GIDA
THE JOURNAL OF FOOD
E-ISSN 1309-6273, ISSN 1300-3070

Research / Araştırma
GIDA (2022) 47 (4) 564-575
doi: 10.15237/gida.GD22028

FUNCTIONAL AND TECHNOLOGICAL PROPERTIES OF *CANDIDA ZEYLANOIDES* STRAINS ISOLATED FROM PASTIRMA

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Received / Geliş: 21.02.2022; *Accepted* / Kabul: 27.05.2022; *Published online* / Online baskı: 22.06.2022

Sayın-Börekçi, B., Kaban, G., Kaya, M. (2022). Functional and technological properties of Candida zeylanoides strains isolated from pastırma. GIDA (2022) 47 (4) 564-575 doi: 10.15237/gida.GD22028

Sayın-Börekçi, B., Kaban, G., Kaya, M. (2022). Pastırmadan izole edilen *Candida zeylanoides* suşlarının fonksiyonel ve teknolojik özellikleri. *GIDA* (2022) 47 (4) 564-575 doi: 10.15237/gida.GD22028

ABSTRACT
The aim of this study was to investigate some of the technological and functional properties of 16 autochthonous *Candida zeylanoides* strains isolated and identified from *pastırma*, a traditional dry-cured meat product. Consequently, it was determined that some strains could grow at high sugar concentrations (45%) while all strains were resistant to 10% NaCl concentration and most strains were tolerant to 10% ethanol and 0.5% bile salt levels. Furthermore, the certain strains showed good growth at pH 3.0 and only 6 strains were able to grow at 42°C. All strains showed catalase activity. It was detected that the strains did not produce hydrogen sulfide (H_2S) and also had no DNase, nitrate reductase, proteolytic, and lipolytic activities. It was found that some strains exhibited urease activity and all strains that could grow at 37°C had β-hemolytic activity and formed biofilm. Moreover, *C. zeylanoides* strains showed sensitivity to nystatin, fluconazole, voriconazole, and ketoconazole.
Keywords: Autochthonous yeast strain, pastırma, technological property, biofilm, antifungal, hemolysis

PASTIRMADAN İZOLE EDİLEN *CANDIDA ZEYLANOIDES* SUŞLARININ FONKSİYONEL VE TEKNOLOJİK ÖZELLİKLERİ

ÖZ
Bu çalışmanın amacı, geleneksel kuru kür edilmiş bir et ürünü olan pastırmadan izole edilen ve tanımlanan 16 yerel *Candida zeylanoides* suşunun bazı teknolojik ve fonksiyonel özelliklerinin araştırılmasıdır. Sonuç olarak, bazı suşların yüksek şeker konsantrasyonlarında (%45) gelişebildiği, tüm suşların %10 NaCl konsantrasyonuna dirençli olduğu ve suşların çoğunun %10 etanol ve %0.5 safra tuzu seviyelerine toleranslı olduğu tespit edilmiştir. Ayrıca, belirli suşlar pH 3.0'te çok iyi gelişme göstermiş ve 42°C'de sadece 6 suş gelişebilmiştir. Tüm suşlar katalaz aktivitesi göstermiştir. Suşların hidrojen sülfür (H_2S) oluşturmadığı ve ayrıca DNaz, nitrat redüktaz, proteolitik ve lipolitik aktivitelerine sahip olmadığı tespit edilmiştir. Bazı suşların üreaz aktivitesi sergilediği ve 37°C'de gelişebilen tüm suşların β-hemolitik aktiviteye sahip olduğu ve biyofilm oluşturduğu belirlenmiştir. Ayrıca, test edilen *C. zeylanoides* suşları nistatin, flukonazol, vorikonazol ve ketokonazole karşı hassasiyet göstermiştir.
Anahtar kelimeler: Yerel maya suşu, pastırma, teknolojik özellik, biyofilm, antifungal, hemoliz

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INTRODUCTION

Many industrial processes such as food, feed ingredients and heterologous protein production, traditional food fermentation, biocatalysis, fundamental biological and biomedical research, bio-control and environmental biotechnology studies are carried out using yeasts (Johnson and Echavarri-Erasun, 2011). One of the important yeast genus *Candida* comprises 150 asporogenous yeasts species and they are mostly classified under the *Deuteromycetes* class (Scully et al., 1994). Yeasts are one of the predominant microorganisms that act during the ripening period of dry-cured meat products. Dry cured meats are one of the oldest products produced based on traditional practices by processes such as salting followed by drying and smoking (Toldrá, 2006). Pastırma is the most typical Turkish dry-cured meat product and has a characteristic flavor which is obtained from the whole muscles located in certain parts of water buffalo and beef carcasses. Lactic acid bacteria and Gram-positive-catalase positive cocci have been identified as the two most important groups of microorganisms in pastırma production. It was also indicated that yeast and mold count increases during drying stages (Kaban, 2013). *Debaryomyces hansenii*, *Candida zeylanoides*, *Yarrowia lipolytica,* and *Rhodotorula* spp. can be isolated from these types of products. Some of them are responsible for the generation of volatile compounds while some others were reported as spoilage microorganisms (Andrade et al., 2006). It is known that *D. hansenii* and *C. zeylanoides* are often isolated from meat products, possibly due to their tolerance to both low temperatures and high salt concentrations (Mortensen et al., 2008). *C. zeylanoides* is especially dominant in the early stages of dry-cured meat production whereas *D. hansenii* dominates late at the ripening stage (Asefa et al., 2009). *C. zeylanoides* have been isolated from different sources such as Spanish fermented sausages (Encinas et al., 2000), dry-cured ham (smoked), Fenalår (unsmoked) (Asefa et al., 2009), Italian salami (Giarratana et al., 2014), sucuk (Öztürk and Sağdıç, 2014), and pastırma (Öztürk, 2015). However, there is little information about the yeasts isolated from pastırma (Öztürk, 2015; Kaya et al., 2017). For this reason, the aim of the present study was to characterize 16 autochthonous *C. zeylanoides* strains, isolated and identified from pastırma from a technological and functional point of view.

MATERIAL AND METHODS

Microorganisms

In this study, 16 *Candida zeylanoides* strains (3.13, 4.1, 4.6, 4.12, 4.14, 7.9, 7.10, 7.12, 8.1, 8.3, 8.4, 11.9, 12.3, 13.5, 13.7, and 13.13) that were isolated and identified from pastırma were used (Kaya et al., 2017). *C. zeylanoides* strains were cryopreserved in glycerol-containing (50% v/v) Malt Extract Broth (MEB) and kept at -80°C until use. Before each experiment, yeast cultures were incubated at 28°C for 48 h in tubes containing MEB (Merck, Darmstadt, Germany).

Methods

Determination of growth characteristics under different conditions

The cultures were inoculated (%2, v/v) into the MEB, which were adjusted to different glucose (30 and 45%), NaCl (5, 10 and 15%), ethanol (5, 10 and 15%), bile salt (0.1, 0.3 and 0.5%) and pH (3.0 and 5.0) levels to determine growth under different conditions. All cultures were incubated for 72 h. In order to evaluate yeast tolerance to bile salt, activated cultures were incubated at 37°C. To determine growth capabilities of yeasts at different temperatures, activated cultures were incubated at 15, 30, 37, and 42°C. For other tests (determination of growth at different glucose, NaCl, ethanol and pH levels), the cultures were incubated at 28°C. Growth of yeasts was measured by a spectrophotometer (Aquamate 9423 AQA 2000E, Thermo Scientific, England) at 600 nm.

Determination of biofilm formation

The Congo red agar method was used to investigate the biofilm formation of yeasts. The medium composition included brain heart infusion broth (37 g/L) (Oxoid, Basingstoke, UK), glucose (80 g/L) (Merck, Darmstadt, Germany), agar (10 g/L) (Merck, Darmstadt, Germany), and Congo red stain (0.8 g/L) (Sigma-Aldrich, Steinheim, Germany). The Congo red stain was prepared as a concentrated aqueous solution and autoclaved at 121°C for 15 min. separately from other medium components

and added when the medium was cooled to 55°C. After inoculation, the cultures were incubated at 37°C for 48 h. The formation of red colonies was regarded as a positive result (Sida et al., 2016).

Determination of hydrogen sulfide (H_2S) formation

For this purpose, the cultures were inoculated on the triple sugar iron agar (Oxoid, Basingstoke, UK) and incubated at 30°C for 14 days. Black color formation was regarded as an indicator of the formation of H_2S by yeasts (Öztürk and Sağdıç, 2014).

Determination of hemolytic activity

The cultures were activated on Sabouraud dextrose agar (SDA; Oxoid, Basingstoke, United Kingdom) and inoculated onto the sheep blood SDA to investigate hemolytic activity. This medium was prepared by adding 7% (v/v) sheep blood to 100 mL of SDA supplemented with 3% (w/v) glucose. After 48 h of incubation at 37°C, zone formation was evaluated (Luo et al., 2001).

Determination of enzyme activities

Catalase activity was determined following the method proposed by Perricone et al. (2014). The formation of bubbles on Petri dishes by 3% hydrogen peroxide (Merck, Darmstadt, Germany) was regarded as a positive result.

Activated cultures were inoculated into the Christensen's urea agar (composition in g/L: agar, 15; NaCl, 5; Na_2HPO_4, 1.2; KH_2PO_4, 0.8; peptone, 1; glucose, 1; and phenol red, 0.012) to determine the urease activity. After autoclaving, 5 mL of sterile urea solution (40%) were added to 100 mL of medium. The strains were incubated for up to 7 days at 25°C. At the end of the incubation, a pink coloration was observed around colonies showing urease activity (Sen and Komagata, 1979).

The DNase Test Agar (Merck, Darmstadt, Germany) was used to analyze the DNase activity. Glucose and yeast extract (1%, w/v) were added to the medium. Then, pH was adjusted to 6.7 before sterilization. The activated cultures were incubated at 25°C for 6 days. Then, 1 N HCl was poured into the Petri dishes and the following transparent zone formation was regarded as an indicator of DNase activity (Sen and Komagata, 1979). The *Staphylococcus aureus* ATCC 29213 was used as the positive control.

Yeast cultures were inoculated into the nitrate broth (Merck, Darmstadt, Germany) and incubated at 30°C for 48 h. At the end of the incubation, 0.6% α-naphthylamine and 0.8% sulfanilic acid solutions, each prepared in 5N acetic acid, were added into the medium and presence of nitrate reductase was assessed with the red color formation. Zinc dust (Sigma-Aldrich, Steinheim, Germany) was added to the tubes where color formation did not occur and the result was regarded as negative in terms of red color formation (Buxton, 2011).

To determine the proteolytic and lipolytic activities, the cultures into 50 mL of MEB in shake flasks were activated in a rotary shaker (JSSI-100, JS Research, Gongju, Korea) at 28°C, 180 rpm for 24 h. Activated cultures were added to the wells as 50 µL. Modified methods by Öztürk and Sağdıç (2014) were used for these tests. Active cultures were inoculated to the pour plates which contained calcium caseinate agar and tributyrin agar (Merck, Darmstadt, Germany), respectively. The cultures were incubated at 30°C for 5 days. The transparent zone formation was associated with the presence of proteolytic and lipolytic activities. Additionally, the yeast extract agar (tryptone 6.0 g/L, yeast extract 3.0 g/L, and agar 15 g/L) containing 1% (v/v) Tween 80 and 0.01% (w/v) phenol red was used to determine the lipase activity. The pH of the medium was adjusted to 7.35 ± 0.05 before autoclave treatment. The active cultures inoculated into the wells were incubated at 37°C for 48 h, and the transformation of the medium from red to yellow was accepted as an indicator of lipase activity (Oliveira et al., 2017).

Determination of antifungal resistance

The antifungal resistance of the strains was evaluated using Mueller-Hinton agar (Merck, Darmstadt, Germany) which contained 2%

glucose and 0.5 µg/mL methylene blue. The cultures that were grown on the SDA were adjusted to 0.5 McFarland level in sterile 0.85% NaCl solution and spread evenly on the surface of the whole Petri dish using a sterile cotton swab. Then, the antifungal discs (Nystatin and Ketoconazole (Himedia, India); Voriconazole and Fluconazole (Oxoid, Basingstoke, United Kingdom)) were placed on the medium and incubated at 35°C for 24 h (Espinel-Ingroff, 2007).

RESULTS AND DISCUSSION

Growth characteristics under different conditions

Table 1 shows the results for the growth at different glucose concentrations (30 and 45%). *C. zeylanoides* 3.13, 4.6, 4.12, 7.10, 13.7 and 13.13 showed good growth both 30% and 45% glucose concentrations. Yeasts that can grow at a sugar level of 40-70% (w/w) are regarded as sugar-tolerant (Ok and Hashinaga, 1997). The cells need to cope with the osmotic stress due to the high sugar concentrations during the production of alcoholic beverages and other industrially-related processes (Gomar-Alba et al., 2015). Karasu-Yalcin et al. (2012) reported that *C. zeylanoides* strains isolated from Tulum cheese could not grow at the 60% glucose level (the growth at 50% glucose level was varied). Additionally, Yalcin and Ucar (2009) determined that all the selected strains of *C. zeylanoides* grew at 50% glucose level. However, Bai et al. (2010) have reached opposing conclusions that *C. zeylanoides* strains that were isolated from fermented milk could not tolerate this glucose level.

In a previous study, it was determined that all *C. zeylanoides* strains which were used in this study had the ability to grow at the 5% to 17.5% pure glycerol concentrations (Sayın Börekçi, 2020). *Aspergillus*, *Rhizopus*, *Yarrowia*, or *Candida* can transform the crude glycerol fraction into bio-chemicals such as organic acids (citric, succinic, and malic acids), polyols (arabitol, erythritol, and mannitol), and single-cell oils. Some of the *Y. lipolytica*, *C. tropicalis*, *C. guilliermondii*, *C. parapsilosis*, *C. oleophila*, and *C. zeylanoides* strains can metabolize hydrophobic substrates and, therefore, can adapt to mediums containing fats, oils, fatty acids, and hydrocarbons (Mitrea et al., 2019). Glycerol is used as a carbon source in industrial processes due to its higher degree of reduction per carbon atom compared to sugars (Xiberras et al., 2019). Mitrea et al. (2019) reported that 12.66 g/L of succinic acid was produced by *C. zeylanoides* 20367 after 96 h using pure glycerol as a substrate.

All strains were resistant to 5% and 10% NaCl concentrations as shown in Table 1. However, *C. zeylanoides* 3.13, 4.6, 4.12, 7.10, 7.12, 8.3, 8.4, 11.9, 13.5, 13.7 and 13.13 were not resistant to 15% NaCl concentration. Öztürk and Sağdıç (2014) and Öztürk (2015) found that *C. zeylanoides* strains were tolerant to 10 and 15% NaCl concentration, respectively. Another study showed that the *C. zeylanoides* strains could grow at 10% NaCl level but not at the 16% level (Yalcin and Ucar, 2009). Yeast growth and growth rate, yield of biomass, lag phase of growth and cell composition are affected by NaCl (Watson, 1970). NaCl tolerance, tolerance to low water activities and low pH values, and properties such as consumption of oxygen improving and accelerating color formation have been reported to be desirable properties for yeast starter cultures in meat fermentation (Sørensen, 1997). In addition, environmental pH is reported to be an important factor that determines the growth of yeasts in the presence of weak organic acids and affects the reaction of yeasts to high salt or sugar concentrations (Praphailong and Fleet, 1997).

The ethanol-tolerant yeasts are preferred in the fermentation industry and they are generally selected from osmotolerant species (Reed and Nagodawithana, 1988). Although brewing yeasts are tolerant to ethanol concentrations of 4-5% (v/v), yeasts that are used in winemaking are tolerant up to 20% (v/v) (Casey and Ingledew, 1986). However, ethanol has an inhibitory effect on growth of microorganism (You et al., 2003), thus, considering its inhibition of cell viability, and fermentation, specifying the parameters that will be used in the examination of ethanol tolerance is necessary (D'amore et al., 1990). In the study, the tolerance to ethanol was examined with respect to

cell growth. As a result, *C. zeylanoides* 13.5 was not resistant to 5% ethanol level while *C. zeylanoides* 8.1, 8.3, 8.4 and 11.9 did not grow at 10% ethanol level. Moreover, *C. zeylanoides* 4.12 strain showed good growth at 15% level of ethanol (Table 1).

Bile and pH tolerance are one of the most important properties that need consideration when selecting a strain since the first biological barriers for the probiotics are stomach acidity and bile salts in the intestine (Shakira et al., 2018). In this study, *C. zeylanoides* 3.13, 4.6, 4.12, 7.10, 13.7 and 13.13 had capability to good grow at 0.1, 0.3, and 0.5% bile salt concentrations while *C. zeylanoides* 4.1, 4.14, 8.1, 8.3, 8.4, 11.9 and 13.5 showed not growth at these levels (Table 1). Bile resistance is necessary for growth of organisms in the intestinal tract (Psomas et al., 2001). Öztürk and Sağdıç (2014) reported that the *C. zeylanoides* strains isolated from *sucuk* (a Turkish dry fermented sausage) were resistant to bile salt concentrations of 0.3%.

The pH value is a parameter affecting yeast growth, fermentation rate, and the formation of fermentation products (Liu et al., 2015). On the other hand, the growth performance at low pH levels can be an important criterion in the selection of the yeast strain for organic acid production, considering that pH values decrease during fermentation. In our study, all *C. zeylanoides* strains could grow at pH 3.0 (some of them albeit poorly). In addition, they showed moderate and good growth at pH 5.0 (Table 1). Öztürk (2015) reported that 48 of the 58 *C. zeylanoides* strains were able to grow at pH 2.5. In another study, no *C. zeylanoides* strains could grow at pH 1.5, and all strains could grow at pH 3.0 (Öztürk and Sağdıç, 2014).

As can be seen in Table 1, *C. zeylanoides* 3.13, 4.1, 4.6, 4.12, 4.14, 7.10, 13.7 and 13.13 showed moderate growth at 15°C. Furthermore, *C. zeylanoides* 3.13, 4.6, 4.12, 7.10, 7.12, 13.7 and 13.13 had good growth at 30 and 37°C. Lastly, only *C. zeylanoides* 3.13 showed good growth at 42°C (Table 1). For *C. zeylanoides* strains, Öztürk (2015) reported that better growth was observed at 10°C compared with 37°C. According to Rajkowska and Kunicka-Styczyńska (2018), food-borne yeasts have ability to grow at body temperature is important but not the only factor when determining their potential virulence. Some researchers specified that *C. zeylanoides* strains isolated from sucuk and Turkish white cheese grew at 37°C (Öztürk and Sağdıç, 2014; Yalcin and Ucar, 2009). Karasu-Yalcin et al. (2012) reported that only some *C. zeylanoides* strains grew at 37°C. In addition, Bai et al. (2010) found that only 1 strain out of the 3 *C. zeylanoides* strains could grow at 37°C and 42°C.

Biofilm formation

As shown in Table 2, all strains that were able to grow at 37°C formed biofilm. Biofilm is defined as an aggregate of cells that grow by attaching to the surface by many microorganisms (Reynolds and Fink, 2001). The adhesion of yeast cells to the surface, formation of separate colonies, cell organization, and producing and secreting extracellular polymeric substances are the stages of biofilm formation (Cavalheiro and Teixeira, 2018). Disinfectant residues are not desired in the food industry and biofilms are resistant to chemical and physical processes. Therefore, biofilm formation should be controlled in the food industry and medical fields (Furukawa, 2015).

H_2S formation

H_2S is an aroma compound which is produced by yeast during fermentation. Off-flavors from volatile compounds derived from yeast metabolism are described as a problem for the wine and other fermented beverages. During fermentation, H_2S is formed in concentrations up to hundreds of μg/L. It is indicated that when the concentration reaches up to 1.6 μg/L in white wine, odor can be described as rotten eggs and putrefaction (Ugliano et al., 2010). As can be seen in Table 2, H_2S tests for all strains were negative. In agreement, Öztürk and Sağdıç (2014) obtained the same findings.

Hemolytic activity

In the present study, all strains that could grow at 37°C (*C. zeylanoides* 3.13, 4.6, 4.12, 7.9, 7.10, 7.12, 12.3, 13.7 and 13.13) had β-hemolytic activity

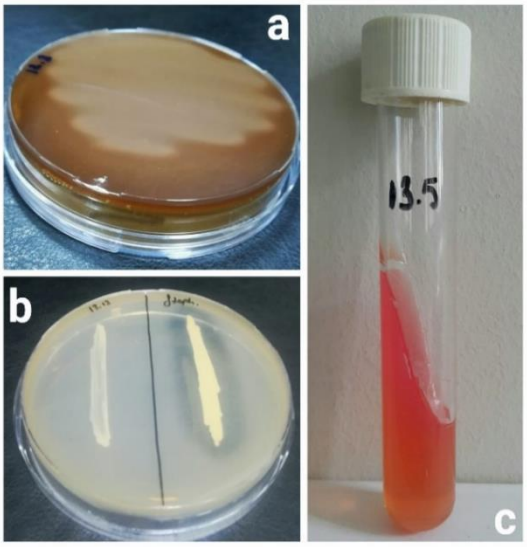
(Table 2). Figure 1a shows the zones in the Petri dishes, indicating the presence of hemolytic activity. The formation of a completely translucent zone is regarded as beta hemolysis while the formation of a greenish-black zone is regarded as alpha hemolysis (Luo et al., 2001). Hemolytic activity has been associated with the release of hemoglobin (Brilhante et al., 2016) and is used to determine the pathogenicity of yeasts (Öztürk and Sağdıç, 2014).

Catalase activity

Catalase is a tetrameric protein which is found in aerobic organisms that are responsible for hydrogen peroxide decomposition (Raveendran et al., 2018). Additionally, methanol, n-alkanes, fatty acids, amines, D-amino acids, and uric acid can increase the catalase levels in yeasts (Verduyn et al., 1988). The 16 strains used in this study were determined to be catalase positive (Table 2). Similarly, Öztürk and Sağdıç (2014) reached the same result and Öztürk (2015) reported that 53 of the 58 *C. zeylanoides* strains were catalase positive.

Urease and DNase activities

Table 2 shows that no strain had DNase activity. Figure 1b shows the transparent zone formed by *S. aureus* on the Petri dish whereas the autochthonous strain did not form a zone. The *C. zeylanoides* 4.1, 8.1, 8.3, 8.4, and 13.5 had urease activity (Table 2). The pink color formation in the medium indicates the presence of urease activity (Figure 1c). At the same time, strains isolated from naturally fermented milks (Bai et al., 2010), Erzincan tulum cheese (Karasu-Yalcin et al., 2012), Turkish white cheese (Yalcin and Ucar, 2009) had negative results for urea hydrolysis. Urease is a key hydrolytic enzyme that is responsible for the uptake and incorporation of carbon and nitrogen sources (Persike et al., 2002). It is a nickel-dependent metalloenzyme catalyzing the hydrolysis of urea, thus producing ammonia and carbamate. Urease is also a cytosolic enzyme and is found in bacteria, yeast, and several higher plants (Bharathi and Meyyappan, 2015). Urease and extracellular DNase activity has been closely associated with taxonomic positions in yeasts. Moreover, it was stated that ascosporogenous yeasts and their imperfect forms generally do not have urease or extracellular DNase activity (Sen and Komagata, 1979).



a: β-hemolytic activity, b: DNase activity, c:Urease activity

Figure 1. Hemolytic, DNase and urease activities of *C. zeylanoides* strains

Nitrate reductase activity

Nitrate reductase is a molybdoprotein that catalyzes the reduction of nitrate to nitrite (Guerrero and Gutierrez, 1977). In this study, no strain had nitrate reductase activity (Table 2). However, there are some studies showing the presence of nitrate reductase activity in some *C. zeylanoides* strains (Öztürk and Sağdıç, 2014; Öztürk, 2015). Although yeasts can use many nitrogen sources, the use of nitrate and nitrite is restricted to a few species of different genera. Besides this, some yeasts can use nitrite but not nitrate (Siverio, 2002).

Proteolytic and lipolytic activities

The strains did not show measurable proteolytic activity (Table 2). Consistently, Corbo et al. (2001) and Öztürk (2015) determined that *C. zeylanoides* strains did not have proteolytic activity. On the contrary, Öztürk and Sağdıç (2014) revealed that the strains had weak proteolytic activity. In another study, some *C. zeylanoides* strains showed proteolytic activity (Pereira-Dias et al., 2000; Yalcin and Ucar, 2009). Otherwise, the strains did not show lipolytic activity in both media (Table 2).

However, there are studies reporting that all or some *C. zeylanoides* strains had lipolytic activity (Corbo et al. 2001; Sağdıç et al., 2010; Öztürk and Sağdıç, 2014; Öztürk, 2015). Some aroma precursors such as amino acids and fatty acid esters are formed by yeasts which have high levels of lipolytic and proteolytic activities (Suzzi et al., 2001). In some food products such as cream, butter, cheese, and ultra-heat-treated products, lipases can cause flavor defects by breaking down fats (Stead, 1986). On the other hand, using yeasts that have high lipolytic activity helps the development of the typical sausage flavor (Durá et al., 2004).

Table 1. Growth characteristics of *C. zeylanoides* strains in different conditions

Strain	Glucose (%)		NaCl (%)			Ethanol (%)			Bile salts (%)	pH		Temperature (°C)			
	30	45	5	10	15	5	10	15	0.1/ 0.3/ 0.5	3.0	5.0	15	30	37	42
3.13	+++	+++	+++	++	N	+++	+++	++	+++	+++	+++	++	+++	+++	+++
4.1	++	+	+++	++	+	++	+	N	N	+	+++	++	+++	N	N
4.6	+++	+++	+++	++	N	+++	+++	++	+++	+++	+++	++	+++	+++	++
4.12	+++	+++	+++	++	N	+++	+++	+++	+++	+++	+++	++	+++	+++	++
4.14	+++	++	+++	+++	+	++	+	N	N	+	+++	++	+++	N	N
7.9	+++	++	+++	+++	+	+++	+++	+	++	++	+++	+	+++	++	N
7.10	+++	+++	+++	++	N	+++	+++	++	+++	+++	+++	++	+++	+++	++
7.12	++	++	+++	+++	N	+++	+++	N	++	+++	+++	+	+++	+++	N
8.1	++	+	+++	+++	+	++	N	N	N	++	+++	+	+++	N	N
8.3	++	+	++	++	N	+	N	N	N	+	++	+	++	N	N
8.4	++	+	++	+	N	+	N	N	N	+	++	+	++	N	N
11.9	++	+	++	++	N	++	N	N	N	++	+++	+	+++	N	N
12.3	++	+	+++	+++	+	+++	+++	N	++	+++	+++	+	+++	++	N
13.5	++	+	++	++	N	N	N	N	N	+	++	+	++	N	N
13.7	+++	+++	+++	++	N	+++	+++	++	+++	+++	+++	++	+++	+++	++
13.13	+++	+++	+++	++	N	+++	+++	++	+++	+++	+++	++	+++	+++	++

Absorbance<0,1=No growth=N, 0,1-0,5= poor growth (+), 0,5-1= moderate growth (++), >1=good growth (+++)

Table 2. Some functional and technological properties of *C. zeylanoides* strains

Properties	Number of positive strains	Number of negative strains
Biofilm formation	9	7
H_2S formation	0	16
Catalase activity	16	0
DNase activity	0	16
Nitrate reductase activity	0	16
Urease activity	5	11
Hemolytic activity	9	7
Proteolytic activity	0	16
Lipolytic activity	0	16

Antibiotic resistance

The evaluation of antifungal susceptibility helps estimate the therapeutic concentrations of antifungal drugs usage for the treatment of *Candida* infections (Ramage et al., 2001). In the study, ketoconazole (15 μg), voriconazole (1 μg), fluconazole (25 μg), and nystatin (50 μg) were used to test antifungal susceptibility. The results were reported in accordance with the Clinical and Laboratory Standards Institute (CLSI) and the disk diffusion method was used for susceptibility testing of *Candida* spp. (NCCLS, 2004). All strains were susceptible to the antifungals. The inhibition zones ranged from 29±1.41 to 31±0.00 mm for nystatin, from 23±1.41 to 37±1.41 mm for fluconazole, from 28±2.83 to 43±1.41 mm for voriconazole, and from 37±1.41 to 44±0.00 for ketoconazole (Table 3). Figure 2 shows the inhibition zones of *C. zeylanoides* 12.3 against antifungals. Shokri (2014) grouped 1 of 14 *C. zeylanoides* strains as genotype A, 2 as genotype B, and 11 as genotype C. It was determined that strain of genotype A was significantly more susceptible to ketoconazole and nystatin than fluconazole. The strains of genotype B were more susceptible to ketoconazole than other antifungal agents. The researchers also found that the strains of genotype C were significantly more susceptible to ketoconazole than fluconazole and nystatin. Öztürk and Sağdıç (2014) revealed that *C. zeylanoides* strains were sensitive to natamycin and nystatin and only two strains were resistant to fluconazole.

Figure 2. Zone diameters for antibiotic resistance test of *C. zeylanoides* 12.3

Table 3. Antifungal susceptibility of *C. zeylanoides* strains

C. zeylanoides	Nystatin	Fluconazole	Voriconazole	Ketoconazole
3.13	29±1.41	35±1.41	37±1.41	39±1.41
4.6	29±1.41	35±1.41	39±1.41	40±0.00
4.12	29±1.41	37±1.41	40±0.00	41±1.41
7.9	29±1.41	26±2.83	32±2.83	38±0.00
7.10	29±1.41	35±1.41	39±1.41	41±1.41
7.12	31±1.41	26±0.00	31±1.41	40±0.00
12.3	30±0.00	23±1.41	28±2.83	37±1.41
13.7	29±1.41	36±0.00	39±1.41	41±1.41
13.13	30±0.00	36±0.00	43±1.41	44±0.00

Susceptible (S), susceptible-dose dependent (S-DD) and resistant (R) species to nystatin (≥15 mm, 10-14 mm and none zone), fluconazole (≥19 mm, 18-15 mm and ≤14 mm), voriconazole (≥17 mm 14-16 mm and ≤13 mm) and ketoconazole (≥28 mm, 27-21 mm and ≤20 mm)

In a previous study, acid production capacities of the strains were measured according to zone formations on modified selective agar. Finally, the highest acid production was obtained by *C. zeylanoides* 7.12 with a formation of a 26 mm zone after 6 days of incubation. Under the same conditions, 12.3 (25 mm), 7.9 and 7.10 (24 mm) strains were found as the strains with the highest acid production capacity after *C. zeylanoides* 7.12 (Sayın Börekçi, 2020). Kamzolova et al. (2011) investigated the acid production capacity of 41 yeast strains that were incubated at 28°C on selective agar medium buffered with $CaCO_3$ for 7 days. It was indicated that *C. zeylanoides* VKM Y-6, VKM Y-14, VKM Y-2324, and VKM Y-2595 could not form any zone. Although, *C. paludigena*

VKM Y-2443 and some *Y. lipolytica* strains formed zones. Considering that the strains can grow even at high sugar concentrations and have acid production capabilities, it is thought that they can be used in microbial organic acid production. For instance, it is known that citric acid production needs a high substrate concentration (120-250 g/L) in the fermentation medium (Yalcin et al., 2010).

CONCLUSION

In the study, 16 *C. zeylanoides* strains were examined in terms of certain characteristics. Some strains were tolerant to high NaCl and sugar concentrations, and some of them were resistant to ethanol concentrations as high as 15%. In this way, it would be possible to select the most adequate strain/strains as producer microorganisms for value-added microbial biotechnological products and starter cultures for the production of foods. Finally, we conclude that future studies can be actualized with certain strains for microbial organic acid production, considering their acid production performances, growth capabilities at low pH, high glucose and high glycerol levels.

ACKNOWLEDGEMENT

The authors would like to thank the Eastern Anatolia High Technology and Research Center (DAYTAM) that helped carry out the analysis.

CONFLICT OF INTEREST

No content conflict of interest.

AUTHOR CONTRIBUTIONS

Bilge Sayın Börekçi: Formal analysis, Writing-original draft, Güzin Kaban: Supervisor, Writing-review & editing, Conceptualization. Mükerrem Kaya: Writing-review & editing, Conceptualization.

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