Sims KA. Citric acid production by *Yarrowia lipolytica*: Effect of nitrogen and biomass concentration on yield and productivity. *Biotechnol Lett*. 1996;**18**(10):1139-44.
11. Wojtatowicz M, Rymowicz W, Kautola H. Comparison of different strains of the yeast *Yarrowia lipolytica* for citric acid production from glucose hydrol. *Appl Biochem Biotechnol*. 1991;**31**(2):165-74.
12. McKay IA, Maddox IS, Brooks JD. High specific rates of glucose utilisation under conditions of restricted growth are required for citric acid accumulation by *Yarrowia lipolytica*. *Appl Microbiol Biotechnol*. 1994;**41**(1):73-8.
13. Kamzolova SV, Morgunov IG, Aurich A, Perevoznikova OA, Shishkanova NV, Stottmeister U, et al. Lipase secretion and citric acid production in *Yarrowia lipolytica* yeast grown on animal and vegetable fat. *Food Technol Biotech*. 2005;**43**(2):113-22.
14. Treichel H, de Oliveira D, Mazutti MA, Di Luccio M, Oliveira JV. A review on microbial lipases production. *Food Bioprocess Tech*. 2010;**3**(2):182-96.
15. Barth G, Gaillardin C. *Yarrowia lipolytica. In: Non-Conventional Yeasts in Biotechnology*. 1996.
16. Barth G, Gaillardin C. Physiology and genetics of the dimorphic fungus *Yarrowia lipolytica*. *FEMS Microbiol Rev*. 1997;**19**(4):219-37.
17. Lopandic K, Zelger S, Banszky LK, Eliskases-Lechner F, Prillinger H. Identification of yeasts associated with milk products using traditional and molecular techniques. *Food Microbiol*. 2006;**23**(4):341-50.
18. Burkholder PR, McVeigh I, Moyer D. Studies on some growth factors of yeasts. *J Bacteriol*. 1944;**48**(4):385.
19. Finogenova TV, Puntus IF, Kamzolova SV, Lunina Iu N, Monastyrskaia SE, Morgunov IG, et al. Obtaining of the mutant *Yarrowia lipolytica* strains producing citric acid from glucose. *Prikl Biokhim Mikrobiol*. 2008;**44**(2):219-24.
20. Fickers P, Nicaud JM, Destain J, Thonart P. Overproduction of lipase by *Yarrowia lipolytica* mutants. *Appl Microbiol Biotechnol*. 2003;**63**(2):136-42.
21. Kouker G, Jaeger KE. Specific and sensitive plate assay for bacterial lipases. *Appl Environ Microbiol*. 1987;**53**(1):211-3.
22. Papanikolaou S, Chevalot I, Galiotou-Panayotou M, Komaitis M, Marc I, Aggelis G. Industrial derivative of tallow: a promising renewable substrate for microbial lipid, single-cell protein and lipase production by *Yarrowia lipolytica*. 2007. **10**(3): 425-35.
23. Mafakher L, Mirbagheri M, Darvishi F, Nahvi I, Zarkesh-Esfahani H, Emtiazi G. Isolation of lipase and citric acid producing yeasts from agro-industrial wastewater. *N Biotechnol*. 2010;**27**(4):337-40.
24. Papanikolaou S, Aggelis G. Lipid production by *Yarrowia lipolytica* growing on industrial glycerol in a single-stage continuous culture. *Bioresour Technol*. 2002;**82**(1):43-9.
25. Darvishi F, Nahvi I, Zarkesh-Esfahani H, Momenbeik F. Effect of plant oils upon lipase and citric acid production in *Yarrowia lipolytica* yeast. *J Biomed Biotechnol*. 2009;**2009**:562943.
26. Papanikolaou S, Muniglia L, Chevalot I, Aggelis G, Marc I. *Yar-*

rowia lipolytica as a potential producer of citric acid from raw glycerol. *J Appl Microbiol*. 2002;**92**(4):737-44.
27. Kurtzman CP, Fell JW. *The yeasts, a taxonomic study*. Elsevier Science; 1998.
28. Vasdinyei R, Deak T. Characterization of yeast isolates originating from Hungarian dairy products using traditional and molecular identification techniques. *Int J Food Microbiol*. 2003;**86**(1-2):123-30.
29. Dlauchy D, Tornai-Lehoczki J, Peter G. Restriction enzyme analysis of PCR amplified rDNA as a taxonomic tool in yeast identification. *Syst Appl Microbiol*. 1999;**22**(3):445-53.
30. White T, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M, Gelfand D, Shinsky J, White T, editors. *PCR Protocols: A Guide to Methods and Applications*: Academic Press; 1990. p. 315-22.
31. Gardner G, editor. Microbial spoilage of cured meats; 1983.
32. Fleet G. Spoilage yeasts. *Crit Rev Biotechnol*. 1992;**12**(1-2):1-44.
33. Nortje GL, Nel L, Jordaan E, Badenhorst K, Goedhart E, Holzapfel WH. The aerobic psychotrophic populations on meat and meat contact surfaces in a meat production system and on meat stored at chill temperatures. *J Appl Bacteriol*. 1990;**68**(4):335-44.
34. Papanikolaou S, Galiotou-Panayotou M, Chevalot I, Komaitis M, Marc I, Aggelis G. Influence of glucose and saturated free-fatty acid mixtures on citric acid and lipid production by *Yarrowia lipolytica*. *Curr Microbiol*. 2006;**52**(2):134-42.
35. Förster A, Aurich A, Mauersberger S, Barth G. Citric acid production from sucrose using a recombinant strain of the yeast *Yarrowia lipolytica*. *Appl Microbiol Biotechnol*. 2007;**75**(6):1409-17.
36. Rywinska A, Rymowicz W, Zarowska B, Wojtatowicz M. Biosynthesis of citric acid from glycerol by acetate mutants of *Yarrowia lipolytica* in fed-batch fermentation. *Food Technol Biotech*. 2009;**47**(1):1-6.
37. Pereira-Meirelles FV, Rocha-Leao MH, Sant Anna GL, Jr. A stable lipase from *Candida lipolytica*: cultivation conditions and crude enzyme characteristics. *Appl Biochem Biotechnol*. 1997;**63-65**:73-85.
38. Corzo G, Revah S. Production and characteristics of the lipase from *Yarrowia lipolytica* 681. *Bioresourc Technol*. 1999;**70**(2):173-80.
39. Lanciotti R, Gianotti A, Baldi D, Angrisani R, Suzzi G, Mastrocola D, et al. Use of *Yarrowia lipolytica* strains for the treatment of olive mill wastewater. *Bioresour Technol*. 2005;**96**(3):317-22.
40. Papanikolaou S, Galiotou-Panayotou M, Fakas S, Komaitis M, Aggelis G. Citric acid production by *Yarrowia lipolytica* cultivated on olive-mill wastewater-based media. *Bioresour Technol*. 2008;**99**(7):2419-28.