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Identification and enzymatic characterization of the yeasts isolated from Erzincan tulum cheese

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Summary

In this study, 146 yeast isolates were obtained from 45 Erzincan tulum cheese samples. By using API ID 32C test system and some complementary morphological, physiological and biochemical tests, 121 of the isolates could be identified at species level, while 12 of them were identified at genus level. The identified yeast isolates belonged to six different genera which were *Candida*, *Geotrichum*, *Kluyveromyces*, *Pichia*, *Saccharomyces* and *Zygosaccharomyces*. The most aboundant species was C. *lambica*, followed by C. *zeylanoides*, C. *famata* var. *famata*, G. *candidum* and C. *kefyr*. Enzymatic characterization of the strains was determined by using API-ZYM test system. All of the isolates had leucin arylamidase activity. Eight strains belonging to *S. cerevisiae*, *Z. mellis*, *G. candidum* and *P. fermentans* were found to have high leucin arylamidase activities. Most of the isolates had β-galactosidase, acid phosphatase and esterase lipase (C8) activities. Eight investigated C. *lambica* strains had high acid phosphatase activities. Such enzymatic properties of investigated yeast isolates could be fundamental factor for their application as starter culture candidates in production of Erzincan tulum cheese. It was demonstrated that the strain C. *lambica* T103 had superior enzymatic characteristics with the potential to be used in further technological investigations as an adjunct starter.

Key words: Erzincan tulum cheese, yeast, isolation, identification, enzymatic activity

Introduction

Yeasts are frequently found within the microflora of many cheese types (Wyder and Puhan, 1999; Padilla et al., 2010). The occurence of yeasts in cheese may be attributed to their ability to grow at low temperatures, the assimilation/fermentation of lactose, the assimilation of organic acids like succinic, lactic and citric acid, their proteolytic and lipolytic activities, resistance against high salt concentrations and resistance to cleaning compounds and sanitizers (Ferreira and Viljoen, 2003). It is reported that composition of yeast flora changes according to the cheese type and technology of cheese ripening. The typical yeast flora of mould-ripened cheeses seems to be mainly composed of *Debaryomyces hansenii*, *Geotrichum candidum*, *Kluyveromyces marxianus* and *Yarrowia lipolytica*. It is also reported that *D. hansenii* and *Kly. marxianus* occur mainly in blue-veined cheeses, and *Kly. marxianus* and *Candida zeylanoides* are usually isolated from fresh cheeses (Fröhlich-Wyder, 2003). The possibility of using yeast strains as adjunct starters for cheese production is proposed due to their positive attributes to cheese ripening such as lactose assimilation and fermentation, proteolytic and lipolytic activity (Fröhlich-Wyder, 2003; De Freitas et al., 2009).

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Erzincan tulum cheese is produced throughout Turkey, mainly in east Anatolia region. It has a white or cream colour, a high fat content, and a crumbly, semi-hard texture; it is dispersible in the mouth and has a buttery and pungent flavour. Studies on Tulum cheese are very limited, but some researches have focused on this cheese during the last decade. Those studies are related mostly to general microbiological characteristics and also on the identification of lactic acid bacteria in this cheese (Hayaloglu et al., 2007). So far, there is no reported study on identification of yeast flora of Erzincan tulum cheese and its technological propertis such as enzymatic activities. The aim of this study was to identify the yeast flora of Erzincan tulum cheese and determine the predominant species present in this flora. Enzymatic characterization of the yeast isolates was a first step of selecting wild strains with good technological properties, which potentially can be used as adjunct starter in the production of Erzincan tulum cheese.

Materials and methods

Cheese samples and chemical analysis

Fifty samples of Erzincan tulum cheese were randomly collected from the markets in Ankara and transported to the laboratory. Chemical analyses were performed and pH, water activity, dry solid content and salt content of all samples were analysed in duplicate. Water activity values of the samples were measured with a water activity measurement device (Aqualab Model CX2, Decagon, USA) based on "dew point" method (Fontana, 1998). Dry solid content was determined according to Bradley (1998) and salt content was determined by Mohr method described by Hendricks (1998).

Isolation of yeasts

Cheese samples were cut into small pieces under aseptic conditions. Twenty five grams of cheese samples were homogenised with 225 mL of 0.1 % (w/v) peptone water in a stomacher (Seward Stomacher 400 Type BA 7021, UK). Appropriate dilutions were inoculated on Yeast Extract Dextrose Chloramphenicol (YDC) agar (Lab M, UK) and Dichloran 18 % Glycerol (DG18) agar plates and incubated at 28 °C for 2-7 days (Deak and Beuchat, 1996; Pitt and Hocking, 1997). DG18 agar was prepared as described by Pitt and Hocking (1997). The colonies grown on both media were randomly selected on the basis of their macroscobic morphology. Colonies with different morphology were selected and subcultured on Yeast Extract Malt Extract (YM) agar (Lab M, UK) at 28 °C for 48 hours to obtain pure cultures. The pure cultures were maintained at 4 °C, until use.

Identification of the yeast isolates

Identifications of the yeast isolates were performed by using rapid miniaturised system API ID 32C (bioMérieux, France), following the suppliers instructions. Evaluation of the results of API ID 32C was performed by using Apilab Plus, a specific computer programme developed for API ID 32C strips and mini API analyser (bioMérieux, France). Some complementary tests used for final identification of the isolates were as follows; macroscobic and microscobic morphologies (Pitt and Hocking, 1997; Kurtzman et al., 2003), growth characteristics in liquid medium (Kurtzman et al., 2003), glucose fermentation (Harrigan, 1998; Yarrow, 2000; Kurtzman et al., 2003), urea hydrolysis (Deak and Beuchat, 1996; Yarrow, 2000; Kurtzman et al., 2003), nitrate assimilation (Deak and Beuchat, 1996; Yarrow, 2000), growth at 50 % and 60 % glucose concentrations (Pitt and Hocking, 1997; Yarrow, 2000), growth at 37 °C, growth in media including 0.5 % and 1 % acetic acid (Pitt and Hocking, 1997), pseudohyphae and ascospore formations (Yarrow, 2000). For some of the isolates, galactose fermentation, growth in medium without vitamin (Yarrow, 2000) and growth in medium including 16 % NaCl and 5 % glucose were also investigated.

Enzymatic characterization

Enzymatic characterizations of the yeast isolates were performed by using API-ZYM test system (bioMérieux, France). API-ZYM is a miniaturized, semiquantitative test system, used for screening 19 different enzyme activities. In order to assay the enzymatic tests, yeast cultures were pre activated on YM agar at 30 °C for 24 hours. Activated yeast cultures were suspended in distilled water until suspensions reached 5 or 6 McFarland turbidity. Those suspensions were inoculated in the microwells on the API-ZYM strip at a level of 65 µL for each cupule. The strips were incubated at 37 °C for 4-4 1/2 hours. Five minutes after addition of ZYM A and

ZYM B reagents to each cupule, strips were put under 1000 W lamp for 10 seconds for prevention of yellow colour formation caused by Fast Blue BB. Enzyme activity was graded from 0 to 5 by comparing developed colour with the API-ZYM colour reaction chart.

Among the 121 identified isolates, 69 of them were selected for screening enzymatic activities. Biochemical and physiological characteristics of the yeast strains belonging to the same species were considered as selection criteria. All strains belonging to the same species with different biochemical and physiological characteristics were enzymatically characterized.

Results and discussion

Chemical analysis

In the selected chemical analysis, pH, water activity (a_w), solid content and salt content of the cheese samples were determined. It was found that the pH and a_w values of cheeses were in the range of 3.33±0.02 - 4.82±0.07 and 0.883±0.003 - 0.967±0.001, respectively. Solid content of the samples were between 52.38±0.05 - 65.46±0.74 %, while salt content on dry basis were changed between 3.61±0.2 - 10.59±0.58 % (data not shown).

Isolation and identification

In the study, yeast growth was obtained 45 out of 50 Erzincan tulum cheese samples. From the cultures of appropriate dilutions of cheese samples, each colony having different macroscobic morphology was selected for further identification. A total of 146 isolates were obtained from 45 Erzincan tulum cheese samples. By using API ID 32C test system and some other supplementary tests, 121 of the isolates could be identified at species level, while 12 of them were identified at genus level. Thirteen (9 %) of the isolates could not be identified by the methods used in this study. The reliable identification of these strains can be further achieved on genotyping level by using molecular methods. Most commercially available yeast identification systems have been reported to provide accurate and reliable results, giving 90 % or more agreement with data obtained by traditional identification methods (Deak and Beuchat, 1996). It is also known that misidentification can occur and traditional methods do not allow fine typing of yeasts at the subspecies level (Van der Vossen et al., 2003). It has been reported that PCR-based molecular methods have permitted both intraspecies differentiation and species identification which can be used for more reliable identification of yeast isolates (Esteve-Zarzoso et al., 1999; Hierro et al., 2004).

One hundered twenty - one identified yeast isolates belonged to six different genus such as C*andida* (97), *Geotrichum* (14), *Kluyveromyces* (1), *Pichia* (1), *Saccharomyces* (7) and *Zygosaccharomyces* (1). The identified yeast species and number of isolates are represented in Table 1. According to the identification results obtained with API ID 32C, species identification levels of the yeasts were changed between 98.1-99.9 %. Identification at genus level was achieved between the range of 47.9-94.8 % (data not shown).

According to the API ID 32C system, identification of the yeast isolates at species level was performed at excellent, very good and good levels. By using this system, 20 isolates could be identified

Table 1. The species and number of identified yeast isolates

Yeast species	Number of isolates
Candida apicola	1
Candida colliculosa	1
Candida famata var. *famata*	15
Candida famata var. *flareri*	2
Candida japonica	1
Candida kefyr	12
Candida krusei	1
Candida lambica	32
Candida lipolytica	2
Candida paludigena	6
Candida rugosa	1
Candida zeylanoides	23
Candida sp.	12
Geotrichum candidum	14
Kluyveromyces lactis var. *lactis*	1
Pichia fermentas	1
Saccharomyces cerevisiae	7
Zygosaccharomyces mellis	1
Not identified	13

at genus level. Two strains gave low discrimination profile, nine of them suspectable profile, while two strains had unacceptable profile and one strain gave no identification result on API ID 32C strips. Additional identification tests were used for the isolates which could not be identified, or identified only at genus level with API ID 32C (Table 2). When the assimilation test results of API ID 32C system and supplementary tests were evaluated together by using identification keys of Barnett et al. (2000), Payne et al. (2000) and Kurtzman and Fell (2000), some of the isolates could be identified at species level. For example, the strain T81 which was identified at genus level as C*andida* genus by API ID 32C, was identified as C. *apicola* by using identification keys of Payne et al. (2000). Similarly, six of the isolates (T31, T32, T51, T145, T162, T170) were identified as C. *paludigena* by the same way**.** Fourteen isolates determined in G*eotrichum* genus by API ID 32C, were identified as G. *candidum* according to identification keys of Payne et al. (2000). Additionally, some of the identification results of the API ID 32C were changed according to the determined ascospore formation characteristics of the isolates. One of the isolates identified as C. *sphaerica* (T2) by API ID 32C was found to form ascospore and identified as its telemorph form; *Kly. lactis*. The isolate belonging to species *P. fermentans* (T9) was also identified by the same way which is the telemorph form of C. *lambica*. The strain T166 which gave unaccepted profile in API ID 32C strips could be identified as C. *japonica* by the identification keys of Payne et al. (2000). A yeast strain (T110) found as belonging to *Zygosaccharomyces* by API ID 32C strips, could only be determined in genus level. By using the identification keys of Kurtzman (2000), it was identified as *Z. mellis*.

Most of the yeast strains (82 %) identified in this study belonged to C*andida* genus. The most aboundant species was C. *lambica*, followed by C. *zeylanoides*, C. *famata* var. *famata*, G*eotrichum candidum* and C. *kefyr* (Table 1). C*andida famata* (telemorph: *D. hansenii*) and G*eotrichum candidum* have also been reported as prevalent yeast species

Table 2. Results of some additional tests used for the identification of the isolates

Additional tests	Yeast species	*C. apicola*	*C. colliculosa*	*C. famata* var. *famata*	*C. famata* var. *flareri*	*C. japonica*	*C. kefyr*	*C. krusei*	*C. lambica*	*C. lipolytica*	*C. paludigena*	*C. rugosa*	*C. zeylanoides*	*G. candidum*	*Kly. lactis* var. *lactis*	*P. fermentans*	*S. cerevisiae*	*Z. mellis*
Glucose fermentation		-	+	v	-	v	v	+	v	-	-	-	v	v	+	-	+	+
Urea hydrolysis		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nitrate assimilation		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth at 50 % glucose		-	+	v	v	v	-	-	-	-	-	+	v	v	-	-	-	-
Growth at 60 % glucose		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth at 37 °C		-	-	-	v	-	v	v	v	v	+	+	v	v	+	-	v	-
Growth at 0.5 % acetic acid		-	-	v	-	-	-	-	v	-	-	+	-	-	-	+	-	-
Growth at 1 % acetic acid		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ascospore formation		-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
Pseudohyphae formation		-	-	v	-	-	v	+	+	+	-	+	+	+	-	+	v	+
Growth on media without vitamin[1]		*	*	*	*	*	*	*	*	*	*	*	*	*	_	*	*	*
Growth on 16 % NaCl-5 % glucose containing media [1]		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	_

(+): Positive. (-): Negative. (v): Variable. ([1]): These tests were used for only some of the isolates. (*): Not used for these isolates.

isolated from other cheeses (Pottier et al., 2008; Del Bove et al., 2009). Aditionally, *D. hansenii* was reported as the predominant yeast at the end of ripening of the artisanal ewe's milk cheeses (Padilla et al., 2010). Frequently isolated *Candida* species from cheeses such as C. *catenulata*, C. *famata*, C. *intermedia*, C. *kefyr*, C. *krusei*, C. *lipolytica*, C. *pseudotropicalis*, C. *robusta*, C. *rugosa*, C. *sake*, C. *sphaerica*, C. *tenius*, C. *utilis*, C. *versatilis* and C. *zeylanoides* have been reported by Fröhlich-Wyder (2003). There are also reports for other yeast species originating from cheeses such as *Kly. marxianus*, *S. cerevisiae*, *Dipodascus capitatus*, *Rhodotorula mucilaginosa*, *Cryptococcus curvatus* (Aponte et al., 2010), *Galactomyces geotrichum* (Wyder et al., 1999), *G. candidum*, *Kly. lactis*, *Dekkera anomala* and *Rhodotorula rubra* (Cosentino et al., 2001). When compared to the results of related studies on yeast flora of cheeses, it is thought that Erzincan tulum cheese may be defined as unique for having predominantly C. *lambica*.

Screening of enzymatic activities

Enzymatic activities of the yeast strains were given in Table 3. According to the results, it was found that none of the isolates had the activities of α-galactosidase, N-acetyl-β-glucoaminidase, α-mannosidase, α-fucosidase, trypsin and α-chymotrypsin. Six strains belonging to C. *kefyr* (T102, T109), C. *lipolytica* (T122, T168), C. *japonica* (T166) and C. *famata* var. *famata* (T7) had β-glucosidase activities at low levels (level 1). β-galactosidase was detected in 24 of the isolates. Among them, one strain of *Kly. lactis* var. *lactis* (T2) and three strains of C. *kefyr* (T80, T102, T155) showed high β-galactosidase activities (level 4). Herreros et al. (2003) reported that β-galactosidase was the key enzyme in the transformation of lactose to lactic acid. Therefore, this enzyme could be important for lactose metabolism in cheese during ripening.

It was determined that all of the isolates had leucin arylamidase activity. Eight strains belonging to *S. cerevisiae* (T125, T106, T91, T50), *Z. mellis* (T110), *G. candidum* (T119, T147) and *P. fermentans* (T9) were found to have high leucin arylamidase activities (level 5). Valine arylamidase and cystine arylamidase activities of the isolates were changed between level 0-3. C. *japonica* (T166) was found to have both enzymes (level 3). The proteolytic enzymes of arylamidases (aminopeptidases) catalyze the hydrolysis of N-terminal aminoacids from peptide, amide or arylamides (Dodor and Tabatabai, 2007). Herreros et al. (2003) reported that these enzymes were important tools in liberation of aminoacids and development of the desirable flavours in cheese. These enzymes were also reported to have a debittering effect during cheese ripening (Herreros et al., 2003). Thus, high leucin arylamydase activity as well as valine and cystine arylamidase activities of the strains determined in the present study could be of great technological importance and could be further investigated as starter culture candidates for production of Erzincan tulum cheese. Additionally, we found that the isolate T2 (*Kly. lactis* var. *lactis*) have leucine arylamidase and cystine arylamidase enzymes (Table 3). This finding could correlate with the study of De Freitas et al. (2009), in which *Kly. lactis* was used as one of the adjunct starters in the production of Cantalet cheese, due to its aminopeptidase activity and ability to produce esters that are associated with the formation of fruity aromas.

It was found that most of the yeast strains had acid phosphatase activity. Eight of ten investigated C. *lambica* strains had high acid phosphatase activities (level 5). Acid phosphatase activities of C. *krusei* (T132), *G. candidum* (T11, T138, T147), C. *rugosa* (T82), C. *zeylanoides* (T60, T27, T135, T12) and C. *apicola* were also high. It is known that phosphatases catalyze the hydrolysis of C-O-P linkage of a wide variety of phosphate esters. They are classified as acid or alkaline phosphatases depending on their pH optima. It was reported that although both acid and alkaline phosphatases were present in cheese, acid phosphatases were more active due to their relatively low optimum pH (Magboul and McSweeney, 1999). Akuzawa and Fox (2004) reported that phosphate-rich peptides, which were resistant to further proteolysis, were produced during cheese ripening. The combined action of acid phosphatases and proteolytic enzymes in cheese was thus required for extensive production of small peptides and free aminoacids. Dephosphorylation of casein and phosphopeptides was reported to have an important impact in cheese ripening (Akuzawa and Fox, 2004). It was also reported that since acid phosphatase enzyme had a direct effect on proteolysis, it could contribute to aroma formation in cheese.

Table 3. Enzyme activities of the yeast strains

Isolate no.	Yeast species	Control	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
T125	*S. cerevisiae*	0	0	1	0	0	5	0	1	0	0	0	2	0	0	0	0	0	0	0	0
T106	*S. cerevisiae*	0	0	1	1	0	5	0	1	0	0	2	1	0	0	0	2	0	0	0	0
T91	*S. cerevisiae*	0	0	1	1	0	5	0	1	0	0	1	1	0	0	0	1	0	0	0	0
T50	*S. cerevisiae*	0	0	1	1	0	5	0	1	0	0	0	1	0	0	0	2	0	0	0	0
T141	*S. cerevisiae*	0	0	0	1	0	3	0	1	0	0	1	1	0	0	0	3	0	0	0	0
T75	*S. cerevisiae*	0	0	1	1	0	4	0	1	0	0	1	1	0	0	0	3	0	0	0	0
T110	*Z. mellis*	0	0	2	3	0	5	2	1	0	0	0	0	0	0	0	0	0	0	0	0
T104	*C. colliculosa*	0	3	1	0	0	3	0	0	0	0	2	1	0	0	0	0	0	0	0	0
T132	*C. krusei*	0	0	1	0	0	3	0	1	0	0	5	2	0	0	0	0	0	0	0	0
T2	*Kly. lactis* var. *lactis*	0	0	1	1	0	4	0	1	0	0	3	3	0	4	0	0	0	0	0	0
T119	*G. candidum*	0	0	0	1	0	5	1	1	0	0	3	3	0	0	0	0	0	0	0	0
T147	*G.candidum*	0	0	1	2	0	5	2	2	0	0	5	2	0	0	0	0	0	0	0	0
T154	*G. candidum*	0	0	1	3	1	2	2	1	0	0	3	1	0	0	0	0	0	0	0	0
T11	*G. candidum*	0	2	0	2	0	3	0	0	0	0	5	1	0	0	0	0	0	0	0	0
T20	*G. candidum*	0	0	0	0	0	2	0	1	0	0	2	2	0	0	0	0	0	0	0	0
T73	*G. candidum*	0	0	0	2	0	2	0	0	0	0	4	1	0	0	0	0	0	0	0	0
T90	*G. candidum*	0	0	1	1	0	3	1	1	0	0	3	1	0	0	0	0	0	0	0	0
T138	*G. candidum*	0	2	1	3	0	2	0	0	0	0	5	2	0	0	0	0	0	0	0	0
T67	*G. candidum*	0	0	0	1	0	2	0	0	0	0	3	1	0	0	0	0	0	0	0	0
T78	*G. candidum*	0	0	1	0	0	3	0	1	0	0	2	1	0	0	0	0	0	0	0	0
T107	*C. kefyr*	0	2	0	0	0	3	0	0	0	0	2	3	0	3	0	0	0	0	0	0
T131	*C. kefyr*	0	0	0	0	0	2	0	1	0	0	0	1	0	3	0	0	0	0	0	0
T158	*C. kefyr*	0	0	0	0	0	3	1	0	0	0	2	1	0	1	0	0	0	0	0	0
T163	*C. kefyr*	0	0	0	0	0	3	0	0	0	0	0	1	0	2	0	0	0	0	0	0
T80	*C. kefyr*	0	0	0	0	0	3	0	0	0	0	3	2	0	4	0	0	0	0	0	0
T112	*C. kefyr*	0	0	2	1	0	4	2	1	0	0	1	1	0	3	0	0	0	0	0	0
T155	*C. kefyr*	0	0	1	1	0	3	0	1	0	0	4	2	0	4	0	0	0	0	0	0
T102	*C. kefyr*	0	0	1	1	0	3	1	1	0	0	4	3	0	4	0	0	1	0	0	0
T120	*C. kefyr*	0	1	2	1	0	4	2	1	0	0	4	2	0	3	0	0	0	0	0	0
T109	*C. kefyr*	0	0	0	0	0	3	1	1	0	0	3	2	0	3	0	0	1	0	0	0
T122	*C. lipolytica*	0	0	0	0	0	1	0	0	0	0	0	2	0	0	0	0	1	0	0	0
T168	*C. lipolytica*	0	2	1	1	0	2	0	1	0	0	3	3	0	0	0	0	1	0	0	0
T82	*C. rugosa*	0	0	0	0	0	1	0	0	0	0	5	3	0	0	0	0	0	0	0	0
T60	*C. zeylanoides*	0	0	1	2	0	2	0	0	0	0	5	3	0	0	0	0	0	0	0	0
T45	*C. zeylanoides*	0	0	1	1	0	3	0	0	0	0	4	1	0	0	0	0	0	0	0	0
T29	*C. zeylanoides*	0	0	2	2	0	3	1	3	0	0	3	2	0	0	0	0	0	0	0	0
T36	*C. zeylanoides*	0	0	2	2	0	3	0	0	0	0	4	2	0	0	0	0	0	0	0	0
T27	*C. zeylanoides*	0	0	1	1	0	3	0	0	0	0	5	1	0	0	0	0	0	0	0	0
T176	*C. zeylanoides*	0	0	1	1	0	3	1	0	0	0	4	1	0	0	0	0	0	0	0	0
T99	*C. zeylanoides*	0	0	1	2	0	2	0	0	0	0	4	1	0	0	0	0	0	0	0	0
T164	*C. zeylanoides*	0	0	1	1	0	2	1	0	0	0	4	1	0	0	0	0	0	0	0	0

Table 3. Enzyme activities of the yeast strains (continues)

Isolate no.	Yeast species	Control	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
T135	*C. zeylanoides*	0	0	1	1	0	3	0	0	0	0	5	1	0	0	0	0	0	0	0	0
T12	*C. zeylanoides*	0	0	1	2	0	2	0	0	0	0	5	1	0	0	0	0	0	0	0	0
T51	*C. paludigena*	0	2	2	1	0	2	0	0	0	0	2	2	0	1	0	1	0	0	0	0
T170	*C. paludigena*	0	2	2	1	0	2	1	1	0	0	3	1	0	2	0	1	0	0	0	0
T145	*C. paludigena*	0	2	2	1	0	2	0	0	0	0	3	2	0	2	0	1	0	0	0	0
T31	*C. paludigena*	0	1	2	1	0	2	0	0	0	0	1	1	0	2	0	0	0	0	0	0
T81	*C. apicola*	0	0	1	1	1	4	3	1	0	0	5	3	0	0	0	0	0	0	0	0
T166	*C. japonica*	0	3	2	1	0	3	3	3	0	0	4	2	0	0	0	3	1	0	0	0
T38	*C. lambica*	0	0	1	0	0	3	1	0	0	0	5	3	0	0	0	0	0	0	0	0
T22	*C. lambica*	0	0	2	1	1	3	1	2	0	0	4	2	0	0	0	0	0	0	0	0
T62	*C. lambica*	0	0	2	1	0	5	2	1	0	0	5	5	0	0	0	0	0	0	0	0
T139	*C. lambica*	0	0	2	1	1	4	2	2	0	0	5	3	0	0	0	0	0	0	0	0
T83	*C. lambica*	0	0	0	0	0	4	2	2	0	0	4	4	0	0	0	0	0	0	0	0
T127	*C. lambica*	0	0	2	1	0	3	2	1	0	0	5	1	0	0	1	0	0	0	0	0
T13	*C. lambica*	0	0	1	1	0	4	1	1	0	0	5	3	0	0	0	0	0	0	0	0
T103	*C. lambica*	0	0	2	1	1	4	1	1	0	0	5	4	0	1	0	0	0	0	0	0
T17	*C. lambica*	0	0	2	1	0	4	2	1	0	0	5	3	0	0	0	0	0	0	0	0
T30	*C. lambica*	0	0	1	1	1	4	1	1	0	0	5	3	0	0	0	0	0	0	0	0
T9	*P. fermentans*	0	1	2	2	0	5	1	1	0	0	4	4	0	0	0	0	0	0	0	0
T56	*C. famata* var. *famata*	0	1	2	1	0	2	0	0	0	0	3	1	0	3	0	1	0	0	0	0
T171	*C. famata* var. *famata*	0	1	2	1	0	3	0	0	0	0	3	1	0	3	0	0	0	0	0	0
T7	*C. famata* var. *famata*	0	1	2	1	0	2	1	0	0	0	2	1	0	3	0	1	1	0	0	0
T172	*C. famata* var. *famata*	0	2	3	1	0	2	0	0	0	0	3	1	0	0	0	0	0	0	0	0
T144	*C. famata* var. *famata*	0	2	1	1	0	3	1	1	0	0	4	3	0	3	0	1	0	0	0	0
T98	*C. famata* var. *famata*	0	2	2	2	0	3	1	0	0	0	2	1	0	3	0	2	0	0	0	0
T116	*C. famata* var. *famata*	0	1	3	1	0	1	0	0	0	0	2	1	0	2	0	2	0	0	0	0
T52	*C. famata* var. *flareri*	0	2	1	1	0	4	1	0	0	0	2	1	0	2	0	0	0	0	0	0
T42	*C. famata* var. *flareri*	0	2	2	1	0	4	1	0	0	0	2	1	0	2	0	0	0	0	0	0

1: Alkaline phosphatase, 2: Esterase (C4), 3: Esterase lipase (C8), 4: Lipase (C14), 5: Leucine arylamidase, 6: Valine arylamidase, 7: Cystine arylamidase, 8: Trypsin, 9: α-chymotrypsin, 10: Acid phosphatase, 11: Naphtol-AS-BI-phosphohydrolise, 12: α-galactosidase, 13: β- galactosidase, 14: β-glucuronidase, 15: α-glucosidase, 16: β-glucosidase, 17: N-acetyl- β-glucoaminidase, 18: α-mannosidase, 19: α-fucosidase

It has been reported that the sources of this enzyme in cheese could be milk or microorganisms (Akuzawa and Fox, 2004). Yeast strains with high acid phosphatase activities may serve as good sources of this enzyme in Erzincan tulum cheese. It was determined that most of the yeast strains (78 %) screened had esterase lipase (C8) activity, while lipase (C14) activity was merely detected. Only five strains (7 %) belonging to *G. candidum* (T154), *C. apicola* (T81), *C. lambica* (T22, T139, T103) had

lipase (C14) activity at low levels. It is known that esterases and lipases catalyze the hydrolysis of ester linkages on lipids. These enzymes contribute to an increase in the concentrations of free fatty acids in cheese. It was reported by Herreros et al. (2003) that low concentrations of free fatty acids could contribute to the flavour of cheese, particularly when they were correctly balanced with the products of proteolysis or other reactions. It is assumed that yeast strains having high esterase, esterase lipase or lipase activities may contribute to lipolysis in cheese ripening.

The results of enzymatic activities of the enzymes important in cheese ripening reveiled that the strain C*andida lambica* T103 had superior enzymatic characteristics. This strain had esterase, esterase lipase, lipase, leucine arylamidase, valine arylamidase, acid phosphatase and β-galactosidase activities. Since this strain was unique considering its enzymatic profiles, it can be selected for further investigations as an adjunct starter.

Conclusions

Yeast flora of Erzincan tulum cheese was characterized for the first time. Our study revealed the presence of C. *lambica*, followed by C. *zeylanoides*, C. *famata* var. *famata*, G. *candidum* and C. *kefyr.* Obtained enzymatic properties of yeast strains such as β-galactosidase, leucin arylamidase, acid phosphatase, esterase lipase could be of great technological importance and could be tested in further studies as starter culture candidates for production of Erzincan tulum cheese.

Identifikacija i enzimatska karakterizacija kvasaca izoliranih iz Erzincan tulum sira

Sažetak

U ovom istraživanju izolirano je 146 kvasaca iz 45 uzoraka Erzincan tulum sira. Pomoću API ID 32C test sustava te nekih komplementarnih morfoloških, fizioloških i biokemijskih testova, 121 izolat kvasca potencijalno je identificiran na razini vrste, dok je 12 izolata identificirano na razini roda. Utvrđeno je da izolirani kvasci pripadaju sljedećim rodovima: *Candida*, *Geotrichum*, *Kluyveromyces*, *Pichia*, *Saccharomyces* i *Zygosaccharomyces*. Najveći broj izolata pripadao je vrstama C. *lambica*, C. *zeylanoides*, C. *famata* var. *famata*, G. *candidum* i C. *kefyr*. Enzimatska karakterizacija sojeva utvrđena je pomoću API-ZYM sustava. Svi izolati pokazali su leucin arilamidaznu aktivnost. Za osam izolata koji pripadaju vrstama *S. cerevisiae*, *Z. mellis*, *G. candidum* i *P. fermentans* utvrđeno je da posjeduju visoke aktivnosti leucin arilamidaze. Većina izolata imala je β-galaktozidaznu, kiselu fosfataznu i esteraznolipaznu (C8) aktivnost. Osam istraživanih sojeva C. *lambica* imalo je visoke aktivnosti kisele fosfataze. Takva enzimatska svojstva istraživanih kvasaca mogla bi biti temelj za njihovu potencijalnu primjenu u pripremi starter kultura u proizvodnji Erzincan tulum sira. Dodatno je utvrđeno da soj C. *lambica* T103 ima izuzetna enzimatska svojstva koja bi se u daljnjim tehnološkim istraživanjima trebala ispitati te uvrstiti kao potencijalni soj u pripremi starter kulture navedenog sira.

Ključne riječi: Erzincan tulum sir, kvasac, izolacija, identifikacija, enzimatska aktivnost

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