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CONTROL OF SOME TOXIGENIC ASPERGILLI IN SOME CHEESE TYPES USING LACTOBACILLUS PLANTARUM

LAMIAA M. TALAAT AL SHRIEF AND RADWA R.S. REFAIE

Certified Food Lab, Animal Health Research Institute, Agriculture Research Center, Egypt

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ABSTRACT

Microbial contamination in dairy products, particularly cheese, significantly affects both product quality and safety. This study investigated the yeast and mold contamination in two types of cheese: Romi, a hard-ripened cheese, and Kariesh, a fresh soft cheese, providing insights into the microbial dynamics of different cheese varieties. Findings indicated that Kariesh cheese, due to its higher moisture content and favorable pH, exhibited significantly elevated yeast and mold counts compared to Romi cheese. The variability in microbial contamination in Kariesh was notably higher, highlighting its vulnerability to fungal growth, specifically *Aspergillus flavus* and *niger*. In contrast, Romi cheese, with lower moisture and higher salt content, showed more controlled contamination levels. PCR and sequencing confirmed the identity of fungal isolates, offering a molecular basis for understanding fungal diversity in these cheese types. Moreover, the study explored the inhibitory effect of *Lactobacillus plantarum* on fungal growth, with higher concentrations (9 Log_{10} cfu/g) showing more significant inhibition of both *A. flavus* and *A. niger*. However, the antifungal activity diminished over time, suggesting a need for combining probiotics with other preservation methods. These findings underline the importance of optimizing preservation strategies in soft cheeses like Kariesh to extend shelf life and ensure safety.

Keywords: *Toxigenic Aspergilli; cheese; Lactic acid bacteria.*

INTRODUCTION

Fungal contamination poses a significant challenge to the dairy industry, impacting cheese production, storage, and distribution (Massarolo *et al.,* 2024). The proliferation of toxigenic fungi, such as *Aspergillus* and *Penicillium*, within cheese matrices can lead to the secretion of mycotoxins, potent compounds detrimental to human health (Pouris *et al.,* 2024). These Mycotoxins, in conjunction with the hydrolytic enzymes produced by these fungi, contribute to accelerated cheese spoilage, resulting in substantial economic losses for the industry (Massarolo *et al.,* 2024; Owolabi *et al.,* 2024).

Consumption of cheese contaminated with mycotoxins has been associated with a range of adverse health effects, including gastrointestinal disturbances, hepato-toxicity, and immunotoxicity (Ahmed & Beshah, 2024 and Alnuimy, 2024). Given the potential health risks and economic

Corresponding author: Lamiaa M. Talaat Al Shrief
E-mail address: lamiaamohamedtalaat@gmail.com
Present address: Certified Food Lab, Animal Health Research Institute, Agriculture Research Center, Egypt

implications (Adam *et al.,* 2024), the development of effective strategies to mitigate fungal contamination in cheese is imperative (Pouris *et al.,* 2024).

The genus *Lactobacillus* encompasses a diverse group of bacteria known for their beneficial roles in various biotechnological and medical applications (Solis-Balandra & Sanchez-Salas, 2024). These bacteria are integral to the production of fermented foods, including dairy products such as cottage cheese, and are renowned for their probiotic properties (Aleksanyan *et al.,* 2024 and Huidrom *et al.,* 2024). In contrast, filamentous fungi, although essential in many industrial processes, can also pose a threat as spoilage organisms or pathogens. Understanding the interactions between these microorganisms is crucial, particularly in the context of food safety and quality (Pouris *et al.,* 2024 and Huidrom *et al.,* 2024).

To address this critical issue, this study aimed to investigate the incidence of toxigenic fungi in commercially available Romi and Kariesh cheese products in the city of Assiut, Egypt. By isolating and characterizing these fungi, this study aimed to identify the predominant species and assess their potential for mycotoxin production. Furthermore, the research explored the efficacy of lactic acid bacteria (LAB) as a biocontrol agent to mitigate fungal growth and mycotoxin contamination in cheese. LAB, known for their antimicrobial properties, and have been widely studied for their potential application in food preservation (Aleksanyan *et al.,* 2024).

This study will evaluate the inhibitory effects of selected LAB strains against isolated toxigenic fungi through in vitro assays. Subsequently, the protective efficacy of LAB will be assessed in a cheese model system by monitoring fungal growth and mycotoxin levels over time. The findings of this research will contribute to a better understanding of fungal contamination in cheese and provide valuable insights for the development of effective control strategies to ensure the safety and quality of dairy products.

MATERIALS AND METHODS

1. Study Area

The study was conducted in Assiut City, Egypt.

2. Samples Collection

A total of 90 cheese samples (45 Romi and 45 Kariesh) were randomly collected from different retail outlets in Assiut City. Samples were collected aseptically in sterile plastic bags and transported to the laboratory under refrigerated conditions for immediate analysis.

3. Fungal Enumeration and Isolation

Twenty-five grams of each cheese sample were homogenized with 225 ml of sterile peptone water using a stomacher for 2 min. Decimal dilutions were prepared from the homogenates. One milliliter of appropriate dilutions was plated in triplicate on Rose Bengal Chloramphenicol agar and incubated at 25°C for 5 days. Fungal colonies were counted, and results were expressed as colony-forming units (CFU/g). For pure culture isolation: Single colonies of morphologically distinct fungi were sub-cultured on Potato Dextrose Agar (PDA) for purification according to (Banjara *et al.,* 2015).

Macroscopic and microscopic characterization: Isolates were characterized based on colony morphology, microscopic features (conidia, hyphae), and cultural characteristics (Kandasamy *et al.,* 2020).

4. DNA confirmation according to (Arteau, M *et al.,* 2012)

DNA extraction from samples was performed using the QIAamp DNeasy Plant Mini Kit (Qiagen, Germany, GmbH). Briefly, 100 mg of each sample was

homogenized in 400 µl Buffer AP1 containing 4 µl RNase A (100 mg/ml) using a Tissue Lyser with tungsten carbide beads for two 1-2 minute intervals at 20-30 Hz. The lysate was incubated at 65°C for 10 minutes, followed by the addition of 130 µl Buffer P3 and incubation on ice for 5 minutes. After centrifugation, the supernatant was transferred to a QIAshredder Mini spin column and centrifuged again. The flow-through was applied to a silica column, washed according to the manufacturer's protocol, and DNA was eluted in 50 µl elution buffer.

PCR Amplification

Primers targeting the ITS region (Table I) were used for PCR amplification. The reaction mixture consisted of 12.5 µl EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer (20 pmol), 4.5 µl water, and 6 µl DNA template. Amplification was performed using an Applied Biosystems 2720 thermal cycler with the conditions outlined in Table 1. PCR products were separated on a 1.5% agarose gel stained with ethidium bromide and visualized using a gel documentation system.

Table I: Primer sequences, target gene, amplicon size, and PCR conditions.

Target gene	Primers	Amplified segment (bp)	PCR Conditions	Reference
ITS	ITS1: TCCGTAGGTGAACCTG CGG ITS4: TCC TCC GCT TAT TGA TAT GC	Variable	94°C for 5 min; 35 cycles of 94°C for 30 sec, 56°C for 40 sec, 72°C for 45 sec; final extension at 72°C for 10 min	Tarini *et al.,* 2010

Sequence Analysis (White *et al.,* 1990)

1. **PCR product purification:** Purify the PCR product using a purification kit.
2. **Sequencing reaction:** Prepare a sequencing reaction using the purified PCR product, sequencing primer, and sequencing reagents.
3. **Sequencing:** Perform sequencing using a DNA sequencer.
4. **Sequence analysis:** Compare the obtained sequences to reference sequences of *A. flavus* and *A. niger* in a database like NCBI GenBank using BLAST.

Additional Considerations

- **Positive and negative controls:** Include positive (known *A. flavus* and *A. niger* DNA) and negative (no template) controls in the PCR reaction.
- **Data analysis:** Use bioinformatics tools to analyze the sequence data and determine the species identity.
- **Quantitative PCR (qPCR):** For quantification of fungal load, consider using qPCR with specific probes for *A. flavus* and *A. niger*.
- **Next-generation sequencing (NGS):** For complex samples or metagenomic analysis, NGS can provide more comprehensive information about the fungal community (Samson *et al.,* 1999)

5. Microbial Strains and Growth Conditions

Lactobacillus plantarum was obtained from the Central Food Safety Lab., Ain Shams University, Egypt., was routinely grown in de Man Rogosa Sharpe (MRS) broth (Oxoid, Basingstoke, UK) at 30°C for 24 hours. Different concentrations of it were prepared by comparing with MacFarland 0.5 to obtain 7, 8, 9, and 10 $\log_{10}$ (Al-Madboly & Abdullah, 2015).

Aspergillus niger, Aspergillus flavus were plated on malt extract agar (Oxoid) and incubated at 24°C for 5 days. A cryopreserved culture was plated on Potato Dextrose Agar (PDA, Oxoid, Basingstoke,

UK) and incubated at 25°C for 5 days. Fungal spore suspensions were then prepared by gently brushing the surface of the plates with a sterile 0.86% NaCl solution (Sigma-Aldrich) containing 0.01% Tween 80 (Sigma-Aldrich) using a sterile swab. The spore suspensions were stored at 4°C for short-term use. The concentration of fungal spores was determined by plating serial dilutions on PDA plates and adjusted to approximately 3 $\log_{10}$ spores/mL. (De Simone *et al.*, 2024).

6. MIC of *L. plantarum* against *Aspergillus niger, Aspergillus flavus*

The overlay method was employed to assess the antifungal activity of LAB strains, following the procedure described by Russo *et al.* (2017). Briefly, 5 µL of bacterial cultures in the mid-exponential phase were spotted on MRS agar plates. After incubating the plates for 24 hours at 30°C, a second layer of medium consisting of 15 mL of Malt Extract soft agar (0.75% agar, Oxoid) supplemented with the fungal spore suspension (1/100 v/v) was poured over the bacterial spots. The plates were then incubated for 2 days at 25°C. The antifungal activity of the *L. plantarum* strains was evaluated based on the presence of inhibition halos around (clear zones) the bacterial colonies (Russo *et al.*, 2017).

7. Experimental Design

Traditionally, Kariesh cheese is made from wormed skimmed buffalo milk (previously pasteurized). Afterward, the following was added as calcium chloride 0.02%, and commercial rennet 0.05%; which is poured directly into special earthenware pots. The skim milk was divided into 7 parts (control negative, without any additives; 2 parts for control positive one of them for *A. flavus* and another one for *A. niger* at 3 $\log_{10}$; 2 parts containing the two fungi at previous concentrations with 8 $\log_{10}$ of *L. planetarium* and the last two parts as previous two parts with 9 $\log_{10}$ of *L. planetarium*) and the curd is transferred onto a mat, which is tied and hung to drain the whey. The process of whey drainage takes two to three days until the cheese achieves the desired texture. Once the cheese has reached the right consistency, it is cut into suitable pieces, salted, and left on the mat for a few more hours until no more whey drains out. The cheese is then ready to be consumed as fresh cheese (Saleh, 2018).

8. Statistical Analysis

The quality descriptors analyzed for data were subjected to a one-way analysis of variance (ANOVA). Pairwise comparisons of treatment means were performed using Tukey's procedure as mean ± SE, with a significance level of $p \leq 0.05$, using SPSS software.

RESULTS

The data presented in Table 1 the total yeast and mold counts in two different types of cheese samples: Romi and Kariesh. For Romi samples, the total yeast count ranged from less than 2 log cfu/g (colony-forming units per gram) to 5.9 log cfu/g, with a mean of 5.2 ± 4.3 log cfu/g, indicating broad variation across the samples. The mold count for Romi samples ranged from less than 2 log cfu/g to 5.2 log cfu/g, with a mean of 3.9 ± 3.3 log cfu/g. In contrast, Kariesh samples exhibited higher yeast and mold counts. The yeast count ranged from less than 2 log cfu/g to 8.4 log cfu/g, with a mean of 7.4 ± 6.7 log cfu/g. The mold count for Kariesh samples ranged from less than 2 log cfu/g to 7.3 log cfu/g, with a mean of 5.9 ± 5.3 log cfu/g. The "<2" values indicated that in some samples, the yeast or mold count was below the detection limit. Overall, Kariesh samples have higher and more variable yeast and mold counts compared to Romi samples.

Table 1: Statistical analytical results of fungi in cheese samples by Log_{10}cfu/gm

Type of samples	Total yeast count			Total mold count		
	Min.	Max.	Mean ± SE	Min.	Max.	Mean ± SE
Romi	<2	5.9	5.2±4.3	<2	5.2	3.9±3.3
Kariesh	<2	8.4	7.4±6.7	<2	7.3	5.9±5.3

The data in Table 2 showed the total counts of *Aspergillus flavus* and *Aspergillus niger* in Romi and Kariesh samples. In Romi samples, the *A. flavus* count ranged from 3 to 4 log cfu/g, with a mean of 3.7 ± 3.3 log cfu/g, while the *A. niger* count ranged from 2 to 4 log cfu/g, with a mean of 3.3 ± 3.2 log cfu/g. In contrast, Kariesh samples show greater variability and higher counts. *The A. flavus* count in Kariesh samples ranged from less than 2 to 6 log cfu/g, with a mean of 5.3 ± 5.1 log cfu/g, and *the A. niger* count ranged from less than 2 to 6 log cfu/g, with a mean of 5.1 ± 4.8 log cfu/g. The "<2" values in Kariesh samples indicated that some counts were below the detection limit. Overall, Romi samples have lower and more consistent *A. flavus* and *A. niger* counts compared to the higher and more variable counts in the Kariesh cheese samples.

Table 2: Statistical analytical results of *A. flavus* and *A. niger* in cheese samples by Log_{10}cfu/gm

Type of samples	Total *A.flavus* count			Total *A.niger* count		
	Min.	Max.	Mean ± SE	Min.	Max.	Mean ± SE
Romi	**3**	**4**	**3.7±3.3**	**2**	**4**	**3.3±3.2**
Kariesh	**<2**	**6**	**5.3±5.1**	**<2**	**6**	**5.1±4.8**

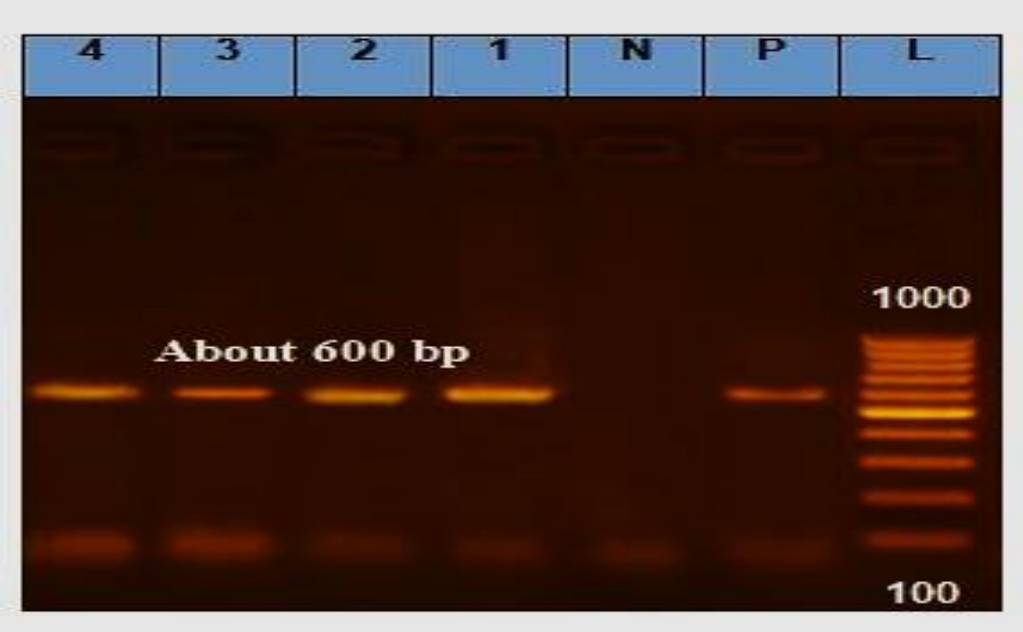

Photo 1: PCR for confirmation of isolates at 600 bp

Photo 1: Agarose gel electrophoresis image showing the ITS (1,4) gene Aspergillus**. L:** ladder**. P:** Positive control for Aspergillus (amplicon size 600 bp)**; Lanes** 1-4**: positive samples. N:** Negative control (Nuclease free water). Zero**: Negative samples.**

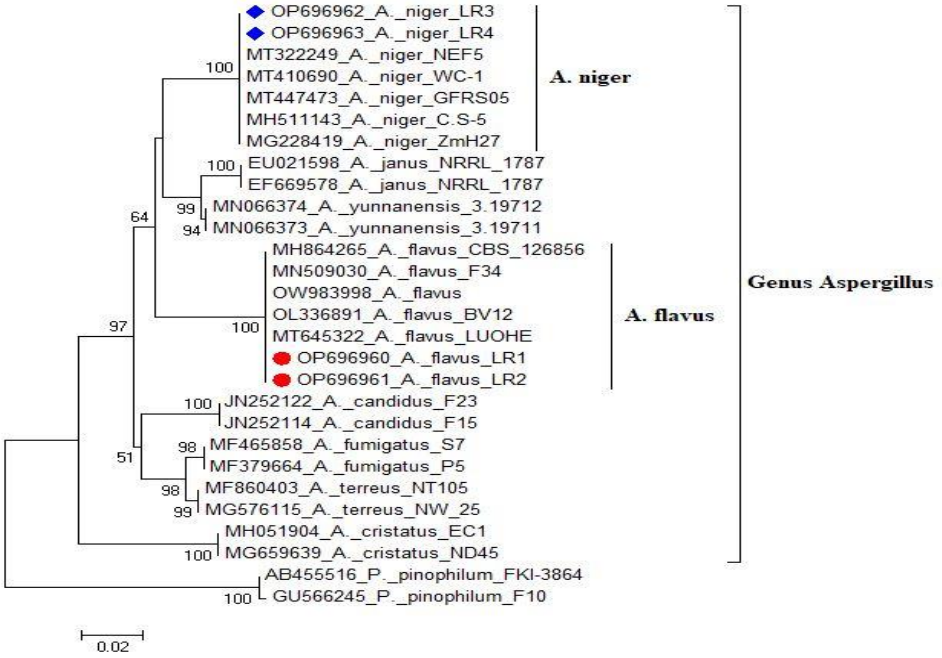

Figure1: Phylogenetic Tree Analysis

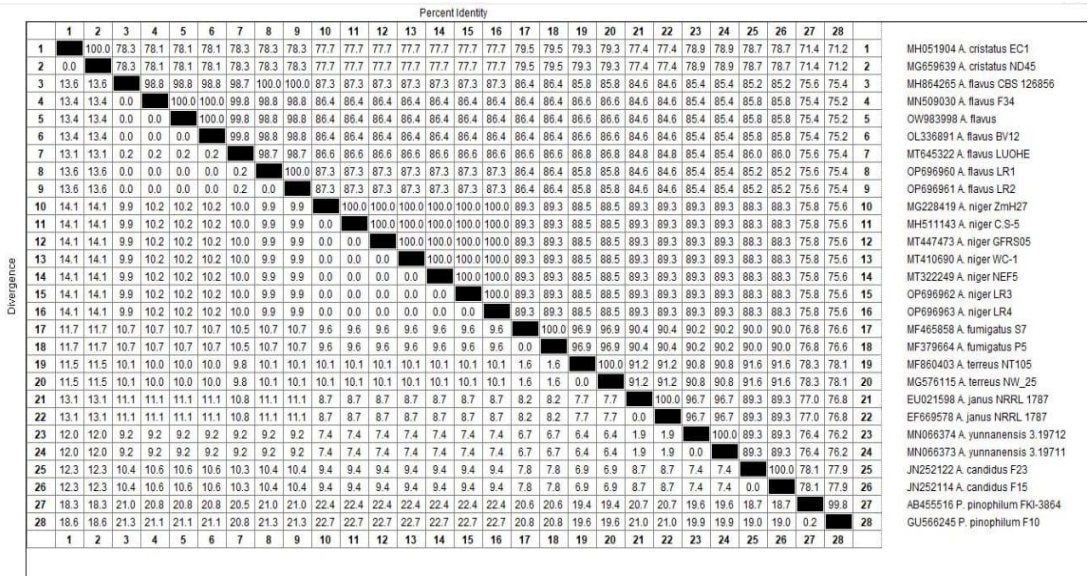

Figure 2: Sequence of *A. flavus* and *A. niger*

Table 3 showed the minimum inhibitory concentration (MIC) of *Lactobacillus plantarum* at different counts (in log10 cfu/g) against *Aspergillus flavus* and *Aspergillus niger*, measured by the zone of inhibition in millimeters. At a count of 7 log10 cfu/g, no inhibition zone was observed for either *A. flavus* or *A. niger*. At 8 log10 cfu/g, the inhibition zones were moderate, with *A. flavus* showing a zone of 5 ± 0.5 mm and *A. niger* showing a zone of 5.3 ± 0.3 mm. When the count increased to 9 log10 cfu/g, the inhibition zones became significantly larger, reaching 8.8 ± 0.9 mm for *A. flavus* and 12 ± 1.2 mm for *A. niger*. These results indicated that higher concentrations of *L. plantarum* were more effective at inhibiting both fungi, with the largest zones of inhibition observed at the highest count tested.

Table 3: MIC of the different count of *L. plantarum* by Log_{10} against *A. flavus* and *A. niger* by zone of inhibition in mm

Different counts of *L. plantarum*	***A. flavus***	***A. niger***
7	NZ	NZ
8	5±0.5	5.3±0.3
9	8.8±0.9	12±1.2
10	12.3±1.5	14.7±0.9

Mean ±SE. * NZ: no zone

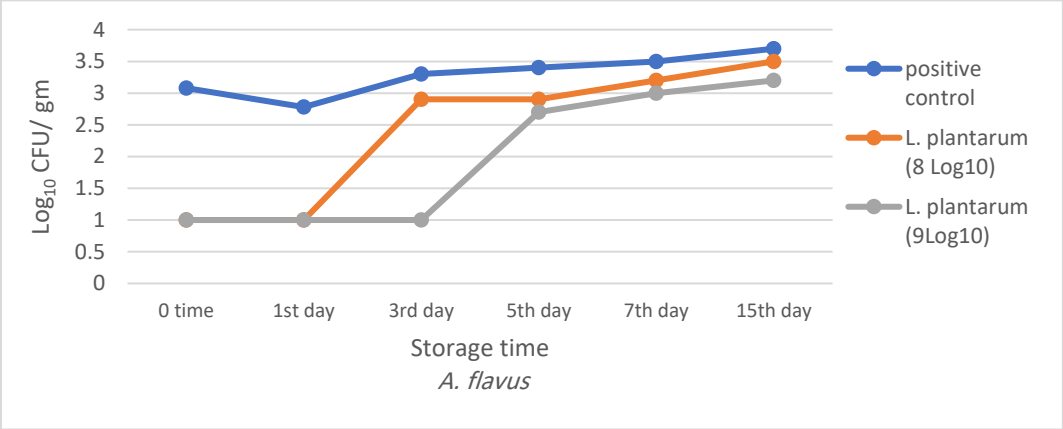

Figure 3: Efficacy of *L. plantarum* at different concentrations against *A. flavus*

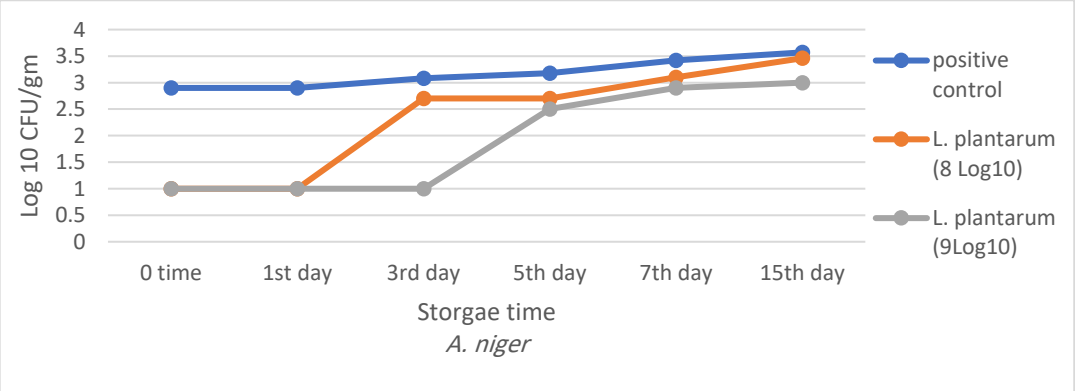

Figure 4: Efficacy of *L. plantarum* at different concentrations against *A. niger*

Fig. (3) presented the effect of two different concentrations of *L. plantarum* (8 Log_{10} and 9 Log_{10}) on the growth of *A. flavus* (3 Log_{10}) over 15 days of inoculated cheese, compared to a positive control. *A. flavus* in the positive control group exhibited continuous growth over time, which is expected in the absence of any inhibitory factor. Overall, while both concentrations of *L. plantarum* appear to initially suppress the growth of *A. flavus*, especially the 9 Log10 concentration, the fungal growth resumes after a few days, indicating that the probiotic's inhibitory effect might be time-limited or less effective as *A. flavus* adapted. While Fig. 4 showed the effect of previous concentrations of *L. plantarum* on the growth of *A. niger* during refrigerated kariesh cheese. In the positive control group, *A. niger* showed continuous growth over time, as expected. While both concentrations of *L. plantarum* exhibited some inhibitory effects on *A. niger* growth, the suppression is temporary, with growth resuming after a few days. The 9 Log_{10} concentration is more effective than the 8 Log_{10} concentration, but neither is sufficient to completely inhibit the fungal growth over the 15-day period. This suggested that while *L. plantarum* has potential as a biocontrol agent against *A. niger*, its efficacy may be limited over extended periods or at lower concentrations.

DISCUSSION

Microbial contamination in food products, particularly dairy, is a critical factor affecting both quality and safety. In cheese, yeast and mold contamination can influence flavor, texture, and shelf life, while also posing health risks if not properly managed (Elsherif, and Al Shrief, 2021). This study investigated the total yeast and mold counts in two distinct types of cheese: Romi, a hard, ripened cheese, and Kariesh, a fresh, soft cheese (Hassanien *et al., 2*021). Due to differences in production, moisture content, and storage conditions, these two cheese varieties provide a unique perspective on microbial contamination levels. By comparing the yeast and mold counts in both cheeses, this analysis offers insights into how different cheese types are susceptible to fungal contamination, potentially impacting their safety and marketability (Ahmed *et al., 20*23).

Yeast and mold counts were both higher in Kariesh cheese than in Romi cheese. The mean yeast and mold count in Kariesh were significantly elevated, which might reflect differences in production methods, storage conditions, or environmental factors between the two types of cheese. The "<2 log cfu/g" values suggested that in some samples, the yeast or mold counts were below the detection limit, indicating a minimal presence of these microbes in certain cases. However, the presence of high counts in other samples, especially for Kariesh, suggested that contamination is inconsistent, but can reach significant levels. The greater variability in Kariesh cheese (indicated by the larger standard deviations) highlights that microbial contamination in this cheese type is less controlled or more susceptible to environmental factors than in Romi cheese.

Hayaloglu *et al. (*2008) and Adam *et al., (*2024) found that yeast and mold counts in hard cheeses were generally lower, with averages around 4-5 log cfu/g for yeast and 3-4 log cfu/g for molds. Brooks *et al. (*2012) and Awad (2016) both documented yeast counts ranging from 6 to 8 log cfu/g in soft cheeses, while mold counts were reported between 4 and 7 log cfu/g.

Previous studies emphasized the role of moisture content and pH in influencing microbial growth. Kariesh cheese, being a fresh soft cheese, has a higher moisture content and a more favorable pH for yeast and mold growth, as noted by Todaro *et al.* (2013). Romi cheese, with its lower moisture and higher salt content, tends to suppress microbial activity (Ayaka *et al., 2*022). Altafini *et al. (*2021) pointed out that improper storage, temperature fluctuations, and exposure to air could significantly increase yeast and mold contamination, especially in soft cheeses like Kariesh. This might explain the broader range of microbial counts observed in the current study. Studies often associated high yeast and mold counts with inadequate hygiene practices during cheese production or poor storage conditions. Nyamakwere *et al.* (2021) noted that artisanal cheeses, including Kariesh, often have higher contamination due to traditional, less controlled production methods.

Kariesh cheese showed significantly higher and more variable counts of both *A. flavus* and *A. niger*. This greater variability and higher mean counts reflected the higher moisture content, lower salt concentration and less acidic environment of soft cheeses, which are more favorable for fungal growth. In their study of traditional Egyptian cheeses, including soft varieties, Adam *et al.* (2024) reported similar findings. They reported higher counts of *A. flavus* and *A. niger* in soft cheeses compared to hard cheeses, aligning with the higher counts found in Kariesh cheese in this study. Hymery *et al. (*2014) indicated that the presence of *A. flavus* and *A. niger* in cheeses often depends on the moisture content and storage conditions.

Soft cheeses, which have higher moisture, typically support greater fungal growth compared to harder cheeses. Benkerroum (2013) found that hard cheeses like Romi tend to have lower fungal contamination due to their drier nature, which inhibits the growth of molds and yeasts. This finding is consistent with the lower and more stable counts of *A. flavus* and *A. niger* observed in Romi cheese.

To scientifically compare *A. flavus* and *A. niger* isolated from Romi and Kariesh cheese, PCR and sequencing are employed to confirm the identities of these fungal isolates. Polymerase chain reaction (PCR) is used to amplify specific genomic regions unique to each species, such as the ITS (Internal Transcribed Spacer) regions, which are then sequenced to obtain nucleotide sequences. The sequences are compared to reference sequences in the NCBI GenBank database using the Basic Local Alignment Search Tool (BLAST), to identify the closest matches and confirm the species of the isolates (Altschul *et al., 199*7). After obtaining the sequences, alignment tools like Clustal Omega or MUSCLE are used to compare them with reference strains, revealing genetic similarities and differences (Larkin e*t al., 2*007). Phylogenetic analysis, using tools such as MEGA or PhyML, constructed a tree to visualize relationships between the isolates and known reference strains, providing insights into their genetic diversity (Tamura *et al.,* 2013). This comparison did not only confirm the species identity, but also highlighted any genetic variability between isolates from Romi and Kariesh cheese. Significant genetic differences could indicate novel strains or adaptations to specific cheese environments, which could have implications for cheese quality and safety. Such analyses are crucial for understanding fungal biodiversity in cheese production and addressing potential impacts on product safety and quality (Pardo *et al.,* 2005).

Table 3 illustrated the effectiveness of *L. plantarum* against *A. flavus* and *A. niger*, measured by the minimum inhibitory concentration (MIC) at different bacterial counts. At a concentration of 7 log10 cfu/g, no inhibition of either fungal species was observed, indicating that *L. plantarum* at this level does not inhibit the growth of *A. flavus* or *A. niger*. However, as the bacterial count increased to 8 log10 cfu/g, moderate inhibition was observed. Specifically, the inhibition zones were 5 ± 0.5 mm for *A. flavus* and 5.3 ± 0.3 mm for *A. niger*, suggesting that a higher concentration of *L. plantarum* begins to exert an inhibitory effect on fungal growth. The inhibition became significantly more pronounced at 9 log10 cfu/g, with zones reaching 8.8 ± 0.9 mm for *A. flavus* and 12 ± 1.2 mm for *A. niger*. This indicated a dose-dependent relationship where higher concentrations of *L. plantarum* are more effective in inhibiting fungal growth, with the largest zones of inhibition observed at the highest count tested. These findings support the notion that increasing the concentration of antimicrobial agents can enhance their efficacy against fungi, as higher microbial loads of *L. plantarum,* resulted in greater inhibition of *A. flavus* and *A. niger* (Nielsen *et al., 2*017; Yang *et al., 20*19).

The application of probiotic strains, such as *L. plantarum* in dairy products is a method of bio preservation, where beneficial bacteria are used to inhibit the growth of spoilage organisms (Corsetti & Settanni, 2012).

L. plantarum is a widely studied lactic acid bacterium (LAB) known for its antimicrobial properties. It can produce bacteriocins, organic acids, and other antimicrobial metabolites that inhibit the growth of spoilage organisms, including molds and yeasts (Mokoena *et al., 2*021).

In both studies, it was observed that higher concentrations of *L. plantarum* (9 Log_{10}) delayed the growth of *A. flavus* and *A.*

niger more effectively than the lower concentration (8 Log_{10}), although neither concentration was able to completely suppress fungal growth beyond the 7th day.

The results showed that *A. flavus* was initially suppressed by both concentrations of *L. plantarum*, particularly the 9 Log_{10} concentration, where fungal growth was completely halted for the first 3 days. This can be attributed to the ability of LABs to lower the pH of the environment and produce organic acids (e.g., lactic acid, acetic acid), which creates an unfavorable condition for mold growth (Ghanbari *et al., 2*013). Similarly, *L. plantarum* was able to suppress *A. niger* growth during the first few days of storage, with the 9 Log_{10} concentration having a more pronounced effect. However, by the 15th day, the growth of both *A. flavus* and *A. niger* resumed, although at a slightly slower rate in the 9 Log_{10} group. This suggested that *L. plantarum* has a transient inhibitory effect, likely due to the depletion of available nutrients or a shift in the microbial ecosystem over time (Dalié et al., 2010).

The antifungal activity of *L. plantarum* is well-documented and can be attributed to several factors: Production of Organic Acids: LABs like *L. plantarum* ferment lactose and other sugars in dairy products, producing organic acids that lower the pH and inhibit the growth of spoilage organisms, including molds (Gänzle, 2015). *L. plantarum* can produce bacteriocins, which are peptides with antimicrobial activity. These bacteriocins target the cell membranes of spoilage microorganisms, causing cell lysis or inhibiting their growth (Soomro *et al.,* 2002). LABs also compete with spoilage organisms for essential nutrients, limiting the resources available to fungi like *Aspergillus* species (Parvez *et al.,* 2006 and Siedler *et al., 2*019).

Although *L. plantarum* showed some success in delaying fungal growth, there are limitations to its effectiveness in long-term storage. The growth of *A. flavus* and *A.* niger resumed after day 5 or 7, indicating that the antifungal activity of *L. plantarum* may diminish over time. This could be due to the short lifespan of active metabolites produced by LABs, which are degraded or lose potency over time. Nutrient depletion or changes in environmental conditions (such as the buildup of lactic acid), might reduce the viability of *L. plantarum* and weaken its inhibitory effects (Bintsis, 2018). Additional factors such as the interaction between *L. plantarum* and other naturally occurring microflora in the cheese matrix, could also play a role in the eventual outgrowth of fungi (El-Ghaish *et al.,* 2011).

The results suggested that while *L. plantarum* can delay fungal growth, it is not sufficient as a standalone preservative for long-term storage of Kariesh cheese. Combining probiotics with other technologies, such as antifungal enzymes, essential oils, or modified atmosphere packaging (MAP), may provide enhanced preservation effects (Dalié *et al., 2*010 and Koleva Gudeva & Trajkova, 2024). The study shows that higher concentrations of *L. plantarum* are more effective in suppressing fungal growth. Further research is needed to determine the optimal concentration and combination of strains to achieve long-term fungal control in refrigerated cheeses. Investigating the stability of the antifungal metabolites produced by *L. plantarum* over time could provide insights into how the duration of fungal suppression can be extended (Gänzle, 2015 and Zavišić *et al., 202*4).

CONCLUSION

In conclusion, *L. plantarum* shows promise as a bio preservative in refrigerated soft cheeses, such as Kariesh cheese, by inhibiting the growth of spoilage fungi like *A. flavus* and *A. niger*. However, the effect is temporary, and growth resumes after a

few days. Future studies should focus on enhancing the stability and effectiveness of probiotic preservation methods, potentially using multiple preservation strategies in combination.

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السيطرة على بعض أجناس الاسيراجيلس المفرزة للسموم في بعض أنواع الجبن باستخدام اللاكتوباسيلس بلنتيرم

لمياء محمد طلعت الشريف ، رضوى رمضان

Email: lamiaamohamedtalaat@gmail.com Assiut University website: www.aun.edu.eg

تُعدّ الملوثات الميكروبية في المنتجات الغذائية، وخصوصاً منتجات الألبان، من العوامل الهامة التي تؤثر على جودتها وسلامتها. من بين هذه المنتجات، الجبن الطري مثل الجبن القريش يتعرض بشكل خاص للتلوث بالخمائر والفطريات نتيجة محتواه العالي من الرطوبة وطبيعته السريعة التلف. الفطريات، مثل *Aspergillus flavus* و *Aspergillus niger*، تُعتبر من الملوثات الشائعة في هذه الأنواع من الأجبان، حيث يمكن أن تؤدي إلى تحلل المنتجات الغذائية وتقلل من فترة صلاحيتها، بالإضافة إلى تأثيراتها الصحية السلبية إذا لم يتم السيطرة عليها بفعالية.

تهدف هذه الدراسة إلى تقييم تلوث الجبن الرومي (وهو جبن صلب) والقريش (وهو جبن طري) بالخمائر والفطريات، مع التركيز على فحص فعالية *Lactobacillus plantarum* كعامل حيوي مثبط لنمو الفطريات، ما يمكن أن يساعد في تحسين جودة وسلامة هذا النوع من المنتجات اللبنية. تم قياس تعداد الخمائر والفطريات في كلا النوعين من الجبن، بالإضافة إلى قياس تأثير *L. plantarum* على تثبيط نمو *A. flavus* و *A. niger* في الجبن القريش. تظهر النتائج أن الجبن القريش يحتوي على نسب أعلى من التلوث بالخمائر والفطريات مقارنةً بالجبن الرومي، مما يعكس أهمية التركيب الفيزيائي والكيميائي لكل نوع في تشجيع نمو الكائنات الدقيقة.

لذلك كان تعداد الخمائر والفطريات في عينات الجبن الرومي قد تراوح من أقل من ٢ إلى ٥,٩ لوج وحدة تكوين مستعمرات/جرام، بمتوسط ٥,٢ ± ٤,٣ لوج وحدة تكوين مستعمرات/جرام، مما يشير إلى تنوع واسع في العينات. تراوح تعداد الفطريات في عينات الرومي من أقل من ٢ إلى ٥,٢ لوج وحدة تكوين مستعمرات/جرام، بمتوسط ٣,٩ ± ٣,٣ لوج وحدة تكوين مستعمرات/جرام. في المقابل، أظهرت عينات القريش تعداداً أعلى من الخمائر والفطريات. تراوح تعداد الخمائر في عينات القريش من أقل من ٢ إلى ٨,٤ لوج وحدة تكوين مستعمرات/جرام، بمتوسط ٧,٤ ± ٦,٧ لوج وحدة تكوين مستعمرات/جرام. أما تعداد الفطريات في عينات القريش فتراوح من أقل من ٢ لوج إلى ٧,٣ لوج وحدة تكوين مستعمرات/غرام، بمتوسط ٥,٩ ± ٥,٣ لوج وحدة تكوين مستعمرات/جرام.

أما ما يخص تعداد *Aspergillus flavus* و *Aspergillus niger* في عينات الرومي والقريش. ففي عينات الرومي، تراوح تعداد *A. flavus* من ٣ إلى ٤ لوج وحدة تكوين مستعمرات/جرام، بمتوسط ٣,٧ ± ٣,٣ لوج وحدة تكوين مستعمرات/جرام، بينما تراوح تعداد *A. niger*من ٢ إلى ٤ لوج وحدة تكوين مستعمرات/جرام، بمتوسط ٣,٣ ± ٣,٢ لوج وحدة تكوين مستعمرات/جرام. على النقيض من ذلك، أظهرت عينات القريش تبايناً أكبر وتعدادات أعلى.

وقد تم قياس التركيز المثبط الأدنى (MIC) لـ *Lactobacillus plantarum*عند تركيزات مختلفة ضد *A. flavus* و *A. niger* باستخدام مناطق التثبيط. كما أوضحت نتائج حقن *Lactobacillus plantarum* فى الجبن القريش المصنع معمليا وتخزينها عند درجة حرارة الثلاجة عند تركيزين ٨ و ٩ لوج ان عند ٩ لوج ١٠ وحدة تكوين مستعمرات/جرام، كانت مناطق التثبيط أكبر، مما يشير إلى فعالية أعلى في تثبيط الفطريات.

وفي الختام، يُظهِر L. plantarum فاعلية باعتباره مادة حافظة حيوية في الجبن الطري المبرد، مثل الجبن القريش، من خلال تثبيط نمو فطريات التلف مثل A. flavus وA. niger. ومع ذلك، فإن التأثير مؤقت، ويستأنف النمو بعد بضعة أيام. وينبغي أن تركز الدراسات المستقبلية على تعزيز استقرار وفعالية طرق الحفظ البروبيوتيكية، باستخدام استراتيجيات حفظ متعددة معًا.