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ENZYMATIC CHARACTERIZATION OF YEAST STRAINS ORIGINATED FROM TRADITIONAL MIHALIC CHEESE

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ABSTRACT

Yeasts, associated with secondary flora of many cheese types, are important microorganisms for cheese ripening process. The aim of this study was to identify the yeasts isolated from traditional Mihalic cheese and to determine their enzymatic activities as a tool for their technological characteristics. Phenotypic identification was performed by using API ID 32C test system and some complementary morphological, physiological and biochemical tests. Enzyme profiles of the isolates were determined by using API-ZYM strips. In this study, 72 yeast isolates were obtained from 29 Mihalic cheese samples. Fifty-six (78%) of the isolates could be identified at species level, and one isolate at genus level. The identified yeast species belonged to three genera; *Candida, Geotrichum* and *Trichosporon*. It was determined that *Candida famata* var. *famata* was the dominant species in Mihalic cheese. The yeast isolates had variable enzyme activities including acid phosphatase, esterase, esterase lipase, lipase, β-galactosidase, leucine arylamidase, valine arylamidase and cysteine arylamidase, which could have important attributes during cheese ripening. *C. famata var. famata* M22, *Candida guilliermondii var. membranefaciens* M54 and *Candida tropicalis* M2 were selected to be superior strains on the basis of their enzyme profiles. Identification and enzymatic characterization of the yeasts originated from Mihalic cheese was performed for the first time in this study.

Keywords: Mihalic cheese, yeast, isolation, identification, enzymatic characterization

INTRODUCTION

Mihalic cheese is one of the traditional cheeses of Turkey, widely produced in the provinces of Bursa, Balıkesir and Canakkale (**Kamber, 2008**). The name "Mihalic" is an old name of Karacabey, county of Bursa, and it is known that the cheese has been produced in these areas for at least 250 years (Hayaloglu *et al*., 2008). Among the inhabitants of the region, the cheese is also known by such different names as Maglic, Mahlic, Kelle or Manyas cheese (**Aday & Karagul Yuceer, 2014**). It is assumed that Mihalic cheese was adopted from Greek cheese-making traditions (**Kamber, 2008**). It is a brined cheese made from raw sheep's or goat's milk and characterized by high levels of salt and dry matter (**Kamber, 2008; Aday & Karagul Yuceer, 2014**). It is ripened in wooden barrels at 15-25°C for three months in brine (**Aday & Karagul-Yuceer, 2014**). It is tough, tightly-structured and ranges from cream to light yellow in color. It has a characteristic sharp taste and odor and has 3-4 mm diameter pores gradually decreasing from the center to the sides in its cross-section (**Kamber, 2008).**
It is known that composition and activity of the microflora in cheese play a critical role in the formation of cheese, leading to production of diverse range of cheese varieties (**Jany & Barbier, 2008**). It was reported that the recovery of yeasts in high numbers from cheeses (e.g. 10^6–10^9 cfu/g) and their ability to hydrolyse milk fat and proteins have suggested that they influence the organoleptic characteristics of cheese. Even in cheeses inoculated with bacterial starters, yeasts are reported to be detected at counts as high as 10^8 cfu/g (**Capece & Romano, 2009).** Yeast species usually represent secondary microbiota in cheeses and among them *Kluyveromyces marxianus*, *Debaryomyces hansenii*, *Saccharomyces cerevisiae*, *Yarrowia lipolytica* (**Golić *et al*., 2013**), *Kluyveromyces lactis* (**Gardini *et al*., 2006**), *Trichosporon cutaneum*, *Candida zeylanoides* and *Geotrichum candidum* (**Padilla *et al*., 2014a**) were reported to be dominant species in various cheeses. It has been reported that the yeasts play an important role in proteolysis, lipolysis, fermentation of residual lactose, and assimilation of lactic and citric acid during the ripening of cheese, contributing to aroma development and to the rheological properties of the final product (**Gardini *et al*., 2006; Padilla *et al*., 2014b**) . Additionally, some cheese yeasts have been recognized by their probiotic character and DNA-bioprotective action against model genotoxins (**Padilla *et al*., 2014b**).

With regard to traditional cheeses in Turkey, with exception of a few varieties, most of the traditional brined cheeses have not yet been industrialized. It has been reported that relatively little is known about the basic and microbiological characteristics of the brined cheeses native to Turkey (**Hayaloglu *et al*., 2008).** There are a few reports about the chemical and microbiological properties of Mihalic cheese (**Solak & Akin, 2013; Aday & Karagul Yuceer, 2014**). The reports concerning microflora of Mihalic cheese were usually focused on pathogenic bacteria (**Cokal *et al*., 2012**). To date, there is no reported study about the yeast flora of Mihalic cheese. The aim of this study was to determine the predominant yeast species in the microflora of Mihalic cheese and to characterize their enzymatic activities.

MATERIALS AND METHODS

Cheese samples and chemical analysis

Thirty-one samples of Mihalic cheese were randomly collected from the markets in Ankara and transported to the laboratory. Most of the cheese samples were packaged in polyethylene bags, and the others were aseptically taken from the newly opened tin boxes in the markets. Water activity, pH, dry solid content and salt content of all samples were analysed in duplicate. Water activity was measured based on "dew point" method (**Fontana, 1998**) by using a water activity measurement device (Aqualab Model CX2, Decagon, USA). 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 and 25 grams from each block (sample) were homogenised with 225 mL of 0.1% (w/v) peptone water in a stomacher (Seward Stomacher 400 Type BA 7021, UK). For isolation of the yeasts, appropriate dilutions were inoculated on Yeast Extract Dextrose Chloramphenicol (YDC) agar (Lab M, UK) and Dichloran 18% Glycerol (DG18) agar plates (**Deák & Beuchat, 1996; Pitt & Hocking, 1997**). After incubation at 28°C for 2-7 days, the colonies grown on both media were randomly selected on

the basis of their macroscobic morphology. Colonies with different morphology were inoculated into Yeast Extract Malt Extract (YM) agar (Lab M, UK) and pure cultures were obtained at 28ºC for 48 hours. The pure cultures were maintained at 4°C, until use.

Identification of the yeast isolates

Yeast isolates were identified by using API ID 32C, a rapid miniaturised identification system (bioMérieux, France), and some complementary tests. API ID 32C strips were used according to the suppliers instructions. The results of API ID 32C was evaluated by using Apilab Plus, a specific computer programme developed for API ID 32C strips and mini API analyser (bioMérieux, France). The complementary identification tests used were as follows; macroscobic and microscobic morphologies **(Pitt & 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 **(Deák & Beuchat, 1996; Yarrow, 2000; Kurtzman *et al*., 2003)**, nitrate assimilation **(Deák & Beuchat, 1996; Yarrow, 2000)** growth at 50% and 60% glucose concentrations **(Yarrow, 2000; Kurtzman *et al*., 2003)**, growth at 37ºC, growth in media including 0.5% and 1% acetic acid **(Pitt & 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.

Determination of enzymatic activities

Enzymatic activities of the yeast isolates were screened by using miniaturized API-ZYM test system (bioMérieux, France), which enables screening 19 different enzyme activities. After activation of the yeast cultures on YM agar at 30ºC for 24 hours, they were suspended in distilled water until suspensions reached 5 or 6 McFarland turbidity. The suspensions were inoculated in the microwells on the API-ZYM strip at a level of 65 □L for each cupule. After incubation at 37ºC for 4 - 4.5 hours, ZYM A and ZYM B reagents were added to each cupule and after five minutes, strips were put under 1000 W lamp for 10 seconds for prevention of yellow colour formation caused by Fast Blue BB. For evaluation of the results, each enzyme activity was graded from 0 to 5 by comparing developed colour with the API-ZYM colour reaction chart. The approximate amount of free hydrolysed substrate (nmol) could be obtained from the colour strength: 0, no activity; 1, liberation of 5 nmol; 2, 10 nmol; 3, 20 nmol; 4, 30 nmol; 5, ≥ 40 nmol. The grades were evaluated as no activity (0), low activity (1), intermediate activity (2-3) and high activity (4-5).

For screening enzymatic activities, 30 of the 56 identified isolates were used. Thirty isolates were selected according to their biochemical and physiological characteristics. All strains belonging to the same species with different biochemical and physiological characteristics were enzymatically characterized.

RESULTS

Results of chemical analysis

The analysed thirty-one Mihalic cheese samples differed in some chemical properties. Water activities and pH values of the samples were found to change between 0.785±0.001-0.954±0.001 and 3.62±0.08 - 4.58±0.10, respectively. Dry solid content of the Mihalic cheese samples were in the range of 55.76±0.12 - 71.67±0.18 (%), while salt content on dry basis was determined to change between 3.46±0.01-13.99±1.63 (%) (data not shown).

Results of yeast isolation and identification

Isolation experiments resulted in obtaining 72 yeast isolates from 29 of the 31 cheese samples. The isolates were coded with "M" and numbers. According to the identification results obtained with API ID 32C, 43 of the isolates could be identified at species level. In species identification, an identification category of "excellent" as established by the manufacturer was obtained for 4 of the isolates. The identification profile was defined as "very good" and "good" for 24 and 15 of the isolates, respectively. Eleven isolates could be identified at genus level as "*Candida*", 2 isolates as "*Geotrichum*". Identification categories of 11 of the isolates were defined as "unacceptable profile", one of the isolates as "acceptable profile", two of them as "doubtful" profile, one of them as "low discrimination", and one of them gave no identification result with the use of API ID 32C strips (data not shown). As a result, ID 32C strips did not give satisfactory identification results for 16 of the isolates. According to the identification results obtained with API ID 32C, species identification levels of the yeasts were changed between 96.6-99.9%. Identification at genus level was achieved between the range of 67.5-94.4% (data not shown). The identified isolates were in the species of; *Trichosporon asahii* (1), *Candida tropicalis* (3), *Candida insconspicua/norvegensis* (3), *Candida famata* (29), *Saccharomyces cerevisiae* (1), *Candida catenulata* (1), *Kodamaea ohmeri* (1), *Candida krusei* (2) and *Candida zeylanoides* (2).

The complementary identification tests were used for the isolates which could not be identified, or identified only at genus level with API ID 32C. The results of these tests were represented in Table 1.

Table 1 Results of some complementary tests used for the identification of the isolates

Tests	**Yeast species**	*C. bertae*	*C. catenulata*	*C. cylindracea*	*C. famata* var *famata*	*C. guilliermondii* var *membranefaciens*	*C. krusei*	*C. norvegensis*	*C. paludigena*	*C. robusta*	*C. tropicalis*	*C. zeylanoides*	*G. candidum*	*T. asahii*
Glucose fermentation		-	+	v	v	+	+	+	-	+	+	v	-	-
Urea hydrolysis		-	-	-	-	-	-	-	-	-	-	-	-	+
Nitrate assimilation		+	-	-	-	-	-	-	-	-	+	-	-	-
Growth at 50% glucose		+	+	v	+	+	-	-	+	+	-	-	-	-
Growth at 60% glucose		v	-	-	v	+	-	-	v	-	-	-	-	-
Growth at 37°C		-	-	-	-	-	v	v	-	-	+	-	v	+
Growth at 0.5% acetic acid		-	-	-	-	-	v	-	-	-	-	-	-	-
Growth at 1% acetic acid		-	-	-	-	-	-	-	-	-	-	-	-	-
Ascospore formation		-	-	-	-	-	-	-	-	-	-	-	-	-
Pseudohyphae formation		-	+	v	v	+	+	v	v	-	+	v	+	-
Growth on media without vitamin[1]		*	*	*	*	*	*	*	*	*	*	*	-	*
Galactose fermentation[1]		*	*	+	*	*	*	*	*	*	*	*	*	*

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

When the assimilation test results of API ID 32C system and complementary identification tests were evaluated together by using identification keys of **Barnett *et al*. (2000)**, **Payne *et al*. (2000)** and **Kurtzman & Fell (2000)**, identification at species level was achieved for some of the isolates. By this way, some of the isolates which could not be identified or identified at genus level could be identified at species level. For example, among the isolates which were identified at genus level as "*Candida*", the strains M53 and M63 could be identified as *Candida bertae* by using identification keys of **Payne *et al*. (2000)**. By the same way, four isolates (M32, M48, M79, M88) could be identified as *Candida paludigena*, while the other four (M73, M77, M78, M83) were identified as *Candida cylindraceae.* For identifying *C. cylindraceae* isolates, galactose fermentation test was used in addition to other complementary tests as recommended by **Payne *et al*. (2000)**. For the strains M3, M52 and M56, identification result of API ID 32C strips were given as *C. insconspicua* or *C. norvegensis*. They were identified as *C. norvegensis* because of their ability to ferment glucose, as described by **Meyer *et al*. (2000)** and **Barnett *et al*. (2000)**. Thirty isolates which were identified as *C. famata*, were in the variety of *C. famata* var. *famata* according to **Meyer *et al*. (2000)**, since they could not grow at 37ºC. The profile of the isolates M6 and M15 were defined as *Geotrichum* spp. by the API ID 32C strips. They were further identified as *G. candidum* by using

the test of growth on the media without vitamin as recommended in the identification keys of **Payne *et al.* (2000)**. By using the ascospor formation tests, the isolate M54 which was identified as *K. ohmeri* by the strips, was determined to be its anamorph *Candida guilliermondii* var. *membranaefaciens*. Similarly, the isolate M8 was identified as *Candida robusta*, the anamorph form of *S. cerevisiae*.
The final identification results were given in Table 2. By using complementary tests in addition to API ID 32C strips, 56 (78%) of the isolates could be identified at species level, and one isolate at genus level as "*Candida*". It was determined that *C. famata* var. *famata* (30) was the dominant species in Mihalic cheese. *C. cylindracea* (4), *C. paludigena* (4), *C. tropicalis* (3), *C. norvegensis* (3), *C. krusei* (2), *C. zeylanoides* (2), *G. candidum* (2), *C. bertae* (2), *C. catenulata* (1), *C. guilliermondii* var. *membranaefaciens* (1), *C. robusta* (1) and *T. asahii* (1) were also among the detected species in the microflora of Mihalic cheese.

Table 2 The species and number of identified yeast isolates

Yeast species	Number of isolates
Candida bertae	2
Candida catenulata	1
Candida cylindracea	4
Candida famata var *famata*	30
Candida guilliermondii var *membranefaciens*	1
Candida krusei	2
Candida norvegensis	3
Candida paludigena	4
Candida robusta	1
Candida tropicalis	3
Candida zeylanoides	2
Candida sp.	1
Geotrichum candidum	2
Trichosporon asahii	1
Not identified	15

Results of enzymatic characterization

The results obtained by API-ZYM strips were given in Table 3. It was found that none of the isolates had α-galactosidase, β-glucuronidase, N-acetyl-β-glucoaminidase, α-mannosidase and α-fucosidase activities. Fifty-percent of the strains had β-galactosidase activity. The highest activity of this enzyme was observed for *C. paludigena* M32. Only two strains; *Trich. asahii* M1 and *C. tropicalis* M2 had β-glucosidase activity. Seventeen (55%) of the isolates showed α-glucosidase activity. Alkaline phosphatase activity was detected for most of the isolates and all of the *C. famata* var. *famata* strains. All of the isolates except two *C. norvegensis* strains, showed acid phosphatase activity. The level of acid phosphatase activity was high (5) for *Trich. asahii* M1, *C. tropicalis* M2, *C. krusei* M57, *C. guilliermondii* var. *membranefaciens* M54, *G. candidum* M15 and *C. zeylanoides* M16 (Table 3). All of the isolates had esterase (C4) and esterase lipase (C8) activities. However, only *C. tropicalis* M2, *C. famata* var. *famata* M22 and *G. candidum* M15 were found to have lipase (C14) activity.
The enzyme leucine arylamidase was detected in all of the isolates, while valine and cysteine arylamidases were found only in a few isolates at very low levels. Among proteases, trypsin and chymotrypsin, which operate most effectively in alcaline pH values, were not detected in most of the isolates of Mihalic cheese, an acidic product. Figure 1 represents the percentage of the yeast strains with certain enzyme activities detected by API-ZYM. The enzymes esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphtol-AS-BI-phosphohydrolise and also alkaline phosphatase were common among the isolates of Mihalic cheese (Table 3).

Table 3 Enzyme activities of the yeast isolates

Isolate no.	Yeast species	Control	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
M1	*T. asahii*	0	3	2	2	0	3	0	0	0	0	5	5	0	0	0	0	2	0	0	0
M2	*C. tropicalis*	0	0	1	2	1	4	1	1	0	0	5	2	0	0	0	4	2	0	0	0
M43	*C. tropicalis*	0	0	2	1	0	3	1	0	0	0	4	2	0	0	0	3	0	0	0	0
M25	*C. catenulata*	0	2	3	2	0	4	1	1	0	0	2	2	0	0	0	0	0	0	0	0
M3	*C. norvegensis*	0	0	1	2	0	3	0	2	0	0	0	1	0	0	0	0	0	0	0	0
M56	*C. norvegensis*	0	0	2	2	0	3	1	2	0	0	0	2	0	0	0	0	0	0	0	0
M57	*C. krusei*	0	0	1	2	0	3	1	2	0	0	5	3	0	0	0	0	0	0	0	0
M54	*C. guilliermondii* var. *membranefaciens*	0	1	2	1	0	5	0	0	0	0	5	1	0	0	0	3	0	0	0	0
M18	*C. famata* var. *famata*	0	3	3	2	0	2	0	1	0	1	3	2	0	2	0	2	0	0	0	0
M91	*C. famata* var. *famata*	0	2	2	2	0	2	0	0	0	0	2	4	0	3	0	1	0	0	0	0
M21	*C. famata* var. *famata*	0	2	2	1	0	3	0	0	0	0	3	1	0	2	0	1	0	0	0	0
M89	*C. famata* var. *famata*	0	2	2	2	0	3	0	1	0	0	3	3	0	1	0	1	0	0	0	0
M22	*C. famata* var. *famata*	0	3	3	2	1	3	1	1	1	1	3	2	0	2	0	2	0	0	0	0
M70	*C. famata* var. *famata*	0	3	2	1	0	2	0	1	0	0	3	1	0	2	0	1	0	0	0	0
M45	*C. famata* var. *famata*	0	1	2	1	0	2	0	0	0	0	1	1	0	3	0	2	0	0	0	0
M12	*C. famata* var. *famata*	0	2	2	1	0	2	0	0	0	0	3	2	0	2	0	1	0	0	0	0
M81	*C. famata* var. *famata*	0	3	1	2	0	2	1	0	0	0	4	2	0	0	0	0	0	0	0	0
M4	*C. famata* var. *famata*	0	1	2	1	0	2	0	0	0	0	1	1	0	2	0	1	0	0	0	0
M6	*G. candidum*	0	0	1	2	0	3	1	1	0	0	4	1	0	0	0	0	0	0	0	0
M15	*G. candidum*	0	1	2	3	2	1	1	0	1	0	5	1	0	0	0	0	0	0	0	0
M8	*C. robusta*	0	1	2	1	0	2	1	0	0	0	2	1	0	3	0	2	0	0	0	0
M16	*C. zeylanoides*	0	0	2	2	0	2	0	0	0	0	5	1	0	0	0	0	0	0	0	0
M76	*C. zeylanoides*	0	0	1	2	0	1	0	0	0	0	3	2	0	0	0	0	0	0	0	0
M73	*C. cylindracea*	0	1	3	2	0	4	1	1	0	0	1	1	0	0	0	0	0	0	0	0
M77	*C. cylindracea*	0	2	2	2	0	5	1	1	0	0	2	2	0	0	0	0	0	0	0	0
M83	*C. cylindracea*	0	2	2	1	0	1	0	0	0	0	3	1	0	3	0	1	0	0	0	0
M79	*C. paludigena*	0	1	2	1	0	2	0	0	0	0	2	2	0	3	0	0	0	0	0	0
M32	*C. paludigena*	0	2	2	1	0	2	0	0	0	0	3	1	0	4	0	1	0	0	0	0
M63	*C. bertae*	0	1	2	1	0	2	0	0	0	0	3	1	0	3	0	1	0	0	0	0
M53	*C. bertae*	0	3	3	2	0	2	0	0	0	0	3	1	0	3	0	1	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

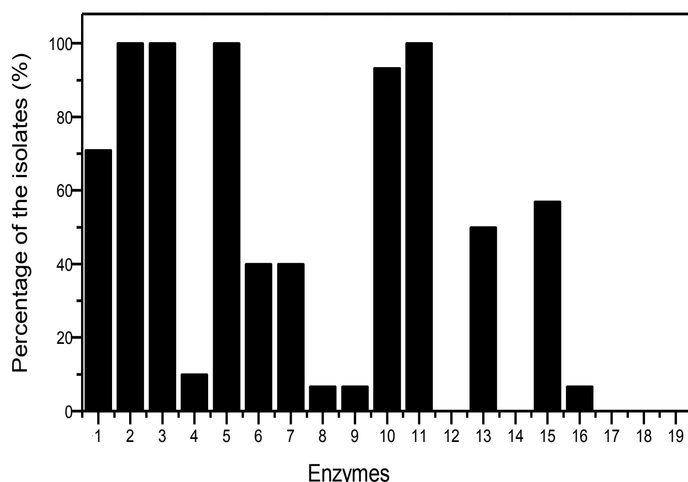

Figure 1 Percentage of the yeast isolates (%) having the certain enzyme activity 1: Alkaline phosphatase, 2: Esterase (C4), 3: Esterase lipase (C8), 4: Lipase (C14), 5: Leucine arylamidase, 6: Valine arylamidase, 7: Cystine arylamidase, 8: Trypsin, 9: α-chymotrypsin, 10: Acid phosphatase, 11: Naphtol-AS-BI-phosphohydrolise, 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

DISCUSSION

C. famata var. *famata* was determined as the dominant yeast in Mihalic cheese. Although the prevalence of different yeast species depends on the type of the cheese considered, *D. hansenii* (anamorph: *C. famata*) was reported to be the most common species found in almost all types of cheeses, especially in most Mediterranian ewes' and goats' cheeses. This was attributed to its ability to grow in the presence of salt at low temperature and to metabolize lactic and citric acids **(Capece & Romano, 2009; Padilla *et al*., 2014b).** *D. hansenii* was also an important component in the microflora of young white pickled cheeses of Serbia **(Golić *et al*., 2013)**, Pecorino di Filiano cheese **(Capece & Romano, 2009)**, Pecorino Crotonese cheese **(Gardini *et al*., 2006)**, Spanish blue-veined Cabrales cheese **(Alvarez-Martin *et al*., 2007)**, Gorgonzola-style and Danish-style blue-veined cheeses **(Viljoen *et al*., 2003)** and Danish surface-ripened cheeses **(Petersen *et al*., 2002)**. *D. hansenii* was defined as a "salt loving" yeast by **Prista & Louerio-Dias (2007)** and this characteristic of the yeast was explained by the capability of membrane potassium carriers to transport potassium into the cells, even in the presence of high concentrations of sodium. It was not unexpected for *D. hansenii* to dominate in the microflora of Mihalic cheese, which is known as a salty local cheese in Turkey.

API-ZYM system was used for detecting general enzyme profiles of the yeast strains. The particular enzymes which are important for cheese ripening are β-galactosidase, α-glucosidase, β-glucosidase, acid and alkali phosphatases, esterase, lipase, trypsin, α-chymotrypsin and arylamidases. According to the enzymatic profiles of the yeasts, the presence of β-galactosidase, α-glucosidase and β-glucosidase suggests that the yeast strains isolated from Mihalic cheese prefer glucose and lactose other than mannose, fructose and glucuronides as carbon and energy sources **(Heperkan *et al*., 2014; Zeng *et al*., 2014)**. Acid and alkali phosphatases are given among the important enzymes for cheese ripening. It is known that although both acid and alkaline phosphatases are present in cheese, acid phosphatases are more active due to their relatively low optimum pH **(Magboul & McSweeney, 1999)**. Acid phosphatase has a greater thermal stability and it is most active at pH values typical of cheese ripening **(Chavarri *et al*., 1998)**. It has been suggested that phosphatase activity could influence cheese flavor because of its effect on proteolysis. Acid phosphatase, acting synergistically with proteolytic enzymes, had been shown to hydrolyze casein molecule **(Akuzawa & Fox, 2004)**. Phosphopeptides in milk have been reported to be resistant to proteolytic attack, and the combined action of acid phosphatases and proteolytic enzymes in cheese was thus required for extensive production of small peptides and free aminoacids **(Chavarri *et al*., 1998; Akuzawa & Fox, 2004)**. It was reported that the high acid phosphatase activity of microorganisms might be useful in metabolizing phosphates and contributing to flavor formation in acidic external environment prevalent in cheese maturation **(Akuzawa & Fox, 2004; Georgieva *et al*., 2009)**.

The strains having esterase and lipase activities are known to have potential to involve in the liberation of free fatty acids during cheese ripening **(Zeng *et al*., 2014)**. It was estimated that yeast strains having high esterase, esterase lipase or lipase activities might contribute to lipolysis in cheese ripening. Lipolysis plays an important role in cheese ripening, and a large number of studies dealing with the acceleration of lipolysis have been published **(Kheadr *et al.*, 2002; El Galiou *et al*., 2013)**. The free fatty acids released during lipolysis contribute, together with the volatile compounds and the proteolysis products, directly to cheese flavor **(El Galiou *et al*., 2013)**. The presence of microorganisms with high aminopeptidase activity was reported as advantageous for cheese ripening and flavor development **(Georgieva *et al*., 2009)**. Arylamidases (aminopeptidases) catalyze the hydrolysis of N-terminal aminoacids from peptide, amide or arylamides **(Dodor & Tabatabai, 2007)**. According to **Zeng *et al.* (2014)**, aldehydes, alcohols and acids, which have very low treshold values, usually originate from the degradation of leucine, valine, phenylalanine and methionine. Aminopeptidases were also reported to have a debittering effect during cheese ripening **(Herreros *et al.,* 2003)**. It was also reported that flavor development during ripening favor the detection of yeast strains with aminopeptidase activity **(Zeng *et al*., 2014)**. The absence of proteases (trypsin and chymotrypsin) and the high activities of peptidases and phosphatases among the strains tested were reported as desireable traits for flavor and texture development in milk fermentations **(Mathara *et al*., 2004; Thapa *et al*., 2006)**. It was also reported that starters with low proteinase and strong peptidase activities were useful in reducing bitterness and improving body and texture defects **(Mathara *et al*., 2004)**. **Zeng *et al.* (2014)** reported that the enzymatic potential of microorganisms can effectively reproduce the characteristic flavour of fermented food rapidly, leading to more diverse flavours. Therefore, enzyme profiling can be used to select suitable strains as starter cultures.

When enzymatic activity results were evaluated in the present study, some yeast strains were found to stand out with their multiple enzyme activities. For selection of the superior strains, both enzyme activities and quantities should be taken into account. It is thought that the premier yeasts having particular enzymes important for cheese ripening could be subject of further investigations for their adjunct starter potential, which could be demonstrated by other technological properties of yeasts in addition to their enzymatic activities. The strain *C. famata* var. *famata* M22 had most of the enzymes (esterase, esterase lipase, lipase, leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, □-chymotrypsin, acid phosphatase, □-galactosidase and □-glucosidase) which may have important attributes during cheese ripening. However, enzyme activities of this strain were generally low. Although low levels of proteases are recommended in some studies, other enzymes such as acid phosphatase and arylamidases may have more critical roles during ripening. Strains lack of proteases, but having high acid phosphatase and arylamidase activities could be also suggested to be potential adjunct starters for further studies. Thus, *C. guilliermondii* var. *membranefaciens* M54 with very high (level 5) acid phosphatase and leucine arylamidase activities could be also selected. Besides, *C. tropicalis* M2 may also be superior because of having all three arylamidases in addition to its high acid phosphatase activity.

CONCLUSION

This study demonstrated the yeast flora unique to Mihalic cheese which is one of the most important traditional cheeses of Turkey and also attracted attention in recent years as slow food. Identification and enzymatic characterization of the yeasts originated from Mihalic cheese was performed for the first time in this study. *C. famata* var. *famata* was determined as the dominant yeast. The yeast isolates had variable enzyme activities including acid phosphatase, esterase, esterase lipase, lipase, β-galactosidase, leucine arylamidase, valine arylamidase and cysteine arylamidase, which could have important attributes during cheese ripening. Enzymatic profiles of the yeast isolates revealed some of their technological properties. Effects of these strains on cheese quality as adjunct starters and change of their enzyme activities during ripening could be investigated in further studies.

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