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ORIGINAL ARTICLE / ÖZGÜN MAKALE

MOLECULAR IDENTIFICATION AND LIPOLYTIC ACTIVITY OF YARROWIA LIPOLYTICA ISOLATED FROM YOGHURT CREAM

YOĞURT KAYMAĞINDAN İZOLE EDİLEN YARROWIA LIPOLYTICA'NIN MOLEKÜLER İDENTİFİKASYONU VE LİPOLİTİK AKTİVİTESİ

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ABSTRACT

Objective: *Yarrowia lipolytica is an aerobic dimorphic yeast that produces various metabolites such as enzymes, organic acids, proteins, surfactants and has industrial potential in some biotransformation and bioremediation reactions. To be a preliminary study to industrial productions, in present study it was aimed to isolate Y. lipolytica strain from yoghurt cream, perform its molecular characterization and investigate the lipolytic activity.*

Material and Method: *A total of 10 samples were taken from the homemade yoghurt cream obtained by fermented milk from local dairy producers in Ankara. Yeast-Peptone-Glycerol (YPG) broth and Yarrowia lipolytica Distinctive (YLD) agar media were used for isolation of the species. Species-level identification was carried out by ITS-Polymerized chain reaction analysis. Lipolytic activity was determined with Rhodamine-B/Olive Oil Agar nutrient medium procedure.*

Result and Discussion: *Yeast colonies were isolated from homemade yoghurt creams by growing in YPG broth. Yeasts were stained with Gram staining method and Gram-positive stained ones were inoculated on YLD agar. At the end of the incubation period, the brown pigmented colony growing on YLD agar was selected as Yarrowia species. According to the molecular characterization results the brown pigmented colony was identified as Yarrowia lipolytica with a total of 358 bases, 100% sequence matching ratio and 100% similarity ratio and the strain was determined as lipase positive. The development of the Y. lipolytica strain isolated in our study as a producer culture that can be used in industrial production should be supported by further studies to benefit from its various properties.*

Keywords: *Industrial yeast, lipolytic activity, Yarrowia lipolytica*

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ÖZ

Amaç: *Yarrowia lipolytica enzimler, organik asitler, proteinler, yüzey aktif maddeler gibi çeşitli metabolitler üreten ve bazı biyotransformasyon ve biyoremediasyon reaksiyonlarında endüstriyel potansiyele sahip olan aerobik dimorfik bir mayadır. Endüstriyel üretimlere bir ön çalışma olması amacıyla, bu çalışmada Y. lipolytica suşunun yoğurt kremasından izole edilmesi, moleküler karakterizasyonunun yapılması ve lipolitik aktivitesinin araştırılması amaçlanmıştır.*

Gereç ve Yöntem: *Ankara'daki farklı yerel süt üreticilerinin sütü fermente ederek elde ettikleri ev yapımı yoğurt kaymaklarından toplam 10 örnek alınmıştır. Yarrowia türlerinin izolasyonunda Yeast-Pepton-Gliserol (YPG) sıvı besiyeri ve Yarrowia lipolytica Distinctive (YLD) agar besiyeri kullanılmıştır. Y. lipolytica'nın tür tanımlaması ITS-Polimerize zincir reaksiyonu analizi ile yapılmıştır. Lipolitik aktivite; Rodamin-B/Zeytin yağı agar besiyeri prosedürü ile belirlenmiştir.*

Sonuç ve Tartışma: *Ev yapımı yoğurt kaymağı örneklerinden YPG besiyerinde gelişen maya kolonileri izole edilmiştir. Maya izolatları Gram boyama yöntemi ile boyanmış ve Gram pozitif boyananlar YLD agar üzerine inoküle edilmiştir. İnkübasyon süresi sonunda YLD agar üzerinde gelişen kahverengi pigmentli koloniler Yarrowia türü olarak seçilmiştir. Moleküler karakterizasyon sonuçlarına göre kahverengi pigmentli bir koloni toplam 358 baz, %100 dizi uyum oranı ve %100 benzerlik oranı ile Yarrowia lipolytica olarak tanımlanmış ve suşun lipaz pozitif olduğu belirlenmiştir. Çalışmamızda izole edilen Y. lipolytica suşunun çeşitli özelliklerinden faydalanmak üzere, endüstriyel üretimlerde kullanılabilecek bir üretici kültür olarak geliştirilmesi, yapılacak ileriki çalışmalarla desteklenmelidir.*

Anahtar Kelimeler: : *Endüstriyel maya, lipolitik aktivite, Yarrowia lipolytica*

INTRODUCTION

Yarrowia lipolytica (*Y. lipolytica*) is an aerobic and dimorphic yeast that found in different types of food (cheeses, yoghurt, kefir, olive oil, soya sauce etc.) naturally and formerly known as *Candida lipolytica*. This yeast has a Generally Recognized as Safe (GRAS) status and produces various amounts of metabolites like proteolytic and lipolytic enzymes, organic acids, and proteins [1, 2]. *Y. lipolytica* prefers to live in lipid-rich environments and can use hydrophobic carbon sources by various mechanisms [3, 4]. It is stated in studies that the efficiency in the production of enzymes (alkaline proteases, lipases, and RNase), which are among their metabolites, varies depending on the substrate preferred by the strain [1]. In addition, based on these properties some studies shown that *Y. lipolytica* has industrial importance and used in fatty acid, single cell protein, flavoring (fruity aroma, γ-decalactone), citric acid, steroid biotransformation reactions [5] and also used for bioremediation, and production of biosurfactants [6]. One of the most important features of *Y. lipolytica* is, it has ability to accumulate lipids at levels exceeding 20-50% of the cell dry weight and to assimilate fatty substances, therefore this yeast is also called oleaginous microorganism [7].

The use of oils and lipids as a carbon source by *Y. lipolytica* has attracted the attention of lipase enzymes produced by yeast. Lipases are a kind of serine hydrolases that hydrolyze long-chain fatty acids to fatty acids and glycerol and are also known as triacylglycerol acylhydrolases [8]. Microbial lipases are among the important enzymes that can contribute to industrial production as a biocatalystits or have applications in food, pharmaceutical areas. *Y. lipolytica* is accepted as a good producer microorganism due to the metabolites it secretes, including lipases [9]. Studies have shown that environmental

components and conditions are effective on the lipase production of the microorganism. For example, olive oil is one of the best raw materials to produce lipase; while some substrats are suitable for high production of extracellular lipase such as casein, peptone, tryptone and yeast extract [10, 11], some of them like glucose, glycerol or mineral nitrogen compounds suppress the lipase production [12] and inorganic compounds do not trigger lipase synthesis [10, 11].

Based on such good properties, it is important to isolate new *Yarrowia* species in order to provide resources and find uses for various industries. The present study as a response to this need was aimed to investigate the molecular characterization and lipolytic activity of *Y. lipolytica* from yoghurt cream.

MATERIAL AND METHOD

Isolation of the yeast

A total of 10 samples were taken from the homemade yoghurt cream obtained by fermenting milk from local dairy producers in Ankara region. Samples transferred into sterile 100 ml sample containers under aseptic conditions and transported to the laboratory by cold chain. The contents of each yoghurt sample were mixed homogeneously and than a 2.5 ml sample was transferred aseptically into 25 ml antibiotic added Yeast-Peptone-Glycerol (YPG) broth and incubated for 48 hours at 30 °C with shaking at 150 rpm. The medium content on a liter scale (pH 7.0) is as follows: 20 g peptone, 10 g yeast extract and 20 g glucose, 20 g agar. After the two-day incubation, samples were taken and inoculated on YPG agar media, and incubated for 48 hours at 30 °C. At the end of the incubation, the morphology of each different colonies formed in the solid medium was examined under the microscope by Gram staining. Yeast colonies were selected according to Gram staining results and inoculated on Yarrowia lipolytica Distinctive (YLD) Agar medium. Petri plates were incubated for 24 hours at 30 °C and at the end of incubation brown pigmented colonies were stocked with the thought of *Y. lipolytica*. The content of YLD Agar medium in liter scale (pH 7.0) is as follows: 5 g peptone, 5 g yeast extract, 1.8 g L-tyrosine, 0.28 g $MnSO_4.7H_2O$, 5 g lactate, 20 g agar. In the presence of manganese ions in the medium, it produces brown pigment from tyrosine [13].

Molecular Characterization

Species identification of the selected yeast sample according to these characteristics was carried out in the BM Laboratory by ITS-Polymerized chain reaction (PCR) analysis. DNA isolation from the sample was performed with the EurX GeneMATRIX Bacterial & Yeast DNA isolation kit. The amount and purity of the obtained DNAs were controlled by spectrophotometric measurement in Thermo Scientific Nanodrop 2000 device. As primers, ITS1 5' TCCGTAGGTGAACCTGCGG 3' and ITS4 5' TCCTCCGCTTATTGATATGC 3' were used. PCR conditions were 5 minutes initial denaturation at 95

°C, 40 cycles (45 seconds denaturation at 95°C, annealing at 57°C for 45 seconds, elongation at 72°C for 60 seconds), and final elongation at 72°C for 5 minutes. To amplify the targeted region, one-step PCR was performed with the Solis Biodyne FIREPol® DNA Polymerase Taq polymerase enzyme. In the purification stage of the PCR product, the MAGBIO "HighPrep™ PCR Clean-up System" (AC-60005) purification kit was used for the single band samples obtained. Sanger Sequencing was performed in the Macrogen Netherlands laboratory, with the ABI 3730XL Sanger sequencing instrument and the BigDye Terminator v3.1 Cycle Sequencing Kit. Reads obtained with the ITS1 – ITS4 primers were contiguous to form a consensus sequence. CAP contig assembly algorithm was used in BioEdit software to perform this process.

Determination of Lipolytic Activity

The determination of the lipolytic activity was carried out on Rhodamine-B/Olive Oil Agar (ROA) nutrient medium. The medium was prepared in two stages, similar to the method of Kumar et al. (2012) [14]. The media containing 8.0 g of Nutrient broth, 4.0 g of NaCl, and 20 g of agar per liter was sterilized in an autoclave and its pH was adjusted to 7.0, cooled to 50°C in a water bath. Ten ml of 1 mg/ml rhodamine-B solution and 31.25 ml olive oil were separately sterilized by filtration and were added to the medium with stirring. The homogenized mixture was poured onto agar plates and allowed to solidify. The yeast suspension, the density of which was adjusted to 0.5 McFarland, was inoculated dropwise into the medium, and incubated at 30°C for 48 hours. Olive oil was expected to hydrolyze into fatty acids because of metabolism and to react with rhodamine-B in the environment.

RESULT AND DISCUSSION

Yeast colonies were isolated from homemade yoghurt cream samples by growing in Yeast-Peptone-Glycerol (YPG) broth. Isolated colonies were stained with Gram procedure and Gram-positive colored colonies were inoculated on Yarrowia lipolytica Distinctive (YLD) Agar medium to distinguish *Yarrowia* colonies from other yeasts. After incubation period (24 hours at 30°C), a brown pigmented colony was selected to be *Yarrowia* species and stored until molecular characterization.

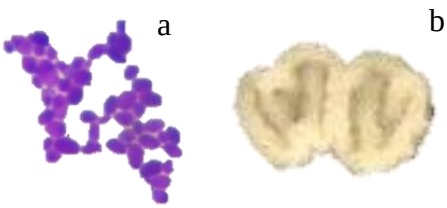

Figure 1. *Yarrowia lipolytica*, (a) Gram morphology under light microscope and (b) colony morphology

According to the molecular analysis results carried out with the ITS1 – ITS4 primers, the sample was identified as *Y. lipolytica* with a total of 358 bases, 100% sequence matching ratio and 100% similarity ratio. Gene sequences presented in Table 1.

Table 1. Gene Sequence of *Yarrowia lipolytica*

Primers	**Gene Sequence of *Yarrowia lipolytica***
ITS1: 5' TCCGTAGGTGAACCTGCGG 3' ITS4: 5' TCCTCCGCTTATTGATATGC 3'	TCCGTAGGTGAACCTGCGGAAGGATCATTATTGATTTTATCTATTTCT GTGGATTTCTGGTATATTACAGCGTCATTTTATCTCAATTATAACTATC AACAACGGATCTCTTGGCTCTCACATCGATGAAGAACGCAGCGAACC GCGATATTTTTTGTGACTTGCAGATGTGAATCATCAATCTTTGAACGC ACATTGCGCGGTATGGCATTCCGTACCGCACGGATGGAGGAGCGTGT TCCCTCTGGGATCGCATTGCTTTCTTGAAATGGATTTTTTAAACTCTCA ATTATTACGTCATTTCACCTCCTTCATCCGAGATTACCCGCTGAACTTA AGCATATCAATAAGCGGAGGA

Lipolytic activity of *Y. lipolytica* was determined according to Kumar et al.'s (2012) method [14]. The reaction was evaluated as positive by observing the formation of an orange color fluorescent zone under UV light. Activity result was shown in Figure 2.

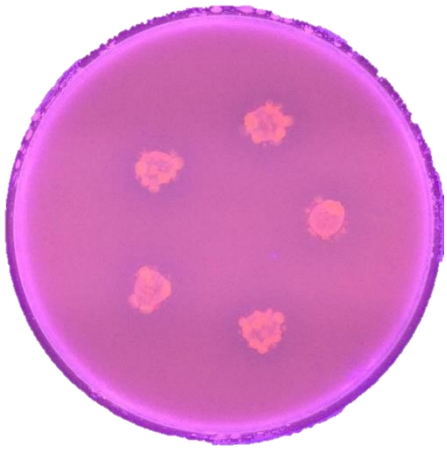

Figure 2. Lipolytic activity of *Yarrowia lipolytica* under UV light.

Microbial enzymes have an important place in the industrial market (including application areas such as food, pharmaceutical and other industries). Lipases are also around 5% in this market in addition to some other enzymes (e.g., proteases) [15]. New biotechnological applications involving lipases include biopolymer production, biodiesel synthesis, and the production of various chemicals and pharmaceuticals [16]. Lipases are also necessary for the physiological requirements of the organism, and this lipase need can be obtained from various sources such as plants, animals, and microorganisms like some bacteria and yeasts [17]. In present study the lipase production of *Y. lipolytica* was determined according to the method of Kumar et al. (2012) [14]. Similar to the results of our study, there are various

lipase-producing *Y. lipolytica* strains in the literature. Louhasakul and Cheirsilp (2022) demonstrated the conversion of crude glycerol to lipid and lipase by *Y. lipolytica* in part of their research [18]. Fraga et al. (2021) investigated the effect of industrial wastes on lipase production of *Y. lipolytica* [19]. Theron et al. (2020) showed that *Y. lipolytica* produced high level extracellular lipase [20]. Kuncharoen et al. (2020) isolated two *Y. lipoytica* strain from fermented rice and determined their lipase activity [21]. de Souza et al. (2019) obtained the lipase production of *Y. lipolytica* strain by solid fermentation process [22]. Yan et al. (2018) also determined the lipolytic activity of *Y. lipolytica* [23]. Darvishi et al. (2009) stated that plant oils can be used in the production of lipase at low cost in *Y. lipolytica* [24].

Enzymes of microbial origin draw attention due to their striking features such as stability (especially in organic solvent) and high specificity to substrates [25]. Extracellular lipases that can be produced by some microorganisms are also considered as a source and *Y. lipolytica* is one of these sources. It is thought that *Y. lipolytica*, which was isolated in our study and whose lipolytic activity was determined, will also constitute a basic source for future industrial production studies. The development of the *Y. lipolytica* strain isolated in our study as a producer culture that can be used in industrial production should be supported by further studies to benefit from its various properties.

AUTHOR CONTRIBUTIONS

Concept: *M.E.K., D.S.*; Design: *M.E.K., D.S.*; Control: *M.E.K., D.S., N.A.*; Sources: *M.E.K., D.S., N.A.*; Materials: *M.E.K., D.S.*; Data Collection and/or processing: *M.E.K., D.S.*; Analysis and/or interpretation: *M.E.K., D.S., N.A.*; Literature review: *M.E.K., D.S.*; Manuscript writing: *M.E.K., D.S.*; Critical review: *M.E.K., D.S., N.A.*; Other: *M.E.K., D.S., N.A.*

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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