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Identification of Previously Unrecorded *Bacills*, *Serratia*, and *Mucor* Strains Isolated from Yogurt

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Abstract: (1) Background: Foodborne illnesses are recognized as a significant threat to public health and the economy in both developed and developing nations. The safety of foods containing microorganisms has consequently become a major worldwide concern. One of the most frequent causes of food deterioration in the world is microbial contamination. (2) Methods: Yogurt containers that were bought commercially in Riyadh, Saudi Arabia during their validity period contained four different species of bacteria and one type of fungus. Using nutritional agar and Czapek-Dox agar medium, the bacteria and fungi were isolated. The isolates of the fungi and bacteria were identified using a scanning microscope. The isolates were further identified and classified for molecular evolutionary analyses using the 16S rRNA and ITS sequences from the bacteria and fungi, respectively, in conjunction with the universal primers 27F, 1492R, ITS1F, and ITS4R. (3) Results: A total of 131 separate strains were identified from 12 yogurt samples based on their phenotypic characteristics. In total, 79 isolates (60.3%) consisted of *Serratia marcescens*, *Bacillus subtilis*, and *Mucor circinelloides*, with 52 isolates (39.7%) being *Bacillus cereus*. While the cells of *Bacillus* and *Serratia* are shaped like rods, the sporangia of *Mucor* are large, round, and black. Each strain was identified by its accession number, which were as follows: MK590996.1: *B. cereus*; MK591144.1: *B. subtilis*; MK591002.1 and MK591014.1: *S. marcescens*; and MK559692.1: *M. circinelloides*. The maximum identification was found to be between 98.64 and 100% when BLAST was used to compare the sequences to the NCBI GeneBank database. (4) Conclusions: Genus and species identification was performed using the similarity score values. Yogurt products containing high concentrations (39.7%) of *Bacillus cereus* isolates carry a significant risk of health hazards due to the potential for spreading pathogenic bacteria to humans.

Keywords: yogurt; isolation; identification; SEM; *Bacillus*; *Serratia*; *Mucor*; KSA

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1. Introduction

Foodborne diseases have been known to be a major public health and economic risk in both rich and poor countries. As a result, the safety of microbial foods has emerged as a major global concern. Microbial contamination is among the most common causes of food degradation worldwide [1]. Food contamination by bacteria can happen at any time as in the industry. Contamination refers to any change that produces unsafe food. During the preservation of most dairy products, various factors might affect their microbiological safety and quality [2]. Between the final manufacturing phase and their use, ready-to-eat foods are not modified [3].

Milk and dairy products are vital components of human nutrition and play a significant role in many people's diets around the world because they include protein, vitamins, and minerals that are essential for the health of people of all ages and genders [1]. Dairy products preserve milk's nutritional content while also making it more attractive to consumers [4]. Fresh milk's microbiome is complex, and it changes depending on a variety

of factors, including cleanliness, seasonality, animals, and others [5]. Denaturation happens when microbes break down milk's carbohydrates, proteins, and lipids, producing a harmful output [4]. Milk produced from healthy cows can be considered bacteria-free, but agricultural and dairy environments can be sources of contamination, especially during milking and in cheese production. In particular, *B. cereus* was previously known to be responsible for the spoilage of raw milk [6]. Moreover, a brief risk assessment of *B. cereus* in the Netherlands predicted that almost 7% of pasteurized milk is characterized by an amount of this pathogen greater than 5 log cfu·mL−1 [7]. Although vegetative *B. cereus* cells cannot survive pasteurization, their spores are resistant to heat treatments, highlighting their viability in pasteurized milk. *Bacillus cereus* has also been found in some dairy products, with their frequency varying from 2 to 52%, depending on the type [8,9].

Bacteria that cause disease, like *Escherichia coli*, *Escherichia faecalis*, and *Bacillus cereus*, can grow in yogurt. These bacteria can enter milk and its derivatives through certain internal and external factors found during the processing of dairy products, such as poor line hygiene. Contaminated equipment in manufacturing, the air flow in production rooms, and the personal hygiene of workers are not guaranteed [10]. Industrial processes have improved the quality, storage, transportation, and marketing of products. Microbiological parameters, especially coliform counts in general, *Escherichia coli* and *Enterococcus bacteria,* are commonly used to verify these conditions [11]. Acidity and temperature standards are also followed to assess the yogurt's ability to preserve its state. Statistics show variations from 0.6 to 1.5 g of lactic acid per 100 g of product, and storage temperatures in dairy markets and industries should not be higher than 100 °C [12].

The presence of foodborne pathogens and microorganisms in yogurt, which include bacteria and fungi, is determined not only by the animal's health, but also via the conditions of its manufacture, storage facilities, and the technologies used [13]. Spore-forming bacteria are a special problem that can penetrate milk production steps because they have the potential to resist abiotic stresses in the spore phase [14,15]. All the same, De Jonghe [2] discovered that *Bacillus cereus* was not involved in food contamination, while cellular tests confirmed the creation and efficiency of *Bacillus circulans*, *Bacillus lentus*, and *Bacillus subtilis* at high temperatures [16,17]. *B. cereus* is found in milk products with varying levels of pollution in Egypt and India [18,19]. *Serratia* spp. can build colonies on abiotic surfaces [20] and create heat-resistant enzymes, allowing them to degrade milk at various stages of production [21,22]. It is really difficult to prevent microbial infections of milk during the preparation of different dairy foods; consequently, the microbiological content of foods is an important factor in establishing their acceptability from a safety standpoint [23]. As a result, it is recommended that pathogens in milk and dairy products be detected using microbiological, immunological, and molecular approaches to achieve human food safety.

Despite advancements in current preparations, fungal spoiling remains a problem for dairy producers, and novel (bio) conservation strategies, such as the utilization of protective cultures, have been developed recently [24]. Types of food might be physically, chemically, or microbially damaged when it comes to their expiration. The principal agents of microbiological degradation include parasites, bacteria, and/or fungi [24]. Due to the diversity of fungus species, food processors suffer significant damage as a result of fungal deterioration. Furthermore, fungal degradation is thought to account for between 5% and 10% of global food output [25,26]. Fungal infections of dairy products can happen at any time throughout the supply chain, from dairy farms to dairy production sites to customers' homes. Rhizopus is the only species in which mucoralean fungi that cause mucormycosis occur more frequently than in *Mucor* species [27,28]. *M. circinelloides* was linked to a nutrition disease outbreak following the consumption of infected yogurt, according to a US FDA investigation. Snyder et al. [29] reported that storage temperature and natamycin dose interact to cause *M. circinelloides* degradation.

Yogurt is among the world's most popular milk products [30]. Even though yeasts and molds are the major pollutants in yogurt, bacterial infection by spore-forming resistant bacteria during commercial thermal processing can cause its value to decline [31]. The aim

of our study is to isolate and identify the bacterial and fungal strains in fresh yogurts in Saudi Arabia by using SEM, 16S rRNA, and ITS.

2. Materials and Methods

2.1. Bacterial and Fungal Isolation

Twelve samples of the same brand of yogurt commercially purchased within their validity period were gathered from Riyadh supermarket in Saudi Arabia and sent to a lab for analysis. For microbiological isolation, the direct inoculation method was chosen. Fungi were isolated using potato dextrose agar, Sabouraud dextrose agar, and Czapek-Dox agar (Oxoid Ltd, Basingstoke, England, UK), all of which were supplemented with chloramphenicol. Nutrient agar (Oxoid, UK) was utilized for bacterial isolation. Two types of culture methods were used: (1) the dilution plate method and (2) the plate method [32]. Before being examined, the dishes were cultured at 28 °C for seven days for fungi and three days for bacteria. Following incubation, each plate was examined again to obtain purified fungus cultures through serial inoculation.

The fungal morphology was examined macroscopically by observing colony features (color, shape, size, and hyphae) and microscopically by a compound microscope equipped with a digital camera and a lactophenol cotton blue-stained (LCB) slide mounted with a small portion of the mycelium. Gram staining was used to examine bacteria macroscopically.

The samples were examined using SEM (scanning electron microscopy) (JEOL 7500FA JEOL, Peabody, MA, USA). The specimens were fixed for 6 h at 4 °C with 2.5% glutaraldehyde; dehydrated in a series of 25, 50, 75, and 100% ethanol (10 min each); and dehydrated in a centrifuge tube at 30 °C. After that, the samples were mounted on aluminum sticks, covered with gold, and observed using SEM at a 10 kV accelerating voltage to determine the samples' color and shape.

2.2. DNA Extraction, PCR Amplification, and Purification

To further identify the bacterial and fungal isolates, ITS and 16S rRNA genes were amplified via polymerase chain reaction (PCR) testing and sequenced as follows: after being freshly grown, a little bit of each isolate was taken and dissolved in autoclaved distilled water in 2 mL sterile Eppendorf tubes, which were then heated for 15 min at 100 °C. Genomic DNA was extracted from fungal and bacterial isolates using the InstaGeneTM Matrix Genomic DNA Kit (Bio-Rad Laboratories, Hercules, CA, USA) following the manufacturer's instructions. These lifeless bacteria and fungi were transported to the SolGent Company in Daejeon, Republic of Korea for DNA isolation using SPINeasy DNA Kit for Tissue & Bacteria (MP Biomedicals Korea, Seoul, Republic of Korea)

To use isolated genomic DNA as a template, PCR amplification of bacterial 16S rRNA and fungal ITS from yogurt specimens was carried out. For bacteria, the primers were 27F and 1492R (5'AGAGTTTGATCMTGGCTCAG3' and 5'TACGGYTACCTTGTTACGACTT3', respectively), while for fungi, they were ITS1F and ITS4R (5'TCCGTAGGTGAACCTGCGG3' and 5'TCCTCCGCTTATTGATATGC3', respectively) [33]. The PCR reaction mixture was prepared using the following steps: 2 µL of 10x Taq PCR Buffer, 1.6 µL of 2.5 mM dNTP mixture, 1.0 µL of F and R primers (10 pmol/µL), 0.2 µL of KOMA Taq (2.5 U/µL), and 2 µL of DNA template (20 ng/µL) were combined, and using distilled water of HPLC grade, the reaction volume was adjusted to 20 µL. Under the following circumstances, amplification was performed in a thermal cycler: initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 0.5 min, annealing at 55 °C for 2 min, and extension at 68 °C for 1.5 min. This was followed by a final polymerization extension at 68 °C for 10 min. Using 1% agarose gel electrophoresis, amplicon was verified. The Montage PCR Cleanup Kit (Millipore Sigma, Burlington, MA, USA) was used to purify the amplifiers.

2.3. DNA Sequencing

The amplification primers and BigDyeTM Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) were used to sequence the purified PCR

products. Macrogen, Inc. used the 3730xl DNA Analyzer automated DNA sequencing system (Applied Biosystems) (Seoul, Republic of Korea) to sequence amplified products.

Sequence analysis: The obtained sequences were edited with Geneious Prime software Version 2020.1.2 [34]. Forward and reverse sequences were combined to form consensus sequences. The sequences were aligned using the National Center for Biotechnology Information's (NCBI) Basic Local Alignment Search Tool (BLAST) with the GenBank database of available nucleotide sequences.

Phylogenetic analyses: The evolutionary history was inferred by using the maximum likelihood method and Tamura–Nei model [35]. The tree with the highest log likelihood (−5755.83) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura–Nei model and then selecting the topology with superior log likelihood value. This analysis involved 6 nucleotide sequences. Codon positions included were 1st + 2nd + 3rd + Noncoding. There were a total of 1106 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [36].

Data availability: This study's data are presented here. The isolates' ITS and 16S rDNA gene sequences have been deposited in the NCBI's GenBank. The fungal strain has accession number MK559692.1, while the bacterial strains have numbers MK590996.1, MK591144.1, MK591002.1, and MK591014.1.

2.4. Statistical Analysis

The data were analyzed using mean ± standard deviation based on samples in PAST 3.2 program [37]. The outcomes were presented in the form of numbers and percentages.

3. Results

3.1. Phenotypic Characteristics of Bacterial and Fungal Isolates

Twelve (12) fresh commercial yogurt specimens were obtained from a Riyadh supermarket. The bacteria were isolated from the nutrient agar medium (NA) following 3 days of incubation at 28 °C. The fungi were recovered from the Czapek-Dox agar which had been cultivated for up to a week at 28 °C. The percentage of frequency of the isolates in the yogurt samples was as follows: *B. cereus* 52 (39.7%), *M. circinelloides* 30 (22.9%), *B. subtilis* 26 (19.8%), and *S. marcescens* 23 (17.6%) (Table 1). The *p*-value of the test is 0.01641, which is less than the significance level alpha = 0.05. We can conclude that the isolation number and mean are significantly correlated with a correlation coefficient of −0.9447834 and a *p*-value of 0.01641.

Table 1. The above output displays the frequency and cumulative frequency of each (a) isolation number, and (b) mean of bacterial and fungal isolates.

Samples	Isolate No.	Isolate %
Bacillus cereus	52	39.70%
Bacillus subtilis	26	19.80%
Serratia marcescens	23	17.60%
Mucor circinelloides	30	22.90%

The SEM images of the bacterial and fungal samples revealed that the investigated strains differed in size and shape (Figure 1; A–C). *B. cereus*, *B. subtilis*, and *S. marcescens* were *Bacillaceae* and *Yersiniaceae* family members with rod-shaped cells, with most of the cells arranged in pairs or chains. The isolates' colonies had a convex form. *Bacillus* colonies were often whitish, while *Serratia* colonies were pale red. Cells could be found individually, in pairs, in chains, or in clusters. *M. circinelloides* belonged to the *Mucoraceae* family and had a morphology characterized by big black sporangia with a mean area of 100%, a 0.202 µm mean circular shape, and a mean solidity of 0.488 µm (Figure 1; D, and Table 2). There is a significant difference between the sample medians ($p = 0.003305$).

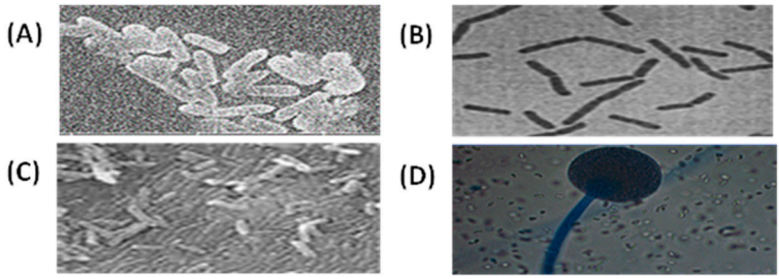

Figure 1. SEM images for (**A**) *Bacillus cereus*, (**B**) *Bacillus subtilis*, (**C**) *Serratia marcescens*, and (**D**) *Mucor circinelloides* detected using SEM at a magnification of 10.00. The scale bar represents 500 µm.

Table 2. The above output displays the frequency and cumulative frequency of each characteristic for Mucor circinelloides isolates.

Characteristic	Mean
Circ.	0.202
Feret	25.403
%Area	100
Feret X	56.769
Feret Y	56.538
Feret Angle	89.393
Min Feret	11.649
AR	2.881
Round	0.413
Solidity	0.488

3.2. Molecular Identification of Bacterial and Fungal Isolates

The isolation of genomic DNA was carried out to obtain pure DNA from the bacterial and fungal isolates. The extracted DNA molecules were used as templates for the PCR amplification of the 16S rRNA and ITS genes using the universal primers 27F, 1492R, ITS1F, and ITS4R, respectively, at an annealing temperature of 55 °C. The amplified products of the bacterial isolates were verified using 1.5% agarose gel stained with ethidium bromide, which revealed a fragment of 1088 bp for the *B. cereus* strain B-191, 1111 bp for *B. subtilis*, 1099 bp for the *S. marcescens* strain B-192, and 1106 bp for the *S. marcescens* strain B-193 when 16S rRNA was used. The *M. circinelloides* amplification was 600 bp when 18S rRNA was used as the ITS region. The sequence of isolates was submitted to the NCBI. The accession numbers for the bacterial sequence isolates were as follows: MK590996.1—*B. cereus* strain B-191, MK591144.1—*B. subtilis* strain B-195, MK591002.1—*S. marcescens* strain B-192, and MK591014.1—*S. marcescens* strain B-193 (Table 3). The accession numbers for fungal isolates were as follows: MK559692.1—*M. circinelloides* isolate (AUMC 10367), identified by internal transcribed spacer 1, partial sequence; the 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and the large subunit ribosomal RNA gene, partial sequence. Sequences from the GenBank nucleotide database were compared to the ones produced. A phylogenetic tree with a 98–100% similarity is used to identify the species. The MEGA 11 program was used to perform the phylogenetic analysis and sequence alignment.

The phylogenetic relation between the *B. cereus* strain B-191, *B. subtilis* strain B-195, *S. marcescens* strains B-192 and B-193, and *M. circinelloides*, as well as members of all other *Bacillus*, *Serratia*, and *Mucor* species are shown in Figures 2–5. The bootstrap values (n = 1000) are shown at the branches. The numbers adjacent to each branch in a tree provide a percentage measure of support for that branch, with 1000 being the maximum support. The 'bootstrapping' approach was performed, and the outcome yielded a high value, indicating that there is significant proof and that the sequences to the side of the branch were grouped exclusively with respect to one another.

Table 3. The molecular identification of isolated bacteria and fungi is shown.

Taxon Name	GenBank	No.
Bacillus cereus strain B-191	MK590996.1	1
Bacillus subtilis strain B-195	MK591144.1	2
Serratia marcescens strain B-192	MK591002.1	3
Serratia marcescens strain B-193	MK591014.1	4
Mucor circinelloides isolate (AUMC 10367)	MK559692.1	5

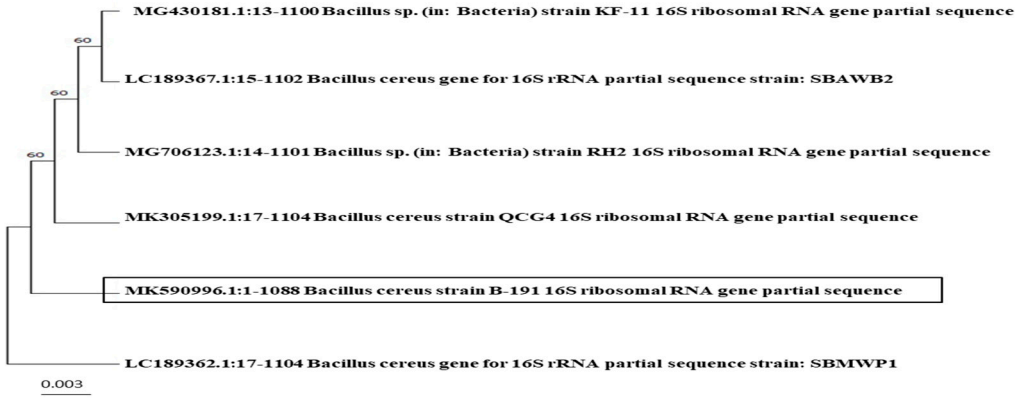

Figure 2. Phylogenic tree of *Bacillus cereus* strain B-191 (MK590996.1) isolated from yogurt and known bacterial relatives (using the neighbor-joining method) with MK305199.1, MG706123.1, MG430181.1, LC189367.1, and LC189362.1.

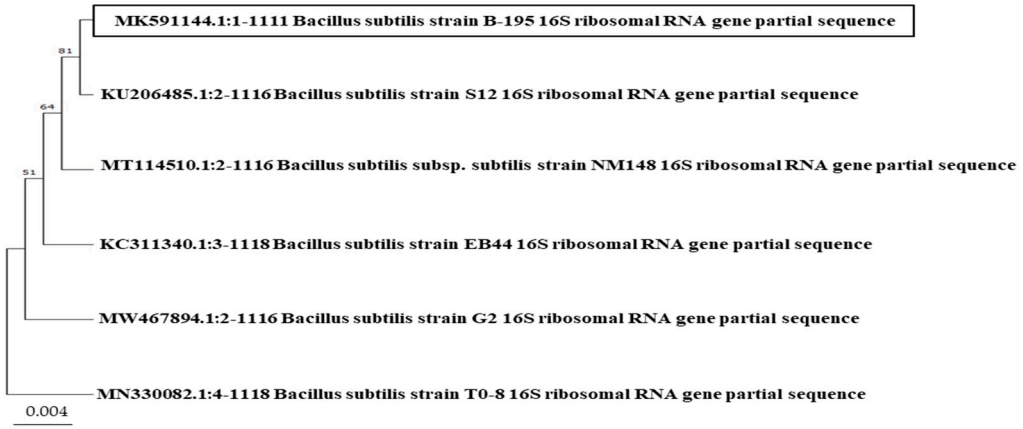

Figure 3. Phylogenic tree of *Bacillus subtilis* strain B-195 (MK591144.1) isolated from yogurt and known bacterial relatives (using the neighbor-joining method) with KU206485.1, KC311340.1, MT114510.1, MW467894.1, and MN330082.1.

Among the nucleotide sequences accessible in the NCBI database, *B. cereus* (MK590996.1) has a strong resemblance to *B. cereus* (MK305199.1), *Bacillus* sp. (MG706123.1), *Bacillus* sp. (MG430181.1), *B. cereus* (LC189367.1), and *B. cereus* (LC189362.1) (Figure 2). *Bacillus subtilis* (MK591144.1) has a high similarity to *B. subtilis* (KU206485.1) (99.10%), (KC311340.1) (99.02%), *B. subtilis subsp. subtilis* (MT114510.1) (99.01%), *B. subtilis* (MW467894.1) (99.01%), and (MN330082.1) (99.01%) (Figure 3). *S. marcescens* (MK591002.1) has a high resemblance to *S. marcescens* (MK591014.1) and *Serratia* sp. (OK067431.1) (98.82%), *S. marcescens* (KR817904.1) (98.73%), *S. marcescens* (GU212864.1), *Serratia* sp. (KF891398.1), and *S. marcescens* (MH669313.1) (Figure 4). *M. circinelloides* (MK559692.1) shared a high degree of similarity (100%) with *M. circinelloides* (MK560184.1) and (99.49%) with *M. circinelloides* (MT603942.1), *M. circinelloides* (MT603901.1), *M. circinelloides* (MT603900.1), and *M. circinelloides* (KX349456.1) (Figure 5).

Figure 4. Phylogenic tree of *Serratia marcescens* strain B-192 (MK591002.1) and Serratia marcescens strain B-193 (MK591014.1) isolated from yogurt and known bacterial relatives (using the neighbor-joining method) with OK067431.1, KR817904.1, GU212864.1, KF891398.1, and MH669313.1.

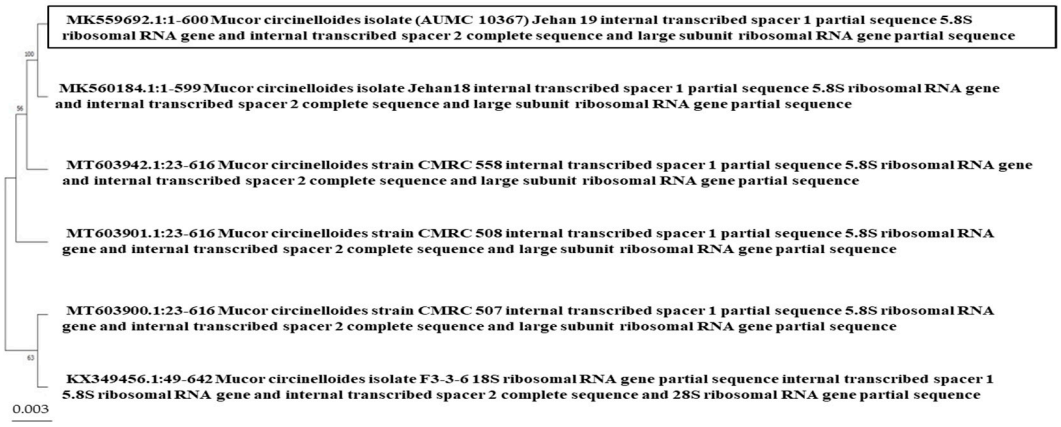

Figure 5. Phylogenic tree of *Mucor circinelloides* (AUMC 10367) (MK559692.1) isolated from yogurt and known fungal relatives (using the neighbor-joining method) with MK560184.1, MT603942.1, MT603901.1, MT603900.1, and KX349456.1.

4. Discussion

4.1. Phenotypic Characteristics of Bacterial and Fungal Isolates

Microbial contamination is one of the most important causes of food spoilage worldwide [1]. The bacterial contamination of food can occur at any stage of the industrial process. When it comes to food expiration, a portion of a food could be physically, chemically, or microbially destroyed. Parasites, bacteria, and/or fungi are the main agents of microbiological deterioration [24]. Spore-forming bacteria are a unique concern among the microorganisms that might enter the milk supply chain on farms or through milk production lines because they can tolerate abiotic stressors while still in the spore phase [14,15]. We isolated four bacterial strains and one fungal strain from 12 commercially available fresh yogurts. The first family was *Bacillaeae* for *B. cereus* and *B. subtilis*, and the second was *Yersiniaceae* for *S. marcescens*. The highest frequencies of the isolates were as follows: 39.7% for *B. cereus*, 22.9% for *M. circinelloides*, 19.8% for *B. subtilis*, and 17.6% for *S. marcescens*. *Bacillus cereus* was recorded as having the highest frequency of the isolates. *Bacillus* and *Serratia* were characterized as rod-shaped. According to Oyeleke [38], the frequency of occurrence of bacterial contaminants in *Bacillus* sp. was 70%. Out of 120 samples (milk, yogurt, and milk contact surfaces), 80 bacteria from nine different species were isolated; the percentage of *S. aureus* contamination was 17 (21.3%) [39]. In Nigeria, Taiwo [40] noted eleven bacterial isolates were found in yogurt; *Lactobacillus* and *Bacillus* spp. made up 16% of the total

microbial load; *Corynebacterium*, *Klebsiela*, *Staphylococcus*, and *Pseudomonas* spp. accounted for 8%; *Proteus*, *Micrococcus*, *Shigella*, *Listeria*, and *Streptococcus* spp. accounted for 4%; and *Mucor* spp. (22%), *Geotrichum* spp. (17%), *Montospora* spp. (11%), and *Aspergillus*, *Rhizopus*, and *Fusanrium* spp. made up 6%. In 200 samples of both balady and brand-name yogurt, 27 isolates (13.5%) of *E. Coli*, 11 isolates of *E. faecalis*, and 8 isolates of *B. cereus* were identified, along with 37 molds (19 *Aspergillus niger*, 5 *Aspergillus fumigatus*, 5 *Mucor* spp., 3 *Aspergillus flavus*, 3 *Penicillium* spp., and 2 *Rhizopus* spp.) and 43 yeasts (23 *C. albicans*, 12 *Rhodotorula* spp., and 8 *C. tropicalis*) [41].

In Egypt and India, *B. cereus* was discovered in milk products with various amounts of contamination [18,19]. *Serratia* spp. can invade abiotic surfaces and produce heat-resistant enzymes, causing them to degrade milk at various stages of production [20–22]. *S. liquefaciens* was found in 27.78% of the positive samples tested [42]. According to a report [42], *S marcescens* had the highest isolation percentage (57.14% out of 21 positive samples). *Serratia* organisms have been linked to human infections such as septicemia, pulmonary and urinary tract infections, and urinary tract abscesses [20].

The third family was *Mucoraceae* for *M. circinelloides*. The morphology of *M. circinelloides* is characterized by big black sporangia with a mean area of 100%, a 0.202 µm mean circular shape, and a mean solidity of 0.488 µm. Numerous *Mucor* species are of high relevance to the field of biotechnology due to their ability to generate proteolytic enzymes and bioactive components [43,44]. Overall, the growth temperature affects the physiological properties of fungi. *Mucor* spp., in particular, can cause human infections [45]. Food processors suffer severe harm as a result of fungal deterioration due to the large spectrum of fungal species. According to a US FDA probe, *M. circinelloides* was related to a nutrition disease outbreak after infected yogurt was consumed. Storage temperature and natamycin interact to produce *M. circinelloides* yogurt deterioration, according to Snyder et al. [29]. Meanwhile, El-Shinawy et al. [46] found no fungal growth in yogurt samples. There are previous reports of isolated and recognized yeast and mold species from yogurt samples [47,48]. The microbial quality of yogurt affects its quality and acceptability. The possibility of microbial contamination due to unhygienic conditions raises the risk of serious consequences for consumer health [13].

A food's shelf life is the amount of time it can be stored without losing its safety, nutritional value, or sensory qualities, making it suitable for human consumption [49]. The product will lose some of its desirable qualities while being stored. Food can be harmed by oxygen, water, light, and harmful microorganisms. It can produce toxins as well as off-flavors and odors. Assessing a food's shelf life is simple; one observes how its colors and odors change. The quantity of microorganisms (bacteria and fungi) and acid produced during starter fermentation determine how long a food can be stored. Pasteurization does not kill all microorganisms. Other bacteria, in addition to a starter, may contribute to a reduction in pH levels [50]. The viability of the microorganisms during storage is influenced by several variables, including acidity, pH, hydrogen peroxide [51], storage temperature, and oxygen content. Microorganism viability will be decreased by unfavorable environmental conditions and poor nutrition [50]. This study revealed that *Bacillus cereus* exhibited the highest frequency of the isolates tested, resulting in bacteria that persist in pasteurized milk and shorten its shelf life.

4.2. Molecular Identification of Bacterial and Fungal Isolates

Our molecular analysis of both the fungal and bacterial isolates using ITS and 16S rRNA sequences revealed that the four isolates belonged to one fungal genus, *Mucor*, and two bacterial genera, *Bacillus* and *Serratia*, respectively. In *M. circinelloides*, a partial sequence consisting of internal transcribed spacers 1 and 2, the large subunit ribosomal RNA gene, and 5.8S ribosomal RNA was found. For a wide range of fungi, the ITS area is one of the indicators with the greatest chance of identification [52]. Several fungal researchers have reported the ITS region as an appropriate fungal barcode [53–55].

The nucleotide sequences of the bacterial and fungal species were matched to those in the GenBank database (NCBI), and a phylogenetic tree was built in MEGA 11 using the neighbor-joining method [36]. In NCBI, there was significant clustering and a high degree of similarity with the associated identified species. *Bacillus cereus* was 99.63% identified; *B. subtilis* was 99.01 to 99.10% identified; *S. marcescens* was 98.64% identical to 98.82% of the nucleotide sequences in the NCBI GenBank, and *M. circinelloides* was 99.49% to 100% identified. The nucleotide sequences that were determined for the five microorganisms helped in the confirmation of their identities. We obtained a sufficient definition of the species within the genera *Bacillus*, *Serratia*, and *Mucor* and our results agreed with those of [56], who proposed describing the classification of the species in the genus *Diaporthe* based on morphological, cultural, and DNA sequences.

The similarity of the strains to linked identified species suggests that these fungi have not been subjected to varied environmental conditions that would otherwise generate increased genetic variation, which is generally referred to as coordinated evolution [57]. A BLAST search indicated that certain isolates from various places were 100% identifiable, such as in *M. circinelloides* (MK559692.1) and *M. circinelloides* (MK560184.1). Teymori et al. [58] found that 23% of yogurt samples were contaminated with harmful microorganisms. To remove and limit the potential for contamination, it is necessary to identify critical limits with automated control systems [58].

5. Conclusions

SEM revealed that the *Bacillus* and *Serratia* cells were rod-shaped, whereas the *Mucor* cells were rounded and circular-shaped. *B. cereus* isolates were found at a high frequency (39.7%) in the yogurt products, causing them to degrade the yogurt at various stages of its production. We used the 16S rRNA and ITS sequences to describe bacterial and fungal isolates from yogurt containers purchased commercially during their validity period from a supermarket in Riyadh, Saudi Arabia. They were found to be *B. cereus* strain B-191, *B. subtilis* strain B-195, *S. marcescens* strain B-192, *S. marcescens* strain B-193 (Accession No: MK590996.1, MK591144.1, MK591002.1, and MK591014.1), and *M. circinelloides* isolates (Accession No: MK559692.1). Thus, the genotyping method based on the 16S rRNA and ITS gene sequences is straightforward and effective in identifying strains. The nucleotide sequences that were determined for the five microorganisms helped in the confirmation of their identities. The importance of such information will be significant for understanding microbial habitat ecology, and it will be simple to develop special protocols to address such microbes. Our research is the first to look specifically at fresh yogurt contaminants in KSA by isolating *Bacillus*, *Serratia*, and *Mucor*, which had been previously unrecorded.

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