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Molecular Identification of Dominant Microorganisms Associated with the Spoilage of Strawberries and Oranges Using Ribosomal RNA Gene Sequencing

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Abstract

Background: This study investigated the molecular identity of key microorganisms responsible for strawberry and orange spoilage. Ribosomal RNA genes (16SrRNA and 18SrRNA) and the nuclear ribosomal internal transcribed spacer (ITS) region were employed to identify the dominant species of microorganism associated with the observed spoilage. **Methods:** Samples of strawberries and oranges exhibiting grey and green molds, respectively, were collected from Al-Obour market. Microbial total counts were determined, and three bacterial isolates (coded Sab-01, Sab-02, and Sab-03) and two fungal isolates (Sa02 (F1) and Sa04 (F2)) were selected. **Results:** The isolates were purified and morphologically identified as Gram-positive bacilli (Sab-01), Gram-negative short rods (Sab-02 and Sab-03), *Botrytis* sp. (Sa02 (F1)), and *Penicillium* sp. (Sa04 (F2)). Molecular identification using the 16SrRNA gene sequence identified the bacterial isolates as *Bacillus subtilis* (Sab-01-RSDS), *Stenotrophomonas maltophilia* (Sab-02-RSDS), and *Pseudomonas putida* (Sab-03-RSDS), while the 18SrRNA and ITS region identified the fungal isolates as *Penicillium digitatum* (Sa04-RODS) and *Botrytis cinerea* (Sa02-RSDS). The strains described were deposited in GenBank under accession numbers LC784320.1 through LC784324.1. Sequence analysis of the 16S rRNA, 18S rRNA genes, and ITS region revealed high sequence identity (close to 100%) with closely related strains in GenBank. Phylogenetic analysis further corroborated the established genetic relationships, reinforcing the utility of these ribosomal RNA regions and the ITS region for precise microbial identification.**Conclusions:** This study provides compelling evidence for the effectiveness of natural plant extracts—specifically licorice (*Glycyrrhiza glabra*) and green tea (*Camellia sinensis*)-along with chitosan and their nanoparticle formulations in controlling postharvest microbial spoilage in strawberries and oranges.

Keywords: Strawberries, Oranges, 16S rRNA, 18S rRNA, ITS region.

1. Introduction

Vegetables, fruits, and cereals are vital components of the human diet due to their nutritional value, which includes vitamins, dietary fiber, minerals, and antioxidants [1]. Despite their health benefits, vegetables and fruits are highly susceptible to spoilage during postharvest handling and storage. This spoilage is often driven by microbial contamination, which can lead to significant losses in global food supply chains [2-4]. Microbial spoilage not only results in food waste but also contributes to customer dissatisfaction and economic losses [5-7].

Microbiological spoilage of fruits and vegetables can be initiated by a variety of microorganisms, including Gram-positive and Gram-negative bacteria, as well as fungi, yeasts, and molds [8-10]. Common spoilage fungi include *Penicillium italicum*, *Penicillium digitatum*, *Aspergillus spp.*, *Fusarium spp.*, *Penicillium spp.*, and *Alternaria spp.*, which can produce mycotoxins harmful to human health [11-12]. Bacteria, such as *Pseudomonas aeruginosa*, *P. putida*, and *P. syringae*, contribute to spoilage by degrading plant tissues using enzymes like cutinase and pectate lyase, leading to various forms of rot in vegetables [13-14]. In a study by Patil *et al.* [15] the most significant postharvest disease of sweet oranges, caused by *Penicillium sp.*, was identified as a major contributor to spoilage, particularly during the rainy season, with losses ranging from 50-60%. *Penicillium italicum* and *P. digitatum* are widely recognized as the primary fungal pathogens affecting citrus fruits during storage [16]. Early detection of these fungi can significantly extend shelf life and improve the quality of stored produce.

Molecular techniques such as nuclear ribosomal internal transcribed spacer (ITS) sequencing have become essential for distinguishing between different fungal species, including *Botrytis spp.* [17]. The use of ribosomal RNA (rRNA) gene sequencing, particularly the 16S rRNA gene for bacteria and the ITS region for fungi, has proven to be an effective tool for microbial identification at the genus and species levels [18-20]. The 16S rRNA gene is widely regarded as a reliable marker for bacterial taxonomy due to its universal presence in bacteria and its stability over time [21]. Similarly, the ITS region and 18S rRNA genes are highly effective for identifying fungal isolates at both the genus and species levels [22.23].

Strawberries, an important soft fruit, are globally cultivated due to their nutritional benefits, including vitamins, micronutrients, and antioxidants [24]. The molecular identification of microbial spoilage agents in strawberries and citrus fruits, using ribosomal RNA genes and the ITS region, is essential for understanding the microbial communities involved and improving postharvest management.

This study investigates the dominant microorganisms responsible for strawberry and orange spoilage through a combined morphological and molecular approach. Ribosomal RNA gene sequencing

and ITS region analysis will be employed to accurately identify the fungal and bacterial species most frequently associated with fruit decay.

2. Methods

Source of Samples: Strawberry and orange samples exhibiting visible grey and green molds, respectively, were collected from a local market **(Figure 1)**.

Microbial Total Counts: Total microbial counts, including bacteria and fungi, were determined following the method outlined by Feroz *et al*. [25], using Nutrient Agar for bacterial enumeration and Potato Dextrose Agar (PDA) for fungal isolation. Bacterial plates were incubated at 37°C for 24 hours, while PDA plates were incubated at 28°C for one week.

Morphological Identification: Morphological identification of the two selected fungal isolates was performed using the slide culture technique described by Prakash and Bhargava, [26], with PDA medium. After 15 days of incubation at 28°C, the slides were examined under light microscopy. This analysis was conducted at the Plant Clinic Unit, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Molecular Identification: The three bacterial isolates, morphologically identified as bacilli and short rods, along with the two fungal isolates identified as *Botrytis* and *Penicillium*, were sent to Macrogen® (908 World Meridian Venture Center, #60-24, Gasan-dong, Geumchun-gu, Seoul 153-781, Korea) for molecular identification **(Figure 2)**. **Nucleotide Sequences of 16S rRNA and 18S rRNA Genes:** The nucleotide sequences of the 16S rRNA gene were amplified by polymerase chain reaction (PCR) using two universal primers: 785F (GGA TTA GAT ACC CTG GTA 3') and 907R (CCG TCA ATT CMT TTR AGT TT 3'). For the 18S rRNA gene, the primers ITS1 (5'–TCC GTA GGT GAA CCT GCG G–3') [27] and ITS4 (5'–ATC CTC CGC TTA TTG ATA TGC–3') [28] were used. DNA extracts from the five microbial isolates served as templates for the amplification. **Sequencing of PCR Products:** The PCR products of both the 16S rRNA and 18S rRNA genes were sequenced using the ABI PRISM BigDye™ Terminator Cycle Sequencing Kit and the ABI PRISM 3730XL Analyzer (96-capillary type, Applied Biosystems). Sequencing was performed with the MJ Research PTC-225 Peltier Thermal Cycler and DNA polymerase (FS enzyme, Applied Biosystems).

Sequencing Analysis: The nucleotide sequences were analyzed and compared with the most similar strains documented in GenBank using the Standard Nucleotide BLAST tool available from the National Library of Medicine, NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi?Program).

3. Results

During storage, strawberries exhibiting grey mold and oranges showing green mold were assessed for microbial contamination. As shown in Table 1, microbial counts were higher in strawberry samples (bacteria: 3.50×10^3 CFU/g; fungi: 4.80×10^4 CFU/g) compared to orange samples (bacteria: 2.75×10^3 CFU/g; fungi: 4.20×10^4 CFU/g). Fungal contamination was more pronounced in both fruits than bacterial contamination.

Key fungal pathogens identified in this study include *Penicillium digitatum* and *Aspergillus niger*, commonly associated with citrus spoilage, and *Botrytis cinerea*, the causative agent of grey mold in strawberries. The isolation of bacterial isolates-Sab-01, Sab-02, and Sab-03-along with two fungal isolates, Sa02 (F1) (*Botrytis sp.*) and Sa04 (F2) (*Penicillium* sp.), confirmed the presence of known spoilage organisms. Morphological identification revealed that Sab-01 was a Gram-positive bacillus, while Sab-02 and Sab-03 were Gram-negative short rods **(Figure 3)**. The fungal isolates were identified as *Botrytis* sp. (Sa02 (F1)) and *Penicillium* sp. (Sa04 (F2)) **(Figure 4)**.

Molecular identification using 16S rRNA and 18S rRNA genes confirmed the bacterial and fungal isolates. The bacterial isolates identified were *Bacillus subtilis* (Sab-01-RSDS) with 99.21% identity to *Bacillus subtilis* strains from GenBank (LC784320.1) **(Figure 5 & Table 2)**, *Stenotrophomonas maltophilia* (Sab-02-RSDS) with 98.19% identity to *Stenotrophomonas maltophilia* strains (LC784321.1) **(Figure 6 & Table 3)**, and *Pseudomonas putida* (Sab-03-RSDS) with 99.93% identity to *Pseudomonas* strains (LC784322.1) **(Figure 7 & Table 4)**. The fungal isolates were identified as *Botrytis cinerea* (Sa02-RSDS) with 99.30% identity to *Botrytis cinerea* strains (LC784324.1) **(Figure 8 & Table 5)** and *Penicillium digitatum* (Sa04-RSDS) with 98.01% identity to *Penicillium digitatum* strains (LC784323.1) **(Figure 9 & Table 6)**. Blast analysis confirmed high identity (99% to 100%) with related strains in GenBank, confirming the accuracy of molecular identification.

Phylogenetic trees generated for the bacterial and fungal strains confirmed the genetic relationship between the isolates and their closest matches in GenBank **(Figure 10)**. This provides additional evidence that ribosomal RNA genes are reliable tools for microbial identification in postharvest studies. The trees show a clear genetic relationship between the identified isolates and their counterparts in GenBank, supporting the conclusion that these species are commonly found in postharvest environments. This method is a valuable tool in the monitoring and identification of microbial pathogens, helping to inform the development of strategies to reduce contamination and spoilage in stored fruits.

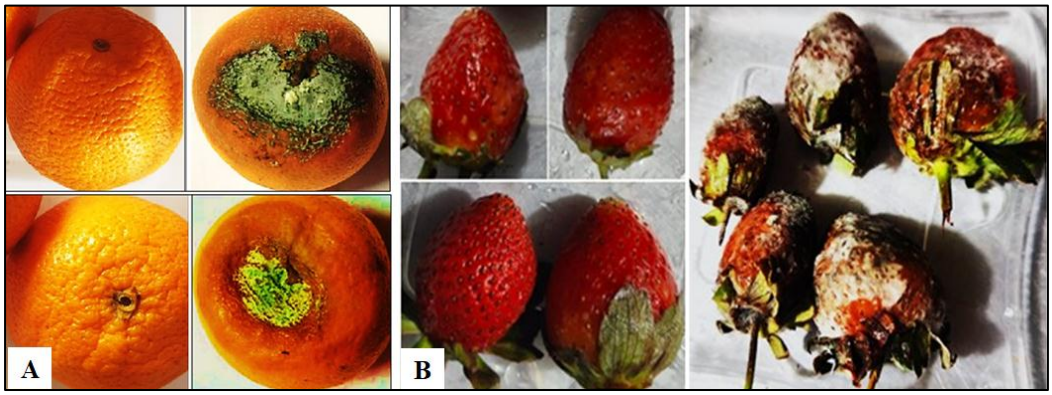

Fig. (1) Orange (A) and strawberry (B) samples exhibiting green and grey molds, respectively, were collected from a local market, alongside healthy control samples for comparison.

Table (1) Microbial total count of strawberry and orange samples collected from a local market, xhibiting green and grey molds, respectively

Samples	Microbial total counts	
	Bacteria	**Fungi**
Strawberries	$3.50x10^3$	$4.80x10^4$
Oranges	$2.75x10^3$	$4.20x10^4$

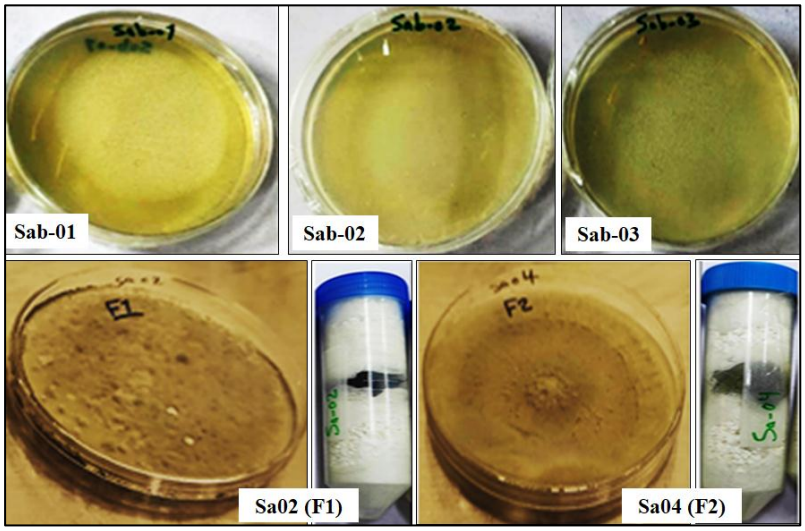

Fig. (2) The microbial isolates, including bacteria (Sab-01, Sab-02, and Sab-03) and fungi (*Sa-02* (F1) and *Sa-04* (F2)) obtained from strawberries and oranges exhibiting green and grey molds, respectively, were sent to Macrogen Center, Korea for sequencing. The isolates were cultured and processed using the calcium chloride dehydration technique.

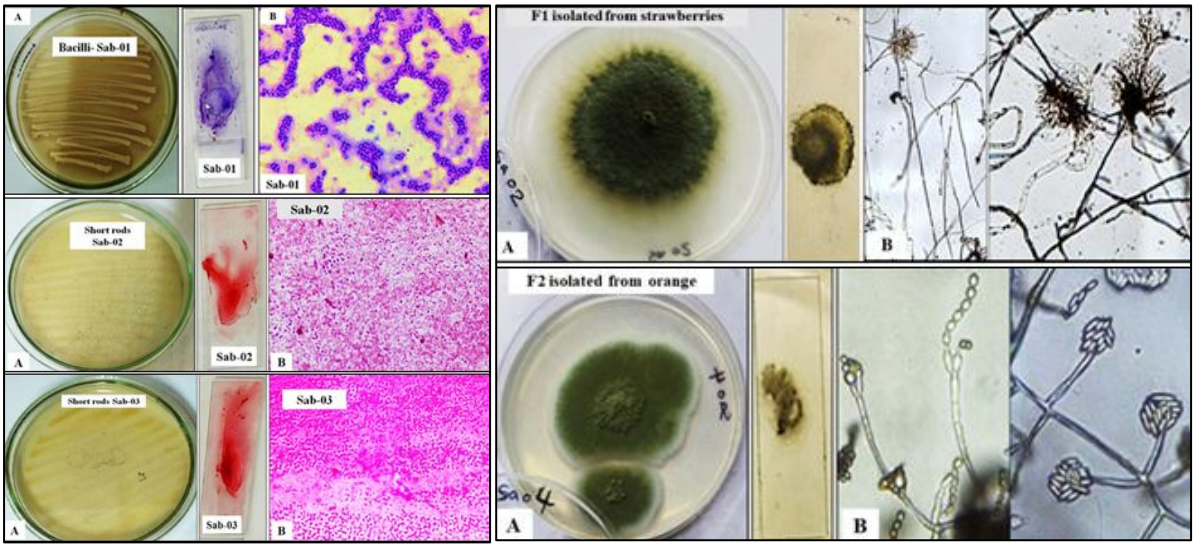

Fig. (3) Purified microbial isolates: Sab-01 (*Bacilli*), Sab-02 (Short rods), Sab-03 (Short rods) & *Botrytis* obtained from a strawberry sample exhibiting grey mold and *Penicillium obtained from* an orange sample exhibiting green mold. A, cultural growth on NA and PDA for bacterial isolates. B, Gram staining and slide culture technique for fungal isolates.

Table (2) The nucleotide sequence of the 16S rRNA gene from *Bacillus subtilis* strain Sab-01-RSDS (LC784320.1), isolated from strawberries exhibiting grey mold, showed significant alignments with the most similar strains in GenBank

Description	Query Cover (%)	Identities (%)	Accession
Bacillus subtilis strain ZZZZ45 16S ribosomal RNA gene, partial sequence	100	99.21	ON624342.1
Bacillus subtilis strain AYEF_9 16S ribosomal RNA gene, partial sequence	100	99.21	OK336472.1
Bacillus subtilis strain AYEC_8 16S ribosomal RNA gene, partial sequence	100	99.21	OK336471.1
Bacillus subtilis strain AYE6_6 16S ribosomal RNA gene, partial sequence	100	99.21	OK336469.1
Bacillus subtilis strain AYEQ_5 16S ribosomal RNA gene, partial sequence	100	99.21	OK336468.1

1 tcaggacgaa cgctggcggc gtgcctaata catgcaagtc gagcggacag atgggagctt
61 gctccctgat gttagcggcg gacgggtgag taacacgtgg gtaacctgcc tgtaagactg
121 ggataactcc gggaaaccgg ggctaatacc ggatgcttgt ttgaaccgca tggttcaaac
181 ataaaaggtg gcttcggcta ccacttacag atggacccgc ggcgcattag ctagttggtg
241 aggtaatggc tcaccaaggc aacgatgcgt agccgacctg agagggtgat cggccacact
301 gggactgaga cacggcccag actcctacgg gaggcagcag tagggaatct tccgcaatgg
361 acgaaagtct gacggagcaa cgccgcgtga gtgatgaagg ttttcggatc gtaaagctct
421 gttgttaggg aagaacaagt accgttcgaa tagggcggta ccttgacggt acctaaccag
481 aaagccacgg ctaactacgt gccagcagcc gcggtaatac gtaggtggca agcgttgtcc
541 ggaattattg ggcgtaaagg gctcgcaggc ggtttcttaa gtctgatgtg aaagcccccg
601 gctcaaccgg ggagggtcat tggaaactgg ggaacttgag tgcagaagag gaaagtggaa
661 ttccacgtgt agcggtgaaa tgcgtagaga tgtggaggaa caccagtggc gaaggcgact
721 ctctggtctg taactgacgc tgaggagcga aagcgtgggg agcgaacagg attagatacc
781 ctggtagtcc acgccgtaaa cgatgagtgc taagtgttag ggggtttccg ccccttagtg

841 ctgcagctaa cgcattaagc actccgcctg gggagtacgg tcgcaagact gaaactcaaa
901 ggaattgacg ggggcccgca caagcggtgg agcatgtggt ttaattcgaa gcaacgcgaa
961 gaaccttacc aggtcttgac atcctctgac aatcctagag ataggacttc cccttcgggg
1021 gcagagtgac aggtggtgca tggttgtcgt cagctcgtgt cgtgagatgt tgggttaagt
1081 cccgcaacga gcgcaaccct tgatcttagt tgccagcatt cagttgggca ctctaaggtg
1141 actgccggtg acaaaccgga ggaaggtggg ggatgacgtc aaatcatcat gccccttatg
1201 acctgggcta cacacgtgct acaatggaca gaacaaaggg cagcgaaacc gcgaggttaa
1261 gccaatccca caaatctgtt ctcagttcgg gatcgcagtc tgcaactcga ctgcgtgaag
1321 ctggaatcgc tagtaatcgc ggatcagcat gccgcgggtg aatacgttcc cgggccttgt
1381 acacaccgcc cgtcacacca cgaagagttt gtaacacccg aagttcggtg aggtaacctt
1441 ttaggagcca gccgccgaag gtgggacaga tgattggggt agtcgtaaca aggtagccgt
1501 atcggaaggt gcggctg

Fig. (4) *Bacillus subtilis* group sp. Sab-01-RSDS, a 16S ribosomal RNA partial sequence gene isolated from strawberries exhibiting grey mold, has been documented in GenBank under accession number LC784320.1.

1 gctcagagtg aacgctggcg gtaggcctaa cacatgcaag tcgaacggca gcacagagga
61 gcttgctcct tgggtggcga gtggcggacg ggtgaggaat acatcggaat ctactctgtc
121 gtgggggata acgtagggaa acttacgcta ataccgcata cgacctacgg gtgaaagcag
181 gggaccttcg ggccttgcgc gattgaatga gccgatgtcg gattagctag ttggcggggt
241 aaaggcccac caaggcgacg atccgtagct ggtctgagag gatgatcagc cacactggaa
301 ctgagacacg gtccagactc ctacgggagg cagcagtggg gaatattgga caatgggcgc
361 aagcctgatc cagccatacc gcgtgggtga agaaggcctt cgggttgtaa agccctttg
421 ttgggaaaga aatccagctg gctaataccc ggttgggatg acggtaccca aagaataagc
481 accggctaac ttcgtgccag cagccgcggt aatacgaagg gtgcaagcgt tactcggaat
541 tactgggcgt aaagcgtgcg taggtggtcg tttaagtccg ttgtgaaagc cctgggctca
601 acctgggaac tgcagtggat actgggcgac tagagtgtgg tagagggtag cggaattcct
661 ggtgtagcag tgaaatgcgt agagatcagg aggaacatcc atggcgaagg cagctacctg
721 gaccaacact gacactgagg cacgaaagcg tggggagcaa acaggattag ataccctggt
781 agtccacgcc ctaaacgatg cgaactggat gttgggtgca atttggcacg cagtatcgaa
841 gctaacgcgt taagtcgccg cctggggagt acggtcgcaa gactgaaact caaaggaatt
901 gacgggggcc cgcacaagcg gtggagtatg tggtttaatt cgatgcaacg cgaagaacct
961 tacctggcct tgacatgtcg agaactttcc agagatggat gggtgccttc gggaactcga
1021 acacaggtgc tgcatggctg tcgtcagctc gtgtcgtgag atgttgggtt aagtcccgca
1081 acgagcgcaa cccttgtcct tagttgccag cacgtaatgg tgggaactct aaggagaccg
1141 ccggtgacaa accggaggaa ggtgggggat gacgtcaagt catcatggcc cttacggcca
1201 gggctacaca cgtactacaa tggtagggac agagggctgc aagccggcga cggtaagcca
1261 atcccagaaa ccctatctca gtccgggatt ggagtctgca actcgactcc atgaagtcgg
1321 aatcgctagt aatcgcagat cagcattgct gcgggtgaaa tacgttcccg ggccttgtac
1381 acaccgcccg tcacacccat gggggagttt tgttgcacca gaaagcaggt agcttaaccc
1441 ttcggggagg gcgcttggcc acgggtgtgg gccgatgact gggggtgaa

Fig. (5) *Stenotrophomonas maltophilia* group sp. Sab-02-RSDS, a partial sequence of the 16S ribosomal RNA gene isolated from strawberries exhibiting grey mold, is documented in GenBank under accession number LC784321.1.

Table (3) Sequences producing significant alignments of the nucleotide sequence of the 16S rRNA gene from *Stenotrophomonas maltophilia* strain Sab-02-RSDS (LC784321.1), isolated from strawberries exhibiting grey mold, compared to the most similar strains in GenBank

Description	Query Cover (%)	Identities (%)	Accession
Stenotrophomonas maltophilia strain Au-Ste59 16S ribosomal RNA gene, partial sequence	100	98.19	OK189604.1
Stenotrophomonas maltophilia strain CEMTC_3670 16S ribosomal RNA gene, partial sequence	100	98.19	MT040045.3
Stenotrophomonas maltophilia strain CEMTC_3659 16S ribosomal RNA gene, partial sequence	100	98.19	MT040043.3
Stenotrophomonas maltophilia strain Lewis_Bac_13 16S ribosomal RNA gene, partial sequence	100	98.19	MH329940.1
Stenotrophomonas maltophilia strain AY2 16S ribosomal RNA gene, partial sequence	99	98.18	MH478205.2

1 attgaacgct ggcggcaggc ctaacacatg caagtcgagc ggatgacggg agcttgctcc
61 ttgattcagc ggcggacggg tgagtaatgc ctaggaatct gcctggtagt gggggacaac
121 gttccgaaag gggcgctaat accgcatacg tcctacggga gaaagtgggg gatcttcgga
181 cctcacgcta tcagatgagc ctaggtcgga ttagctagtt ggtgaggtaa aggctcacca
241 aggcgacgat ccgtaactgg tctgagagga tgatcagtca cactggaact gagacacggt
301 ccagactcct acgggaggca gcagtgggga atattggaca atgggcgaaa gcctgatcca
361 gccatgccgc gtgtgtgaag aaggtcttcg gattgtaaag cactttaagt tgggaggaag
421 ggcagtaagt taataccttg ctgttttgac gttaccgaca gaataagcac cggctaactc
481 tgtgccagca gccgcggtaa tacagagggt gcaagcgtta atcggaatta ctgggcgtaa
541 agcgcgcgta ggtggttcgt taagttggat gtgaaagccc cgggctcaac ctgggaactg
601 catccaaaac tggcgagcta gagtatggta gagggtggtg gaatttcctg tgtagcggtg
661 aaatgcgtag atataggaag gaacaccagt ggcgaaggcg accacctgga ctgatactga
721 cactgaggtg cgaaagcgtg gggagcaaac aggattagat accctggtag tccacgccgt
781 aaacgatgtc aactagccgt tggaatcctt gagattttag tggcgcagct aacgcattaa
841 gttgaccgcc tggggagtac ggccgcaagg ttaaaactca aatgaattga cgggggcccg
901 cacaagcggt ggagcatgtg gtttaattcg aagcaacgcg aagaacctta ccaggccttg
961 acatgcagag aactttccag agatggattg gtgccttcgg gaactctgac acaggtgctg
1021 catggctgtc gtcagctcgt gtcgtgagat gttgggttaa gtcccgtaac gagcgcaacc
1081 cttgtcctta gttaccagca cgttatggtg ggcactctaa ggagactgcc ggtgacaaac
1141 cggaggaagg tggggatgac gtcaagtcat catggccctt acggcctggg ctacacacgt
1201 gctacaatgg tcggtacaga gggttgccaa gccgcgaggt ggagctaatc tcacaaaacc
1261 gatcgtagtc cggatcgcag tctgcaactc gactgcgtga agtcggaatc gctagtaatc
1321 gcgaatcaga atgtcgcggt gaatacgttc ccgggccttg tacacaccgc ccgtcacacc
1381 atggggagtg ggttgcacca gaagtagcta gtctaacctt cgggaggacg gttaccacgg
1441 tgtgattcat gactggggtg a

Fig. (6) *Pseudomonas putida* group sp. Sab-03-RSDS, a partial sequence of the 16S ribosomal RNA gene isolated from strawberries exhibiting grey mold, is documented in GenBank under accession number LC784322.1.

Table (4) Sequences producing significant alignments of the nucleotide sequence of the 16S rRNA gene from *Pseudomonas putida* strain Sab-03-RSDS (LC784322.1), isolated from strawberries exhibiting grey mold, compared to the most similar strains documented in GenBank

Description	Query Cover (%)	Identities (%)	Accession
Pseudomonas putida strain IEC33019, complete genome	100	99.93	CP016634.1
Pseudomonas sp. B4(2012) 16S ribosomal RNA gene, partial sequence	100	99.93	JN828798.1
Pseudomonas sp. BCRC 17752 16S ribosomal RNA gene, partial sequence	100	99.93	GU370392.1
Pseudomonas sp. SMIC-5 16S ribosomal RNA gene, partial sequence	100	99.93	FJ877156.1
Pseudomonas sp. strain QAUO6 16S ribosomal RNA gene, partial sequence	99	99.86	KX644133.1

1 gttaaaactt tcaacaacgg atctcttggt tctggcatcg atgaagaacg cagcgaaatg
61 cgatacgtag tgtgaattgc agaattcagt gaatcatcga atctttgaac gcacattgcg
121 ccctttggta ttccgggggg catgcctgtt cgagcgtcat ttgaaccctc aagcttagct
181 tggtattgag tctatgtcag taatggcagg ctctaaaatc agtggcggcg gcgctgggtc
241 ctgaacgtag taatatctct cgttacaggt tctcggtgtg cttctgccaa aacccaaatt
301 tttctatggt tgaccacgga tcaggtaggg atagccgctg aacttaagca ta

Fig. (7) *Botrytis cinerea* Sa02-RSDS, a gene sequence encompassing 18S rRNA, ITS1, 5.8S rRNA, ITS2, and 28S rRNA (partial and complete sequences), isolated from strawberries exhibiting grey mold, is documented in GenBank under accession number LC784324.1.

Table (5) Sequences producing significant alignments of the nucleotide sequence of 18S rRNA, ITS1, 5.8S rRNA, ITS2, and 28S rRNA from *Botrytis cinerea* strain Sa02-RSDS (LC784324.1), isolated from strawberries exhibiting grey mold, compared to the most similar strains documented in GenBank

Description	Query Cover (%)	Identities (%)	Accession
Botrytis cinerea strain ATCC 11542 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene	100	98.01	KU729081.1
Botrytis cinerea isolate 19-4d-2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene	100	98.01	KX074008.1
Botrytis fabae isolate 19-3d internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene	100	98.01	KX074007.1
Botrytis cinerea strain P5_B8_425 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene	100	98.01	KU325214.1
Botrytis cinerea strain P1_G4_40 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene	100	98.01	KU324964.1

1 ctgcggagac attaccgagt gagggccctc tgggtccaac ctcccacccg tgtttatttt
61 accttgttgc ttcggcgggc ccgcctttac tggccgccgg ggggctcacg ctcccgggcc
121 cgcgcccgcc gaagacaccc ccgaactctg tctgaagatt gcagtctgag tgaaaacgaa
181 attatttaaa actttcaaca acggatctct tggttccggc atcgatgaag aacgcagcga
241 aatgcgatac gtaatgtgaa ttgcaaattc agtgaatcat cgagtctttg aacgcacatt
301 gcgccccctg gtattccggg gggcatgcct gtccgagcgt cattgctgcc ctcaagcccg
361 gcttgtgtgt tgggccccgt cccccgatcc cgggggacgg gcccgaaagg cagcggcggc
421 accgcgtccg gtcctcgagc gtatggggct ttgtcacccg ctccgtaggc ccggccggcg
481 cctgccgatc aaccccaaat ttttaatcca ggttgacctc ggatcaggta gggatacccg
541 ctgaacttaa gcatatcaat aaagcggag

Fig. (8) *Penicillium* sp. Sa04-RODS genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, and 28S rRNA (partial and complete sequences), isolated from oranges exhibiting green mold, and documented in GenBank under accession number LC784323.1.

Table (6) Sequences producing significant alignments of the nucleotide sequence of 18S rRNA, ITS1, 5.8S rRNA, ITS2, and 28S rRNA (partial and complete sequences), isolated from oranges exhibiting green mold and documented in GenBank under accession number LC784323.1, compared to the most similar strains in GenBank.

Description	Query Cover (%)	Identities (%)	Accession
Penicillium digitatum isolate CMV010G4 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene	100	99.30	MK450692.1
Penicillium digitatum strain 74 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene	100	99.30	MF568039.1
Penicillium digitatum strain PdVN1 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene	100	99.30	MF527231.1
Penicillium digitatum strain FRR 1313 18S ribosomal RNA gene	100	99.30	AY373910.1
Penicillium sp. 13 BRO-2013 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene	100	99.30	KF367511.1

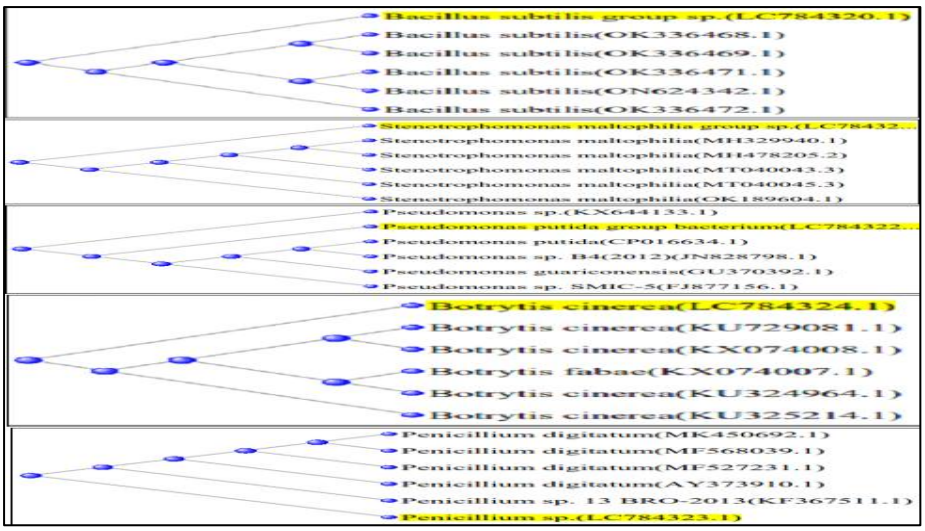

Fig. (9) Dendrogram showing the genetic relationship between the partial nucleotide sequence of 16S rRNA of the three bacterial isolates (LC784320.1, LC784321.1 and (LC784322.1) and 18S rRNA of each of *Botrytis cinerea* (LC784324.1) and *Penicillium digitatum* (LC784323.1) strains isolated from strawberries and orange exhibiting molds compared to the most similar strains documented in GenBank.

4. Discussion

The results demonstrated that fungal contamination was more dominant than bacterial contamination in both strawberries and oranges during storage. This aligns with previous findings by Beuchat and Ryu [29], who reported that the acidic pH of fruits favors fungal growth, making fungi the primary agents of postharvest spoilage. The identification of *Botrytis cinerea* in strawberries and *Penicillium digitatum* in oranges supports previous reports by Moss [30], which highlighted these species as the major fungal pathogens in stored soft fruits and citrus. Morphological and cultural characteristics further confirmed the typical traits of these spoilage organisms. Molecular identification using 16S and 18S rRNA genes proved effective, yielding high identity matches with known sequences in GenBank. These findings are consistent with studies by Lu *et al*. [31-12], which emphasized the reliability of ribosomal RNA genes for accurate microbial identification in food spoilage contexts. Moreover, phylogenetic analyses provided additional confirmation of the close taxonomic relationships between the isolates and their respective reference strains. This approach proves valuable in tracking microbial pathogens in postharvest environments and may support the development of strategies to mitigate fruit spoilage and economic losses during storage.

5. Conclusion

Spoiled strawberries and oranges from Al-Obour market yielded three bacteria (*Bacillus subtilis* Sab-01-RSDS, *Stenotrophomonas maltophilia* Sab-02-RSDS, *Pseudomonas putida* Sab-03-RSDS) and two fungal isolates (*Penicillium digitatum* Sa04-RODS, *Botrytis cinerea* Sa02-RSDS), identified *via* 16S rRNA, 18S rRNA, and ITS sequencing. These isolates, exhibiting high sequence similarity (close to 100%) to existing GenBank strains, were deposited under accession numbers LC784320.1 to LC784324.1. Phylogenetic analyses corroborate species identification, affirming the utility of ribosomal RNA and ITS regions for accurate isolate identification.

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