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A rapid spectroscopic method for the identification of the filamentous fungi isolated from Turkish traditional mold-ripened cheeses

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ABSTRACT

Fourier transform infrared spectroscopy (FTIR) is an alternative microbial identification technique due to its faster analysis times and lower cost compared to molecular methods. In this study, forty-three fungal strains isolated from different Turkish traditional mold-ripened cheeses representing nine different *Penicillium* species (*P. roqueforti*, *P. corylophilum*, *P. before*, *P. crustosum*, *P. spinulosum*, *P. rubens*, *P. brevicompactum*, *P. paneum,* and *P. solitum*) were analyzed by using FTIR HTS-XT (High Throughput Screening Extension) method in the 4000–400 cm^{-1} wavenumber range. The spectra of the isolates were evaluated, and the chemical structures corresponding to the fungus-specific spectral regions were determined as fatty acids (3600–2800 cm^{-1}), amide I and amide II of proteins and peptides (1740–1500 cm^{-1}), polysaccharides (1200–900 cm^{-1}) and carbohydrates (900–600 cm^{-1}). The isolates were grouped according to the hierarchical clustering analysis (HCA) by applying chemometrics combined with FTIR spectroscopy. Results showed that FTIR spectroscopy has a high capability for rapid determination of cheese fungi based on their FTIR spectra.

1. Introduction

Turkish mold-ripened cheeses are generally obtained by ripening in the cool and humid atmosphere of cellars or caves, allowing the growth of filamentous fungi on the cheese surface. Erzurum Kuflu Civil (Chechil), Kars Chechil, Konya Kuflu Tulum (Gok), Karaman Divle Cave, Hatay Kuflu Surk, Elazig-Bingol Tomas, Mediterranean Tulum are among the main these cheeses (Cakmakci et al., 2012; Gunduz, 1982; Ozkalp and Durak, 1998; Hayaloglu and Kirbag, 2007; Yalman et al., 2016; Kirtil et al., 2021).

Penicillium roqueforti is the dominant filamentous fungal species in mold-ripened cheeses. In addition to *Geotrichum candidum* in some varieties, other *Penicillium* spp., *Fusarium* spp., *Thamnidium* spp., and *Aspergillus* spp. may also be involved in the cheese mycobiota. Especially, the other *Penicillium* species such as *P. biforme, P. chrysogenum, P. rubens, P. polonicum, P. crustosum* come into prominence (Parente and Cogan, 2004; Cakmakci et al., 2012; Fox et al., 2016; Ozturkoglu-Budak et al., 2016; Metin, 2018; Kirtil et al., 2021).

Polymerase chain reaction (PCR)-based identification methods could provide reliable and accurate results, but generally, these techniques are time-consuming, laborious, costly, and require experienced staff. In this context, there is a need for fast, effective, and economical techniques that can be used for similar purposes. The intricacy of the PCR process, the high cost of the reagents, the necessity to select particular primers for each species, and the sensitivity of the species to mutations make PCR-based approaches challenging. The ideal method for replacing these time-consuming processes would be rapid, automated, at least reasonably affordable analyses requiring minimal sample preparation and, preferably, analyzing samples directly without reagents (Santos et al., 2010). It is possible to collect good results rapidly and with little experience in FTIR spectroscopy. Results are obtained shortly after single colonies are removed from a microbial cell plate. Although the equipment is pricey for the spectroscopic techniques, the routine operation is inexpensive compared to genotaxonomic systems identifying microorganisms (Alvarez-Ordóñez and Prieto, 2012). Compared to the PCR-based methods, the spectroscopic techniques' per-sample assay cost is considerably lower (less than $3 per sample) (Singh et al., 2020). FTIR spectroscopy provides the opportunity to obtain biochemical fingerprinting data that reveal the molecular structure and composition in a non-destructive and rapid manner. The FTIR spectroscopy's organism fingerprints are incredibly accurate and show the sample's chemical composition. Because it is a non-destructive technique, it is performed

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without damaging the integrity or any sample alteration. When working with delicate and small sample sizes, the non-destructive characteristics of FTIR spectroscopy are very appealing (Wenning and Scherer, 2013; Barnes et al., 2023). Since the 1950's, infrared spectroscopy has been used to distinguish microorganisms (Sivakesava and Irudayaraj, 2001). Although the investment made for the FTIR system is costly, routine studies can be performed much more economically than the genotaxonomic systems used to identify microorganisms. It also requires minimal sample preparation and small amounts of sample (less than a few milligrams) for analysis (Alvarez-Ordóñez and Prieto, 2012; Wenning and Scherer, 2013). FTIR technique can be used as a highly compatible tool in the taxonomic classification of microbial groups. Classification analyses can be carried out at very different taxonomic levels. When the FTIR technique is applied together with chemometrics, classification can be achieved successfully at the genus, species, and strain level (Alvarez-Ordóñez and Prieto, 2012).

While most of the studies on the identification of microorganisms using FTIR are about bacteria or yeasts, filamentous fungi have been included as a research subject in fewer studies (Dziuba et al., 2007; Grewal et al., 2015; Arici et al., 2018; Farouk et al., 2022). A limited number of studies have shown that the technique can be successfully applied for the identification of fungi as well (Erukhimovitch et al., 2010; Shapaval et al., 2010, 2013; Lecellier et al., 2014, 2015; Fantin et al., 2022).

In a study, a method to distinguish *Aspergillus* and *Penicillium* species (*P. expansum, P. roqueforti, P. crustosum, P. brevicompactum,* and *P.verrucosum*) was developed that would form a basis for characterizing microfungi (Fischer et al., 2006). In this study, only 5 *Penicillium* species were used. FTIR technique was used successfully as an alternative to molecular techniques, different *Aspergillus* species, such as *A. ochraceus* and *A. westerdijkiae* (Tralamazza et al., 2013), and *A. flavus, A. fumigatus,* and *A. parasiticus* (Garon et al., 2010). A significant development for the identification of 486 fungal strains (43 genera and 140 species), including 15 *Penicillium* species using the FTIR technique was presented by Lecellier et al. (2015), which involved PLS-DA (partial least squares method and differential analysis) chemometric combined with FTIR HTS-XT technique. Using this analysis, 92.3% identification was achieved at the species level (Lecellier et al., 2015).

In this study, FTIR spectra of forty-three fungal strains previously isolated from various Turkish traditional mold-ripened cheeses and identified by ITS or *benA* were analyzed by using the FTIR HTS-XT (High Throughput Screening Extension) method combined with HCA (hierarchical clustering analysis) as a chemometric technique.

2. Material and methods

2.1. Fungal isolates

In this study, 43 isolates selected from a culture collection harboring 169 fungal strains previously isolated from various traditional Turkish mold-ripened cheeses (Culture Collections of Yildiz Technical University and Istanbul Sabahattin Zaim University) were used (Kirtil et al., 2021; Kirtil et al., 2022; Seri and Metin, 2021). The culture stocks were refreshed in 40% glycerol and were kept at −80 °C for long-term storage (Kirtil et al., 2021).

2.2. Genomic DNA isolation

Genomic DNA from the isolates was purified using the phenol-chloroform-isoamyl alcohol (PCI) method, according to Kirtil et al. (2021). The purity (ratio of 260/280 nm) and the quantity (260 nm) of the purified DNA were determined using a BioSpec Nano-spectrophotometer (Shimadzu, Kyoto, Japan).

2.3. PCR and sequencing

Forty-three fungal isolates were identified by ITS or *benA* sequencing (Metin, 2020; Kirtil et al., 2021). The PCR mix was prepared, and the PCR conditions were performed as given in Kirtil et al. (2021). PCR amplicons were run using the OWL A2 gel electrophoresis system (Thermo Fisher Scientific, St. Louis, MO, USA) at 100 *V* for 50 min on a 1% agarose gel with EtBr staining solution and visualized using the Gel Doc EZ imaging system (Bio-Rad Laboratories, Hercules, CA, USA). PCR amplicons were purified according to the instructions of the Vivantis GF-1 PCR purification kit (Vivantis, Malaysia) and submitted for sequence analysis to a commercial company (MedsanTek, Istanbul, Turkey). The sequences were analyzed using NCBI BLAST.

2.4. Phylogenetic analysis

The phylogenetic tree was created in the MEGA X software (version 10.1) (Kumar et al., 2018) using an alignment of the *benA* sequences of reference (type) strains: *Penicillium solitum* CBS 424.89 (MN969398.1), *Penicillium biforme* CBS 297.48 (MN969373.1), *Penicillium crustosum* CBS 115503 (MN969379.1), *Penicillium paneum* CBS 101032 (KM503670.1), *Penicillium roqueforti* CBS 221.30 (MN969396.1), *Penicillium rubens* ATCC 9178 (KU897012.1), *Penicillium brevicompactum* NRLL 2011 (DQ645784.1), *Penicillium spinulosum* CBS 374.48 (KJ834493.1), *Penicillium corylophilum* CBS 312.48 (JX141042.1). *Aspergillus niger* NRLL 326 (EF661089.1) was used as the outgroup. The evolutionary history was inferred using the Maximum Likelihood method and the Kimura 2-parameter model, the best-fit model determined with 1000 bootstrap replicates (Kimura, 1980).

2.5. FTIR spectroscopy

2.5.1. Sample preparation

Stock cultures were cultivated on potato dextrose agar (Merck, Germany) at 25 °C for five days. The spores were collected with a sterile loop and were suspended in 300 μL of 0.9% NaCl to obtain homogeneous suspensions in microcentrifuge tubes before FTIR analysis (Lecellier et al., 2015; Oberle et al., 2015; Dzurendova et al., 2020). For each strain, three independent cultures were prepared on three different days (biological replications).

2.5.2. Spectral acquisition

FTIR measurements were carried out using a High Throughput Screening Extension (HTS-XT) unit coupled to a Tensor 27 spectrometer (Bruker Optics GmbH, Ettlingen, Germany). Twenty-five microliters of the spore suspensions were transferred to a 96-well ZnSe plate. Measurements were performed in two replicates to verify repeatability. The ZnSe plate was dried in the incubator at 40 °C for 45 min (Lecellier et al., 2015; Arici et al., 2018). The measurements were performed in 64 accumulations, 4 cm^{-1} resolution values, and 4000–400 cm^{-1} wavelength ranges. Before each measurement, the background was measured using an empty well on the plate. Each measurement was repeated twice for each sample.

2.5.3. Chemometrics and HCA

Hierarchical cluster analysis (HCA) was performed using OPUS software (version 7.2). In chemometric analysis, the Ward algorithm was applied with the “normal to reprolevel” distance. Spectral ranges of 1767–1589 cm^{-1}, 1472–1269 cm^{-1}, 1171–999 cm^{-1}, and 941–714 cm^{-1} were preferred to distinguish the fungal strains (Fischer et al., 2006). The weighting factor was chosen as “1” in the HCA analysis for each spectral region.

3. Results

3.1. The diversity of fungal isolates and the phylogenetic analysis

The 43 isolates used consisted of 9 *Penicillium* species as *P. roqueforti* (21 isolates, 48.8%), *P. corylophilum* (6 isolates, 14%), *P. biforme* (5 isolates, 11.6%), *P. crustosum* (3 isolates, 7%), *P. spinulosum* (3 isolates, 7%), *P. rubens* (2 isolates, 4.7%), *P. brevicompactum* (1 isolate, 2.3%), *P. paneum* (1 isolate, 2.3%) and *P. solitum* (1 isolate, 2.3%) (Table 1). To compare the molecular identification with the results of FTIR spectroscopy, the phylogenetic tree in Fig. 1 was constructed using the *benA* gene sequences of the reference (type) strains of these species obtained from NCBI to represent each *Penicillium* species. The reference strains were selected from the American Type Culture Collection (ATCC), Agricultural Research Service (ARC) Culture Collection (NRRL), and the Centraalbureau voor Schimmelcultures (CBS) Collection. Although the ribosomal RNA (rRNA) and/or internal transcribed spacer (ITS) regions are the most widely used markers for fungi, these are not discriminative for some *Penicillium* species (Houbraken et al., 2010; Visagie et al., 2014). The *benA* gene is more variable than the rRNA and/or ITS regions; therefore, for distinguishing closely related species within the *Penicillium* genus, *benA* is the preferred marker (Houbraken et al., 2010; Visagie et al., 2014). This tree showed the relationship of *Penicillium* species with each other and their compatibility with the FTIR dendogram.

3.2. Interpretation of fungal species spectrum

FTIR spectra of different fungal strains were classified based on FTIR fingerprint spectra using hierarchical clustering analysis (HCA). Since the interpretation of the infrared spectrum of a microorganism creates a more complex structure than a chemical component, four infrared spectrum regions were used in the identification and classification processes of fungal samples. The spectral regions selected for classification contain chemical information in the form of fingerprints specific to fungal species. In this classification process, Ward's algorithm and primary derivative applications were used to provide the most specific and descriptive classification. This analysis showed the similarity relationship between spectra graphically on the dendogram with a hierarchical clustering analysis. Thanks to the obtained dendogram, grouping strains belonging to different species was possible according to similarity relations. Fig. 2 presents FTIR spectra of different mold strains and chemical structures corresponding to the spectral regions. The regions were fatty acids (3600–2800 cm^{-1}), amide I and amide II of proteins and peptides (1740–1500 cm^{-1}), polysaccharides (1200–900 cm^{-1}), and carbohydrates (900–600 cm^{-1}) bands due to spectral properties of the fungal strains originating from stress vibrations.

Fourier transform infrared spectroscopy has become valuable and

Table 1
Molecular identification results of the isolates from Turkish traditional mold-ripened cheeses.

Isolate Code	ITS Sequencing	*benA* Sequencing	Isolation Source	References
IZU1K (1K4)		*Penicillium solitum*	Rize-Ardesen Golot Cheese	Metin (2020)
YTU10 (1Y5D)	*Penicillium roqueforti*		Konya Kuflu Tulum Cheese-1	Kirtil et al. (2021)
YTU11 (2Y5F)	*Penicillium roqueforti*		Konya Kuflu Tulum Cheese −2	Kirtil et al. (2021)
YTU12 (3Y5A)	*Penicillium roqueforti*		Konya Kuflu Tulum Cheese −3	Kirtil et al. (2021)
YTU13 (4Y5A)	*Penicillium roqueforti*		Konya Kuflu Tulum Cheese-4	Kirtil et al. (2021)
YTU14 (4Y5B)	*Penicillium roqueforti*		Konya Kuflu Tulum Cheese −4	Kirtil et al. (2021)
YTU15 (4Y5D)	*Penicillium crustosum*		Konya Kuflu Tulum Cheese-4	Kirtil et al. (2021)
YTU16 (5B)	*Penicillium biforme*		Other*	Kirtil et al. (2021)
YTU17 (5Y5A)	*Penicillium roqueforti*		Konya Kuflu Tulum Cheese-5	Kirtil et al. (2021)
YTU18 (5Y5C)	*Penicillium crustosum*		Konya Kuflu Tulum Cheese-5	Kirtil et al. (2021)
YTU19 (6)	*Penicillium corylophilum*		Other*	Kirtil et al. (2021)
YTU20 (6Y5B)	*Penicillium roqueforti*		Konya Kuflu Tulum Cheese-6	Kirtil et al. (2021)
YTU21 (6Y5C)	*Penicillium roqueforti*		Konya Kuflu Tulum Cheese-6	Kirtil et al. (2021)
YTU22 (6Y5E)	*Penicillium roqueforti*		Konya Kuflu Tulum Cheese-6	Kirtil et al. (2021)
YTU23 (7Y5E)	*Penicillium roqueforti*		Konya Kuflu Tulum Cheese-7	Kirtil et al. (2021)
YTU24 (8Y5B)	*Penicillium roqueforti*		Konya Kuflu Tulum Cheese-8	Kirtil et al. (2021)
IZU2K (9CA5C)		*Penicillium paneum*	Erzurum Kuflu Civil Cheese	Kirtil et al. (2022)
YTU26 (9Y5B)	*Penicillium roqueforti*		Konya Kuflu Tulum Cheese-9	Kirtil et al. (2021)
YTU27 (10D)	*Penicillium spinulosum*		Other*	Kirtil et al. (2021)
YTU28 (10Y5C)	*Penicillium roqueforti*		Konya Kuflu Tulum Cheese −10	Kirtil et al. (2021)
YTU29 (11C)	*Penicillium biforme*		Other*	Kirtil et al. (2021)
YTU30 (11Y5B)	*Penicillium roqueforti*		Konya Kuflu Tulum Cheese-11	Kirtil et al. (2021)
YTU31 (12D)	*Penicillium biforme*		Other*	Kirtil et al. (2021)
YTU32 (12F)	*Penicillium roqueforti*		Other*	Kirtil et al. (2021)
YTU33 (12Y5D)	*Penicillium crustosum*		Divle Cave Tulum Cheese-1	Kirtil et al. (2021)
YTU34 (13B)	*Penicillium corylophilum*		Other*	Kirtil et al. (2021)
IZU3K (13N6B)		*Penicillium brevicompactum*	Erzurum Kuflu Civil Cheese	Kirtil et al. (2022)
YTU35 (13Y6E)	*Penicillium rubens*		Divle Cave Tulum Cheese-2	Kirtil et al. (2021)
YTU36 (14Y5B)	*Penicillium roqueforti*		Erzurum Kuflu Civil Cheese-1	Kirtil et al. (2021)
YTU37 (16Y4B)	*Penicillium roqueforti*		Erzurum Kuflu Civil Cheese-3	Kirtil et al. (2021)
YTU38 (17Y5C)	*Penicillium roqueforti*		Divle Cave Tulum Cheese-3	Kirtil et al. (2021)
YTU39 (17Y5D)	*Penicillium roqueforti*		Divle Cave Tulum Cheese-3	Kirtil et al. (2021)
YTU40 (21C)	*Penicillium corylophilum*		Other*	Kirtil et al. (2021)
YTU41 (22E)	*Penicillium biforme*		Other*	Kirtil et al. (2021)
YTU42 (23A)	*Penicillium corylophilum*		Other*	Kirtil et al. (2021)
YTU43 (23B)	*Penicillium corylophilum*		Other*	Kirtil et al. (2021)
YTU44 (23C)	*Penicillium roqueforti*		Other*	Kirtil et al. (2021)
YTU45 (25C)	*Penicillium corylophilum*		Other*	Kirtil et al. (2021)
YTU46 (25D)	*Penicillium rubens*		Other*	Kirtil et al. (2021)
YTU47 (29D)	*Penicillium roqueforti*		Other*	Kirtil et al. (2021)
YTU48 (30C)	*Penicillium spinulosum*		Other*	Kirtil et al. (2021)
YTU49 (30D)	*Penicillium spinulosum*		Other*	Kirtil et al. (2021)
YTU50 (31A)	*Penicillium biforme*		Other*	Kirtil et al. (2021)

* Other: Previously isolated fungal strains from Kars Kuflu Chechil, Elazig-Bingöl Tomas cheese, Hatay Surk cheese and Mediterranean Tulum cheese.

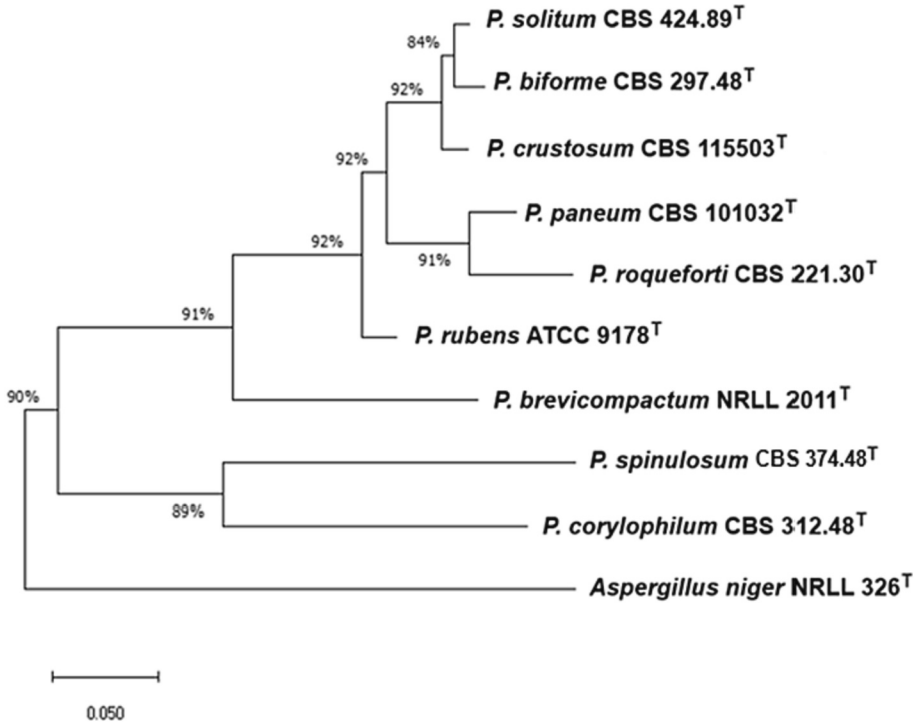

Fig. 1. Phylogenetic tree performed with Maximum Likelihood approach using *benA* gene of type strains belonging to *Penicillium* species in this study

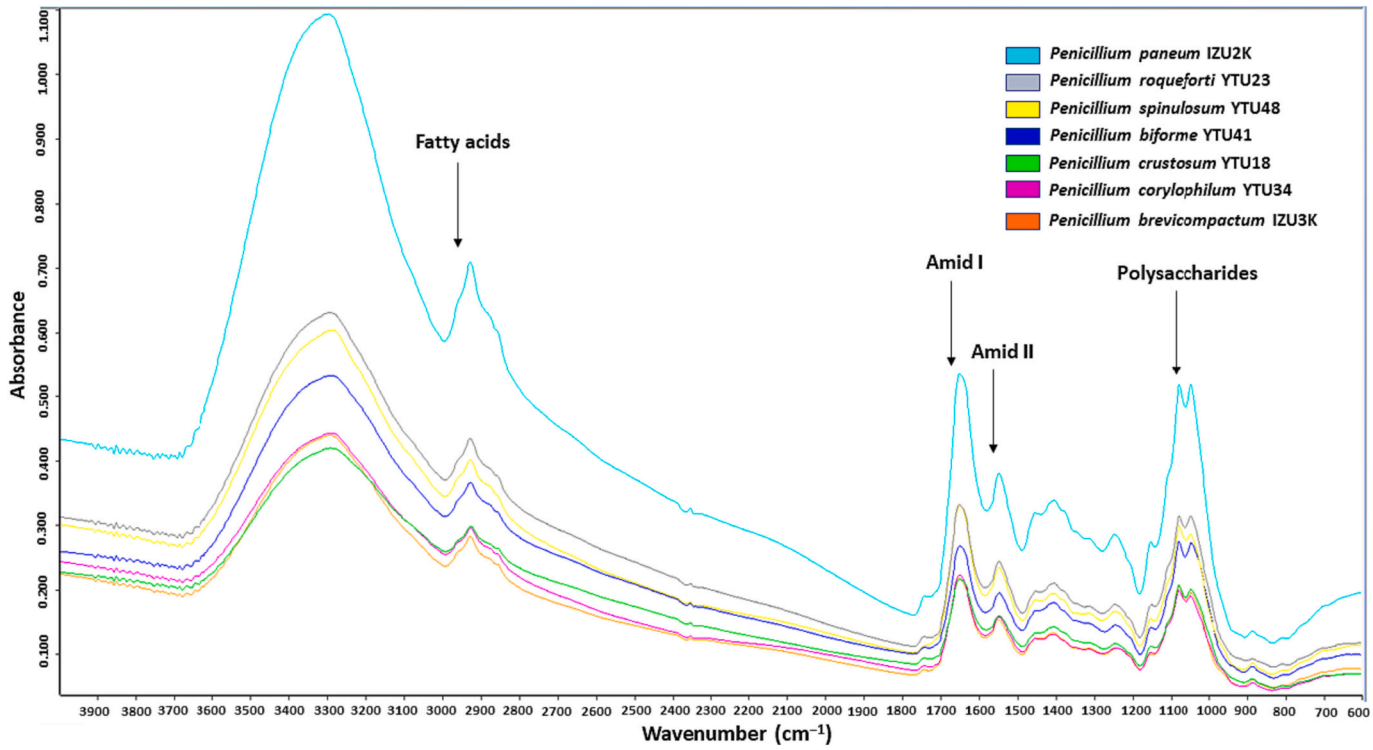

Fig. 2. FTIR spectra for different fungal species (*Penicillium paneum, Penicillium roqueforti, Penicillium spinulosum, Penicillium biforme, Penicillium crustosum, Penicillium corylophilum, Penicillium brevicompactum*). The FTIR spectrum of the isolates yielded major peaks in 3600–2800 cm^{-1} (Fatty acids), 1740–1500 cm^{-1} (Amide-*I* and Amide-II), and 1200–900 cm^{-1} (Polysaccharides) regions.

useful for the fast and accurate identification of microorganisms due to the generally time-consuming nature of traditional identification methods and the difficulty of interpretation (Taha et al., 2013). Although molecular techniques are still used as the gold standard for identifying fungi, they are only partially suitable for many samples due to their cost and complexity. Using the FTIR technique makes it possible to obtain fast and accurate results economically without using expensive reagents or chemicals (Arici et al., 2018). The basic identification principle in infrared spectroscopy is the absorption of IR radiation by the components of the cell under examination, and the generation of a

spectrum as a fingerprint that reflects the entire chemical structure of the cell. The spectrum data must be analyzed to provide an identification system based on the FTIR spectra. Spectral characteristics of microorganisms resulting from CH stretch vibrations in the 3000–2800 cm^{-1} spectral region, from the Amide *I* and Amide II interactions of proteins and peptides in the 1800–1500 cm^{-1} spectral region, resulting from C = O stress vibrations around 1740 cm^{-1}, arising from the stress vibrations of polysaccharides in the 1200–900 cm^{-1} and the other carbohydrates around 900–600 cm^{-1} are observed (Alvarez-Ordóñez and Prieto, 2012). Various chemometric methods are used to benefit from the identification and classification processes from spectral data (Wenning and Scherer, 2013).

3.3. Discrimination and clusterization of mold species

The dendogram obtained using HCA grouped the fungal strains in two main branches (Fig. 3). While the phylogenetically closely related *P. roqueforti* and *P. paneum* clustered together in one branch, the rest of the species grouped in the other cluster. The hierarchical cluster analysis result showed a clear discrimination pattern between species. The dendogram obtained using chemometric analysis together with FTIR spectroscopy separates the fungal species in different clusters successfully in most cases. For example, *P. corylophilum, P. brevicompactum, P. solitum, P. spinulosum*, and *P. rubens* species could be well-separated. However, when the results were compared with the *benA* phylogenetic tree in Fig. 3, *P. paneum* closely related to *P. roqueforti* could not be separated from the *P. roqueforti* group. Only one *P. paneum* strain was used in this study. Repeating the FTIR analysis with more *P. paneum* isolates to be obtained in future studies may be beneficial to make this distinction more accurate. Similarly, one of the *P. biforme* isolates (YTU29) could not be separated from *P. crustosum*, and another *P. biforme* isolate (YTU31) was settled on a separate branch near *P. spinulosum*.

4. Discussion

The current research showed the capability of FTIR spectroscopy combined with chemometrics for the classification of 43 different cheese-originated fungal strains based on their similarities and differences. In the study, standardized methods were used for sample preparation and data acquisition. The hierarchical clustering analysis (HCA) of the fungal strains (Fig. 3) classified the *Penicillium* species based on their FTIR spectra. Specifically, *P. roqueforti* species were distinctly classified apart from the other fungal species on the main right arm of the dendogram. However, one *P. biforme* isolate (IZU2K) was located in a different cluster than the other isolates and another one (YTU29) was clustered with *the P. crustosum* group. Additionally, the discrimination of *P. paneum* from *P. roqueforti* was problematic, which could be attributed to the close phylogenetic relationship (Fig. 1) between the two species. Although FTIR spectroscopy distinguishes phenotypes, identifying closely related species could be difficult due to the morphological similarities and the closeness of biochemical structures (Lecellier et al., 2014). Besides, it may be necessary to use precise discriminant spectral ranges to identify the species included in each *Penicillium* section (Lecellier et al., 2014). Repeating the FTIR analysis with more *P. paneum* isolates may also be helpful for discrimination.

The spectral regions of 3600–2800 cm^{-1}, 1740–1500 cm^{-1}, 1200–900 cm^{-1}, and 900–600 cm^{-1} were used to build a reliable and robust classification pattern of fungal species. The region (900–600 cm^{-1}) is called the true fingerprinting region because it contains specific spectral patterns (Alvarez-Ordóñez and Prieto, 2012). Different fungal species isolated from cheeses were clearly and reliably classified mainly based on fingerprint spectral regions. FTIR-based classification of fungal species was in good agreement with those obtained by molecular identification results, proving the reliability and precision of the FTIR method. There is a need for rapid, reliable, and cost-effective methodologies to ensure the quality of traditional products such as mold-ripened cheeses. As a cost-effective and analytical technique, FTIR spectroscopy promises rapid, uncomplicated, and robust identification of fungal species compared to conventional methodologies. Previous research studies reported the high capability of FTIR spectroscopy combined with chemometrics for the identification of fungal species, and our results are in parallel with those obtained in previous studies (Bastert et al., 1999; Garon et al., 2010; Lecellier et al., 2014). To the best of our knowledge, current research is the first attempt at 2-D visualization of the discrimination pattern of 9 *Penicillium* species (*P. roqueforti*, *P. corylophilum*, *P. biforme*, *P. crustosum*, *P. spinulosum*, *P. rubens*, *P. brevicompactum*, *P. paneum,* and *P. solitum)* isolated from mold-ripened cheeses using the FTIR spectroscopy combined with chemometrics of HCA.

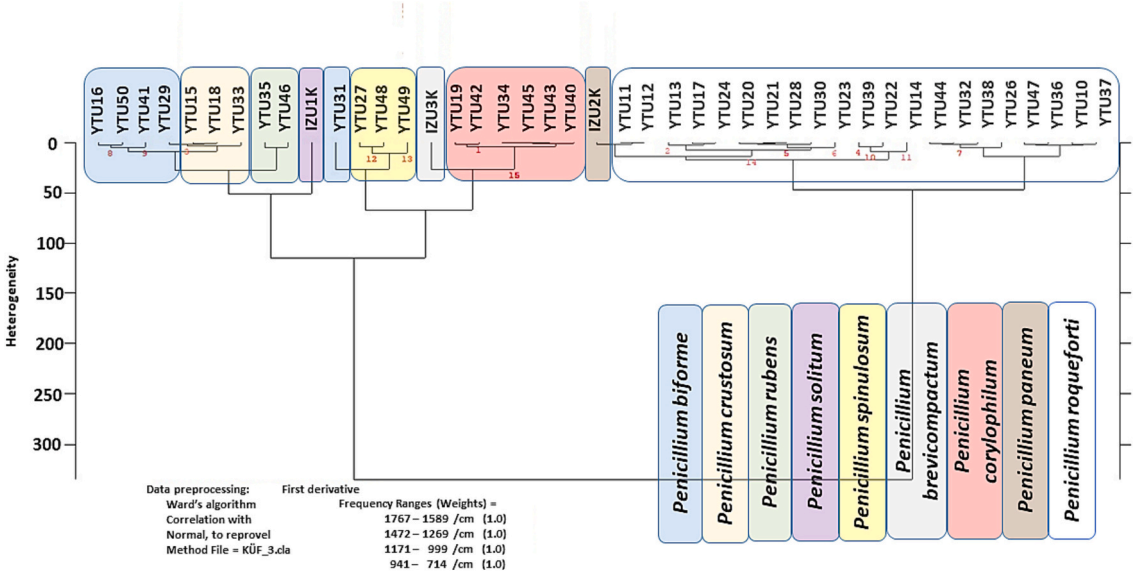

Fig. 3. Hierarchical clustering analysis (HCA) of the fungal strains (Ward's algorithm, spectral regions are 1767–1589 cm^{-1}, 1472–1269 cm^{-1}, 1171–999 cm^{-1}, 941–714 cm^{-1}). The top panel indicates the designation of the fungal strains.

5. Conclusion

The traditional identification of fungal species requires time-consuming and arduous protocols in the food quality control laboratories and food industry. There is a need for rapid, easy, reliable, and reproducible analytical approaches and new methodologies to ensure the quality of foods from farm to fork. In the present study, 43 cheese-originated fungal strains identified by molecular methods representing 9 *Penicillium* species were analyzed by the FTIR HTS-XT method. According to HCA, by applying chemometry with FTIR spectroscopy, most *Penicillium* species could be successfully differentiated by FTIR analysis, except the species *P. roqueforti / P. paneum* and *P. biforme / P. crustosum*. Future studies conducted with more strains from these species may allow better discrimination. In addition, the FTIR library will benefit from being enriched with fungal strains belonging to different genera and species. FTIR can be used to identify cheese fungi as a fast and cost-effective identification technique. Additionally, the current research findings may shed light on further studies that will identify different fungal or bacterial species.

The ethics statement

Ethical approval is not applicable for this article.

The declaration of generative AI and AI-assisted technologies in the writing process

No AI and AI-assisted Technologies were used in the writing process.

CRediT authorship contribution statement

Hatice Ebrar Kirtil: Investigation, Writing – original draft, Formal analysis. **Nur Cebi:** Investigation, Methodology, Writing – original draft, Writing – review & editing. **Rusen Metin Yildirim:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft. **Banu Metin:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. **Muhammet Arici:** Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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