Food Bioscience 43 (2021) 101253

Contents lists available at ScienceDirect

Food Bioscience

journal homepage: www.elsevier.com/locate/fbio

Evaluating starter culture potential of wild *Penicillium roqueforti* strains from moldy cheeses of artisanal origin

Murat Doğan [a,*], İsmail Hakkı Tekiner [b,c]

[a] Gastronomy and Culinary Arts Department, Fine Arts Faculty, Istanbul Gelişim University, TR-34310, Istanbul, Turkey
[b] Nutrition and Dietetics Department, Health Sciences Faculty, Istanbul Sabahattin Zaim University, TR-34303, Istanbul, Turkey
[c] Food and Nutrition Department, School of Graduate Studies, Istanbul Sabahattin Zaim University, TR-34303, Istanbul, Turkey

ARTICLE INFO

Keywords:
Moldy cheese
P. roqueforti
Starter culture

ABSTRACT

Penicillium roqueforti from native food habitats can provide more insights into moldy cheese production. The objective of this study was to evaluate the starter culture potential of wild *P. roqueforti* strains from moldy cheese samples of artisanal origin. Their starter culture potential was studied after culturing, morphological analysis, and PCR-identification of *P. roqueforti* isolates. Overall, 17 of 623 cultured fungal strains were identified as *P. roqueforti* by PCR. The identified strains showed high proteolytic activity (8.9 ± 8.4), followed by amylolytic (0.413 ± 0.289) and lipolytic (0.29 ± 0.28) activities, with an optimum acid pH of 6.0. In addition, the strains were inhibitory on *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, and S. typhimurium,* susceptible to TE, CPD, and CTX whereas resistant to CAZ, CN, K, VA, and C, and antagonistically suppressed by some lactic acid bacteria species. The mean highest viability was detected as 7.4 ± 1.2 g/CFU in a medium of sucrose, peptone, KH_2PO_4, $MgSO_4{\cdot}7H_2O$, KCl, whey, and milk powder under batch-fermentation conditions of at 25 °C for 72–96 h, with pH 6.0 and 10% dissolved oxygen. In summary, our results showed that wild *P. roqueforti* isolates could meet specific requirements for becoming fungal starter cultures for moldy cheese production.

1. Introduction

Cheese is a complex microbial ecosystem comprising a broad range of different microorganisms (Khattab, Guirguis, Tawfik, & Farag, 2019). For example, in cheese production, lactic acid bacteria (LAB) is used as a starter culture for acid production, whereas yeasts, molds, other bacteria, and heterofermentative *lactobacilli* are involved in the ripening process (Irlinger, Helinck, & Jany, 2017; Pereira et al., 2019). In addition, fungal strains contribute to specific sensorial characteristics (Bockelmann, 2007; Copetti, 2019a).

The involvement of visible fungal mycelium was one of the earliest approaches in cheese production 4000 years ago (Ghorai et al., 2009). Moldy cheeses are popular but not mass-produced (3% of the total cheese production globally, and up to 8% of the whole cheese produced in Europe) (McSweeney, Ottogalli, & Fox, 2017; Spinnler, 2017). They have a characteristic appearance, typical aroma, and taste due to their complex ripening patterns (Al-Otaibi, Haddadin, & Haddadin, 2016). Moldy cheeses are divided into two subtypes. The first is the surface mold-ripened cheeses such as Camembert and Brie, generally ripened by *P. camemberti*, forming a velvety white rind. The second is the internal mold-ripened (blue-veined) cheeses (Danablu, Roquefort, Stilton and Gorgonzola, ripened by *P. roqueforti*) (Copetti, 2019b; Desmasures, 2014; Dumas et al., 2020).

P. roqueforti is one of the thirteen filamentous fungal species in the dairy industry (Bourdichon et al., 2012). It is one of the most extensively studied species for its technological use (Kure & Skaar, 2019). Its major reservoir is the native pool of food habitat but cannot always meet specific requirements of fabricated food production (Geisen, 1993; Steensels, Gallone, Voordeckers, & Verstrepen, 2019). In the last years, the studies have focused on its morphological, metabolic, and genetic characteristics as well as its adaptation to the cheese matrix and domestication process (Kırtıl, Metin, & Arıcı, 2020). *P. roqueforti* is selected based on morphological and physiological properties and pigmentation (Roostita & Fleet, 1996). Taxonomically, it is recognized

Abbreviations: AM, Ampicillin; C, Chloramphenicol; CAZ, Ceftazidime; CD, Colony Diameter; CDA, Czapek-Dox Agar; CN, Cefalexin; CPD, Cefpodoxime; CTX, Cefotaxime; CYA, Czapek Yeast Autolysate Agar; DA, Clindamycin; E, Erythromycin; K, Kanamycin; LAB, Lactic Acid Bacteria; MEA, Malt Extract Agar; MHA, Mueller Hinton Agar; PCR, Polymerase Chain Reaction; PDA, Potato Dextrose Agar; S, Streptomycin; TE, Tetracycline; VA, Vancomycin; ZD, Zone Diameter.

* Corresponding author.
E-mail address: mdogan@gelisim.edu.tr (M. Doğan).

https://doi.org/10.1016/j.fbio.2021.101253
Received 3 May 2021; Received in revised form 8 July 2021; Accepted 13 July 2021
Available online 20 July 2021

as a single species despite having morphological and intraspecific diversity (Pitt & Hocking, 2009, p. 520; Ropars et al., 2014). By the 19th century, milk or curd was inoculated with this fungus for Roquefort cheese production. Following World War II, it was selected in terms of technological and organoleptic impacts (Dumas et al., 2020; Ndlovu, van Jaarsveld, & Caleb, 2019).

P. roqueforti is conventionally characterized by morphological properties and colony morphology when grown on specific growth mediums. It generally exhibits high macroscopic variability on Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA), colony color and texture, as well as margin width (Coton, Coton, Hymery, Mounier, & Jany, 2020). However, the Potato Dextrose Agar is the most discriminative one for macroscopic and microscopic evaluation (Gillot et al., 2015). Morphology of the organism itself is based on features of the brush-shaped fruiting head; size, shape, and number of conidia; size and number of sterigmata; whether there is branching; length and surface markings of the conidiophore; overall dimensions; and like characters (Belén Flórez, Álvarez-Martín, López-Díaz, & Mayo, 2007). Its conidia are not affected at 55 °C for 10 min, even though its hydration time is extended to 48 h (Dantigny & Nanguy, 2009). The optimum temperature and pH on the germination of *P. roqueforti* conidia showed 26.9 °C and pH 5.6 (Kalai, Anzala, Bensoussan, & Dantigny, 2017).

Natural variants of *P. roqueforti* may thrive better in fabricated environments than others. However, it seems unclear whether *P. roqueforti* from native food habitats can meet the performance characteristics in the food sector. Therefore, assessing its starter culture potential can provide more insights into moldy cheese production (Steensels et al., 2019).

This study aimed to evaluate the starter culture potential of wild *P. roqueforti* strains from moldy cheese samples of artisanal origin.

2. Materials and methods

2.1. Materials

2.1.1. Sampling

From May 2019 to June 2020, 100 moldy cheese samples of artisanal origin were collected randomly from different districts of Turkey (22 in Marmara, 41 in Central, and 37 in Eastern Anatolia). All the samples were put into sterile sampling bags to prevent cross-contamination (EC Samancta SAM-110, 2012) and transported to the laboratory in a thermobox container at 4 °C until sample preparation and analysis.

2.1.2. Chemicals and reagents

Potato Dextrose Agar (PDA) (Merck 1.10130, Darmstadt, Germany), Malt Extract Agar (MEA) (Merck 1.05398), Czapek Yeast Autolysate Agar M2061 (CYA Agar), Czapek-Dox Agar (CDA) (Merck 105460), and 0.1% peptone solution (Merck 107228) were prepared for isolation, cultivation, and identification of pure *Penicillium* species in the samples, and lactophenol blue solution stain (fungal) (Sigma-Aldrich 61335, Taufkirchen, Germany) for macroscopic and microscopic testing. All chemicals and reagents were selected according to ISO 11133 (2014), Cakmakci et al. (2015), and Gillot et al. (2015).

2.2. Mold identification

For sample preparation and conventional analysis, 10 g of each sample was homogenized with 90 mL of 0.1% peptone solution in a sterile stomacher bag (Interscience Bag System) for 2 min using a stomacher (AES Laboratoire, Chemunex, France). Serial dilutions (10^{-1} to 10^{-4}) of homogenized samples were inoculated onto PDA and CDA plates and incubated at 25 °C and 35 °C for five days (ISO 11133, 2014).

Following incubation, the macroscopic colony morphology of the isolates was examined on PDA and CDA mediums after seven days of incubation at 25 °C and 35 °C by recording colony diameters (CD) every 24-h. After incubation, fungal cultures were stained with Lacto cotton blue solution, and their macroscopic images were photographed. Exudate production on CYA agar was considered, and the mycelium layer was selected as droplet sweating (Palacios-Cabrera, Taniwaki, Hashimoto, & Menezes, 2005; Pitt & Hocking, 2009, p. 520; Raper & Thom, 1949, pp. 1–875). Microscopic colony morphology was also evaluated by observing the spores of pure isolates under the microscope, considering spial after metula forming phialides as *Penicillium* species (Pitt & Hocking, 2009, p. 520; Tiwari, Jadhav, & Kumar, 2011).

After that, genomic DNA was extracted from fresh mycelium for each isolate after 5–7 days of growth on 20 g/L malt extract, 3 g/L yeast extract, and 15 g/L agar using Fast DNA SPIN Kit (MP Biomedicals, Illkirch, France), according to the manufacturer's instructions. Quantitative determination of total DNA was determined using NanoDrop 2000 Micro volume Spectrophotometer (Thermo Scientific™, USA) at 350 nm. Stock solutions (100 ng/μL) were prepared for PCR testing, and all DNA samples were stored at −20 °C.

Following the DNA isolation, the amplification of the β-tubulin gene was conducted for all *Penicillium* isolates to ensure that they belonged to the *P. roqueforti* species by using Bt2a and Bt2b primers. The primers were designed as Bt2a (5′-GGTAACCAAATCGGTGCTGCTTTC-3′) and Bt2b (5′-ACCCTCAGTGTAGTGACCCTTGGC-3′), as described by Glass and Donaldson (1995). The amplification was performed by Thermal Cycler PTC-0200G DNA Engine (BioRad, USA) with one cycle for 3 min at 94 °C, 30 cycles for 40 s at 94 °C, 40 s at 55 °C, 1 min at 72 °C, and final elongation at 72 °C for 15 min. Gel-electrophoresis was performed on a 1.5% agarose gel at 75 V for 45 min. The amplicons were photographed with UV illumination by Biorad GelDoc 2000 imaging system and analyzed by Biorad Quantity one 4.6.3 GelDoc XR Software.

2.3. Determination of proteolytic, amylolytic, and lipolytic activities

Proteolysis is the most important biochemical event during the ripening of most cheese varieties, with a significant impact on flavor and texture. The effect of casein on the qualitative proteolytic activity was studied by inoculating the cultures on the MEA plate containing 10% skim milk and incubating them for 72 h at 27-30 °C. Finally, proteolytic strains were recognized by their clear halo in the plates, and calculated was obtained by the ratio of zone diameter (ZD) to colony diameter (CD) (Pereira, Crespo, & San Romao, 2001). The proteolytic activity was quantified according to the method described by Keay and Wildi (1970), using casein as a substrate. One unit (U) of protease activity was defined as the increase in absorbance of 0.001 for casein per min and per mL of the suspension containing 10^8 spores from 6-day-old colonies, incubated at 37 °C for 20 min. Finally, proteolytic activity (IU/mL) was read at 660 nm by a spectrophotometer (Cecil CE 3021 3000 series, Cambridge, UK).

An amylolytic microorganism can decompose starchy material through the amylases production during the fermentation processes. The effect of amylase medium soluble starch on the qualitative amylolytic activity was studied by inoculating the cultures on the petroleum-containing amylase medium and incubating them for 72 h at 27-30 °C. After that, the petri dish was sprayed with iodine solution, and amylolytic strains were recognized by dark blue color. The amylolytic activity was obtained by the ratio of ZD to CD. The amylolytic activity was quantified using starch as a substrate. The suspension containing 10^8 spores from 6-day-old colonies was incubated at 25 °C and pH 5.0 for six days, at 100×*g*. After that, 20 mL of each cultured medium was centrifuged at 4750×*g* for 20 min, and the supernatant was recognized as the crude enzyme solution. Finally, amylolytic activity (IU/mL) was read by a spectrophotometer (Cecil CE 3021 3000 series) (Møller, Sharif, & Olsson, 2004).

Lipolysis is the enzymatic hydrolysis of triglycerides to fatty acids and glycerol and mono- or diglycerides significantly impacting flavor development. The qualitative lipolytic activity was studied by inoculating the cultures on Tween 80 agar, followed by incubation at 27–30 °C for 72 h. First, the lipolytic strains were recognized by the opaque zone. Next, the lipolytic activity was measured by the ratio of ZD

to CD. Next, the lipolytic activity was quantified by inoculating the cultures containing 10^8 spores in a medium, including $NaNO_3$, $MgSO_4.7H_2O$, KCI, KH_2PO_4, yeast extract, peptone, olive oil, with a pH of 5.5. Then, after incubation at 37 °C for 20 min, 20 mL of each cultured medium was centrifuged at 4750×*g* for 20 min, and the supernatant was recognized as the crude enzyme solution. Finally, lipolytic activity was obtained based on the absorbance value of p-nitrophenol (PNF) at 404 nm by spectrophotometer (Cecil CE 3021 3000 series), as previously described by Haba et al. (2000).

2.4. Determination of metabolic activity

The metabolic activity was studied by the litmus milk reaction method. For this, one drop of the 24-h cultured isolate was inoculated into a 5 mL of broth-type medium containing litmus milk and incubated at 35 °C for seven days. The red or white color turn was recognized as litmus positive, whereas blue was considered negative for litmus milk reaction (Aspri, Bozoudi, Tsaltas, Hill, & Papademas, 2017; Welsh, 2017).

2.5. Determination of antibacterial activity

The antibacterial activity was determined by well diffusion method, using *S. aureus* (ATTC® 29213), *E. coli* (ATTC®35218), *B. subtilis* (ATTC®6663), and *S. typhimirium* (ATTC®13311) as the indicator microorganisms. A 200 μL-aliquot of each strain cultured in the nutrient broth for 72 h (approximately with a population of 10^8 CFU/mL) was pipetted into the bored well of 8 mm in diameter and incubated for 72 h at 25 °C. Finally, the breakpoints with zone diameters were evaluated according to CLSI (2015) and Todorov and Dicks (2006).

2.6. Determination of antibiotic susceptibility

The antibiotic susceptibility testing was studied by Kirby-Bauer Disc Diffusion Method. First, a 200 μL-aliquot of each strain was spread over Mueller Hinton Agar (MHA). After that, antibiotic discs of S (10 μg), CAZ (30 μg), VA (30 μg), CN (30 μg), E (15 μg), TE (30 μg), DA (2 μg), AM (10 μg), K (30 μg), CTX (30 μg) μg), CPD (30 μg), and C (30 μg) were placed on the plate surface. Afterward, the disc-inserted plates were incubated at 25 °C for 48 h. Finally, the inhibition zone diameters were measured and evaluated as susceptible (S), Intermediate (I), or Resistant (R) according to the criteria by CLSI (2015) and Hudzicki (2009).

2.7. Determination of antagonistic activity

The antagonistic activity of each strain was tested using the well diffusion method. The indicator lactic acid bacteria (LAB) species, including *L. lactis, L. plantarum*, and a combination of *L. bulgaricus and S. thermophilus*, were obtained from the culture collection of Farmapark Biotechnology R & D Company in Konya, Turkey. After the incubation of the plates at 32 °C for 96 h, the inhibition zones around LAB streaks were scaled according to "no growth suppression (−)" and "1–5 mm growth suppression (+) (CLSI, 2015; Todorov & Dicks, 2006).

2.8. Determination of viability under different growth conditions

The cultures' viability was monitored in a 5 L batch-cut fermenter (New Brunswick® BioFlo Fermentor, USA). For this, four different growth mediums (designated as A, B, C, and D), autoclaved at 121 °C for 15 min, were prepared (Table 1). First, five percent of the medium and 3% of the culture inoculum were filled into the fermenter. Then, the optimum fermenter conditions were applied as 120×*g*, pH 6, and 25 °C for 72–96 h, with 10% dissolved O_2. Afterward, 10 mL of the fermenter solution was taken and homogenized in 90 mL maximum recovery diluent, serially diluted (up to 10^{-10}). Finally, 100 μL of this aliquot was spread on Dichloran Rose Bengal Chloramphenicol (DRBC) agar, incubated at 25 °C for five days, and finally, the viability was recorded, as previously suggested by Klich (2002).

Table 1
Different growth conditions in the fermenter.

Component/Media	A	B	C	D
	Quantity (g/L)			
Sucrose	30			
Peptone	5	1		
KH_2PO_4	1			
$MgSO_4.7H_2O$	0.5		150	50
KCI	0.5			50
Whey Powder	33			
Milk Powder	30			
Malt Extract		20		
Glucose		20		
dH_2O		1000	885	1000
Difco™ Yeast Extract			20	
Trace elements stock solution			1	
$NaNO_3$				300
$FeSO_4.7H_2O$				1
$ZnSO_4.7H_2O$				1
$CuSO_4.7H_2O$				1
dH_2O				1000

3. Results and discussion

This study evaluated the fungal starter culture potential of wild *P. roqueforti* from moldy cheese samples. Of 623 cultured and morphologically identified fungal isolates, 55 isolates were classified as *Penicillium* species, and 17 isolates were identified as *P. Roqueforti*. Overall, our technological assessments on the fungal starter culture potential showed that wild *P. roqueforti* strains could meet specific requirements for becoming fungal starter culture for moldy cheese production (Table 2) (see Fig. 1).

3.1. Mold identification results

In this study, we hydrated fungi species on PDA medium for seven days and cultivated 623 fungal isolates. Among them, 17 were classified as *P. roqueforti* morphologically (Fig. 2 & Fig. 3), as previously described by Belén Flórez et al. (2007). Fourteen isolates were isolated from the different samples (2 from Marmara, 7 from Central, and 5 from Eastern Anatolia), while 3 were from the same samples from the Marmara, Central, Eastern regions. The optimum hydration temperature for germination of *P. roqueforti* conidia was kept at 25 °C during the cultivation, similar to the previously given temperatures of 23.9 °C and 26.9 °C as Brancato and Golding (1953) and Kalai et al. (2017). The average colony diameter of *P. roqueforti* strains on PDA medium after seven days incubation was 35.2 ± 5.4 mm. Our results indicated that our isolates grew slower than those by Gillot et al. (2015) (66.6 mm) and Brancato and Golding (1953) (56 mm) but grew faster than Camardo Leggieri, Pietri, and Battilani (2020) (23 mm), and Punt et al. (2020) (3.94 mm). Temperature influences growth rate and metabolic efficiency differently, although mold fungus had the highest growth rate at 25 °C (Dantigny & Nanguy, 2009; Li, Wadsö, & Larsson, 2009; Samson, Houbraken, Thrane, Frisvad, & Andersen, 2019), and our isolates were slightly more resistant to the growing conditions (see Fig. 4).

The genotypic testing revealed that 17 (2.7%) of *Penicillium* species were identified as *P. roqueforti* by PCR analysis. In the literature, Gillot et al., 2015 screened *P. roqueforti* from 120 local moldy cheese varieties by genomic studies. Similarly, Belén Flórez et al. (2007) examined 35 white and blue-greenish filamentous fungi during ripening of Cabrales cheese to identify *P. roqueforti*. In addition, it is well known that the *P. roqueforti* group has recently been split into three species, *P. roqueforti*, *P. carneum*, and *P. paneum*, based on differences in ribosomal DNA sequences and secondary metabolite profiles (Boysen, Jacobsson, &

Table 2
Technological performance results of *P. roqueforti* strains.

Strain no	Sporulation activity (mm, 25 °C)				Proteolytic Activity			Amylolytic Activity			Lipolytic Activity			Viability (CFU/mL)															
														48 h				72 h				96 h				120 h			
	Day 3	Day 4	Day 5	Day 6	Day 7	CD (mm)	ZD (mm)	Z/C	Tyrosine (IU/mL)	CD (mm)	ZD (mm)	Z/C	Amylase (IU/mL)	CD (mm)	ZD (mm)	Z/C	Lipase (IU/mL)	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
1	21	28	31	34	35	23.4	25	1.07	1.65	18.5	–	–	–	15.5	21	1.4	0.718	4.4	3.36	3.52	4.04	5.77	4.46	5.51	5.34	6.74	5.65	5.32	6.36	7.81	7.53	5.28	7
2	19	25	28	33	38	25	28.4	1.14	1.35	21	–	–	–	10.2	13	1.3	0.643	5.34	3.28	4.3	4.56	5.56	4.46	4.32	5.4	7.64	6.53	4.75	5.15	7.58	5.56	5.04	6.2
3	16	22	26	31	36	26.6	–	–	1.15	17	–	–	–	18.6	–	–	0.422	5.23	4.43	3.79	3.26	5.46	4.52	4.79	5.08	7.7	8.51	4.72	5.11	8.04	7.36	5.7	6.23
4	20	25	30	35	37	18	21	1.17	2.83	21.5	–	–	–	19	–	–	0.492	4.3	5.34	4.51	4.04	5.46	5.52	4.7	4.26	7.52	6.8	4.68	4.4	7	6.3	4.61	5.48
5	17	26	25	35	34	22	23	1.05	0.53	22	26	1.2	0.022	22	–	–	0.341	5.75	4.15	3.04	4.52	6.46	5.2	4.68	5.56	8.65	6.72	4.74	6.72	8.08	4.2	4.56	7.77
6	18	23	32	35	40	22.3	26.2	1.17	4.75	18	–		0.125	28	31	1.1	0.623	5.36	4.08	5.36	4.64	6.46	5.51	4.46	5.68	7.6	5.15	7.6	5.72	7.51	5	7.51	6.8
7	18	19	23	27	31	26	28	1.08	1.78	21.2	23.8	1.1	0.178	18.5	20	1.1	0.115	6.57	5.65	4.48	4.74	7.46	6.4	5.53	5.51	8.7	6.41	5.52	7.3	9.08	6.46	5.48	7.28
8	19	28	34	42	47	24	–	–	0.03	20.5	–	–	–	15.5	17.5	1.1	0.354	3.52	4.75	3.38	4.32	5.46	5.78	4.51	5.34	5.59	6.36	5.51	5.51	5.6	6.08	5.48	6.72
9	14	16	23	28	33	19.8	25.2	1.27	2.93	19	20.5	1.1	0.137	18	–	–	0.003	3.3	4.5	4.7	5	5.5	5.7	4.7	6	5.5	5.6	4.7	6.2	6.7	5.3	4.7	7
10	17	22	26	32	37	21	25.5	1.21	3.75	16	18	1.1	0.606	16	–	–	0.115	5.3	4	4.5	4.6	6.6	6.4	5.5	5.6	8	6.6	5.4	6.3	8	6.5	5.4	7.5
11	12	15	20	23	26	28	31	1.11	0.35	17.5	–	–		17.5	23	1.3	1.018	4.6	3.3	5.2	4.3	5.6	4.4	5.3	5.2	7.6	5.1	5.3	5.4	8.1	6.3	6.6	6.3
12	16	17	21	27	31	21	25	1.19	4.55	21	–	–	–	21	–	–	0.453	4	4.6	3.6	4.2	4.6	5.1	4.1	6.3	5.5	5	5.1	5.3	5	4.1	4	5.3
13	15	22	26	29	32	18	26	1.44	0.53	18	–	–	–	18	–	–	0.115	5.7	4.5	3.8	4.6	7.7	5.5	4.5	4.7	8.4	5.4	5.5	5.4	7.3	4.3	5.3	5.3
14	16	20	26	27	31	20	24.8	1,24	3.9	20	21	1.1	0.181	25.7	–	–	0.348	5.7	3	3.8	4.5	7.2	5.3	4.6	5.3	8.6	5.1	5.3	5.6	8.3	5	6.3	6.8
15	17	21	26	27	31	16.5	24.7	1,5	0.05	16.5	–	–	–	16.5	18	1.1	0.457	6.7	4.7	4.6	4.2	8.5	5.7	5.6	5.2	9.1	5.5	5.7	6.3	8	5.3	6.7	6.3
16	15	21	25	30	32	14	–	–	0.03	16	–	–	–	23	–	–	0.805	4.5	3.2	4.5	4.1	5.5	3.4	4.5	5.4	6.5	3.6	4.3	5.3	5.5	3.6	4.1	5.3
17	19	26	33	42	47	17.5	19.6	1,12	0.03	17	24	1.4	0.790	26.5	–	–	0.001	6.4	4.7	4.6	5.6	7.5	5.7	4.3	6.7	8.5	6.6	4.2	6.6	9	6.4	4.5	8.4
Median	*17*	*22*	*27*	*32*	*35*	21.5	25.6	1.3		18.3	22.3	1.2	0.29	20.6	21.0	1.2	0.413	5.1	4.2	4.2	4.4	6.3	5.2	4.8	5.4	7.5	5.9	5.2	5.8	7.4	5.6	5.4	6.6
Sdv±	*2.3*	*3.9*	*4.1*	*5.2*	*5.6*	4.1	3.3	0.2		2.2	3.5	0.2	0.28	3.7	5.9	0.1	0.289	1.0	0.8	0.6	0.5	1.1	0.8	0.5	0.6	1.2	1.1	0.8	0.7	1.2	1.1	1.0	0.9

Schnürer, 2000). Thus, molecular techniques enable us to screen different environments and analyze extensive *P. roqueforti* strains other than traditional morphological examination.

3.2. Proteolytic, amylolytic, and lipolytic activity results

In our qualitative proteolytic activity study, the mean ratio of ZD (mean: 25.6 ± 3.3 mm) to CD (mean: 21.5 ± 4.1 mm) was obtained as 1.3 ± 0.2 mm, whereas the mean quantitative proteolytic activity on tyrosine level was determined to be 8.9 ± 8.4 IU/mL. Overall, all *P. roqueforti* isolates showed quantitative proteolytic activity with tyrosine as a more sensitive ripening criterion than soluble protein values, as previously explained by Silverman and Kosikowski (1955). The proteolytic action of *P. roqueforti* is derived from hydrolysis of the casein matrix and a decrease in curd's water activity. The catabolism of tyrosine is the result of the formation of peptides and amino acids by proteolytic activity, contributing to flavor (e.g. bitterness) (Diezhandino et al., 2016). In the literature, the role of *P. roqueforti* in proteolysis has been reviewed. For instance, in Danish blue cheese, *P. roqueforti* proteinases became apparent during nine weeks' ripening at pH 5.8 (Mane, Ciocia, Beck, Lillevang, & McSweeney, 2019). In Valdeón blue-veined cheese, proteolysis was very high and showed great complexity at pH 4.6 (Diezhandino, Fernández, González, McSweeney, & Fresno, 2015), and this 'Valdeón-industrial' strain showed high levels of proteolytic activity than *P. roqueforti* strains from local varieties of moldy cheese in Spain and *P. roqueforti* CECT 2905 (ATCC 10110) (Fernández-Bodega, Mauriz, Gómez, & Martín, 2009). Similarly, wild *P. roqueforti* NRRL 849 showed low proteolytic activity, making them suitable for recombinant protein production and other biotechnological applications (Chávez et al., 2010). Thus, *P. roqueforti* is a well-known multifunctional cell factory of high added-value biomolecules (Beresford, Fitzsimons, Brennan, & Cogan, 2001; Mioso, Toledo Marante, & Herrera Bravo de Laguna, 2015; Gillot et al., 2017; Hamlyn, Wales, & Sagar, 1987; Molimard & Spinnler, 1996). In our study, proteolytic activity results indicated that wild *P. roqueforti* strains were adequate for speed of growth and differences apart from the morphology.

In our study, the mean ratio of ZD (mean: 22.3 ± 3.5 mm) to CD (mean: 18.3 ± 2.2 mm) was obtained as 1.2 ± 0.2 mm for qualitative amylolytic activity, whereas the mean quantitative amylolytic activity on amylase level was determined to be 0.29 ± 0.28 IU/mL. These results showed that only 35% of *P. roqueforti* isolates exhibited amylolytic activity. Amylases were first widely marketed in the early 1960s, after a wide range of proteases and lipases (Saxena, Gupta, Saxena, & Gulati, 2001). Amylase catalyzes the hydrolysis of starch and leads to glucose, maltose, and maltotriose units (Konkit & Kim, 2016). Our amylolytic activity findings revealed that the degree of amylase production varied among the *P. roqueforti* isolates, possibly depending on the microbe's origin, as well as the growth factors.

In our qualitative lipolytic activity study, the mean ratio of ZD (mean: 21.0 ± 5.9 mm) to CD (mean: 20.6 ± 3.8 mm) was obtained as 1.2 ± 0.1 mm, whereas the mean quantitative lipolytic activity on lipase level was determined to be 0.413 ± 0.289 IU/mL. The findings revealed that only 41% of *P. roqueforti* isolates showed lipolytic activity. Our results revealed that some *P. roqueforti* strains showed lipolytic activity with high proteolytic activity, as previously described Ozturkoglu-Budak, Wiebenga, Bron, and de Vries (2016). Lipases improve the flavor profile of the moldy cheese (Khattab et al., 2019), and some are commercialized (Li & Zong, 2010). *P. roqueforti* possesses two lipases, one with a pH optimum of 7.5–8.0, the other with a more alkaline pH optimum (9–9.5) (Collins, McSweeney, & Wilkinson, 2003; Geoffry & Achur, 2018), and hence the lipases produced by our strains needs further analysis to clarify the technical detail given by Collins et al. (2018). Various media compositions stimulate *P. roqueforti* for the production of lipase, stable up to 55 °C within a broad pH range (Hasan, Shah, & Hameed, 2009). In our study, we have tested four different complex mediums under batch fermenter conditions. In the literature,

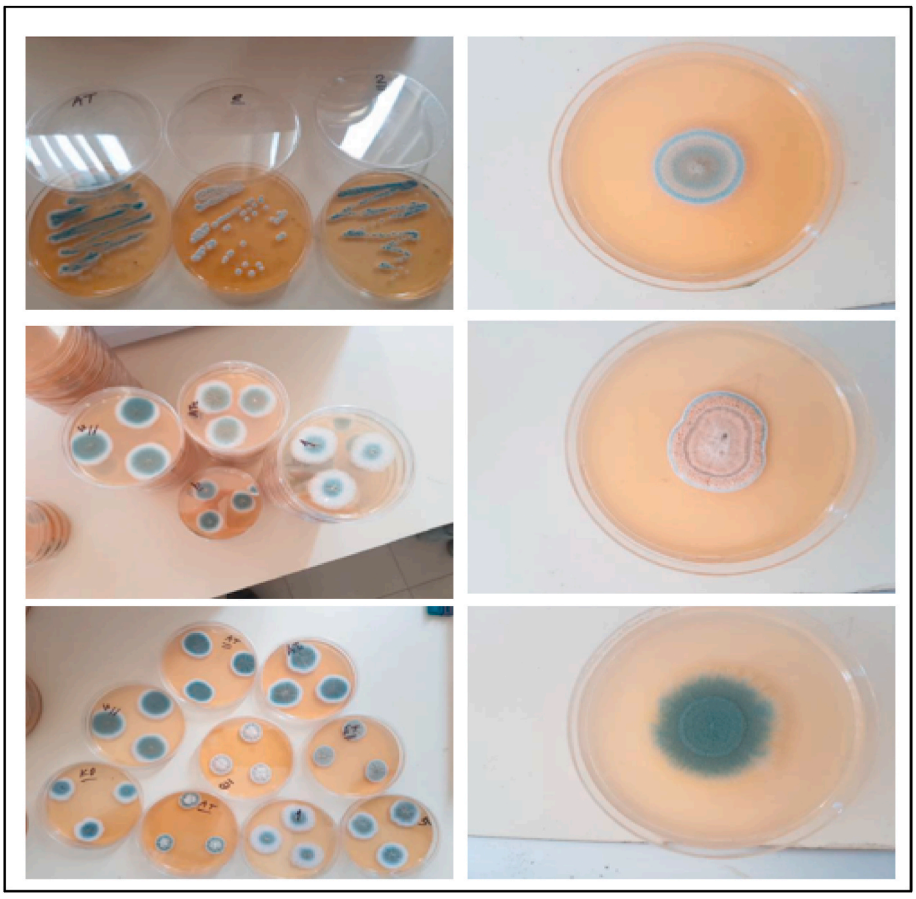

Fig. 1. Macroscopic views of fungi species on PDA medium.

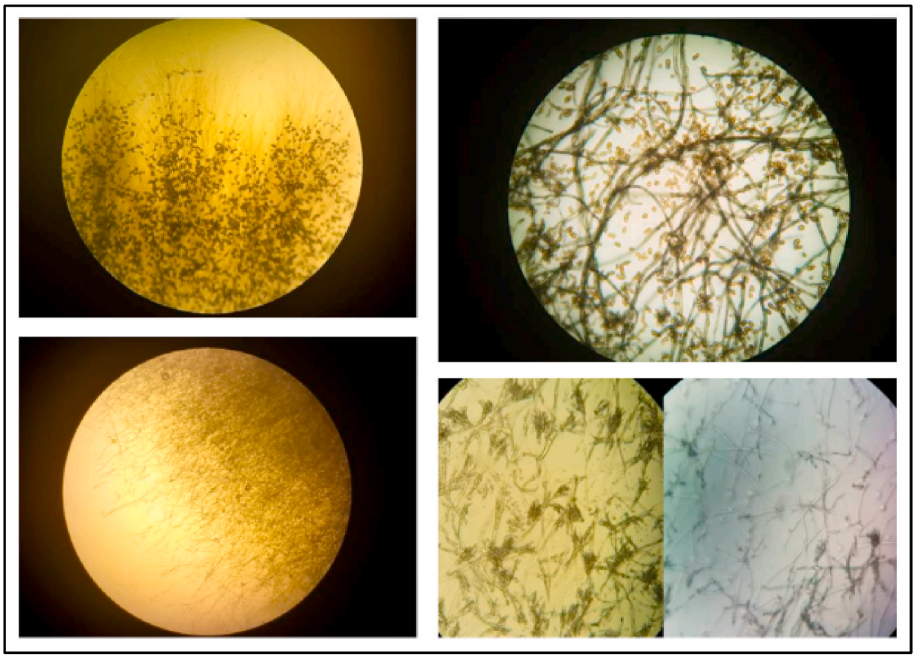

Fig. 2. Microscopic views of *Penicillium* species.

lipolytic activity of wild *P. roqueforti* strains were similar to *P. roqueforti* CECT 2905 (ATCC 10110) and a strain 'Valdeón-industrial' (Fernández-Bodega et al., 2009). Overall, our results proved that *P. roqueforti* strains were much more proteolytic than lipolytic and amylolytic, concordant with Kinsella, Hwang, and Dwivedi (1976), confirming that there was a negative correlation between proteolysis and lipolysis in different strains of *P. roqueforti* (Fig. 3).

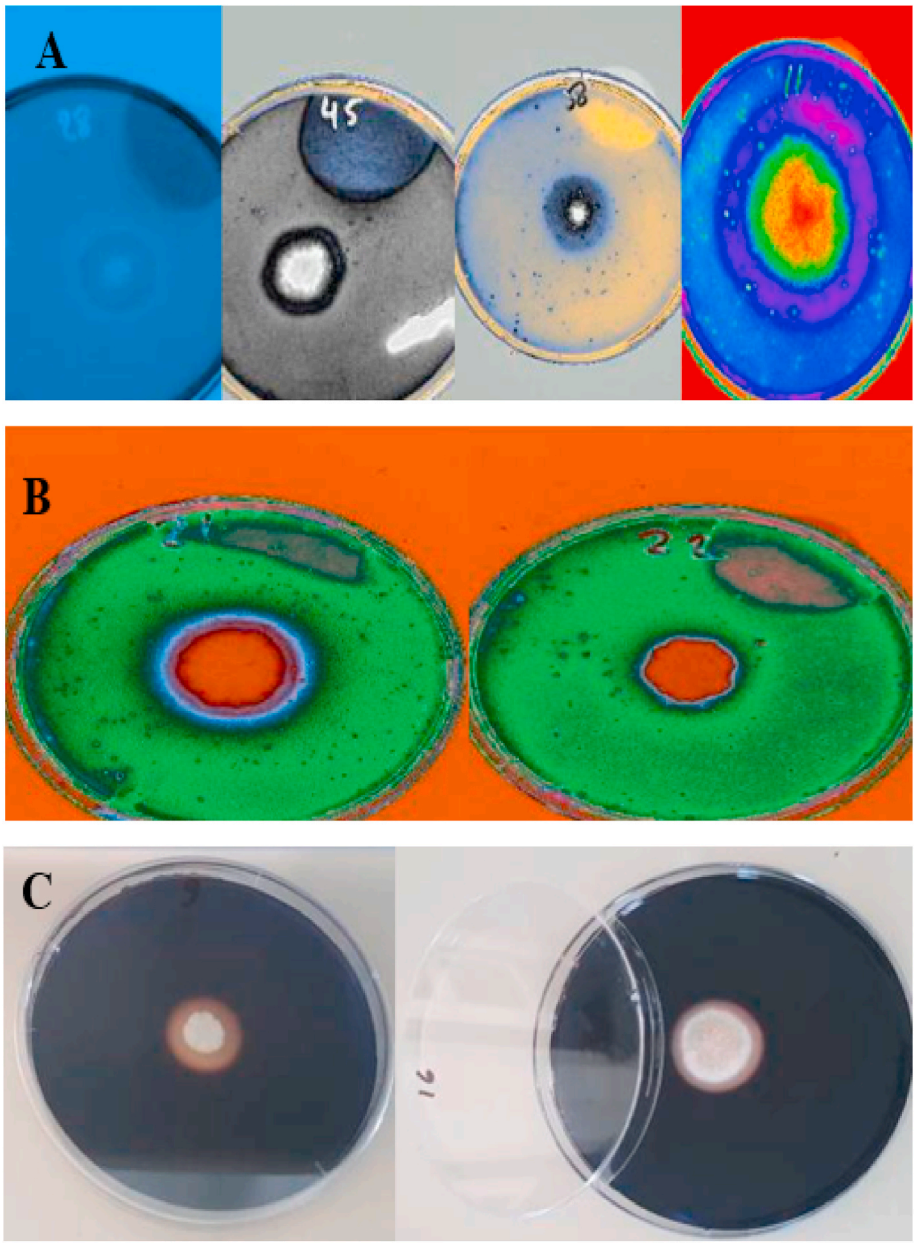

Fig. 3. Proteolytic (A), amylolytic (B) and lipolytic (C) activities of *P. roqueforti* strains.

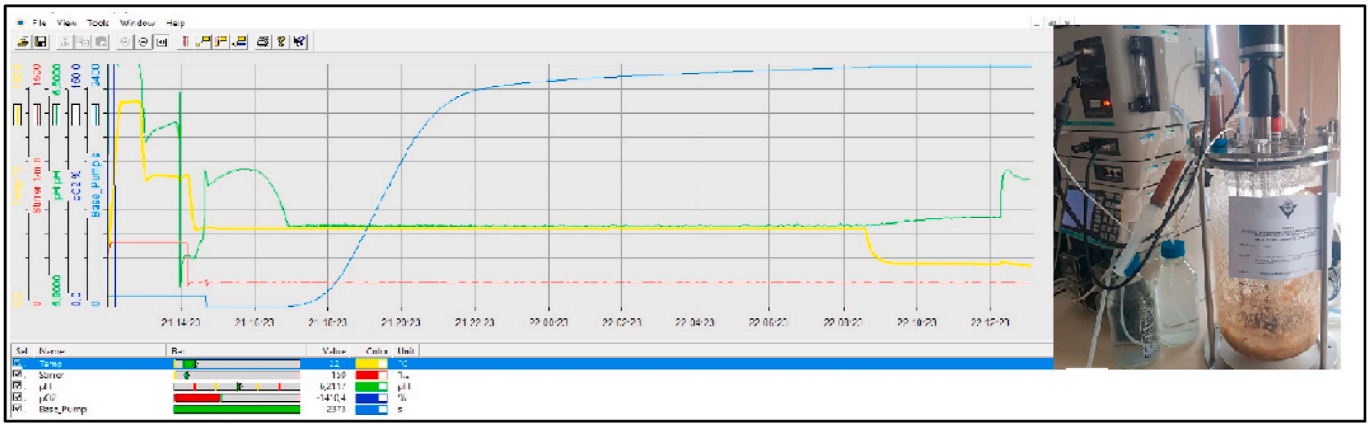

Fig. 4. Fermenter used and the data throughput.

3.3. Metabolic activity results

Our metabolic activity results indicated the fermentation of lactose, and 94% of the strains produced acid, changing color to pink-pinkish with a good acidification activity after 96 h of incubation at 25 °C, and pH ranged from 5.6 to 6.0. Only one strain altered the color to white with a pH of 5.5. Coagulation was observed in 3 strains (18%), indicating sufficient acid production, whereas no gas formation was detected. Fermentations initiated by natural milk contaminants are generally undesirable for industrial purposes (Veljovic et al., 2007). Differentiating *P. roqueforti* strains based on metabolic reactions was performed by the litmus milk reduction test (Khemariya, Singh, Nath, & Gulati, 2013). We also examined the metabolic activity using this technique. In addition, our pH findings were matching with an acid optimum pH 5.6 of the commercial culture of *P. roqueforti* by Danisco (Kalai et al., 2017).

3.4. Antibacterial activity results

S. aureus, *E. coli*, *B. subtilis,* and *S. typhimirium* were used as reference cultures to test the antibacterial activity of the isolates. Our results revealed that nine isolates were inhibitory on *S. aureus* and *E. coli*, three on *B. subtilis*, and one on *S. typhimirium*. Of them, one isolate performed the highest performance with ZDs of 51.3 mm for *S. aureus*, 43.6 mm for *E. coli*, 43.3 mm for *B. subtilis*, and 55.1 mm for *S. typhimirium*. It is known that the *Penicillia* species are capable of producing antibacterial compounds (Wilkowske & Krienke, 1954). For instance, a study by Muhiddin, Yanti, and Asni (2018) reported that the highest zone of inhibition in molds was 16.7 mm for *E. coli* and 17.4 mm for *S. aureus*. Overall, we can say that our isolates showed higher performance in antibacterial influence on the indicator microbes. Still, antibacterial preserving additives should not be neglected for protection in moldy cheese production.

3.5. Antibiotic susceptibility results

One of the most common characteristics for the evaluation of starter culture potential is the susceptibility to antibiotics. In our study, the breakpoints with zone diameters were determined as 17.1 ± 1.2 mm for TE, 16.8 ± 1.2 mm for CPD, 16.7 ± 1.0 mm for CTX, 12.1 ± 2.1 mm for CAZ, 7.4 ± 1.1 mm for CN, 7.1 ± 0.8 mm for K, 5.9 ± 0.8 mm for VA, and 2.1 ± 1.1 mm for C, respectively. Accordingly, the mean ZD measurements revealed that the selected *P. roqueforti* isolates had moderate susceptibility to TE, CPD, and CTX and were resistant to CAZ, CN, K, VA C, not susceptible to S, E, DA, and AM. In the dairy industry, chemical preservatives such as weak organic acids and natamycin prevent spoilage. For instance, the inhibitory effect of sorbic acid on *P. roqueforti* was found as 0.05% (w/w) at 0.90 a_w (Garnier, Valence, & Monuier, 2017), and moldy isolates were resistant to clindamycin only (Čanžek Majhenič, Rogelj, & Perko, 2005). Our results revealed that some examined moldy cheese samples would potentially become a reservoir for antibiotic susceptible strains.

3.6. Antagonistic activity results

In our study, the antagonistic activity of the isolates was tested for some LAB species, including *L. lactis*, *L. plantarum*, and a combination of *L. bulgaricus* and *S. thermophilus*. Accordingly, the mean inhibition zone diameters were found as 8.9 ± 5.2 mm for *L. lactis*, 7.6 ± 3.2 mm for *a* combination of *L. bulgaricus* and *S. thermophilus*, and 6.8 ± 1.7 mm for *L. plantarum*. *L. plantarum* is an antifungal microorganism (Leyva Salas et al., 2017), when used as adjuncts during cheese production (Coda et al., 2008). It can inhibit *P. roqueforti* (Ruiz Rodríguez et al., 2016). Similarly, antifungal activities of *L. lactis* and *L. plantarum* strains on *P. roqueforti* were determined to be 81.3% and 91.5% (Valerio et al., 2009). Contrary to these previous findings, *P. roqueforti* can also be a spoiler in other products such as grated or fresh cheese due to its tolerance to high acid levels (Valerio et al., 2009). Overall, our antagonistic results indicated that LAB cultures could inhibit wild *P. roqueforti* strains with a growth suppression (>5 mm).

3.7. Viability results under different growth conditions

In our study, *P. roqueforti* strains were tested in a batch fermenter using four different mediums (designated as A, B, C, and D). The optimum fermenter conditions were set to 120×*g*, pH 6.0, and 25 °C for 72–96 h, with 10% dissolved oxygen. Afterward, an aliquot of the fermenter output was spread on DRBC agar, incubated at 25 °C for five days, and finally, the viability was recorded. Accordingly, the mean viable count of the strains was determined to be 7.4 ± 1.2 g/CFU for Media A, 6.6 ± 0.9 g/CFU for Media D, 5.6 ± 1.1 g/CFU for Media B, and 5.4 ± 1.0 g/CFU for Media C. Compositionally, and medium A was comprised of sucrose, peptone, KH_2PO_4, $MgSO_4{\cdot}7H_2O$, KCl, whey, and milk powder. Thus, a milk-based medium was one of the first industrial flavor bioprocesses for the production of blue cheese, as Copetti (2019a) said. Similarly, Ismaiel, Ahmed, and El-Sayed (2014) suggested the appropriate fermentation conditions as 25 °C for ten days, pH 6.0, 120×*g* in a medium of sucrose, peptone, KH_2PO_4, $MgSO_4{\cdot}7H_2O$ and KCl. Overall, *P. roqueforti* strains showed high performance in milk whey-based medium rich in carbohydrates and protein as nutrient sources. Furthermore, the fermentation conditions we set were in concordance with other previous works.

4. Conclusions

This study evaluated the starter culture potential of wild *P. Roqueforti* strains from moldy cheese samples of artisanal origin. We concluded that wild-type *P. roqueforti* can be used as a starter culture to manufacture the cheese Roqueforti. Therefore, evaluation of technological characteristics of fungal species from diverse natural origins can provide more insights into moldy cheese production.

CRediT authorship contribution statement

MD: Investigation, Methodology, Project administration, Data curation, Formal analysis, Writing - original draft, Writing – review & editing. **İHT**: Conceptualization, Methodology, Writing - review & editing.

Declaration of competing interest

The authors declare that there is no conflict of interest.

Acknowledgments

This work was supported by The Scientific and Technological Research Council of Turkey (TÜBİTAK) with project accession number 1150273. The authors would like to thank Mr. Erdal Alsancak and Farmapark R&D Biotechnology Company for allocating the experimental infrastructure to perform the test.

References

Al-Otaibi, M. M., Haddadin, J. S., & Haddadin, M. S. Y. (2016). Mold-ripened soft cheeses fortified with date palm fruit product as functional dairy products. *Pakistan Journal of Biological Sciences, 19*, 11–25. https://doi.org/10.3923/pjbs.2016.11.25

Aspri, M., Bozoudi, D., Tsaltas, D., Hill, C., & Papademas, P. (2017). Raw donkey milk as a source of Enterococcus diversity: Assessment of their technological properties and safety characteristics. *Food Control, 73*(Part A), 81–90. https://doi.org/10.1016/j.foodcont.2016.05.022

Belén Flórez, A., Álvarez-Martín, P., López-Díaz, T. M., & Mayo, B. (2007). Morphotypic and molecular identification of filamentous fungi from Spanish blue-veined Cabrales cheese, and typing of Penicillium roqueforti and Geotrichum candidum isolates. *International Dairy Journal, 17*(4), 350–357. https://doi.org/10.1016/j.idairyj.2006.04.002

Beresford, T. P., Fitzsimons, N. A., Brennan, N. L., & Cogan, T. M. (2001). Recent advances in cheese microbiology. *International Dairy Journal, 11*(4–7), 259–274. https://doi.org/10.1016/S0958-6946(01)00056-5

Bockelmann, W. (2007). Cheeses with secondary cultures: Mould-ripened, smear-ripened, and farmhouse cheeses. In B. C. Weimer (Ed.), *Improving the flavour of cheese* (pp. 494–519). Sawston, Cambridge: Woodhead Publishing.

Bourdichon, F., Casaregola, S., Farrokh, C., Frisvad, J. C., Gerds, M. L., Hammes, W. P., et al. (2012). Food fermentations: Microorganisms with technological beneficial use. *International Journal of Food Microbiology, 154*, 87–97. https://doi.org/10.1016/j.ijfoodmicro.2011.12.030

Boysen, M. E., Jacobsson, K. G., & Schnürer, J. (2000). Molecular identification of species from the Penicillium roqueforti group associated with spoiled animal feed. *Applied and Environmental Microbiology, 66*(4), 1523–1526. https://doi.org/10.1128/aem.66.4.1523-1526.2000

Brancato, F. P., & Golding, N. S. (1953). The diameter of the mold colony as a reliable measure of growth. *Mycologia, 45*(6), 848–864. https://doi.org/10.1080/00275514.1953.12024321

Cakmakci, S., Gurses, M., Hayaloglu, A. A., Cetin, B., Sekerci, P., & Dagdemir, E. (2015). Mycotoxin production capability of Penicillium roqueforti in strains isolated from mould-ripened traditional Turkish civil cheese. *Food Additives & Contaminants: Part A, 32*(2), 245–249. https://doi.org/10.1080/19440049.2014.997808

Camardo Leggieri, M., Pietri, A., & Battilani, P. (2020). Modelling fungal growth, mycotoxin production and release in grana cheese. *Microorganisms, 8*(1), 69. https://doi.org/10.3390/microorganisms8010069

Čanžek Majhenič, A., Rogelj, I., & Perko, B. (2005). Enterococci from Tolminc cheese: Population structure, antibiotic susceptibility and incidence of virulence determinants. *International Journal of Food Microbiology, 102*(2), 239–244. https://doi.org/10.1016/j.ijfoodmicro.2004.12.021

Chávez, R., Roa, A., Navarrete, K., Trebotich, J., Espinosa, Y., & Vaca, I. (2010). Evaluation of properties of several cheese-ripening fungi for potential biotechnological applications. *Mycoscience, 51*(1), 84–87. https://doi.org/10.1007/S10267-009-0004-4

CLSI, C. (2015). *Performance standards for antimicrobial susceptibility testing: 25th informational supplement. CLSI document M100-S25*. Clinical and Laboratory Standards Institute.

Coda, R., Rizzello, C. G., Nigro, F., De Angelis, M., Arnault, P., & Gobbetti, M. (2008). Long-term fungal inhibitory activity of watersoluble extracts of Phaseolus vulgaris cv. Pinto and sourdough lactic acid bacteria during bread storage. *Applied and Environmental Microbiology, 74*, 7391e7398. https://doi.org/10.1128/AEM.01420-08

Collins, Y. F., McSweeney, P. L. H., & Wilkinson, M. G. (2003). Lipolysis and free fatty acid catabolism in cheese: A review of current knowledge. *International Dairy Journal, 13*(11), 841–866. https://doi.org/10.1016/S0958-6946(03)00109-2

Copetti, M. V. (2019a). Fungi as industrial producers of food ingredients. *Current Opinion in Food Science, 25*, 52–56. https://doi.org/10.1016/j.cofs.2019.02.006

Copetti, M. V. (2019b). Yeasts and molds in fermented food production: An ancient bioprocess. *Current Opinion in Food Science, 25*, 57–61. https://doi.org/10.1016/j.cofs.2019.02.014

Coton, E., Coton, M., Hymery, N., Mounier, J., & Jany, J.-L. (2020). Penicillium roqueforti: An overview of its genetics, physiology, metabolism and biotechnological applications. *Fungal Biology Reviews, 34*(2), 59–73. https://doi.org/10.1016/j.fbr.2020.03.001

Dantigny, P., & Nanguy, S. P.-M. (2009). Significance of the physiological state of fungal spores. *International Journal of Food Microbiology, 134*(1–2), 16–20. https://doi.org/10.1016/j.ijfoodmicro.2009.02.005

Desmasures, N. (2014). Mold-ripened varieties. *Encyclopedia of Food Microbiology, 2*, 409–416.

Diezhandino, I., Fernández, D., González, L., McSweeney, P. L. H., & Fresno, J. M. (2015). Microbiological, physico-chemical and proteolytic changes in a Spanish blue cheese during ripening (Valdeón cheese). *Food Chemistry, 168*, 134–141. https://doi.org/10.1016/j.foodchem.2014.07.039

Diezhandino, I., Fernández, D., Sacristán, N., Combarros-Fuertes, P., Prieto, B., & Fresno, J. M. (2016). Rheological, textural, colour and sensory characteristics of a Spanish blue cheese (Valdeón cheese). *Lebensmittel-Wissenschaft und -Technologie-Food Science and Technology, 65*, 1118–1125. https://doi.org/10.1016/j.lwt.2015.10.003

Dumas, E., Feurtey, A., Rodriguez de la Vega, R., Le Prieur, S., Snirc, A., Coton, M., et al. (2020). Independent domestication events in the blue-cheese fungus Penicillium roqueforti. *Molecular Ecology, 29*(14), 2639–2660. https://doi.org/10.1111/mec.15359

Fernández-Bodega, M. A., Mauriz, E., Gómez, A., & Martín, J. F. (2009). Proteolytic activity, mycotoxins and andrastin A in Penicillium roqueforti strains isolated from Cabrales, Valdeón and Bejes–Tresviso local varieties of blue-veined cheeses. *International Journal of Food Microbiology, 136*(1), 18–25. https://doi.org/10.1016/j.ijfoodmicro.2009.09.014

Garnier, L., Valence, F., & Mounier, J. (2017). Diversity and control of spoilage fungi in dairy products: An update. *Microorganisms, 5*(3), 42. https://doi.org/10.3390/microorganisms5030042

Geisen, R. (1993). Fungal starter cultures for fermented foods: Molecular aspects. *Trends in Food Science & Technology, 4*(8), 251–256. https://doi.org/10.1016/0924-2244(93)90140-6

Geoffry, K., & Achur, R. N. (2018). Screening and production of lipase from fungal organisms. *Biocatalysis and Agricultural Biotechnology, 14*, 241–253. https://doi.org/10.1016/j.bcab.2018.03.009

Ghorai, S., Banik, S. P., Verma, D., Chowdhury, S., Mukherjee, S., & Khowala, S. (2009). Fungal biotechnology in food and feed processing. *Food Research International, 42*(5–6), 577–587. https://doi.org/10.1016/j.foodres.2009.02.019

Gillot, G., Jany, J.-L., Coton, M., Le Floch, G., Debaets, S., Ropars, J., … Coton, E. (2015). Insights into Penicillium roqueforti morphological and genetic diversity. *PloS One, 10*(6), Article e0129849. https://doi.org/10.1371/journal.pone.0129849

Gillot, G., Jany, J.-L., Poirier, E., Maillard, M.-B., Debaets, S., Thierry, A., … Coton, M. (2017). Functional diversity within the Penicillium roqueforti species. *International Journal of Food Microbiology, 241*, 141–150. https://doi.org/10.1016/j.ijfoodmicro.2016.10.001

Glass, N. L., & Donaldson, G. C. (1995). Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology, 61*(4), 1323–1330. https://doi.org/10.1128/AEM.61.4.1323-1330.1995

Haba, E., Bresco, O., Ferrer, C., Marques, A., Busquets, M., & Manresa, A. (2000). Isolation of lipase-secreting bacteria by deploying used frying oil as selective substrate. *Enzyme and Microbial Technology, 26*(1), 40–44. https://doi.org/10.1016/S0141-0229(99)00125-8

Hamlyn, P. F., Wales, D. S., & Sagar, B. F. (1987). Extracellular enzymes of *Penicillium*. In J. F. Peberdy (Ed.), *Penicillium and acremonium. Biotechnology handbooks* (Vol. 1). Boston, MA: Springer. https://doi.org/10.1007/978-1-4899-1986-1_7.

Hasan, F., Shah, A. A., & Hameed, A. (2009). Methods for detection and characterization of lipases: A comprehensive review. *Biotechnology Advances, 27*(6), 782–798. https://doi.org/10.1016/j.biotechadv.2009.06.001

Hudzicki, J. (2009). Kirby-Bauer disk diffusion susceptibility test protocol. Retrieved from https://www.asmscience.org/docserver/fulltext/education/protocol/protocol.3189.pdf?expires=1614717616&id=id&accname=guest&checksum=0142F46334A025139D5348A605609B26. (Accessed 2 March 2021).

Irlinger, F., Helinck, S., & Jany, J. L. (2017). Secondary and adjunct cultures. In P. L. H. McSweeney, P. F. Fox, P. D. Cotter, & D. W. Everett (Eds.), *Cheese.* (pp. 273–300). San Diego, CA: Academic Press. https://doi.org/10.1016/B978-0-12-417012-4.00011-9.

Ismaiel, A. A., Ahmed, A. S., & El-Sayed, E.-S. R. (2014). Optimization of submerged fermentation conditions for immunosuppressant mycophenolic acid production by Penicillium roqueforti isolated from blue-molded cheeses: Enhanced production by ultraviolet and gamma irradiation. *World Journal of Microbiology and Biotechnology, 30*(10), 2625–2638. https://doi.org/10.1007/s11274-014-1685-1

ISO 11133. (2014). Microbiology of food, animal feed and water — preparation, production, storage and performance testing of culture media. Retrieved from https://www.iso.org/standard/53610.html. (Accessed 2 March 2021).

Kalai, S., Anzala, L., Bensoussan, M., & Dantigny, P. (2017). Modelling the effect of temperature, pH, water activity, and organic acids on the germination time of Penicillium camemberti and Penicillium roqueforti conidia. *International Journal of Food Microbiology, 240*, 124–130. https://doi.org/10.1016/j.ijfoodmicro.2016.03.024

Keay, L., & Wildi, B. S. (1970). Proteases of the genus Bacillus. I. Neutral proteases. *Biotechnology and Bioengineering, 12*(2), 179–212. https://doi.org/10.1002/bit.260120205

Khattab, A. R., Guirguis, H. A., Tawfik, S. M., & Farag, M. A. (2019). Cheese ripening: A review on modern technologies towards flavor enhancement, process acceleration and improved quality assessment. *Trends in Food Science & Technology, 88*, 343–360. https://doi.org/10.1016/j.tifs.2019.03.009

Khemariya, P., Singh, S., Nath, G., & Gulati, A. K. (2013). Isolation, identification and antibiotic susceptibility of nis+ Lactococcus lactis from dairy and non-dairy sources. *Czech Journal of Food Sciences, 31*(4), 323–331.

Kinsella, J. E., Hwang, D. H., & Dwivedi, B. (1976). Enzymes of Penicillium roqueforti involved in the biosynthesis of cheese flavor. *CRC Critical Reviews in Food Science & Nutrition, 8*(2), 191–228. https://doi.org/10.1080/10408397609527222

Klich, M. A. (2002). *Identification of common Aspergillus species*. CBS.

Konkit, M., & Kim, W. (2016). Activities of amylase, proteinase, and lipase enzymes from Lactococcus chungangensis and its application in dairy products. *Journal of Dairy Science, 99*(7), 4999–5007. https://doi.org/10.3168/jds.2016-11002

Kure, C. F., & Skaar, I. (2019). The fungal problem in cheese industry. *Current Opinion in Food Science, 29*, 14–19. https://doi.org/10.1016/j.cofs.2019.07.003

Kırtıl, H. E., Metin, B., & Arıcı, M. (2020). Taxonomy, morphological, genetic and metabolic characteristics of Penicillium roqueforti as A cheese mold. *Gıda, 45*(6), 1188–1200. https://doi.org/10.15237/gida.GD20091

Leyva Salas, M., Mounier, J., Valence, F., Coton, M., Thierry, A., & Coton, E. (2017). Antifungal microbial agents for food biopreservation—a review. *Microorganisms, 5*(3), 37. https://doi.org/10.3390/microorganisms5030037

Li, Y., Wadsö, L., & Larsson, L. (2009). Impact of temperature on growth and metabolic efficiency of Penicillium roqueforti- correlations between produced heat, ergosterol content and biomass. *Journal of Applied Microbiology, 106*(5), 1494–1501. https://doi.org/10.1111/j.1365-2672.2008.04110.x

Li, N., & Zong, M.-H. (2010). Lipases from the genus Penicillium: Production, purification, characterization and applications. *Journal of Molecular Catalysis B: Enzymatic, 66*(1–2), 43–54. https://doi.org/10.1016/j.molcatb.2010.05.004

Mane, A., Ciocia, F., Beck, T. K., Lillevang, S. K., & McSweeney, P. L. H. (2019). Proteolysis in Danish blue cheese during ripening. *International Dairy Journal, 97*, 191–200. https://doi.org/10.1016/j.idairyj.2019.05.017

McSweeney, P. L., Ottogalli, G., & Fox, P. F. (2017). Diversity and classification of cheese varieties: An overview. In P. L. H. McSweeney, P. F. Fox, P. D. Cotter, & D. W. Everett (Eds.), *Cheese: Chemistry, physics and microbiology* (pp. 781–808). San Diego, CA: Academic Press. https://doi.org/10.1016/B978-0-12-417012-4.00031-4.

Mioso, R., Toledo Marante, F. J., & Herrera Bravo de Laguna, I. (2015). Chemical constituents of the fermentation broth of the marine-derived fungus Penicillium

roqueforti. *Revista Iberoamericana De Micologia, 32*(3), 147–152. https://doi.org/10.1016/j.riam.2014.01.004

Molimard, P., & Spinnler, H. E. (1996). Review: Compounds involved in the flavor of surface mold-ripened cheeses: Origins and properties. *Journal of Dairy Science, 79*(2), 169–184. https://doi.org/10.3168/jds.S0022-0302(96)76348-8

Møller, K., Sharif, M. Z., & Olsson, L. (2004). Production of fungal α-amylase by Saccharomyces kluyveri in glucose-limited cultivations. *Journal of Biotechnology, 111*(3), 311–318. https://doi.org/10.1016/j.jbiotec.2004.04.013

Muhiddin, N. H., Yanti, N. A., & Asni, N. (2018). Antibacterial activity of mold isolate from "wikau maombo" based on incubation period. *Journal of Physics: Conference Series, 1028*, Article 012017. https://doi.org/10.1088/1742-6596/1028/1/012017

Ndlovu, T., van Jaarsveld, F., & Caleb, O. J. (2019). French and Mediterranean-style diets: Contradictions, misconceptions and scientific facts-A review. *Food Research International, 116*, 840–858. https://doi.org/10.1016/j.foodres.2018.09.020

Ozturkoglu-Budak, S., Wiebenga, A., Bron, P. A., & de Vries, R. P. (2016). Protease and lipase activities of fungal and bacterial strains derived from an artisanal raw Ewe's milk cheese. *International Journal of Food Microbiology, 237*, 17–27. https://doi.org/10.1016/j.ijfoodmicro.2016.08.007

Palacios-Cabrera, H., Taniwaki, M. H., Hashimoto, J. M., & Menezes, H. C. D. (2005). Growth of Aspergillus ochraceus, A. carbonarius and A. Niger on culture media at different water activities and temperatures. *Brazilian Journal of Microbiology, 36*(1), 24–28. https://doi.org/10.1590/S1517-83822005000100005

Pereira, C. I., Crespo, M. B., & San Romao, M. V. (2001). Evidence for proteolytic activity and biogenic amines production in Lactobacillus curvatus and L. homohiochii. *International Journal of Food Microbiology, 68*(3), 211–216. https://doi.org/10.1016/S0168-1605(01)00534-7

Pereira, G. V. D. M., Neto, D. P. D. C., Junqueira, A. C. D. O., Karp, S. G., Letti, L. A. J., Júnior, A. I. M., et al. (2019). Review of selection criteria for starter culture development in the food made from ovine milk. *Fermentation Industry. Food Reviews International, 36*, 135–167. https://doi.org/10.1080/87559129.2019.1630636

Pitt, J., & Hocking, A. (2009). *Fungi and food spoilage* (3rd ed.). London: Blackie Academic and Professional. https://doi.org/10.1007/978-0-387-92207-2

Punt, M., van den Brule, T., Teertstra, W. R., Dijksterhuis, J., den Besten, H. M. W., Ohm, R. A., et al. (2020). Impact of maturation and growth temperature on cell-size distribution, heat-resistance, compatible solute composition and transcription profiles of Penicillium roqueforti conidia. *Food Research International, 136*, 109287. https://doi.org/10.1016/j.foodres.2020.109287

Raper, K. B., & Thom, C. (1949). *A manual of the Penicillia*. Baltimore: The Williams and Wilkins Co.

Roostita, R., & Fleet, G. H. (1996). The occurrence and growth of yeasts in Camembert and blue-veined cheeses. *International Journal of Food Microbiology, 28*(3), 393–404. https://doi.org/10.1016/0168-1605(95)00018-6

Ropars, J., Lopez-Villavicencio, M., Dupont, J., Snirc, A., Gillot, G., Coton, M., et al. (2014). Induction of sexual reproduction and genetic diversity in the cheese fungus Penicillium roqueforti. *Evolutionary Applications, 7*(4), 433–441. https://doi.org/10.1111/eva.12140

Ruiz Rodríguez, L., Vera Pingitore, E., Rollan, G., Cocconcelli, P. S., Fontana, C., Saavedra, L., et al. (2016). Biodiversity and technological-functional potential of lactic acid bacteria isolated from spontaneously fermented quinoa sourdoughs. *Journal of Applied Microbiology, 120*(5), 1289–1301. https://doi.org/10.1111/jam.13104

Samancta, E. C. (2012). EC Sampling procedure cards – SAM-110 Cheese sampling. Retrieved from https://ec.europa.eu/taxation_customs/dds2/SAMANCTA/EN/SamplingProcedure/FoodstuffCheese_EN.htm Accessed November 12, 20212.

Samson, R. A., Houbraken, J., Thrane, U., Frisvad, J. C., & Andersen, B. (2019). *Food and indoor fungi*. Centraalbureau voor Schimmelcultures (Chapter 3).

Saxena, R. K., Gupta, R., Saxena, S., & Gulati, R. (2001). Role of fungal enzymes in food processing. *Agriculture and Food Production,* 353–386. https://doi.org/10.1016/S1874-5334(01)80015-0

Silverman, G. J., & Kosikowski, F. V. (1955). Tyrosine in cheddar cheese. *Journal of Dairy Science, 38*(9), 950–958. https://doi.org/10.3168/jds.S0022-0302(55)95063-3

Spinnler, H. E. (2017). Surface mold–ripened cheeses. In P. L. H. McSweeney, P. F. Fox, P. D. Cotter, & D. W. Everett (Eds.), *Cheese.* (pp. 911–928). San Diego, CA: Academic Press. https://doi.org/10.1016/B978-0-12-417012-4.00036-3.

Steensels, J., Gallone, B., Voordeckers, K., & Verstrepen, K. J. (2019). Domestication of industrial microbes. *Current Biology, 29*(10), R381–R393. https://doi.org/10.1016/j.cub.2019.04.025

Tiwari, K. L., Jadhav, S. K., & Kumar, A. (2011). Morphological and molecular study of different Penicillium species. *Middle-East Journal of Scientific Research, 7*(2), 203–210.

Todorov, S. D., & Dicks, L. M. T. (2006). Screening for bacteriocin-producing lactic acid bacteria from boza, a traditional cereal beverage from Bulgaria: Comparison of the bacteriocins. *Process Biochemistry, 41*(1), 11–19. https://doi.org/10.1016/j.chroma.2013.11.059

Valerio, F., Favilla, M., De Bellis, P., Sisto, A., de Candia, S., & Lavermicocca, P. (2009). Antifungal activity of strains of lactic acid bacteria isolated from a semolina ecosystem against Penicillium roqueforti, Aspergillus Niger and Endomyces fibuliger contaminating bakery products. *Systematic & Applied Microbiology, 32*(6), 438–448. https://doi.org/10.1016/j.syapm.2009.01.004

Veljovic, K., Terzic-Vidojevic, A., Vukasinovic, M., Strahinic, I., Begovic, J., Lozo, J., & Topisirovic, L. (2007). Preliminary characterization of lactic acid bacteria isolated from Zlatar cheese. *Journal of Applied Microbiology, 103*(6), 2142–2152. https://doi.org/10.1111/j.1365-2672.2007.03450.x

Welsh, C. T. (2017). *Microbiology: A laboratory manual* (Global Edition). Pearson Education Limited (Part 5).

Wilkowske, H. H., & Krienke, W. A. (1954). Assay of various mold-ripened cheeses for antibiotic activity. *Journal of Dairy Science, 37*(10), 1184–1189. https://doi.org/10.3168/jds.S0022-0302(54)91388-0