Antibacterial activity of Mexican oregano (Lippia graveolens H.B.K.) essential oil against of bacteria associated with postharvest spoilage of strawberries and tomatoes

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Research Article

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Abstract

Strawberries (*Fragaria × ananassa*) and tomatoes (*Solanum lycopersicum*) are highly perishable plant-based foods that are susceptible to postharvest spoilage caused by bacteria. The accurate identification of microorganisms associated with spoilage and the evaluation of natural antimicrobial compounds are essential for developing sustainable preservation strategies. This study aimed to isolate and molecularly characterize native bacterial strains associated with postharvest spoilage of strawberries and tomatoes, as well as to evaluate the *in vitro* antibacterial activity of Mexican oregano (*Lippia graveolens* H.B.K.) essential oil against the isolated strains. Four bacterial strains were characterized by colony morphology, Gram staining, partial 16S rRNA gene sequencing, and phylogenetic analysis using the Neighbor-Joining method with 1,000 bootstrap replicates. The strains were classified into the genera *Enterobacter* and *Bacillus*, showing the greatest similarity to *Enterobacter cloacae*, *Enterobacter hormaechei*, *Bacillus subtilis*, and *Bacillus mycoides*. Although partial 16S rRNA gene sequences provided limited resolution at the species level for some strains, phylogenetic analysis supported their preliminary taxonomic classification. The antibacterial activity of Mexican oregano essential oil formulated as an emulsion was evaluated against the bacterial isolates. Complete inhibition of the *Enterobacter* isolates (SB1 and TB1) and one *Bacillus* isolate (SB2) was observed at concentrations as low as 0.05%, whereas the second *Bacillus* isolate (TB2) showed greater tolerance within the conditions evaluated. These findings expand current knowledge of the bacterial diversity associated with postharvest spoilage of strawberries and tomatoes and support the potential use of Mexican oregano essential oil as a promising natural alternative for controlling bacteria associated with postharvest spoilage.

Highlights

- Native bacteria associated with postharvest spoilage were molecularly characterized.
- Phylogenetic analysis assigned isolates to the genera *Enterobacter* and *Bacillus*.
- Mexican oregano essential oil inhibited bacterial isolates at low concentrations.
- Mexican oregano essential oil is a promising natural preservative.

Introduction

Tomato (*Solanum lycopersicum*) is one of the most important and widely consumed vegetables worldwide and can be considered the number one vegetable [36]. Another important crop is the strawberry (*Fragaria × ananassa*), which is widely distributed due to its genetic diversity, intense red color, juicy texture, and flavor [38]. Tomatoes and strawberries are economically important horticultural crops with extensive global production and commercialization. However, they are highly perishable due to intrinsic properties such as their high water activity (Aw) and a pH close to neutrality. These characteristics make them susceptible to mechanical damage and deterioration by bacteria, filamentous fungi, and yeasts, which are the main agents responsible for postharvest deterioration [19]. Many microorganisms associated with spoilage cause significant organoleptic deterioration, including changes in texture, aroma, flavor, and appearance, leading to product rejection [33]. Visual symptoms often include softening of tissues, watery appearance, soft texture, and lesion formation. Several bacterial genera have been associated with the spoilage of plant-based foods, including *Erwinia*, *Enterobacter*, *Serratia*, *Pseudomonas*, *Lactobacillus, Leuconostoc*, *Bacillus*, *Xanthomonas*, and

Ralstonia [3, 19, 41]. Therefore, accurate identification of spoilage-associated bacteria is essential for understanding postharvest microbiota and for developing effective preservation strategies. Conventional phenotypic characterization offers several advantages, as this method is easy to observe, evaluate, and replicate without the need for highly specialized equipment [5]. Nowadays, bacterial identification has improved considerably through the use of molecular methods based on genotypic markers, particularly 16S ribosomal RNA gene sequencing, which has become a standard method for determining bacterial diversity and taxonomy and is widely accepted as a reference marker for phylogenetic analysis. This gene contains highly conserved regions, suitable for the design of universal primers, along with hypervariable regions that allow for reliable taxonomic discrimination, especially at the genus level, making it one of the most widely used molecular markers for bacterial identification and phylogenetic analysis [18, 42, 43].

Although significant progress has been made in identifying the microorganisms that cause spoilage, effective strategies are necessary to control bacterial spoilage of plant-based foods. In recent years, plant-derived antimicrobial compounds have attracted growing interest as natural alternatives to synthetic preservatives. Essential oils are complex mixtures of volatile compounds with well-documented antimicrobial activity against a broad spectrum of microorganisms. Among them, Mexican oregano essential oil (*Lippia graveolens* H.B.K.) is characterized by a high content of phenolic monoterpenes, particularly carvacrol and thymol, which disrupt the integrity of the bacterial membrane and inhibit microbial growth [12, 14, 22]. Although essential oils have been reported to exhibit antimicrobial activity against a wide variety of microorganisms, much fewer data are available on their activity against native bacteria associated with postharvest spoilage of plant-based foods. Despite growing interest in natural antimicrobial compounds, there remains a lack of information that combines the molecular characterization of native bacteria associated with spoilage with an evaluation of Mexican oregano essential oil against these strains.

Therefore, the objective of this study was to isolate and molecularly characterize native bacterial strains associated with the postharvest spoilage of strawberries and tomatoes, as well as to evaluate the *in vitro* antibacterial activity of Mexican oregano (*Lippia graveolens* H.B.K.) essential oil against these isolates.

Materials and methods

Collection of samples and taxonomic identification

Samples of strawberries (*Fragaria × ananassa*) and tomatoes (*Solanum lycopersicum*) showing visible symptoms of postharvest deterioration were collected at the local “El Refugio” market (Durango, Mexico) (Fig. 1). Fresh samples with no visible signs of spoilage, used for taxonomic identification, and spoiled samples, used for microbiological analysis, were transported separately under aseptic conditions to prevent cross-contamination.

Taxonomic identification of strawberry and tomato was performed by Dr. Socorro González-Elizondo, and voucher specimens (No. 61895 and 471, respectively) were stored at the Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional del Instituto Politécnico Nacional, Unidad Durango, México (CIIDIR) for future reference.

The spoiled samples were individually packaged, labeled, and aseptically transported to the Microbiology and Biotechnology Laboratory at the Instituto Tecnológico de Durango, where the bacteria were immediately isolated.

Isolation and purification of bacteria from naturally infected sample

The isolation of bacteria was carried out from naturally infected strawberry and tomato fruits to obtain axenic cultures throughout three consecutive subcultures of individual colonies, according to the method described in [31], with some modifications. In the first isolation, in a laminar flow cabinet (Laminar Flow Cabinet ESCO®, Singapore) the affected part was taken with a sterile scalpel, approximately 1 cm^3 of visibly deteriorated tissue of strawberry or tomato, areas with symptoms such as bruises or softening were selected and placed in the center of Petri dishes containing sterile Luria Broth (Miller's LB Broth) 6 g/L (w/v) (Condalab, Spain) and agar 6 g/L (w/v) (Mcd lab, Mexico) and incubated at 37 ± 2°C for 24 h. For the second isolation, bacterial growth was observed, and morphologically different colonies (shape, size, color, brightness) were selected for re-isolation by the cross-streak method. A third and final isolation was carried out by subsequent re-streaking on sterile LB medium. This process allowed for the progressive purification of native bacterial isolates. Four native bacterial strains were isolated from deteriorated plant-based foods. From strawberry samples, two strains were obtained and coded as SB1 and SB2, while for tomato samples, also two strains were isolated and labeled as TB1 and TB2. The bacteria were isolated from naturally deteriorated tissues; therefore, the isolated strains were considered microorganisms associated with deterioration, rather than confirmed causative agents of deterioration.

Phenotypic characterization of isolated bacteria

Bacterial colonies were examined for colony morphology; observations were made after 24 h of incubation in LB medium. Macroscopic characterization was based on the color, shape, borders, texture and elevation of the bacterial colonies, following criteria described in the literature [5] For microscopic characterization, Gram staining was performed according to the method described by [25]. Slides were observed under a light microscope (LEICA CME, Germany) using a 100x oil immersion objective.

Molecular characterization of isolated bacteria

Genomic DNA extraction

Genomic DNA was extracted from bacterial strains following the protocol described by [8], with minor modifications. DNA concentration and purity were determined using a NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific, USA), and DNA integrity was confirmed by 1% (w/v) agarose gel electrophoresis, according to the protocol described by [37]. The corresponding bands were observed in an ENDURO™ UV Transilluminator (UVP) (Labnet, USA).

PCR amplification of 16S rRNA genes

For the molecular identification of the bacteria, the amplification of the partial 16S rRNA gene was performed following the protocol described by [34]. The universal primers used were 356F (5'-CCTACGGGAGGCAGCAG-3') and 517R (5'-ATTACCGCGGCTGCTGG-3'), as originally described by [26]. For one isolate, primer 805R (5'-GACTACAAGGGTATCTAATCC-3') was additionally used to improve sequence coverage. Primers were synthesized by T4OLIGO® (Mexico). Each PCR reaction was prepared in a sterile 0.25 mL Eppendorf microtube with a final volume of 25 µL, the components and specific amounts of each reagent were 5 µL of 5× amplification buffer, 0.5 µL of dNTPs (20 mM), 0.5 µL of forward primer (20 µM), 0.5 µL of reverse primer (20 µM), 0.25 µL of thermostable DNA polymerase, 1.5 µL of $MgCl_2$, 14.75 µL of nuclease-free water, 2 µL of genomic DNA. PCR was carried out in a T100 Thermal Cycler (Bio-Rad, USA) under the following conditions: initial denaturation at 95°C for 3 min, followed by 30 cycles of denaturation at 70°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min; followed by a final extension at 72°C for 5 min. Amplification products were confirmed by electrophoresis on 1% (w/v) agarose gel using a 1 kb molecular marker, as described by [37]. The amplified fragments were observed in an ENDURO™ UV Transilluminator (UVP) (Labnet, USA). PCR products were purified with the Wizard® SV Gel and PCR Clean-Up System (Promega, USA).

Sequencing and data analysis

PCR products were sequenced at the Biotechnology Institute of the Universidad Nacional Autónoma de México (UNAM) located in Cuernavaca, Morelos, Mexico. Bidirectional sequencing was performed to obtain high-quality complementary reads. Raw sequence data were processed and aligned using BioEdit software version 7.2.5 [16]. The sequences were compared against the National Center for Biotechnology Information (NCBI) non-redundant database (http://www.ncbi.nlm.nih.gov/) using the BLASTn algorithm. Only results with identity above 97%, an E-value close to zero, and consistent results across multiple databases were considered valid. The resulting 16S rRNA gene sequences were aligned using MUSCLE. A phylogenetic tree was constructed using the Neighbour-joining (NJ) method with 1000 bootstrap replicates, implemented in MEGA software version 11.0.13 [35, 40]. The sequences obtained were compared with reference sequences deposited in GenBank using the BLASTn algorithm, and the closest matches were used to establish a provisional taxonomic classification at the genus level. The partial 16S rRNA gene sequences generated in this study were deposited in the GenBank database under accession numbers PZ625925 (SB1), PZ625926 (SB2), PZ625927 (TB1), and PZ625928 (TB2).

Antimicrobial activity of Mexican oregano essential oil

Preparation of oregano essential oil emulsion

Mexican oregano (*Lippia graveolens* H.B.K.) essential oil produced in the municipality of Mezquital, Durango, Mexico, was kindly provided by Oreganic S.P.R. de R.L. According to the chromatographic profile provided by the supplier, carvacrol (90.31%) and thymol (9.69%) were the predominant constituents of the essential oil.

A 10% (v/v) stock emulsion of the essential oil was prepared using Tween 80 (5%, v/v) purchased from Hycel as the emulsifying agent and sterile distilled water as the continuous phase. The mixture was homogenized using a high-speed homogenizer (FSH-2A, Hinotek, China) at 12,500 rpm for 2 min, according to the

methodology described by [17]. From this stock emulsion, working emulsions containing 0.01, 0.05, 0.1, 0.5, and 1.0% (v/v) of essential oil were prepared.

In vitro antibacterial activity

The antimicrobial activity of Mexican oregano essential oil (*Lippia graveolens* H.B.K.) against the bacterial isolates was evaluated using an agar incorporation assay adapted from [39]. Essential oil emulsions were aseptically incorporated into sterile LB agar that had been cooled to approximately 45–50°C before being poured into sterile Petri dishes. LB agar supplemented with essential oil emulsions at final concentrations of 0.01, 0.05, 0.1, 0.5 and 1.0% (v/v), together with an untreated control, was poured into sterile Petri dishes.

The bacterial suspensions were adjusted to 1×10^6 CFU/mL, and 10 µL of each suspension was inoculated using the droplet technique onto the surface of the agar. Each treatment was performed in duplicate.

The plates were incubated at 37 ± 2°C for 24 h, and antimicrobial activity was assessed by visual observation of bacterial growth. The presence or absence of visible colonies on the plates containing essential oil was compared with the untreated control.

Results and discussion

Botanical identification of plant-based foods

The strawberry sample was identified as *Fragaria × ananassa* (Weston) Duchesne *ex* Rozier (Rosaceae) and deposited under voucher number 61895. The tomato sample was identified as *Solanum lycopersicum* L. (Solanaceae) and deposited under voucher number 471.

Isolation and phenotypic characterization of native bacterial isolates

The four native bacterial isolates were subjected to phenotypic characterization based on colony morphology and microscopic observation following Gram staining. These analyses provided an initial taxonomic framework for the preliminary classification of the isolates prior to molecular characterization. Figure 2 shows the macroscopic characteristics of the colonies grown on LB agar after 24 h and the associated microscopic observations.

Strain SB1 (Fig. 2a) produced irregular colonies, without elevation, cream to yellowish opaque coloration, and diffuse margins. Based on these macroscopic features and Gram-negative staining, the strain was preliminarily classified within the genus *Enterobacter* [4, 23]. Strain SB2 (Fig. 2b) formed small, creamy to milky translucent colonies with defined margins, a viscous appearance, and slight elevation. Gram staining revealed Gram-positive rod-shaped cells. These phenotypic characteristics are consistent with those commonly described for members of the genus *Bacillus* [24, 45]. The TB1 strain (Fig. 2c) exhibited creamy white, circular colonies with entire margins, a smooth surface, and slight elevation. Based on these macroscopic traits and Gram-negative staining, the strain was preliminarily classified within the genus *Enterobacter* [28, 44]. Finally, strain TB2 (Fig. 2d) formed small, circular, compact, whitish colonies with smooth edges and a slightly shiny surface. Gram staining revealed the presence of Gram-positive rod-shaped

cells. These morphological characteristics supported preliminary classification within the genus *Bacillus* [19, 27].

Although phenotypic characterization alone is not adequate for accurately identifying species, these observations provided an initial taxonomic framework that was subsequently confirmed through PCR amplification, sequencing of a partial 16S ribosomal RNA gene, and phylogenetic analyses.

PCR amplification of 16S rRNA genes

Genomic DNA extracted from the four native bacterial isolates was successfully amplified using primers targeting a partial region of the 16S ribosomal RNA gene. Agarose gel electrophoresis confirmed the presence of the expected PCR products in the four strains (Fig. 3), indicating that the amplified fragments were suitable for subsequent sequencing and molecular identification.

In the case of the bacterial strains isolated from strawberries (Fig. 3a), clear amplification products for strains SB1 and SB2 were observed. Similarly, the strains isolated from tomatoes (Fig. 3b) produced well-defined PCR products for strains TB1 and TB2. In all cases, the amplified fragments corresponded to the expected size generated by primers targeting conserved regions of the bacterial 16S rRNA gene [26]. The absence of non-specific amplification products or additional bands indicates that the PCR conditions were sufficiently specific for the amplification of the target region. The resulting amplicons were therefore considered suitable for DNA sequencing and subsequent molecular identification [7].

Molecular identification and phylogenetic analysis of native bacterial isolates

The four bacterial isolates recovered in this study were subjected to molecular characterization based on partial sequences of the 16S ribosomal RNA gene, in order to determine their preliminary taxonomic affiliation and establish their relationship to bacterial taxa previously described in spoiled plant-based foods. PCR amplification resulted in fragments ranging from 163 to 414 bp. The sequences obtained were compared with reference sequences deposited in the NCBI database using the BLASTn algorithm. The results of the molecular identification are summarized in Table 1.

The partial sequences of the 16S ribosomal RNA gene obtained in this study have been deposited in GenBank under the accession numbers PZ625925–PZ625928. The highest sequence similarities were observed with members of the genera *Enterobacter* and *Bacillus*. The closest BLAST matches were with *Enterobacter cloacae*, *Enterobacter hormaechei*, *Bacillus subtilis*, and *Bacillus mycoides*. However, due to the relatively short length of the sequences obtained, the isolates were conservatively classified at the genus level.

Table 1
Molecular characterization of native bacterial isolates based on partial 16S rRNA gene sequences

Source	Isolate	Sequence length (bp)	Closest BLAST match	Sequence identity (%)	Query coverage (%)	GenBank reference sequences	GenBank accession (this study)
Strawberry	SB1	173	*Enterobacter* sp.	99.39	95	KX698106.1	PZ625925
	SB2	174	*Bacillus* sp.	100	99	MT254842.1	PZ625926
Tomato	TB1	163	*Enterobacter* sp.	99.39	100	PV915452.1	PZ625927
	TB2	414	*Bacillus* sp.	99.48	94	KC153124.1	PZ625928

Although partial sequences of the 16S ribosomal RNA gene showed high sequence similarity to reference sequences available in GenBank (Table 1), the relatively short length of the sequences prevented reliable taxonomic classification at the species level for some isolates. Therefore, phylogenetic analyses were performed to evaluate in greater detail the evolutionary relationships between the bacterial isolates and their closest reference taxa. The resultant phylogenetic trees are shown in Fig. 4.

Phylogenetic analysis based on partial sequences of the 16S ribosomal RNA gene revealed that strain SB1 was grouped within the genus *Enterobacter* (Fig. 4a). The isolate clustered with reference sequences from *Enterobacter cloacae*, *Enterobacter hormaechei*, and *Enterobacter ludwigii*, while *Serratia marcescens* served as an outgroup and formed a separate branch. The relatively low bootstrap support values observed among *Enterobacter* species likely reflect the limited phylogenetic resolution provided by the short partial 16S rRNA sequence analyzed (173 bp). Therefore, the isolate was conservatively assigned to the genus *Enterobacter*, and further molecular markers or multilocus approaches would be needed to achieve reliable identification at the species level, particularly within the *Enterobacter cloacae* complex, where it is often not possible to distinguish closely related species using only partial 16S rRNA sequences [20, 32, 46]. Species of the genus *Enterobacter*, particularly those belonging to the *Enterobacter cloacae* complex, have frequently been isolated from various plant-based foods including onions, papaya, macadamia nuts, dragon fruit and chili pepper [4, 13, 23, 29, 30]. The isolation of an *Enterobacter* strain from spoiled strawberry samples in this study is consistent with previous studies describing the presence of species of this genus in spoiled plant-based foods.

Phylogenetic analysis based on partial 16S rRNA gene sequences revealed that strain SB2 clustered within the genus *Bacillus* (Fig. 4b). The isolate clustered with reference sequences belonging to members of the *Bacillus subtilis* species complex, which includes *Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Bacillus velezensis*, while *Paenibacillus polymyxa* was included as an outgroup and clustered separately. Although the closest BLAST match was to *Bacillus* sp., the relatively short sequence analyzed (178 bp) provided limited phylogenetic resolution within the *B. subtilis* species complex. Members of the *Bacillus subtilis* species complex comprise several closely related species that exhibit a very high similarity in the sequence of the 16S ribosomal RNA gene, making it difficult to distinguish them based exclusively on this marker [11]. Consequently, to define the limitations between species within this complex, it usually becomes necessary to resort to multilocus sequence analysis or genome-based approaches [11, 20, 46]. Species belonging to the genus *Bacillus*, including members of the *Bacillus subtilis* species complex, have been isolated from various

plant-based foods, including pawpaw fruit, tomato, watermelon and cabbage [10, 24, 31, 45]. The isolation of a *Bacillus* sp. strain from spoiled strawberries in this study is consistent with previous studies that report the presence of *Bacillus* species in spoiled plant-based foods.

Phylogenetic analysis based on partial sequences of the 16S ribosomal RNA gene revealed that strain TB1 clustered within the genus *Enterobacter* (Fig. 4c). The isolate clustered with reference sequences from *Enterobacter hormaechei*, *Enterobacter cloacae*, and *Enterobacter ludwigii*, while *Serratia marcescens* was included as an outgroup and formed a distinct branch. The relatively low bootstrap support values observed among *Enterobacter* species likely reflect the limited phylogenetic resolution afforded by the short partial 16S rRNA sequence analyzed (163 bp). Hence, strain TB1 was conservatively assigned to the genus *Enterobacter*, and additional molecular markers or a multilocus sequence analysis would be required for reliable identification at the species level, especially among closely related species of the *Enterobacter cloacae* complex [20, 32, 46]. Members of the genus *Enterobacter*, including the bacterium *E. hormaechei*, have frequently been isolated from various plant-based foods, such as sweet peppers, tomatoes, and various fresh products intended for human consumption [1, 21]. The isolation of *Enterobacter* strains from samples of spoiled strawberries and tomatoes in this study is consistent with previous studies describing the presence of species of this genus in plant-based foods.

Phylogenetic analysis based on partial sequences of the 16S ribosomal RNA gene revealed that strain TB2 was grouped within the *Bacillus cereus* species group (Fig. 4d). The isolate clustered most closely with the reference sequence for *Bacillus mycoides*, supported by a bootstrap value of 89%, while *Bacillus cereus* and *Bacillus paramycoides* formed a closely related sister clade. *Lysinibacillus sphaericus* was incorporated as an outgroup and was clearly separated from the *Bacillus* species, providing a suitable root for the phylogenetic tree. The close phylogenetic relationship observed among *B. mycoides*, *B. cereus*, and *B. paramycoides* is consistent with previous studies documenting high genetic similarity within the *Bacillus cereus* species group, where partial 16S rRNA gene sequences often provide limited resolution for species-level discrimination [15, 20, 46]. Bacteria in the *Bacillus cereus* group comprise a diverse bacterial complex that includes species with distinct ecological niches and pathogenic potential [2, 6]. Compared to other members of this group, *B. mycoides* has been more frequently associated with environmental niches than with foodborne illnesses [6]. In the present study, a strain very similar to *B. mycoides* was isolated from spoiled tomatoes. Although this species has been described predominantly in environmental sources, the recovery of this strain from spoiled tomatoes expands knowledge about the bacterial diversity associated with postharvest spoilage of plant-based foods.

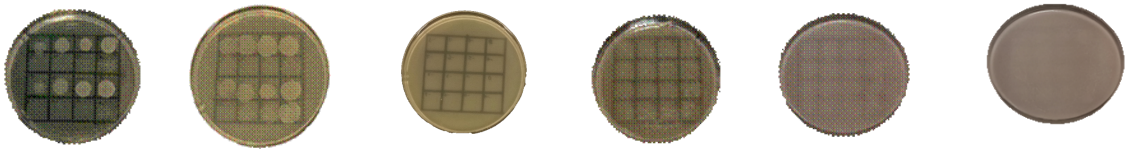
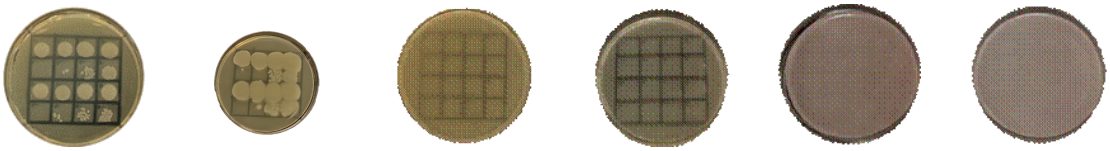
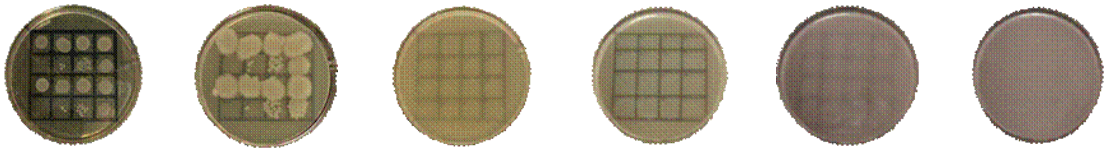
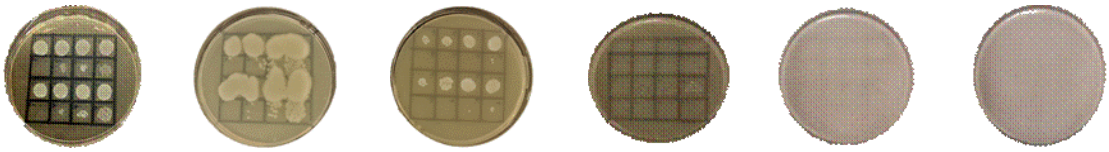
In overall, the phylogenetic analyses based on partial sequences of the 16S ribosomal RNA gene revealed that the isolated bacterial strains belonged to the genera *Enterobacter* and *Bacillus* and clustered closely with reference sequences from *Enterobacter cloacae*, *Enterobacter hormaechei*, *Bacillus subtilis*, and *Bacillus mycoides*. Although these phylogenetic relationships were consistent with the sequence similarity results obtained using BLAST, the short, partial fragments of the 16S rRNA gene analyzed in this study provided limited resolution for reliable species-level discrimination within several bacterial groups. Nevertheless, the combination of sequence similarity analysis and phylogenetic reconstruction provided a robust preliminary taxonomic framework for the recovered isolates. These findings contribute to current knowledge regarding the bacterial diversity associated with postharvest deterioration of strawberries and tomatoes and are consistent

with bacterial taxa previously described in plant-based foods and environmental sources. Future studies incorporating higher-resolution approaches, such as multilocus sequence analysis, genome-based comparisons (ANI or digital DNA-DNA hybridization), MALDI-TOF mass spectrometry, or housekeeping gene sequencing, could further enhance our understanding of the microbial communities involved in these processes.

In vitro antibacterial activity of Mexican oregano essential oil

The *in vitro* antibacterial activity of Mexican oregano essential oil emulsions against the four native bacterial isolates recovered from spoiled strawberries and tomatoes is summarized in Table 2. Growth inhibition depended on the concentration of the essential oil and varied among the different bacterial strains. Three isolates (SB1, SB2, and TB1) showed complete inhibition at 0.05% (v/v), whereas isolate TB2 required a higher concentration (0.1%) to completely inhibit bacterial growth.

Table 2 *In vitro* antibacterial activity of Mexican oregano essential oil emulsions against native bacterial isolates recovered from spoiled strawberries and tomatoes

Isolate	Control	Concentration (%)				
		0.01	0.05	0.1	0.5	1.0
SB1						
SB2						
TB1						
TB2						

These results indicate that Mexican oregano essential oil exhibited antibacterial activity against both Gram-negative and Gram-positive bacterial isolates. Although Gram-negative bacteria are generally considered to be less sensitive to essential oils due to the presence of an outer membrane rich in lipopolysaccharides, complete inhibition was achieved at low concentrations in both *Enterobacter* sp. strains (SB1 and TB1). Likewise, *Bacillus* sp. (SB2) was inhibited at the same concentration, while *Bacillus* sp. (TB2) showed comparatively greater tolerance.

The antibacterial activity observed may be related to the high content of carvacrol (90.31%) and thymol (9.69%) reported for the essential oil used in this study. These phenolic compounds are known to disrupt the integrity of the bacterial membrane, increase its permeability, promote the leakage of intracellular components, and ultimately inhibit bacterial growth [12]. Similar antibacterial effects of Mexican oregano essential oil against both Gram-positive and Gram-negative bacteria have been reported previously, although the inhibitory concentrations vary depending on the bacterial species, the composition of the essential oil, and the experimental methodology [14, 22]. The higher tolerance observed in the case of *B. mycoides* suggests that antimicrobial susceptibility may vary even among phylogenetically related *Bacillus* species, highlighting the importance of individually evaluating native microorganisms that cause spoilage.

Overall, these results indicate that Mexican oregano essential oil formulated as an emulsion inhibited the bacterial isolates evaluated in this study. These findings support the potential of Mexican oregano essential oil as a natural antimicrobial alternative for postharvest preservation of plant-based foods.

Conclusions

Native bacterial strains associated with the postharvest spoilage of strawberries and tomatoes were successfully isolated and characterized through phenotypic analysis, partial 16S rRNA gene sequencing, and phylogenetic analysis. The isolates were assigned to the genera *Enterobacter* and *Bacillus*, providing valuable information on the bacterial taxa associated with the postharvest deterioration of these plant-based foods. Although partial sequences of the 16S rRNA gene provided sufficient information for a reliable preliminary taxonomic classification, their limited length did not always allow definitive species-level identification, highlighting the need for complementary molecular approaches to achieve definitive taxonomic resolution.

Mexican oregano (*Lippia graveolens* H.B.K.) essential oil, formulated as an emulsion significant antibacterial activity against all bacterial isolates evaluated in this study. Complete growth inhibition was achieved at concentrations of 0.05% (v/v) in three isolates, whereas one isolate required 0.1% (v/v), demonstrating variability in antimicrobial susceptibility among the bacterial isolates. The antibacterial activity observed may be associated with the high content of carvacrol (90.31%) and thymol (9.69%), the major constituents of the essential oil used in this study.

Overall, these results contribute to current knowledge regarding the bacterial diversity associated with postharvest spoilage of strawberries and tomatoes produced in Mexico and support the potential of Mexican oregano essential oil emulsions as a natural antimicrobial alternative for controlling bacterial spoilage in plant-based foods. Future studies incorporating genome-based identification methods, pathogenicity assays, and *in vivo* assessments will contribute to a more comprehensive understanding of the microorganisms involved and to the practical application of this preservation strategy.

Declarations

CRediT authorship contribution statement

Conceptualization: M.I.G.R., J.A.R.C., and J.M.C.; data curation: C.A.E.R.; formal analysis: C.A.E.R.; funding acquisition: M.I.G.R., and J.A.R.C.; investigation: J.M.C., and C.I.G.G.; methodology: J.A.R.C., M.I.G.R., and

C.A.E.R.; project administration: M.I.G.R., and J.A.R.C.; resources: D.R.J., J.A.R.C., and M.I.G.R.; software: C.A.E.R.; supervision: D.R.J., P.G.V.O., J.M.C., and M.I.G.R.; validation: J.A.R.C., and M.I.G.R.; visualization: C.A.E.R., and M.I.G.R.; writing - original draft: C.A.E.R, and M.I.G.R.; writing - reviewing and editing: P.G.V.O., D.R.J and C.I.G.G. All authors have read and approved the final version of the manuscript.

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Declaration of competing interest

The authors declare that they have no competing interests.

Author Contribution

Conceptualization: M.I.G.R., J.A.R.C., and J.M.C.; data curation: C.A.E.R.; formal analysis: C.A.E.R.; funding acquisition: M.I.G.R., and J.A.R.C.; investigation: J.M.C., and C.I.G.G.; methodology: J.A.R.C., M.I.G.R., and C.A.E.R.; project administration: M.I.G.R., and J.A.R.C.; resources: D.R.J., J.A.R.C., and M.I.G.R.; software: C.A.E.R.; supervision: D.R.J., P.G.V.O., J.M.C., and M.I.G.R.; validation: J.A.R.C., and M.I.G.R.; visualization: C.A.E.R., and M.I.G.R.; writing - original draft: C.A.E.R, and M.I.G.R.; writing - reviewing and editing: P.G.V.O., D.R.J and C.I.G.G. All authors have read and approved the final version of the manuscript.

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Data Availability

The original contributions presented in this study are included in the article/supplementary data. The partial 16S rRNA gene sequences generated during this study have been deposited in GenBank under accession

numbers PZ625925 (SB1), PZ625926 (SB2), PZ625927 (TB1), and PZ625928 (TB2). Additional data are available from the corresponding author upon reasonable request.

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Figures

Figure 1

Representative strawberry and tomato samples showing post-harvest deterioration before microbiological analysis. (a) Strawberry (*Fragaria × ananassa*) and (b) tomato (*Solanum lycopersicum*)

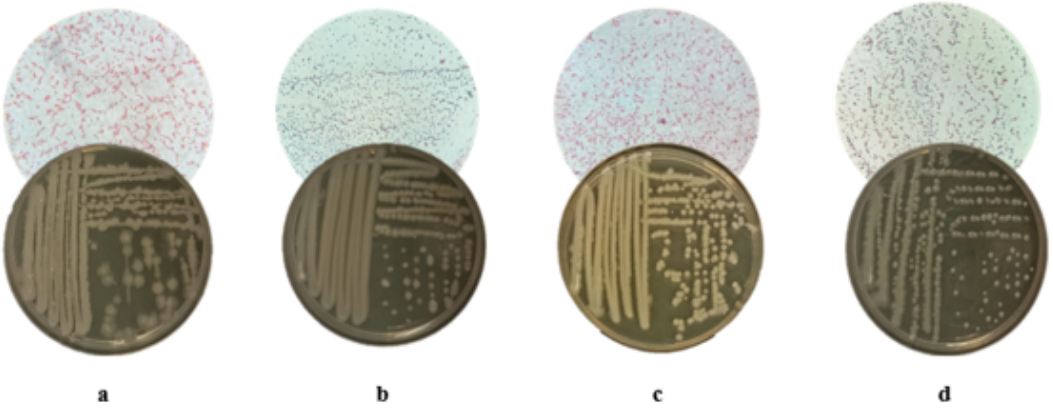

Figure 2

Phenotypic characterization of native bacterial isolates. Macroscopic morphology of colonies on LB agar after 24 h of incubation and microscopic observation following Gram staining (100× objective). (a) SB1, (b) SB2, (c) TB1, and (d) TB2

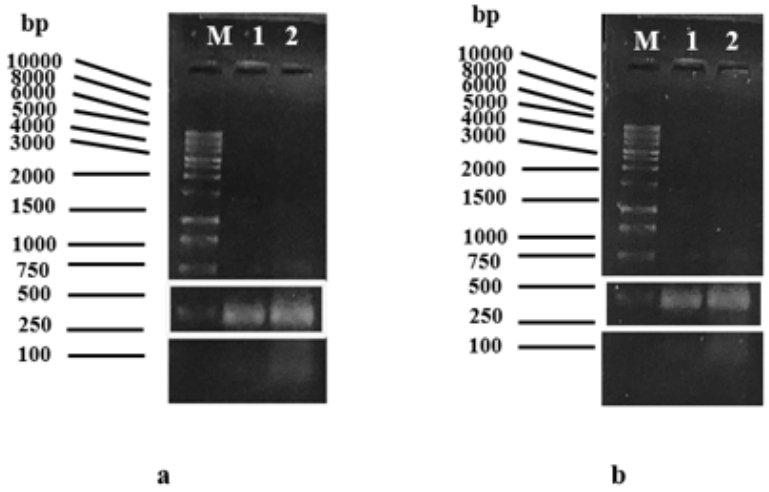

Figure 3

Agarose gel electrophoresis of PCR-amplified partial 16S rRNA gene fragments from native bacterial isolates. PCR products are indicated by white boxes and compared with a 1-kb DNA ladder (M). Lane assignments: (a) 1 = SB1, 2 = SB2; (b) 1 = TB1, 2 = TB2

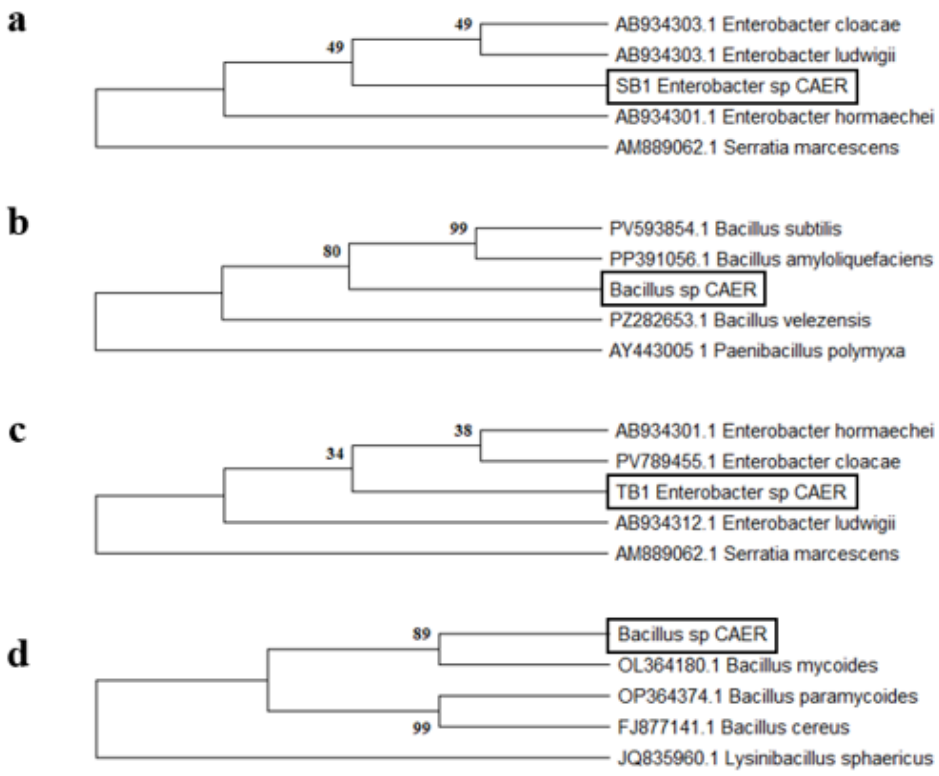

Figure 4

Phylogenetic analysis based on partial sequences of the 16S ribosomal RNA gene from bacterial strains isolated from spoiled strawberries and tomatoes. The phylogenetic trees were constructed using the Neighbor-Joining method with 1,000 bootstrap replicates in MEGA version 11. The strains characterized in this study are highlighted in black boxes: (a) SB1, (b) SB2, (c) TB1, and (d) TB2

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