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Research Article

Isolation, Identification, and Characterization of the Native Yeast Strains from Homemade Cheese to Assess their Eliminating Impact on the Aflatoxin B1 and M1 of the Simulated Gastrointestinal Fluid

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Background: The occurrence of aflatoxins in food products is a silent threat to human health worldwide. A range of strategies has been introduced to address the bioavailability of aflatoxins, which are considered microbial tools to provide a low-cost and promising approach.

Objectives: The present study focused on the separation of yeast strains from the homemade cheese rind layer to investigate the ability of native yeasts to eliminate AB1 and AM1 from simulated gastrointestinal fluids.

Material and Methods: Homemade cheese samples were prepared from different locations in Tehran provinces and yeast strains were isolated and identified through the biochemical methods and molecular analysis of internal transcribed spacer and D1/D2 domain of 26S rDNA regions. Isolated strains were screened using simulated gastrointestinal fluids, and the ability of yeast strains to absorb aflatoxin was evaluated.

Results: Out of 13 strains, 7 yeast strains were not affected by 5 ppm AFM1 while 11 strains did not show any significant response to 5 $mg.L^{-1}$ (ppm) of AFB1. On the other hand, 5 strains were able to successfully tolerate 20 ppm AFB1. Candidate yeasts showed different abilities to remove aflatoxins B1 and M1. In addition, *C. lusitaniae, G. geotrichum, G. candidum,* and *C. sanyaensis* exhibited a significant ability to detoxify aflatoxins from the gastrointestinal fluid, respectively.

Conclusion: Our data suggest that yeast communities with essential effects on the quality of homemade cheese appear to be precise candidates for the potential elimination of aflatoxins from the gastrointestinal fluid.

Keywords: AB1, Aflatoxin elimination, AM1, Gastrointestinal fluid, Homemade cheese, Yeast

1. Background

Aflatoxins belong to a group of polyketide-derived furanocoumarins that can contaminate a wide range of food products (1). There are many determinants and effectors such as meteorological and environmental factors, micro and macro, which cause the release of aflatoxin-producing fungi (2). AFB1 and AFM1 are classified as human carcinogenic secondary metabolites due to the formation of DNA adducts (3). The carcinogenicity and immunosuppression capacity of aflatoxin B1 (AFB1) causes a broad spectrum of disorders in livestock and human health worldwide. Due to the hydroxylation of AFB1, milk products are mainly contaminated by AFM1 and therefore milk

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plays an important role in transferring aflatoxins residing in human food (4). The health of more than 4.5 billion people is threatened by foodstuffs contaminated with various levels of aflatoxins (5, 6). Previous studies have shown that the concentration of AFM1 in cheese can be up to 1.9 fold higher than the milk used to produce it (7, 8).

The ripening and maturation process of fresh cheese reduced AFM1 concentration, which was inversely correlated with plate counts of lactic acid bacteria (9). *Lactobacillus sp*. plays a key role in the process of turning milk into yogurt by significantly reducing the level of AFM1 compared to its initial value in milk (10). Among the methods used to reduce the bioavailability of aflatoxins, microbial tools are a low-cost method and a promising strategy to eliminate these metabolites from food and feeds (11).

As mentioned earlier, the detoxification ability of yeast cells has been attributed to their cell wall composition (16-17). Some yeast strains such as *Pichia kudriavzevii* and *Lachancea thermotolerans* were able to act as biocontrol agents against aflatoxin-producing fungi (18) and others such as *Saccharomyces cerevisiae* could detoxify AFB1 (19).

In the present study, homemade cheese samples were collected from four different geographical areas of Tehran province. These regions have a long experience in producing homemade cheese from ewe milk called Liqvan cheese. Homemade cheeses are generally made from unpasteurized milk, which is affected by the natural microbiota of milk and the production environment, which plays an important role in preserving taste, aroma, and texture. After lactic acid bacteria, yeast species are the predominant microbiota in dairy products (20).

2. Objective

Therefore, this study focused on the natural yeast populations of cheese samples to find some isolates with relatively strong AFB1 and AFM1 detoxification capabilities.

3. Materials and Methods

3.1. Sample Collection

Homemade cheese samples were randomly prepared from four different geographical regions of Tehran province, including Shahriar, Garmsar, Firoozkooh, and Chetan cities, whose geographical characteristics are presented in **Table 1**. From each sampling location, eight pieces of 20- 25 days ripened cheese at 4 °C were sampled by scraping the rind surface with a sterile blade and the samples from each region were pooled and kept at a cool place and immediately transferred to the lab. The total fresh weight of the pooled samples was about 200g.

3.2. Yeast Isolation

Yeast isolation was performed based on Yalcin and Ucar's studies with some modifications (21). In summary, homemade cheese samples (3 g) were placed in 20 mL of peptone water (0.1%) medium and homogenized. Then decimal dilutions were prepared and cultured in plates containing Chloramphenicol Yeast Glucose Agar (CYG) (20 $g.L^{-1}$ dextroses, 5.0 $g.L^{-1}$ yeast extract, 0.1 $g.L^{-1}$ chloramphenicol, 14.9

Table 1. Climate and geographical features of cheeses sampling sites. These are selected from different climatic and geographic regions to probably show the effect of regional parameters on yeast populations and characteristics.

Location	Rainfall (mm/year)	Geographical profile		Climate	Altitude (m)	Relative humidity (%)
		N	E			
Firoozkooh	264.00	35°45'17.95"	52°46'20.48"	Very cold and semi-humid	1975.5	67.00
Garmsar	71.32	35°13'54.30"	52°20'29.09"	Warm and dry	856.0	30.12
Chetan	450.00	36°20'44.73"	51°28'3.75"	Mid weather and humid	1500.0	79.00
Shahriar	279.10	35°40'14.44"	51° 1'21.93"	Cold and semi-dry	1140.0	59.91

g.L^{-1} agar, pH: 6.9 ± 0.2) (Sigma). The plates were then incubated at 30 °C for five days. The growth of microorganisms was monitored every 24 hours. Colonies with distinct morphologies were selected and purified on CYG medium by streaking method. Finally, the purified strains were kept at 4 °C for further investigation.

3.3. Polymerase Chain Reaction

The purified strain's DNA extraction was processed through the Higher Purity Yeast Genomic DNA Extraction kit (Canvax) according to manufacturer instructions. To distinguish yeasts from other isolates, ITS1-5.8S rDNA-ITS4 (internal transcribed spacer) region was amplified (21). Primers for the amplification were ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC). The DNA was amplified in a 25 µL reaction mixture containing 5 ng extracted DNA, 1.5 U Taq DNA polymerase, 0.5 µM each of primers, 1.6 mM dNTPs, 20 mM Tris-HCl (pH 8.8), 1.5 mM $MgCl_2$, and 50 mM KCl. The PCR conditions were as follows: initial denaturation at 95 °C for seven min, followed by 35 cycles of denaturation at 94 °C for 30 min, 54 °C for one min, extension at 72 °C for two min, and final stage at 72 °C for five min in a thermal cycler (Eppendorf). The DNA fragments were separated by 1.5% agarose gel electrophoresis staining with erythrogel and visualized with a transilluminator.

3.4. Amplification of D1/D2 Domain of 26S rDNA Region

Identification of yeast isolates was carried out by amplifying and analyzing the sequences of the D1/D2 region of the 26S rDNA encoding gene using the primers NL1 (GCATATCAAT AAGCGGAGGAAAAG) and NL4 (GGTCCGT GTTTCAAGACGG). The amplification mixture was prepared as abovementioned and the amplification condition was as follows: initial denaturation at 94 °C for 2 min, followed by 40 cycles of denaturation at 95 °C for 45 s, annealing at 55 °C for 45 s, extension 72 °C for 35 s, and a final stage of extension 72 °C for 10 min (22). Amplified DNA fragments were purified using a QIAEXII Gel extraction kit (QIAGEN), and their nucleic acid sequence was determined at the DNA Sequencing Service of Pishgam Bio-Tech (Tehran, Iran). The DNA sequences were checked through the BLASTN program of The National Center for Biotechnology database (www.ncbi.nlm.nih.gov/BLAST) and were analyzed using MEGA 7 software (23).

3.5. Preparation of Aflatoxin Stock Solution

AFB1 and AFM1 standard solutions (10 µg.mL^{-1}) were purchased from Faroogh Life Sciences Research Laboratory, Iran. To prepare the stock solution, the standards were dissolved in benzene: acetonitrile (98:2 v/v) (24-25). The stock solution was stored in an amber glass at 4 °C until use.

3.6. Screening of Yeast Isolates

The medium used in shake flask assays was CYG broth. Fifty mL of the medium-plus 5, 10, and 20 ppm aflatoxin B1 and 5 ppm aflatoxin M1 were added to 250 mL Erlenmeyer flasks. The experiment was performed in five replications. In each flask, 10^{12} cells of each yeast strain were inoculated and immediately incubated at 37 °C and shaken at 120 rpm. Chloramphenicol Yeast Glucose Agar was used for the isolation and enumeration of yeast cells.

3.7. Preparation of Digestive Fluids

Simulated stomach fluid containing 1.2 g.L^{-1} sodium bicarbonate, 2.2 g.L^{-1} potassium chloride, 6.2 g.L^{-1} sodium chloride, 0.22 g.L^{-1} calcium chloride and 0.3% pepsin and intestinal fluid containing 0.45% bile salt, 5 g.L^{-1} sodium chloride, 0.3 g.L^{-1} calcium chloride, 0.6 g.L^{-1} potassium chloride and 0.1% pancreatin were prepared and sterilized by filtration of 0.2 µm. Stomach and intestinal fluid pH was adjusted to 2.5 and 7.5 using hydrochloric acid 0.1 N and 0.1 N sodium hydroxide solutions (Sigma), respectively.

3.8. Aflatoxin Removal Assessment

Four candidate yeast strains were cultured separately in fluids prepared to simulate gastric conditions. In the first step, 1×10^{10} yeast cells were added to 10 mL of simulated stomach fluid contaminated with 5 ppm AFB1 or AFM1, vortexed for 15 s, and incubated for two hours at 37 °C. Next, one milliliter of the resulting fluid was added to nine mL of simulated intestinal fluid, vortexed, and incubated for two hours at 37 °C. Finally, each sample was centrifuged at 12000 g for 15 min and the amount of aflatoxin remaining in the supernatant solution was determined. The result was considered to calculate the ability of yeast strains to absorb aflatoxin compared to the amount of aflatoxin in the control solution.

Figure 1. Homemade cheeses sampling sites around Tehran province in Iran. The circles were indicated 1: Shahriar, 2: Chetan, 3: Garmsar, 4: Firoozkooh and star shape were showed the center of Tehran province.

3.9. Quantification of Aflatoxins
ELISA kits AFB1 and AFM1 (R-Biopharm; Darmstadt, Germany) were used to detect the amount of AFB1 and AFM1 content of solutions as previously described (19). Quantitative analysis was performed by competitive enzymatic immunoassay. To determine the residual aflatoxin, different concentrations (0, 1, 5, 10, 20, and 50 ng.mL^{-1}) of standard AFB1 and AFM1 solutions were prepared. Aflatoxin standards and samples were then repeatedly added to microtiter plate wells precoated with antibodies against AFB1 and AFM1 and incubated at 25 °C in the dark for half an hour. Then 50 µL of anti-aflatoxin antibodies and enzymatically conjugated solution were added to the wells and incubated at 25 °C for one hour in a dark place. The washing step removed the unbound conjugate and then 50 µL of each substrate and chromogen were added to the wells. The plates were incubated in the dark at 25 °C for 15 minutes and finally, the process was stopped by adding 100 µL of stop solution, and the absorbance was measured at 450 nm on an ELISA plate reader (Bio Tek ELx800, USA).

3.10. Statistical Analysis
The distance matrices of the identified yeast isolates were performed using the UPGMA method (Unweighted Pair Group Method with Arithmetic mean) available at http://genomes.urv.es/UPGMA (26). Dendrograms were visualized with MEGA7 software. Data analysis was performed using a Statistical Package for Social Sciences (SPSS) version 18.0. Comparison of multiple means and analysis of variance between all samples was performed using one-way analysis of variance (ANOVA) and Fisher's least significant difference (LSD) test, respectively. Quantitative data were expressed as mean ± standard error of the mean (SEM) of four independent tests. All experiments were performed in four replicates unless otherwise stated.

4. Results

4.1. Climate and Geographical Features of Sampling Sites
As shown in **Table 1**, Firoozkooh is the highest in terms of altitude and Garmsar is placed in the lowest region. Garmsar is a representative of the semi-desert regions of Iran and Chetan has a mid-weather and humid climate. Shahriar is a region with an approximate average of geographical features among the sampling sites studied. As shown in **Figure 1**, all sampling sites are in the northern region of Iran, therefore it is expected that the significant difference in the climatic feature of the sampling sites is due to topographic cues.

4.2. Yeast Isolation, Identification, and Characterization
Cheese layers were collected, and yeast strains were isolated using CYG media. In total, 13 isolates were obtained from 4 different cheese samples. The sequences D1/D2 and ITS domains of 26S rDNA gene were analyzed for identification of the isolated yeasts as described by Knutsen *et al* (28) (**Fig. 2A**). Our results

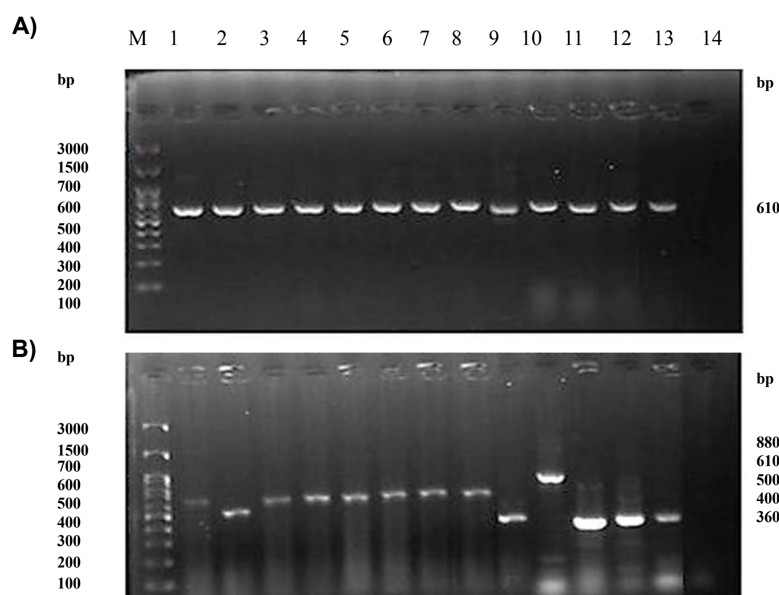

Figure 2. Analysis of D1/D2 and ITS domains of 26S rDNA gene of the isolated yeasts. A) Amplification of D1/D2 domain of 26S rDNA region. Lane M: DNA ladder (100 bp). Lines 1 to 13 presented different yeast strains (610 bp). Line 14: negative control. **B**) PCR products of the ITS regions of different yeast isolates. Lane M: DNA ladder (100 bp). Lines 1, 6, 7, and 8: *Debaryomyces hansenii* strains. Line 2: *Candida sanyaensis*. Lines 3, 4, and 5: *Candida zeylanoides* strains. Line 9: *Calvispora lusitaniae*. Line 10: *Saccharomyces cerevisiae*. Lines 11 and 13: *Galactomyces geotrichum* strains. Line 12: *Geotrichum candidum*. Lane 14: negative control.

showed that D1/D2-PCR resulted in a single fragment of 610 bp, which means that all isolates are yeast (22). ITS region sequencing has been used repeatedly to isolate and identify yeasts in dairy products, wine, and other foods (29) (30) (31). Electrophoresis of ITS region PCR products provided different visual bands from 360 to 880 bp. The largest DNA fragment was produced by isolating *Saccharomyces cerevisiae* while the smallest fragment produced by isolating *Galactomyces geotrichum* and *Geotrichum candidum*. Based on the presented results, *Candida sanyaensis* and *Calvispora lusitaniae* isolates produced 500 and 400 bp fragments and *Debaryomyces hansenii* isolates produced 610 bp fragments in length (**Fig. 2B**).

The D1/D2 sequence of each isolate was analyzed and compared with the homolog sequences in the NCBI databases. Sequence alignment of yeast strains showed that twelve yeast strains had a homology score higher than 95% (**Table 2**). The identified yeast isolates belonged to 6 and 7 different genera and species, respectively.

To determine the evolutionary position of yeast strains, all sequences were used to construct a phylogenetic tree according to the UPGMA method. As shown in **Figure 3**, 3 clade clusters were formed with different members. The biggest cluster consisted of 9 yeast strains from 3 genera. 3 strains of Debaryomyces hansenii along with 2 out of 3 strains of Candida zeylanoides were classified in the same group. On the other hand, 2 strains of Galactomyces geotrichum were placed in the same class. Candida sanyaensis, Saccharomyces cerevisiae, and Geotrichum candidum made a cluster, and Candida zeylanoides and Calvispora lusitaniae made a distinct class of phylogeny. According to the phylogenetic tree, the earliest ancestral roots of the yeast strains had confidence levels greater than 99% which means there were high-confidence evolutionary relationships in the same clade cluster.

There were two strains, *D. hansenii* and *C. zeylanoides*, which showed a higher occurrence in homemade cheese samples (**Table 3**). Yeast strain, *D. hansenii* was absent in the Garmsar cheese samples while *C. zeylanoides* did not find in the Firoozkooh samples. The relative optimum temperature was 28 ^{o}C for yeast strains while the optimum pH showed a big variation among strains.

Table 2. Identification of yeast strains based on homology analysis of D1/D2 domain sequence.

Lane No.	Yeast code	Alignment results			GenBank information	
		Recommended strain	Accession No.	Homology (%)	Accession No.	Strain Name
1	FI1	*D. hansenii*	KY512397.1	97.60	OK184810.1	SBU1
2	FI2	*C. sanyaensis*	MK110136.1	98.45	OK184817.1	SBU5
6	CI1	*D. hansenii*	KY512103.1	99.83	OK184811.1	SBU2
12	CI2	*G. candidum*	MT151654.1	95.49	OK184515.1	SBU11
7	CI3	*D. hansenii*	MK358164.1	99.65	OK184512.1	SBU3
10	CI4	*S. cerevisiae*	HM101472.1	99.32	OK184514.1	SBU10
11	CI5	*G. geotrichum*	JF262195.1	98.34	OK184516.1	SBU12
5	CI6	*C. zeylanoides*	MF462796.1	98.92	OK184519.1	SBU6
9	GI1	*C. lusitaniae*	KY106929.1	91.90	OK184518.1	SBU9
4	GI2	*C. zeylanoides*	MF462810.1	99.65	OK184520.1	SBU7
8	SI1	*D. hansenii*	KY512185.1	99.83	OK184513.1	SBU4
13	SI2	*G. geotrichum*	JF262196.1	99.28	OK184522.1	SBU13
3	SI3	*C. zeylanoides*	MT422087.1	98.67	OK184521.1	SBU8

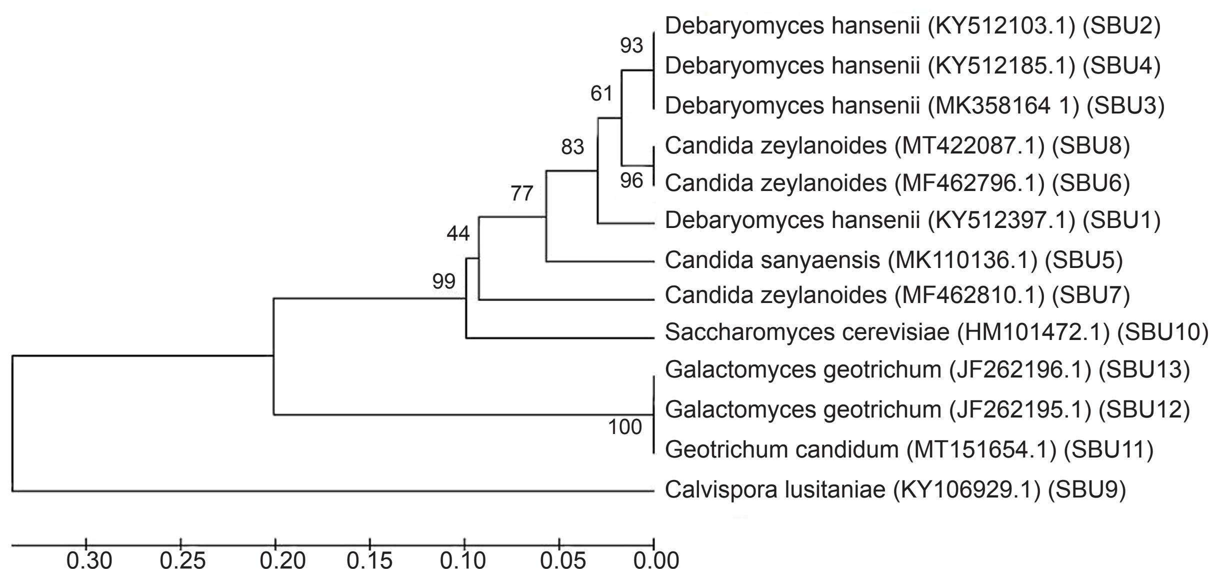

Figure 3. **The estimation of phylogenetic relatedness among 13 different yeast isolates was determined using UPGMA statistical method with 1000 bootstrap replications.** The substitution model was a maximum composite likelihood. Numbers are bootstrap values as confidence level.

The optimum amount of pH and temperature did not demonstrate any correlation with sampling locations.

4.3. Yeast Strains' Responsiveness to Aflatoxins

Of the 13 strains, 5 strains were able to successfully tolerate 20 ppm AFB1. On the other hand, 7 strains of yeast had no effect on 5 ppm AFM1 while 11 strains did not show any significant response to 5 ppm AFB1. As shown in **Table 4**, aflatoxin M1 strongly inhibits yeast growth relative to aflatoxin B1. Four strains of D. *hansenii* illustrated large differences in response to AFM1 while all of them were strongly

Table 3. Optimization of media pH and Temperature for yeast strains

Sampling site	Location code	Optical Density at different temperature and pH levels									CFU (mL)
		°C at optimum pH				pH at 28 °C					
		20	25	28	37	3.5	4.5	5.5	6.0	6.5	
Firoozkooh	FI1	0.38	0.54	0.62	0.42	0.38	0.45	0.35	0.34	0.35	7.8(10^7)
	FI2	0.15	0.36	0.21	0.23	0.15	0.61	0.31	0.40	0.23	3.7(10^7)
Chetan	CI1	0.50	0.88	0.92	0.91	0.50	0.41	0.40	0.50	0.71	4(10^7)
	CI2	0.51	0.97	0.91	0.82	0.51	0.35	0.40	0.56	0.61	4(10^9)
	CI3	0.29	1.00	1.01	1.07	0.29	0.22	0.22	0.21	0.24	4.9(10^6)
	CI4	0.50	1.00	1.01	1.05	0.50	0.44	0.26	0.27	0.26	9.4(10^8)
	CI5	0.50	1.07	1.05	1.17	0.50	0.38	0.35	0.53	0.53	5.3(10^8)
	CI6	0.57	1.03	0.87	1.03	0.57	0.46	0.52	0.57	0.63	8.3(10^5)
Garmsar	GI1	0.20	0.38	0.44	0.25	0.20	0.29	0.23	0.25	0.27	3.8(10^8)
	GI2	0.29	0.89	0.86	0.86	0.29	0.49	0.42	0.57	0.33	3.6(10^9)
Shahriar	SI1	0.72	0.35	0.46	0.31	0.52	0.68	0.64	0.76	0.74	3.9(10^9)
	SI2	0.76	0.86	0.95	1.01	0.76	0.83	0.61	0.76	0.77	5.7(10^8)
	SI3	0.64	1.02	1.00	0.93	0.64	0.76	0.63	0.71	0.72	3.1(10^{10})
	SI4	0.44	0.74	0.66	0.52	0.38	0.45	0.35	0.34	0.35	9.8(10^9)

captured by AFTB1 at 20 ppm. Unlike D. *hansenii*, the C. *zeylanoides* strain did not follow the path of D. *hansenii*. To evaluate the ability of aflatoxin removal of candidate yeasts in simulated gastrointestinal conditions, 3 indicators were considered. First, the highest mean value and the lowest standard deviation were calculated with the growth rate under aflatoxin treatments (**Table 4**) and then the candidate species should not be repeated among other candidates.

Candidate yeast strains exhibited different removal abilities for aflatoxins B1 and M1 (**Fig. 4**). Despite the yeast growth response to AFB1 (**Table 4**), there was a significant difference among all candidate strains in their ability in simulated gastrointestinal conditions. C. *lusitaniae* and C. *sanyaensis* strains illustrated the lowest and highest removal ability, respectively. In addition, the removal ability of C. *lusitaniae*, G. *geotrichum* and G. *candidum* was remarkable against AFB1. On the other hand, the reaction of the yeast strains to AFM1 was completely different and C. *sanyaensis* and C. *lusitaniae* showed the highest inhibitory potential against AFM1. Furthermore, G. *candidum* showed a noteworthy performance in removing AFM1 from the gastrointestinal fluid.

5. Discussion

Climatic and geographical parameters are among the main natural driving forces that can create the pressure of effective selection for the formation of native microbial communities in each region. Cheese production is adapted to different environmental conditions (27) and as a result, microbial communities of cheese are strongly influenced by climatic conditions and geographical features. In this study, homemade cheese samples were collected from different places that have experienced different climatic and geographical conditions. As reported, the highest prevalence of the yeast species on the cheese rind was belong to *Debaryomyces hansenii* and *Geotrichum candidum* (32) and also *Penicillium roqueforti* and *Debaryomyces hansenii* (33). As expected from previous studies, the evaluation of yeast diversity of the homemade cheeses along with different ecological behaviors of yeast isolates from the same species strongly highlighted the influence of the local manufacturing process on the yeast diversity and ecology of the cheeses (34) (35) (20).

According to previous studies on aflatoxin contamination in Iranian dairy products (36) (37) (38), cheese is one of the main products with the highest rate of

Table 4. Growth response of yeast strains to different concentrations of AFB1 (5, 10, and 20 ppm) and AFM1 (5 ppm) in comparison with control conditions during two-time points (24 and 48 hours). Growth of yeast under control conditions was considered 100 percent and according to that growth under aflatoxin, treatments were calculated. As Fisher's least significant difference procedure, the smallest significant difference between the two means was 23.5.

Location code	Strain Name	AFB1 (ppm)						AFM1 (ppm)		Ave.	Std.
		5		10		20		5			
		24	48	24	48	24	48	24	48		
FI1	*D. hansenii*	100	100	100	100	50	63	50	46	76	26
FI2	***C. sanyaensis***	**93**	**97**	**123**	**114**	**117**	**100**	**65**	**67**	**97**	**22**
CI1	*D. hansenii*	0	71	0	100	20	44	100	127	58	49
CI2	***G. candidum***	**94**	**111**	**175**	**100**	**103**	**111**	**100**	**125**	**115**	**26**
CI3	*D. hansenii*	0	71	0	125	20	33	136	106	61	56
CI4	*S. cerevisiae*	100	107	100	100	85	71	111	133	101	18
CI5	*G. geotrichum*	88	100	100	100	90	100	121	97	100	10
CI6	*C. zeylanoides*	100	107	100	97	50	46	5	63	71	36
GI1	***C. lusitaniae***	**100**	**100**	**104**	**100**	**88**	**95**	**90**	**63**	**93**	**13**
GI2	*C. zeylanoides*	100	139	625	130	100	79	91	86	169	186
SI1	*D. hansenii*	0	100	0	100	40	56	74	90	58	41
SI2	***G. geotrichum***	**100**	**109**	**100**	**100**	**110**	**120**	**133**	**160**	**117**	**21**
SI3	*C. zeylanoides*	80	125	625	115	81	53	106	116	163	189

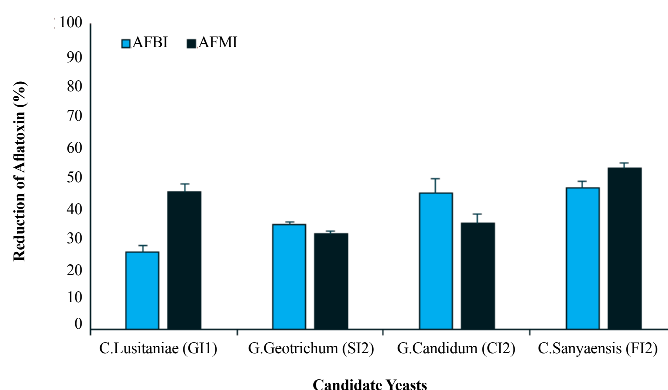

Figure 4. Assessment of the binding ability of yeast isolates against 5 ppm aflatoxin M1 and B1 in simulated gastrointestinal conditions.

aflatoxin contamination. On the other hand, it seems that the natural yeast population of cheese surfaces can play a decisive role in reducing the destructive effect of aflatoxins. In this study, evaluation of the response of yeast strains showed different growth responses to different concentrations of aflatoxins.

Previous studies have shown that aflatoxin binding to the polysaccharide part of its cell wall is a physical phenomenon that occurs on the surface of yeast (39). Shetty *et al*, observed that 75% of the binding strength of yeast is related to substances extracted from the cell wall (40, 41). Zhou *et al* research indicated that binding to the cell wall was the main reason for the detoxification of aflatoxin B1 by *Zygosaccharomyces rouxii* and *Streptococcus thermophilus* in liquid fermentation. The amount of aflatoxin bound by these two microorganisms

was more than 50% (42). These data seem to be in line with the findings of this study because the isolated yeast strains retained the ability to absorb aflatoxin even after being killed.

In another study, Hamad *et al* investigated the reduction of aflatoxin in milk with a combination of probiotic bacteria and yeast strains and showed that the combination of *Saccharomyces cerevisiae* yeast strains with probiotic *Lactobacillus Plantarum*, *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *Kluyveromyces lactis* can significantly reduce the amount of aflatoxin M1 in milk (25). Similar to our findings, yeast strains were able to reduce both AFB1 and AFB2; 54% with *S. cerevisiae* and 42% with *K. lactis*. The minimal difference in the percentage of aflatoxin reduction can be attributed to the aflatoxin absorption mechanism and the binding capacity of yeasts to these toxins.

6. Conclusions

All geographical samples of homemade cheeses comprise different yeast isolates with distinct diverse biological characteristics. Some species of yeast, D. *hansenii*, and C. *zeylanoides*, despite their extensive ecological presence, show extensive growth changes against aflatoxins. On average, candidate yeast strains did not have a negative effect on different aflatoxin concentrations and successfully removed AFB1 and AFM1 from gastrointestinal fluid in the range of 25 to 50%. Yeast communities appear to provide accurate candidates for potential aflatoxin removal from the gastrointestinal fluid, with a significant impact on the quality of homemade cheese.

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