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Unravelling the Post–Harvest Pathobiome of Underground Vegetables: Phenotypic, Biochemical and Molecular Characterization of Fungal and Bacterial Pathogens from Warangal, India

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ABSTRACT

Postharvest microbial deterioration considerably affects the quality, storability and marketability of root and tuber vegetables. The present study investigated fungal and bacterial microorganisms associated with deteriorated carrot (Daucus carota L.), beetroot (Beta vulgaris L.), sweet potato (Ipomoea batatas L.) and elephant foot yam (Amorphophallus paeoniifolius) collected from Warangal district, Telangana, India, using cultural, morphological, biochemical and molecular approaches. Fungal isolation on Potato Dextrose Agar revealed three predominant morphotypes belonging to Aspergillus, Fusarium and Sclerotium. Cultural and microscopic examination showed characteristic conidial heads in Aspergillus, curved multicellular macroconidia and microconidia in Fusarium, and dense septate mycelia with sclerotial development in Sclerotium. Partial 18S rRNA sequencing and phylogenetic analysis confirmed the isolates as Aspergillus niger var. niger, Fusarium oxysporum and Agroathelia rolfsii (Sclerotium rolfsii), with sequence lengths of 1,680, 1,680 and 1,400 bp, respectively. Their sequences were deposited in GenBank under accession numbers PZ830939.1, PZ830943.1 and PZ830973.1. Four distinct bacterial isolates were recovered through serial dilution and culture-based purification. All were Gram-positive, rod-shaped and endospore-forming, and showed positive reactions for catalase, oxidase, citrate utilization, Voges–Proskauer, nitrate reduction, starch hydrolysis, glucose, sucrose and mannitol utilization, while all were indole-negative. Differential MR, urease and lactose reactions enabled preliminary discrimination. Partial 16S rRNA sequencing confirmed the carrot isolate as Bacillus cereus (1,400 bp; PZ840803.1), beetroot isolate as Bacillus licheniformis (1,470 bp; PZ840805.1), sweet potato isolate as Bacillus pumilus (1,400 bp; PZ840806.1) and elephant foot yam isolate as Priestia megaterium (1,400 bp; PZ840807.1). Phylogenetic placement of the fungal and bacterial isolates was strongly supported by bootstrap values reaching 99% and 98%, respectively. These findings establish the molecular identity and diversity of microorganisms associated with postharvest deterioration of major underground vegetables in Warangal and provide baseline information for pathogen diagnostics and subsequent disease-management studies.

Keywords: *Underground vegetables; postharvest deterioration; fungal pathogens; Fusarium; Aspergillus; Bacillus Sps; 16S rRNA; 18S rRNA; molecular identification.*

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1. Introduction

Root and tuber vegetables constitute an important component of Indian horticulture because of their contribution to food security, dietary diversity and agricultural livelihoods. Among these crops, carrot (*Daucus carota* L.), beetroot (*Beta vulgaris* L.), sweet potato (*Ipomoea batatas* L.) and elephant foot yam (*Amorphophallus paeoniifolius*) are widely consumed for their nutritional value, particularly as sources of carbohydrates, minerals, dietary constituents and antioxidant compounds. Unlike many above-ground vegetables, the edible portions of these crops develop in direct contact with soil and its diverse microbial communities. Mechanical injuries occurring during harvesting, washing, transportation and marketing can further expose nutrient-rich internal tissues to microorganisms. Their relatively high moisture content and limited postharvest processing consequently create favourable conditions for microbial colonization and deterioration [1]. Fungal infection represents a major biological factor responsible for deterioration of roots and tubers during postharvest handling and storage. Investigations of diseased sweet potato storage roots have demonstrated that several fungal taxa may occur simultaneously, with *Fusarium* species frequently representing an important component of the recovered mycobiota [2]. Fungi belonging to *Fusarium*, *Aspergillus* and *Sclerotium* are particularly relevant because members of these genera can persist in production environments and colonize susceptible plant tissues under favourable conditions.

Their involvement has been associated with diseases including wilts, dry rots, collar rots and sclerotial rots, ultimately reducing produce quality, storage potential and commercial value [3].

Among these fungi, *Aspergillus niger* is frequently encountered on stored agricultural commodities, including fleshy roots, tubers and bulbs. Its extensive sporulation and capacity to develop under warm storage environments favour rapid establishment on damaged or physiologically weakened tissues. In culture, the fungus can be recognized by the development of conspicuous dark to black, powdery conidial masses, although microscopic and molecular examination is required for reliable taxonomic confirmation [4]. Another important soil-associated fungus is *Sclerotium rolfsii*, currently recognized as *Agroathelia rolfsii*. This organism possesses a broad host range and has been associated with collar, sprout and sclerotial rots in several economically important crops. Its dense white mycelial growth and subsequent production of compact, mustard-seed-like sclerotia facilitate both recognition and long-term survival under adverse environmental conditions [5,6]. *Fusarium oxysporum* is similarly widespread in agricultural soils and includes numerous pathogenic lineages associated with vascular wilt, root deterioration and storage-related diseases of vegetable crops [3]. Postharvest microbial communities are not restricted to these dominant fungal groups. *Alternaria*, *Rhizoctonia*, *Rhizopus* and *Botrytis* species have also been associated with deterioration of carrot, beetroot, sweet potato and related commodities under different production and storage environments [15]. The composition and predominance of spoilage fungi can vary according to crop species, geographic location, temperature, relative humidity, harvesting practices and storage conditions. Consequently, pathogen profiles established in one production region may not accurately represent those occurring elsewhere. Such variability highlights the importance of generating geographically specific information on the microorganisms associated with postharvest disease. Elephant foot yam deserves particular attention because its underground corm is highly exposed to soil-associated inoculum throughout crop development. Collar and sclerotial rot associated with *S. rolfsii* (*A. rolfsii*) has been recognized as an important constraint affecting elephant foot yam production in India. Infection may occur during different stages of crop development, while contaminated or infected corms can continue to deteriorate during storage and transportation. The ability of the fungus to produce persistent sclerotia further contributes to its survival in cultivated soils and its recurrence in subsequent crops [16,17]. Bacteria may also participate in the deterioration of harvested underground vegetables, particularly following tissue injury or physiological weakening. Aerobic, Gram-positive and endospore-forming bacteria belonging to *Bacillus* and related genera are commonly encountered in soil and plant-associated environments. Some strains possess extracellular hydrolytic enzyme systems capable of degrading structural components of plant tissues and may therefore contribute to secondary colonization and tissue softening. However, species within the *Bacillus* group frequently exhibit overlapping colony, cellular and biochemical characteristics, making identification based exclusively on conventional phenotypic traits unreliable at the species level [7]. Accurate identification of *Bacillus*-group isolates also has implications beyond postharvest pathology. Certain species, particularly *Bacillus cereus*, have food-safety significance because they may contaminate fresh or minimally processed vegetables and some strains are capable of producing toxins associated with food-borne illness [18].

At the same time, advances in bacterial systematics have substantially altered the classification of the traditional genus *Bacillus*. Phylogenomic analyses have resulted in the transfer of several former *Bacillus* species into newly defined genera. Notably, organisms belonging to the former *Bacillus megaterium* lineage are now classified within *Priestia*, illustrating why contemporary molecular evidence is necessary when reporting bacterial identities [19]. Conventional characterization remains valuable as an initial step in pathogen identification. Colony colour, growth pattern, pigmentation, surface characteristics, sporulation and microscopic structures provide useful diagnostic information for fungal isolates, while colony morphology, Gram reaction, cellular shape, endospore production and biochemical responses assist in differentiating bacterial isolates. Nevertheless, phenotypic plasticity and overlapping morphological characteristics among related microorganisms can limit the discriminatory power of these approaches when used independently [8]. Integrating conventional observations with sequence-based identification therefore provides greater taxonomic resolution. Ribosomal DNA markers have become particularly useful for confirming microorganisms associated with plant diseases. The bacterial 16S rRNA gene provides conserved and variable sequence regions suitable for taxonomic comparison, whereas small-subunit 18S rRNA and internal transcribed spacer (ITS) regions are extensively employed in fungal identification. Sequence comparison against authenticated or curated reference records through the NCBI Basic Local Alignment Search Tool (BLAST), together with phylogenetic analysis, enables isolates to be positioned relative to closely related taxa and strengthens species-level assignments [9,10,11]. Such molecular information also generates reproducible sequence records that can support future diagnostic and epidemiological investigations. The importance of reliable pathogen identification is further emphasized by the magnitude of postharvest losses in horticultural supply chains. In developing regions, deterioration may occur at multiple stages between harvest and consumption because of mechanical injury, inadequate sanitation, inappropriate packaging, insufficient temperature management and limitations in transportation and storage infrastructure. These conditions can accelerate physiological deterioration and facilitate fungal and bacterial invasion [12]. Roots and tubers are particularly vulnerable because their fleshy storage organs contain substantial moisture and nutrients and can sustain wounds during removal from soil and subsequent handling. Improved postharvest management therefore requires knowledge of both the environmental factors promoting deterioration and the microorganisms associated with diseased produce [13,14].

2. Materials and Methods

2.1 Study area, sample collection and preparation of diseased samples

A systematic survey was conducted at selected local markets and farm-level storage sites across Warangal district, Telangana, to obtain naturally deteriorated samples of carrot (*Daucus carota* L.), beetroot (*Beta vulgaris* L.), sweet potato (*Ipomoea batatas* L.) and elephant foot yam (*Amorphophallus paeoniifolius*). Collected specimens were visually inspected, and samples showing distinct signs of postharvest deterioration, including soft or dry rotting, surface discoloration, dark to black necrotic lesions, water-soaked patches, tissue softening or maceration and visible fungal development, were selected for microbiological examination.

The location, appearance and characteristic features of each affected region were documented prior to laboratory processing. Under aseptic conditions, tissue sections measuring approximately 3–5 mm were excised with a sterile scalpel from the actively progressing lesion margins so that each section contained both symptomatic and adjacent apparently unaffected tissue. To minimize interference from superficial microorganisms, the excised tissues were immersed in 1% sodium hypochlorite for 1–2 min, followed by two to three successive washes with sterile distilled water to remove traces of the disinfectant. Surface-sterilized tissue pieces were then placed on sterile filter paper under aseptic conditions and allowed to dry briefly before being transferred to the appropriate culture media for subsequent isolation of the associated fungal and bacterial microorganisms.

2.2 Isolation of fungal pathogens

Fungi associated with symptomatic tissues of the selected underground vegetables were recovered using an aseptic tissue-plating procedure. Following surface sterilization and drying, the prepared tissue sections were placed directly onto sterile Potato Dextrose Agar (PDA) containing an appropriate antibacterial supplement to minimize bacterial interference. The inoculated Petri plates were maintained at 25 ± 2 °C for 5–7 days and examined periodically for fungal growth emerging from the tissue margins. Colonies displaying differences in colour, texture, growth pattern, pigmentation or other cultural characteristics were selected separately, and actively growing hyphal tips from each colony were transferred onto fresh PDA medium. Repeated subculturing was carried out until discrete, morphologically consistent cultures free from visible contamination were established. Each purified isolate was provided with a unique identification code corresponding to the vegetable host and sampling source to ensure traceability throughout the investigation. The resulting pure fungal cultures were transferred to PDA slants and stored at 4 °C for subsequent examination of colony and microscopic characteristics, taxonomic identification, molecular characterization and further experimental analyses.

2.3 Morphological identification of fungal isolates

Purified fungal cultures were subjected to both macroscopic and microscopic examination for preliminary taxonomic identification. For cultural characterization, individual isolates were grown on Potato Dextrose Agar (PDA), and diagnostic colony features, including colour, radial growth pattern, surface texture, margin characteristics, pigmentation, mycelial density and appearance, and extent of sporulation, were systematically recorded. For microscopic characterization, a small portion of actively growing mycelium was aseptically removed from each culture, mounted in lactophenol cotton blue, and examined under a compound microscope. Particular attention was given to diagnostically relevant structures such as hyphal morphology and septation, branching pattern, conidiophores, conidiogenous structures, conidia, spores, sporangia and other reproductive or survival structures when present. The combination of cultural and microscopic characteristics was compared with descriptions and diagnostic criteria available in established fungal taxonomic keys and standard mycological monographs. Based on the collective phenotypic features, each fungal isolate was provisionally assigned to the most appropriate taxonomic group, genus or species before confirmation through molecular characterization.

2.4 Molecular identification of fungal pathogens

Purified fungal isolates representing distinct cultural and microscopic characteristics were selected for molecular identification. Genomic DNA was extracted from actively growing pure fungal cultures using a standard fungal DNA extraction procedure, and the quality and concentration of the extracted DNA were assessed prior to amplification. The 18S rRNA gene was amplified by polymerase chain reaction (PCR) using appropriate universal fungal primers. The amplified products were purified and subjected to nucleotide sequencing. The resulting sequences were examined for quality, trimmed to eliminate ambiguous regions and assembled into consensus sequences. Each processed 18S rRNA sequence was compared with related fungal sequences available in the NCBI nucleotide database using the Basic Local Alignment Search Tool (BLAST). Taxonomic identification was determined by considering sequence similarity, query coverage and agreement with closely related reference sequences. Relevant homologous sequences were retrieved from the NCBI database and aligned with the sequences generated in the present study. Phylogenetic trees were constructed using Molecular Evolutionary Genetics Analysis (MEGA) software to determine the phylogenetic placement and evolutionary relationships of the fungal isolates with reference taxa. The validated 18S rRNA nucleotide sequences were submitted to the NCBI GenBank database, and the accession numbers assigned to the isolates were recorded. Molecular findings were subsequently considered together with the cultural and microscopic characteristics for final identification of the fungal isolates.

2.5 Isolation of bacterial pathogens

Bacteria associated with postharvest deterioration were recovered from carrot, beetroot, sweet potato and elephant foot yam samples displaying symptoms such as tissue softening, water-soaked areas, maceration, discoloration and visible bacterial exudation. Under aseptic conditions, small tissue sections were removed from the margins of actively developing lesions with a sterile scalpel and transferred into sterile distilled water or physiological saline. The tissues were gently crushed to release the associated bacterial cells into the suspension. Serial dilutions were subsequently prepared from each suspension, and suitable dilutions were inoculated onto Nutrient Agar (NA) and other appropriate bacteriological media when required. The inoculated plates were incubated at 28–30 °C for 24–48 h, after which bacterial colonies were examined for differences in colour, size, form, elevation, margin, surface appearance, consistency and opacity. Each isolate was subsequently maintained on agar slants under suitable refrigerated conditions for further cellular, biochemical and molecular characterization.

2.6 Characterization of bacterial isolates

Purified bacterial cultures were characterized by cultural, microscopic and biochemical methods. Colony morphology was examined on Nutrient Agar by recording colour, size, form, elevation, margin, surface appearance, consistency and opacity. Gram staining was performed on freshly grown cultures, followed by microscopic examination to determine Gram reaction, cell shape and cellular arrangement. Endospore formation was also examined using standard staining procedures. Biochemical characterization was carried out using catalase, oxidase, citrate utilization, indole production, methyl red (MR), Voges–Proskauer (VP), urease, nitrate reduction and starch hydrolysis tests.

Carbohydrate utilization was evaluated separately using glucose, sucrose, lactose and mannitol as substrates. All assays were performed under aseptic conditions following standard bacteriological procedures, and the reactions were recorded as positive or negative after the recommended incubation period. The resulting cultural, cellular and biochemical profiles were compared with the diagnostic characteristics described in standard bacteriological identification manuals for preliminary identification of the bacterial isolates.

2.7 Molecular identification of bacterial isolates

Purified bacterial isolates were selected for molecular identification based on their distinct cultural, microscopic and biochemical characteristics. Genomic DNA was extracted from actively growing pure bacterial cultures using a standard bacterial DNA extraction procedure, and DNA quality and concentration were evaluated prior to amplification. The 16S rRNA gene was amplified by polymerase chain reaction (PCR) using universal bacterial primers. The amplified PCR products were purified and subjected to nucleotide sequencing. The resulting sequences were checked for quality, trimmed to remove ambiguous nucleotide regions and assembled into consensus sequences. Each processed 16S rRNA sequence was compared with related bacterial sequences available in the NCBI nucleotide database using the Basic Local Alignment Search Tool (BLAST). Species-level assignments were made by considering sequence similarity, query coverage and taxonomic correspondence with closely related reference sequences. Representative homologous sequences were retrieved from the NCBI database and aligned with the sequences obtained in the present investigation. Phylogenetic trees were constructed using Molecular Evolutionary Genetics Analysis (MEGA) software to determine the phylogenetic placement and relationships of the bacterial isolates with closely related reference taxa. The validated 16S rRNA nucleotide sequences generated for the bacterial isolates were subsequently submitted to the NCBI GenBank database, and the assigned accession numbers were recorded for future reference. Molecular identification was interpreted together with the phenotypic and biochemical characteristics of the isolates to establish their final taxonomic identity.

3. Results

3.1 Collection and Visual Assessment of Diseased Underground Vegetables

Field and market sampling in Warangal district yielded visibly deteriorated specimens of carrot (*Daucus carota* L.), beetroot (*Beta vulgaris* L.), sweet potato (*Ipomoea batatas* L.) and elephant foot yam (*Amorphophallus paeoniifolius*). Considerable variation in the external appearance and severity of deterioration was observed among the collected samples. Carrot roots exhibited localized darkening, brown-to-black necrotic areas, surface abrasions, irregular discoloured patches and occasional whitish fungal growth. In several roots, deterioration was concentrated around the crown and terminal regions, whereas other specimens showed scattered lesions extending along the root surface (Figure 1A).

Beetroot samples displayed extensive dark brown to black discoloration accompanied by irregular necrotic patches and conspicuous cream-to-white fungal growth on affected surfaces. Some roots showed larger deteriorated areas with progressive tissue breakdown, indicating advanced postharvest colonization (Figure 1B).

Sweet potato storage roots exhibited heterogeneous symptoms ranging from superficial lesions and localized tissue damage to circular necrotic spots, skin discoloration, abrasions and darkened regions. Differences in symptom expression were evident among individual storage roots, suggesting variation in the extent and stage of deterioration (Figure 1C).

Elephant foot yam corms showed prominent surface deterioration characterized by brown-to-black patches, irregular lesions, damaged epidermal regions and localized areas of exposed or discoloured internal tissue. Several corms exhibited substantial surface injury and tissue degradation, particularly around naturally irregular or wounded portions of the corm (Figure 1D). Overall, all four underground vegetables displayed clear visual evidence of postharvest deterioration, and symptomatic tissues representing the advancing margins of these lesions were subsequently selected for microbiological isolation and characterization.

Figure 1(A) *Figure 1(B)*

Figure 1(C) *Figure 1(D)*

Figure 1: Postharvest deterioration symptoms observed in underground vegetables collected from Warangal district, Telangana, India

(A) Carrot (Daucus carota L.) showing surface discoloration, necrotic lesions and localized deterioration; (B) beetroot (Beta vulgaris L.) exhibiting extensive darkened lesions and visible fungal growth; (C) sweet potato (Ipomoea batatas L.) showing variable surface lesions, discoloration and localized tissue damage; and (D) elephant foot yam (Amorphophallus paeoniifolius) exhibiting irregular brown-to-black lesions and surface tissue deterioration.

3.2 Primary isolation and cultural diversity of fungi associated with diseased underground vegetables

Primary isolation on Potato Dextrose Agar (PDA) revealed substantial fungal growth from the diseased tissues of all four underground vegetables. The recovered colonies displayed clear variation in colour, texture, pigmentation, growth pattern and sporulation, indicating the occurrence of multiple fungal morphotypes. Carrot-derived cultures produced rapidly expanding colonies ranging from white and cottony to pale pink, together with green to dark-green powdery colonies. These contrasting cultural features were consistent with the preliminary recovery of *Fusarium*- and *Aspergillus*-like fungi from carrot tissues (Figure 2A). Beetroot samples produced particularly distinct fungal growth, including extensive dark brown to black, heavily sporulating colonies as well as rapidly spreading white, cottony mycelial growth. These characteristics indicated the presence of *Aspergillus*- and *Sclerotium*-like morphotypes (Figure 2B).

Fungal growth recovered from sweet potato was predominantly white to cream and exhibited dense cottony, fluffy or clustered mycelial development. Differences in colony architecture, including diffuse aerial mycelium and compact aggregated growth, suggested the presence of more than one fungal morphotype, provisionally corresponding to *Fusarium* and *Sclerotium* groups (Figure 2C). Similarly, elephant foot yam yielded abundant cream-to-white fungal colonies characterized by extensive spreading mycelia and numerous compact mycelial aggregates. Distinct differences in colony density and growth form were evident among the cultures, supporting the occurrence of multiple fungal types associated with deteriorated yam tissues (Figure 2D). Overall, primary PDA isolation demonstrated a heterogeneous fungal population associated with the four vegetable hosts. Morphologically distinct colonies were therefore selected individually and subjected to repeated purification before detailed macroscopic, microscopic and molecular characterization.

Figure 2(A)

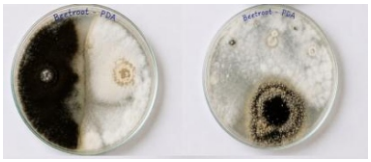


Figure 2(B)

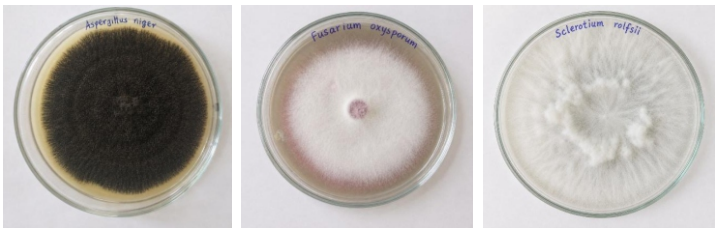
Figure 2(C)

Figure 2(D)

Figure 2. Primary fungal isolation from diseased underground vegetables collected from Warangal district, Telangana, India.
(A) Fungal colonies isolated from carrot (Daucus carota L.) on PDA; (B) fungal colonies isolated from beetroot (Beta vulgaris L.) on PDA; (C) fungal colonies isolated from sweet potato (Ipomoea batatas L.) on PDA; and (D) fungal colonies isolated from elephant foot yam (Amorphophallus paeoniifolius) on PDA.

3.3 Purification and cultural characteristics of fungal isolates

Morphologically distinct fungal colonies obtained during primary isolation were repeatedly subcultured on Potato Dextrose Agar (PDA) until uniform, contamination-free cultures were established. Three predominant fungal isolates were successfully purified and provisionally identified as *Aspergillus niger*, *Fusarium oxysporum* and *Sclerotium rolfsii* based on their characteristic cultural features (Figure. 3). *A. niger* developed rapid radial growth with dense, dark brown to black, powdery sporulation covering most of the agar surface, with a narrow pale peripheral zone. *F. oxysporum* produced a rapidly expanding, cottony to floccose colony that was predominantly white with pale pink to pinkish-purple pigmentation, particularly toward the central and peripheral regions. In contrast, *S. rolfsii* exhibited extensive white, dense and cottony mycelial growth that spread across the PDA surface, producing a characteristic fluffy appearance. The consistent colony morphology observed following successive subculturing indicated successful purification of the three fungal isolates. These pure cultures were subsequently maintained separately and used for microscopic characterization, molecular identification, sequence analysis, phylogenetic evaluation and further experimental investigations.



(A) Aspergillus niger ***(B) Fusarium oxysporum*** ***(C) Sclerotium rolfsii***

Figure 3. Cultural characteristics of purified fungal isolates recovered from diseased underground vegetables on Potato Dextrose Agar (PDA)
(A) Aspergillus niger showing dense black sporulating growth; (B) Fusarium oxysporum exhibiting white cottony mycelium with pinkish pigmentation; and (C) Sclerotium rolfsii displaying rapidly spreading, dense white cottony mycelial growth.

3.4 Morphological characterization and preliminary identification of fungal Isolates

Macroscopic and microscopic characterization demonstrated distinct fungal morphotypes associated with the four underground vegetables (Table 1; Figure 4). The isolates were provisionally grouped as *Fusarium*, *Aspergillus* and *Sclerotium* based on their colony characteristics on PDA and diagnostic microscopic structures. In carrot, *Fusarium* sp. produced rapidly spreading white-to-pale-pink colonies with cottony to floccose mycelium, irregular margins, pale pink to reddish pigmentation and moderate-to-abundant sporulation. Microscopic examination revealed septate hyphae, slender conidiophores, numerous microconidia and characteristic curved, multicellular macroconidia. A second carrot-associated isolate exhibited *Aspergillus*-like characteristics, including rapid radial growth, green-to-dark colonies with a powdery or granular surface and abundant sporulation. Microscopically, erect conidiophores terminating in vesicles and bearing phialides with chains of conidia were clearly observed (Figure 4A).

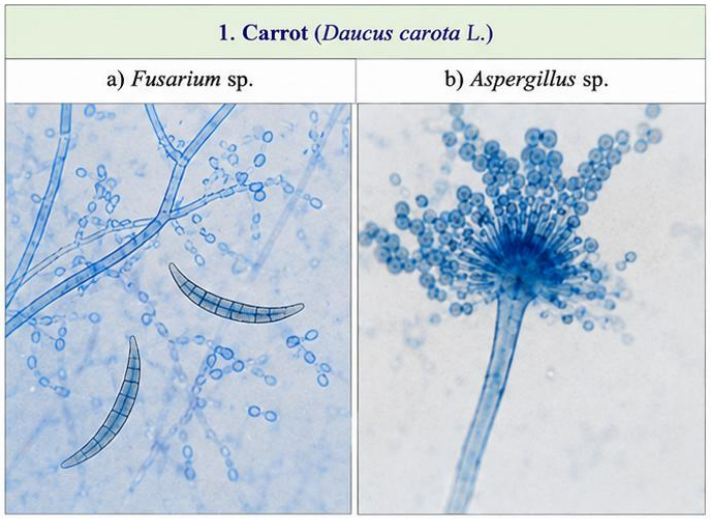


Figure 4A. Microscopic characteristics of Fusarium sp. and Aspergillus sp. isolated from diseased carrot (Daucus carota L.)

Beetroot yielded two morphologically distinguishable fungal groups. The *Aspergillus niger*-like isolate initially developed pale mycelial growth that subsequently became dark brown to black with extensive sporulation. Microscopy showed septate hyphae and long conidiophores terminating in globose vesicles surrounded by conidiogenous structures and numerous dark conidia. The second isolate exhibited *Sclerotium*-like morphology, characterized by rapidly spreading white-to-cream, dense cottony mycelium and formation of compact mycelial aggregates.

Microscopic observations revealed an extensive network of hyaline, branched septate hyphae associated with dense sclerotia development (Figure 4B).

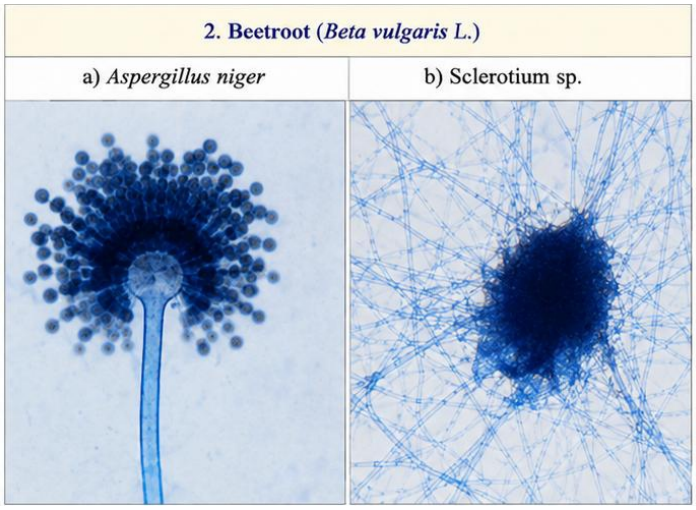


Figure 4B. Microscopic characteristics of Aspergillus niger and Sclerotium sp. isolated from diseased beetroot (Beta vulgaris L.)

Sweet potato tissues similarly yielded *Fusarium*- and *Sclerotium*-like fungi. The *Fusarium* isolate showed rapidly expanding white-to-cream or pale-pink cottony growth with irregular margins and moderate-to-abundant sporulation. Hyaline septate hyphae, microconidia and curved septate macroconidia were prominent microscopic features. The *Sclerotium* isolate developed dense white-to-cream cottony or fluffy mycelium with compact sclerotial bodies becoming evident during maturation (Figure 4C).

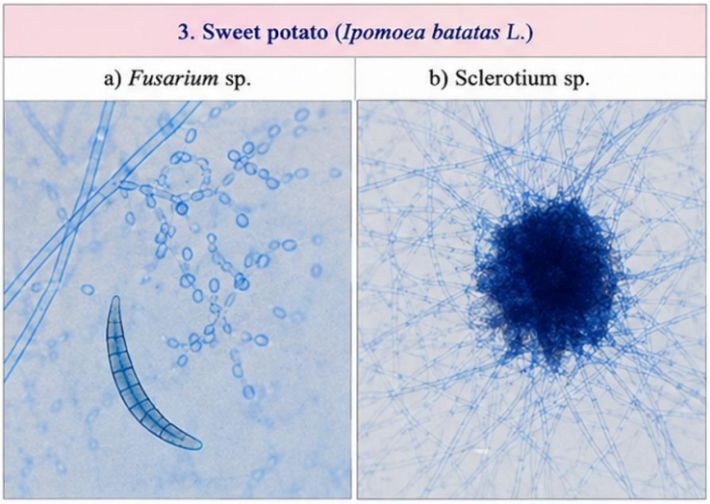


Figure 4C. Microscopic characteristics of Fusarium sp. and Sclerotium sp. isolated from diseased sweet potato (Ipomoea batatas L.)

The elephant foot yam isolates displayed comparable morphological differentiation. *Fusarium* sp. developed white-to-pale-pink, cottony colonies and produced septate hyphae, microconidia and curved multicellular macroconidia. In contrast, *Sclerotium* sp. exhibited rapidly spreading dense white mycelium that gradually developed cream to light-brown pigmentation, together with compact sclerotia structures surrounded by extensively branched septate hyphae (Figure 4D)

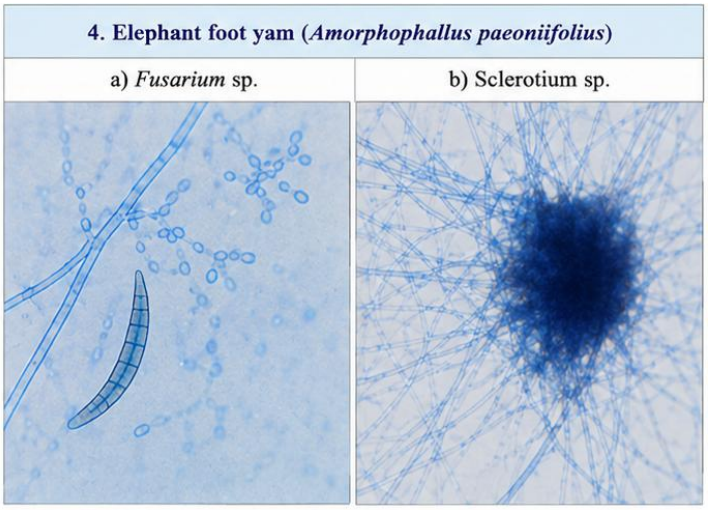


Figure 4D. Microscopic characteristics of Fusarium sp. and Sclerotium sp. isolated from diseased elephant foot yam (Amorphophallus paeoniifolius)

Collectively, the morphological observations demonstrated recurring *Fusarium*, *Aspergillus* and *Sclerotium* morphotypes among the diseased vegetables. These preliminary identifications were subsequently subjected to molecular characterization for species-level confirmation.

Table 1: Cultural and microscopic characteristics of fungal isolates recovered from diseased underground vegetables

Host vegetable	Probable fungal isolate	Colony colour on PDA	Growth pattern	Surface texture / mycelium	Colony margin	Pigmentation	Sporulation	Major microscopic characteristics
Carrot (*Daucus carota* L.)	*Fusarium* sp.	White to pale pink	Rapid, spreading	Cottony to floccose	Irregular	Pale pink to reddish	Moderate to abundant	Septate hyphae; slender conidiophores; microconidia and curved, multicellular macroconidia
	Aspergillus sp.	Initially green, later green to dark black	Rapid, radial	Powdery to granular	Entire to slightly irregular	Greenish to dark	Abundant	Septate hyphae; erect conidiophores terminating in vesicles; phialides and chains of conidia

Beetroot (*Beta vulgaris* L.)	*Aspergillus niger*	White initially, becoming dark brown to black	Rapid, radial	Powdery to granular	Entire	Dark brown to black	Abundant	Septate hyphae; long conidiophores; globose vesicles bearing phialides and chains of dark conidia
	Sclerotium sp.	White, becoming cream with age	Rapid, spreading	Dense, cottony mycelium	Irregular	White to cream	Sclerotial structures present	Hyaline, branched septate hyphae; compact mycelial aggregates/sclerotia; typical conidia generally absent
Sweet potato (*Ipomoea batatas* L.)	*Fusarium* sp.	White to cream/pale pink	Rapid, spreading	Cottony to woolly	Irregular	Pale pink	Moderate to abundant	Hyaline septate hyphae; microconidia and characteristically curved, septate macroconidia
	Sclerotium sp.	White to cream	Rapid, radial spreading	Dense, cottony to fluffy	Irregular	White to cream	Sclerotia formed with maturation	Branched septate hyphae with dense mycelial development and formation of compact sclerotial bodies
Elephant foot yam (*Amorphophallus paeoniifolius*)	*Fusarium* sp.	White to pale pink	Spreading	Cottony to floccose	Irregular	Cream to pinkish	Moderate	Septate hyphae; microconidia and curved, multicellular macroconidia
	Sclerotium sp.	White to cream	Rapid, spreading	Dense cottony mycelium	Irregular	White to light brown with age	Sclerotial development	Hyaline, branched septate hyphae and development of compact sclerotial structures

3.5 Molecular Identification and Phylogenetic Analysis of Fungal Isolates

Molecular characterization of the representative fungal isolates using partial small-subunit 18S rRNA gene sequencing confirmed the identities initially indicated by cultural and microscopic observations. Sequence comparison with reference records and subsequent phylogenetic analysis resolved the three isolates as *Aspergillus niger* var. *niger*, *Fusarium oxysporum* and *Agroathelia rolfsii* (*Sclerotium rolfsii*). The validated nucleotide sequences were deposited in the NCBI GenBank database under accession numbers PZ830939, PZ830943 and PZ830973, respectively. The *Aspergillus* isolate generated a 1,680 bp partial small-subunit rRNA gene sequence and was confirmed as *Aspergillus niger* var. *niger*, with GenBank accession PZ830939.1. Phylogenetic analysis placed the study isolate in a strongly supported lineage with closely related members of the *Aspergillus* group. The isolate clustered most closely with *Aspergillus brasiliensis* isolate Himedia_414, with a 99% bootstrap value. The next hierarchical nodes connecting this lineage with *A. luchuensis* and *A. niger* strain IMT received bootstrap support of 92% and 85%, respectively. A second lineage containing *A. niger*, *A. awamori* and *Aspergillus* sp. strain ZSEFL14 showed internal support values of 96% and 80%, while the major separation between the two groups was supported by 67% (Figure 5A).

The *Fusarium* isolate also yielded a 1,680 bp partial sequence and was identified as *Fusarium oxysporum* (GenBank accession PZ830943.1). In the phylogenetic tree, the study isolate grouped directly with *F. oxysporum* f. sp. *lycopersici* 4287 with 98% bootstrap support, providing strong evidence for its placement within the *F. oxysporum* lineage. Additional nodes involving *F. oxysporum* f. sp. *lycopersici* and isolate K9 showed 93% support, whereas other *Fusarium* reference sequences formed a separate cluster with bootstrap values ranging from 77–95%. The broader division of the principal lineages showed 64% support (Figure 5B).

The third fungal isolate produced a 1,400 bp partial small-subunit rRNA sequence and was identified as *Agroathelia rolfsii*, the currently accepted taxonomic placement of *Sclerotium rolfsii*, with GenBank accession PZ830973.1.

The study isolate formed a highly supported sister grouping with *A. rolfsii* isolate ZZ-3, supported by a 97% bootstrap value. The associated lineage showed 86% support, while other *A. rolfsii*-related groups exhibited bootstrap values of 91%, 94%, 79% and 75%. The basal separation represented in the tree received 62% bootstrap support (Figure 5C). The molecular and phylogenetic results supported the identification of the three principal fungal isolates and were consistent with their previously observed cultural and microscopic characteristics.

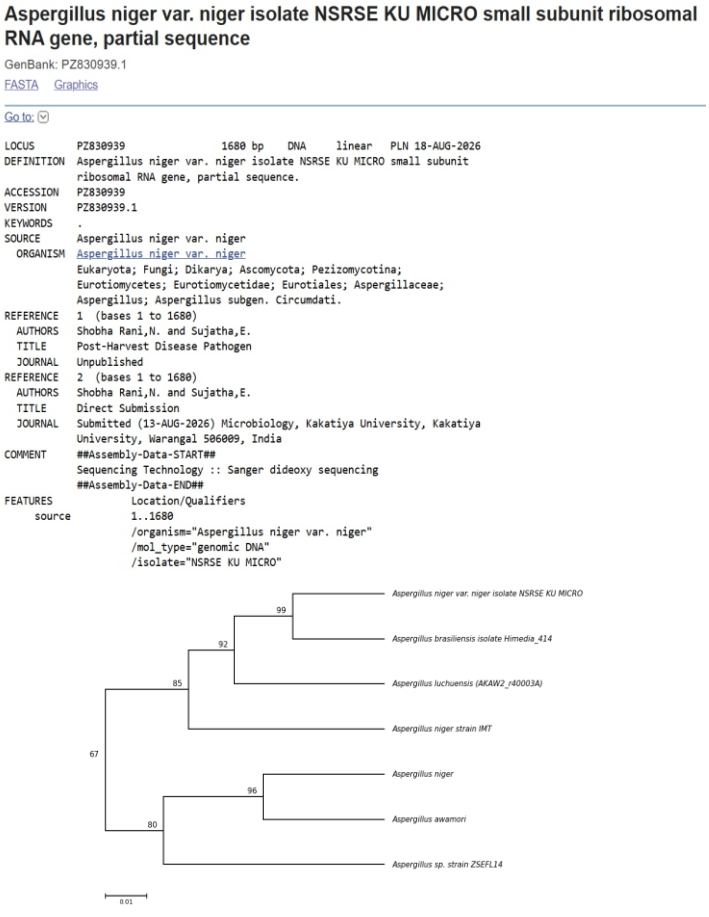


Figure 5(A) Phylogenetic placement of Aspergillus niger var. niger isolate NSRSE KU MICRO (GenBank accession PZ830939.1)

Fusarium oxysporum isolate NSRSE KU MICRO small subunit ribosomal RNA gene, partial sequence

GenBank: PZ830943.1

FASTA Graphics

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  ORGANISM  Fusarium oxysporum
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  AUTHORS   Shobha Rani,N. and Sujatha,E.
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  JOURNAL   Unpublished
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  TITLE     Direct Submission
  JOURNAL   Submitted (13-AUG-2026) Microbiology, Kakatiya University, Kakatiya
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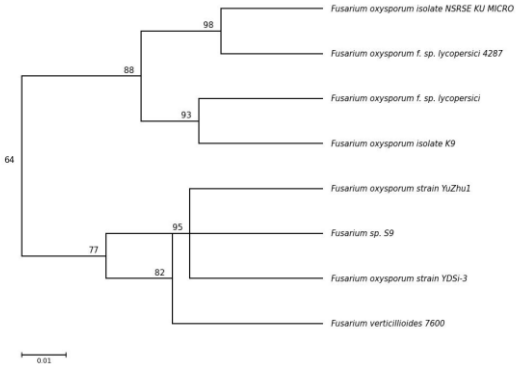

Figure 5(B) phylogenetic placement of Fusarium oxysporum isolate NSRSE KU MICRO (GenBank accession PZ830943.1)

Agroathelia rolfsii isolate NSRSE KU MICRO small subunit ribosomal RNA gene, partial sequence

GenBank: PZ830973.1

FASTA Graphics

Go to:

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KEYWORDS    .
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            Amylocorticiaceae; Agroathelia.
REFERENCE   1  (bases 1 to 1400)
  AUTHORS   Shobha Rani,N. and Sujatha,E.
  TITLE     Post-Harvest Disease Pathogen
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1400)
  AUTHORS   Shobha Rani,N. and Sujatha,E.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-AUG-2026) Microbiology, Kakatiya University, Kakatiya
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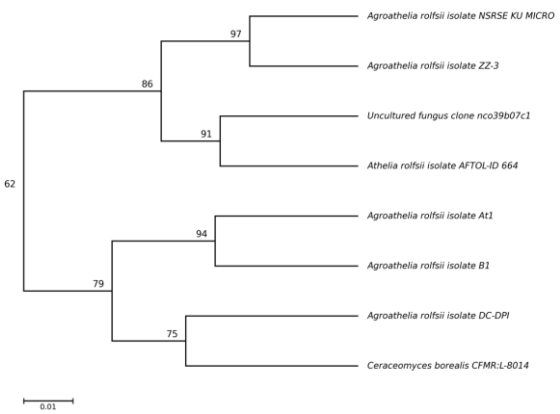

Figure(C) phylogenetic placement of Agroathelia rolfsii isolate NSRSE KU MICRO (GenBank accession PZ830973.1)

3.6 Isolation of bacteria from diseased underground vegetables

Bacterial populations associated with deteriorated carrot, beetroot, sweet potato and elephant foot yam were successfully recovered by serial dilution and agar plating. Visible bacterial colonies developed from all four vegetable samples, confirming the presence of cultivable bacterial populations in the affected tissues (Figure 6). A clear dilution-dependent difference in colony density was evident between the 10^{-1} and 10^{-7} dilutions. In general, the 10^{-1} plates exhibited dense bacterial growth with numerous closely distributed colonies, whereas the 10^{-7} plates produced comparatively fewer and more spatially separated colonies, facilitating the selection of individual colonies for purification. Carrot samples showed abundant bacterial growth at both dilution levels, with colonies differing visibly in size and appearance, suggesting the occurrence of more than one bacterial morphotype (Figure 6A). Beetroot exhibited a high and heterogeneous colony density at 10^{-1}, while the 10^{-7} dilution yielded markedly fewer discrete colonies suitable for subsequent isolation (Figure 6B). Sweet potato also produced extensive bacterial growth at the lower dilution, followed by a substantial reduction in colony density at 10^{-7}, where individual colonies were readily distinguishable (Figure 6C). A similar trend was observed for elephant foot yam, with densely distributed colonies at 10^{-1} and clearly separated colonies at 10^{-7} (Figure 6D). Representative colonies displaying distinct cultural characteristics were selected from the dilution plates and repeatedly streaked onto fresh medium to obtain pure bacterial cultures for morphological, biochemical and molecular characterization. The reduction in colony density at higher dilution facilitated the recovery of spatially separated colonies for purification and subsequent characterization.

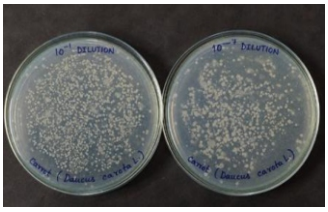

Figure 6 (A) carrot (Daucus carota L.)

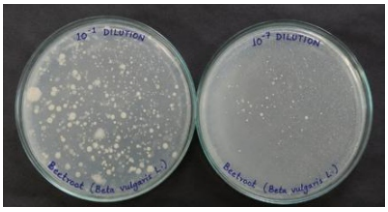

Figure 6 (B) beetroot (Beta vulgaris L.)

Figure 6(C) sweet potato (Ipomoea batatas L.)

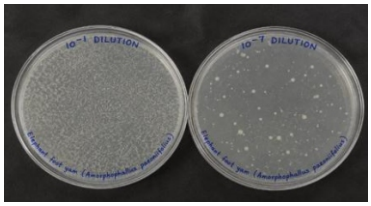

Figure 6(D) elephant foot yam (Amorphophallus paeoniifolius)

Figure 6. Isolation of culturable bacteria from diseased underground vegetables collected from Warangal district, Telangana, India, using serial dilution plating. Representative bacterial growth at 10^{-1} and 10^{-7} dilutions isolated from, (A) carrot (Daucus carota L.), (B) beetroot (Beta vulgaris L.), (C) sweet potato (Ipomoea batatas L.) and (D) elephant foot yam (Amorphophallus paeoniifolius)

3.7 Purification and colony morphology of bacterial isolates

Following primary isolation, morphologically distinct bacterial colonies were repeatedly streaked on fresh agar medium to obtain pure cultures. Four representative isolates were successfully purified and designated according to their vegetable source as Isolate 1 from carrot (*Daucus carota* L.), Isolate 2 from beetroot (*Beta vulgaris* L.), Isolate 3 from sweet potato (*Ipomoea batatas* L.) and Isolate 4 from elephant foot yam (*Amorphophallus paeoniifolius*) (Figure 7).

The purified isolates showed clear differences in colony architecture, surface characteristics, consistency and opacity (Table 2).

Isolate 1 produced creamy-white, circular colonies with convex elevation, entire margins and a smooth, glistening surface. The colonies were butyrous in consistency and opaque. Isolate 2 also exhibited creamy-white pigmentation but was readily differentiated by its irregular form, raised elevation, undulate margins and slightly rough surface; its colonies were butyrous and opaque. Isolate 3 formed white, circular colonies characterized by convex elevation, entire margins and a smooth surface. Unlike the other isolates, it displayed a mucoid consistency and translucent appearance. Isolate 4 showed the most distinctive cultural morphology, producing creamy-white, irregular colonies with raised elevation, lobate margins and a rough, wrinkled surface. Its colonies were dry in consistency and opaque. The reproducibility of these characteristics following repeated subculturing indicated successful establishment of four phenotypically distinct pure bacterial cultures. These isolates were retained separately for subsequent microscopic, biochemical and molecular characterization.

Figure. 7: Isolate 1 – Carrot (Daucus carota L.), Isolate 2 – Beetroot (Beta vulgaris L.), Isolate 3 – Sweet potato (Ipomoea batatas L.) and Isolate 4 – Elephant foot yam (Amorphophallus paeoniifolius)

Table 2: Colony morphology of purified bacterial isolates recovered from diseased underground vegetables

Isolate	Vegetable source	Colony colour	Form	Elevation	Margin	Surface appearance	Consistency	Opacity
Isolate 1	Carrot (*Daucus carota* L.)	Creamy white	Circular	Convex	Entire	Smooth, glistening	Butyrous	Opaque
Isolate 2	Beetroot (*Beta vulgaris* L.)	Creamy white	Irregular	Raised	Undulate	Slightly rough	Butyrous	Opaque
Isolate 3	Sweet potato (*Ipomoea batatas* L.)	White	Circular	Convex	Entire	Smooth	Mucoid	Translucent
Isolate 4	Elephant foot yam (*Amorphophallus paeoniifolius*)	Creamy white	Irregular	Raised	Lobate	Rough, wrinkled	Dry	Opaque

3.8 Cellular and biochemical characterization of bacterial isolates

Cellular and biochemical characterization revealed a common set of phenotypic features among the four bacterial isolates, together with several differential reactions useful for their preliminary discrimination. All isolates were Gram-positive, rod-shaped, endospore-forming bacteria occurring predominantly as single cells or short chains. Each isolate showed positive reactions for catalase, oxidase, citrate utilization, Voges–Proskauer (VP), nitrate reduction and starch hydrolysis, whereas indole production was consistently negative. All four isolates utilized glucose, sucrose and mannitol, indicating comparable utilization of these carbohydrate substrates. Distinct biochemical differences were observed for the methyl red (MR), urease and lactose utilization tests. Isolates 1 and 3, recovered from carrot and sweet potato, respectively, were MR-positive and urease-negative, whereas isolates 2 and 4, obtained from beetroot and elephant foot yam, were MR-negative and urease-positive. Lactose utilization further differentiated the isolates, with a positive reaction recorded only for Isolate 2, while isolates 1, 3 and 4 were negative. Based on the collective cellular characteristics and biochemical reaction profiles, all four isolates were initially assigned to the genus *Bacillus*. Comparison of their differential phenotypic characteristics suggested that Isolate 1 was consistent with *Bacillus cereus*, isolate 2 with *Bacillus licheniformis*, isolate 3 with *Bacillus pumilus* and isolate 4 with *Bacillus megaterium* (Table. 3). These assignments were considered tentative pending confirmation through 16S rRNA gene sequencing and phylogenetic analysis.

Table 3: Cellular and biochemical characteristics of bacterial isolates recovered from underground vegetables

Characteristic / test	Isolate 1 – Carrot	Isolate 2 – Beetroot	Isolate 3 – Sweet potato	Isolate 4 – Elephant foot yam
Gram reaction	Positive	Positive	Positive	Positive
Cell morphology	Rod	Rod	Rod	Rod
Cell arrangement	Single/short chains	Single/short chains	Single/short chains	Single/short chains
Spore formation	+	+	+	+
Catalase	+	+	+	+
Oxidase	+	+	+	+
Citrate utilization	+	+	+	+
Indole production	–	–	–	–
Methyl red (MR)	+	–	+	–
Voges–Proskauer (VP)	+	+	+	+
Urease	–	+	–	+
Nitrate reduction	+	+	+	+
Starch hydrolysis	+	+	+	+
Glucose utilization	+	+	+	+
Sucrose utilization	+	+	+	+
Lactose utilization	–	+	–	–
Mannitol utilization	+	+	+	+
Preliminary genus identification	*Bacillus cereus*	*Bacillus licheniformis*	*Bacillus pumilus*	*Bacillus megaterium* known *as Priestia megaterium*

Key: + positive reaction; – negative reaction

3.9 Molecular identification and phylogenetic analysis of bacterial isolates

Molecular characterization based on partial 16S rRNA gene sequencing confirmed the taxonomic identity of the four bacterial isolates recovered from the selected underground vegetables. Sequence analysis identified isolate 1 from carrot as *Bacillus cereus* strain NSRSE KU MICRO C1, isolate 2 from beetroot as *Bacillus licheniformis* strain NSRSE KU MICRO BR2, isolate 3 from sweet potato as *Bacillus pumilus* strain NSRSE KU MICRO SP3, and isolate 4 from elephant foot yam as *Priestia megaterium* strain NSRSE KU MICRO EFY4. The sequences were deposited in GenBank under accession numbers PZ840803.1, PZ840805.1, PZ840806.1 and PZ840807.1, respectively. The carrot isolate produced a 1,400 bp partial 16S rRNA sequence and was identified as *B. cereus*. Phylogenetic analysis positioned strain NSRSE KU MICRO C1 directly with *B. cereus* strain 1-55, supported by a 97% bootstrap value. The associated cluster containing other *B. cereus*-related sequences showed internal support values of 86% and 91%, while additional *B. cereus* lineages displayed bootstrap values of 94%, 83% and 78%. The major lineage separation was supported by 74%, corroborating placement of the carrot isolate within the *B. cereus* group (Figure 8A). The beetroot isolate generated a 1,470 bp sequence and was confirmed as *B. licheniformis*. Strain NSRSE KU MICRO BR2 formed a strongly supported grouping with *Bacillus* sp. strain B0879.18, with a 98% bootstrap value. This lineage was connected with reference *B. licheniformis* strains KUBOTAB1 and DSM 13 through nodes supported at 94% and 96%. Other *B. licheniformis* reference groups showed bootstrap support of 93%, 90%, 88% and 85%, while the principal division of the tree received 82% support (Figure 8B). The sweet potato isolate yielded a 1,400 bp sequence and was molecularly assigned to *B. pumilus*. Strain NSRSE KU MICRO SP3 clustered most closely with *B. pumilus* strain GL15 with 98% bootstrap support and was further associated with *B. pumilus* strain GBWR33 at a 95% supported node. The remaining reference taxa formed distinct lineages supported by 92%, 88% and 84%, while the major phylogenetic separation showed 81% bootstrap support. These relationships provided strong support for the molecular placement of the sweet potato isolate within the *B. pumilus* lineage (Figure 8C).

The elephant foot yam isolate generated a 1,400 bp partial sequence and was identified as *Priestia megaterium*, corresponding to the organism historically classified as *Bacillus megaterium*. Strain NSRSE KU MICRO EFY4 grouped directly with *P. megaterium* strain sample_60 with 98% bootstrap support. This cluster was associated with *P. megaterium* R5-3 and R5-2 through nodes receiving 91% and 94% support. Additional *Priestia* lineages showed bootstrap values of 89%, 83% and 76%, whereas the broader phylogenetic separation received 79% support (Figure 8D). Overall, the 16S rRNA-based phylogenetic results supported the phenotypic and biochemical identification of all four bacterial isolates and provided species-level molecular assignments.

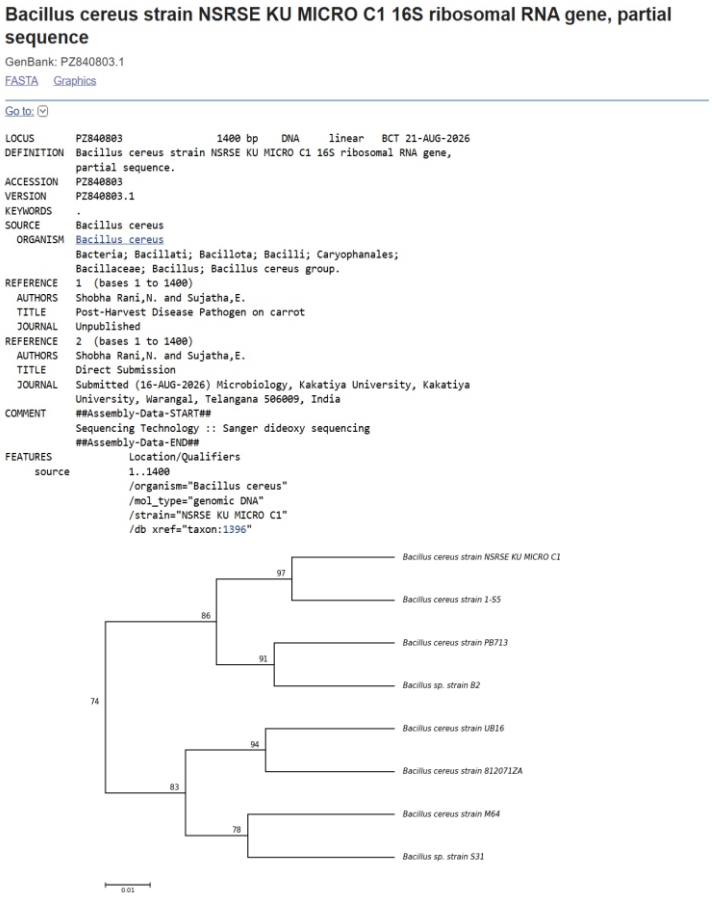

Figure 8 (A) Bacillus cereus strain NSRSE KU MICRO C1 from carrot (GenBank PZ840803.1)

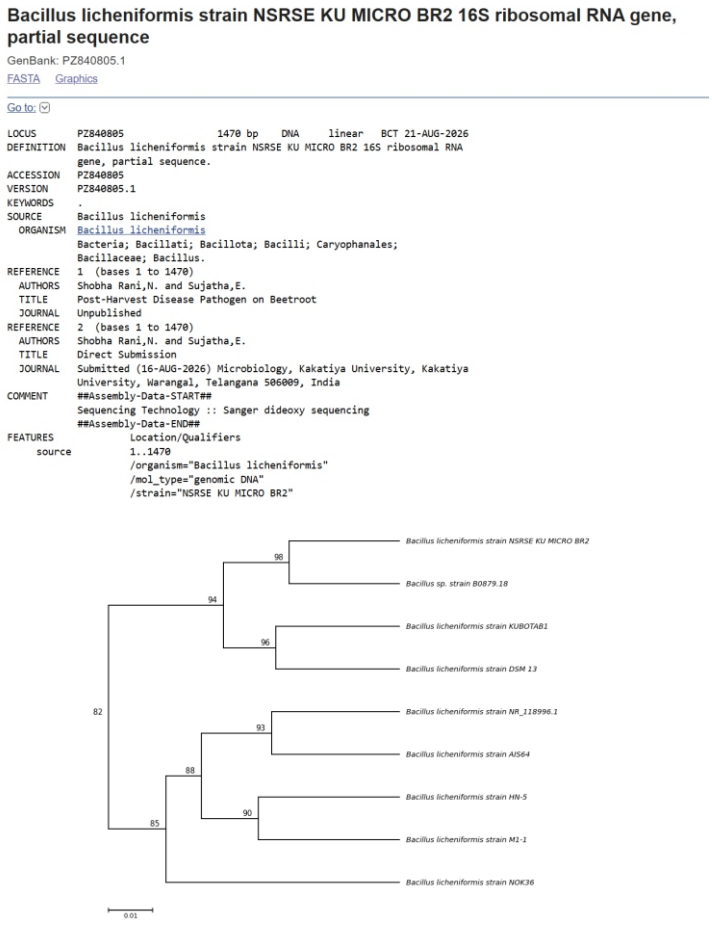

Figure 8 (B) Bacillus licheniformis strain NSRSE KU MICRO BR2 from beetroot (PZ840805.1)

Bacillus pumilus strain NSRSE KU MICRO SP3 16S ribosomal RNA gene, partial sequence

GenBank: PZ840806.1

FASTA Graphics

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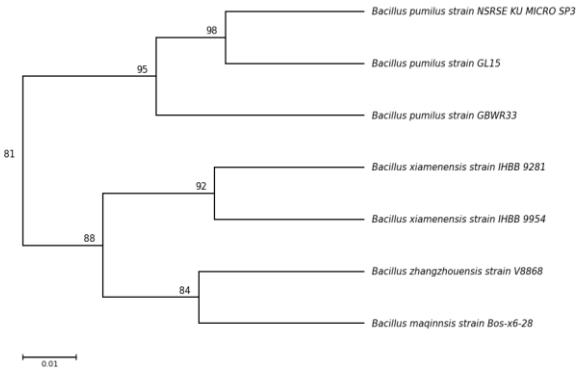

Figure 8 (C) Bacillus pumilus strain NSRSE KU MICRO SP3 from sweet potato (PZ840806.1)

Priestia megaterium strain NSRSE KU MICRO EFY4 16S ribosomal RNA gene, partial sequence

GenBank: PZ840807.1

FASTA Graphics

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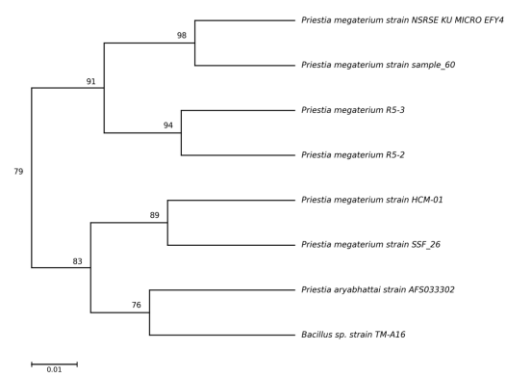

Figure 8(D) Priestia megaterium strain NSRSE KU MICRO EFY4 from elephant foot yam (PZ840807.1)

4. Discussion

The present investigation demonstrated that postharvest deterioration of carrot, beetroot, sweet potato and elephant foot yam in Warangal district was associated with a diverse assemblage of culturable fungi and bacteria. Integration of cultural characteristics, microscopy, biochemical profiling and ribosomal gene sequencing identified three principal fungal taxa *Aspergillus niger* var. *niger*, *Fusarium oxysporum* and *Agroathelia rolfsii* (*Sclerotium rolfsii*) together with four bacterial species, namely *Bacillus cereus*, *Bacillus licheniformis*, *Bacillus pumilus* and *Priestia megaterium*. The occurrence of these microorganisms in visibly deteriorated tissues emphasizes the microbial complexity that may develop in nutrient-rich underground storage organs following harvest. Previous investigations have similarly demonstrated that roots and tubers can harbour multiple fungal taxa, with *Fusarium*, *Aspergillus* and other soil-associated fungi frequently recovered from deteriorating produce [14,15]. Among the recovered fungi, *F. oxysporum* was readily distinguished by its rapidly expanding white-to-pale-pink colonies, septate hyphae, microconidia and characteristic curved, multicellular macroconidia. The recovery of *Fusarium* from carrot, sweet potato and elephant foot yam is biologically plausible because members of this genus are persistent inhabitants of agricultural soils and can colonize underground plant organs through wounds generated during harvesting and handling. *Fusarium* species are widely associated with root, tuber and storage rots, and their persistence in crop residues and soil increases the possibility of infection both before and after harvest [14,16]. The present morphological observations therefore correspond well with established descriptions of *Fusarium* and reinforce its importance within the postharvest fungal complex of underground vegetables [17].

The *Aspergillus* isolates were characterized by rapid radial development, powdery-to-granular colonies and abundant production of conidia. Molecular analysis ultimately resolved the representative isolate as *A. niger* var. *niger*. Dark conidial pigmentation and well-developed conidial heads were particularly useful phenotypic characteristics supporting its preliminary identification. *A. niger* is frequently encountered on harvested plant commodities and can readily colonize injured tissues when temperature and moisture conditions favour fungal development [18]. Consequently, its occurrence in the present samples may reflect the combined influence of soil exposure, harvesting injuries and subsequent storage conditions. Nevertheless, recovery from deteriorated tissue alone does not establish whether the fungus initiated the lesion or colonized tissue already weakened by another organism or physiological damage. The third major fungal group exhibited extensive white cottony mycelium and compact sclerotial development, leading initially to its identification as *Sclerotium* sp. Molecular characterization subsequently confirmed the representative isolate as *Agroathelia rolfsii*, historically and widely reported as *Sclerotium rolfsii*. The ability of this fungus to form resistant sclerotia is epidemiologically important because these structures permit prolonged survival in soil and plant debris and can provide inoculum for subsequent crops [19]. Its broad host range and association with collar, root and storage rots further support its recovery from multiple underground vegetable hosts. The occurrence of *A. rolfsii* in elephant foot yam is particularly relevant because sclerotial rot represents an important constraint affecting corm-producing crops under warm tropical and subtropical environments [20].

Molecular characterization provided substantial support for the fungal identifications established initially through cultural and microscopic observations. The partial 18S rRNA sequences of *A. niger* var. *niger*, *F. oxysporum* and *A. rolfsii* were 1,680, 1,680 and 1,400 bp, respectively, and were deposited in GenBank as PZ830939.1, PZ830943.1 and PZ830973.1. Phylogenetic reconstruction placed the study isolates within their respective taxonomic lineages, with the closest nodes receiving bootstrap support of 99% for the *Aspergillus* isolate, 98% for the *Fusarium* isolate and 97% for the *Agroathelia* isolate. These results demonstrate the advantage of supplementing morphology with sequence-based evidence because colony appearance and microscopic structures may vary with culture conditions, isolate age and growth medium [21,22]. The bacterial component also showed considerable phenotypic diversity despite several shared characteristics. All four isolates were Gram-positive, rod-shaped and endospore-forming and occurred predominantly as single cells or short chains. They were uniformly positive for catalase, oxidase, citrate utilization, Voges–Proskauer reaction, nitrate reduction and starch hydrolysis and utilized glucose, sucrose and mannitol, while indole production was consistently negative. These common properties initially supported their placement among *Bacillus*-related bacteria [23]. However, differential reactions provided additional discriminatory information. Isolates 1 and 3 were methyl-red positive and urease negative, whereas isolates 2 and 4 exhibited the opposite pattern. Lactose utilization was restricted to Isolate 2. Such biochemical variation illustrates the value of employing a panel of diagnostic reactions rather than relying on an individual phenotypic characteristic [23,24].

The preliminary biochemical assignments were subsequently clarified by 16S rRNA gene sequencing. Isolate 1 from carrot was identified as *B. cereus* strain NSRSE KU MICRO C1 (1,400 bp; PZ840803.1), Isolate 2 from beetroot as *B. licheniformis* strain NSRSE KU MICRO BR2 (1,470 bp; PZ840805.1), Isolate 3 from sweet potato as *B. pumilus* strain NSRSE KU MICRO SP3 (1,400 bp; PZ840806.1) and Isolate 4 from elephant foot yam as *P. megaterium* strain NSRSE KU MICRO EFY4 (1,400 bp; PZ840807.1). The corresponding phylogenetic analyses provided strong support for these placements, with the nearest relationships receiving bootstrap values of 97%, 98%, 98% and 98%, respectively. Ribosomal gene sequencing therefore provided a more objective basis for species assignment than phenotypic characterization alone, particularly for closely related aerobic endospore-forming bacteria [24,25]. The molecular identification of the elephant foot yam isolate as *Priestia megaterium* is also taxonomically significant. This organism was historically reported as *Bacillus megaterium*, but phylogenomic restructuring of *Bacillus* sensu lato resulted in its transfer to the genus *Priestia*. Such taxonomic revisions demonstrate that conventional identification schemes may retain outdated nomenclature unless they are interpreted alongside contemporary sequence-based classifications [26]. Accordingly, reporting the isolate as *P. megaterium* provides a taxonomically current interpretation of the molecular data while acknowledging its former designation as *B. megaterium*.

The recovery of *B. cereus* from carrot also warrants attention because members of the *B. cereus* group occur widely in soil and may contaminate raw vegetables during cultivation, harvesting or postharvest handling. Some strains possess recognized food-safety relevance, although identification of an isolate as *B. cereus* by 16S rRNA sequencing does not by itself demonstrate toxigenicity or foodborne risk [27].

Further strain-level characterization, including detection of relevant virulence or toxin-associated genes, would therefore be required before any conclusion regarding public-health significance could be drawn. An equally important consideration is that isolation of *Bacillus* or *Priestia* species from diseased tissue does not necessarily demonstrate that these bacteria function as primary pathogens. Several members of these genera occur naturally in soil, rhizospheres and plant tissues and may exhibit saprophytic, plant-beneficial or antagonistic behaviour. *B. licheniformis*, for example, has been investigated for plant-growth-promoting and antifungal properties, including production of extracellular enzymes, siderophores and antimicrobial metabolites [28]. Similarly, various *Bacillus* species are extensively studied as biological control organisms. Their recovery from deteriorated vegetables in the present investigation could therefore represent primary infection, opportunistic invasion of damaged tissues or secondary colonization following fungal infection. Experimental pathogenicity assays and successful re-isolation from artificially inoculated hosts would be necessary to distinguish among these possibilities [28,29]. The agreement between conventional and molecular approaches nevertheless demonstrates the usefulness of an integrated identification strategy. Cultural morphology and microscopy efficiently separated the major fungal groups, while Gram staining and biochemical profiling provided an initial framework for differentiating the bacterial isolates. Ribosomal gene sequencing, BLAST comparison and phylogenetic reconstruction then provided greater taxonomic resolution and independently supported the phenotypic assignments. Combining these complementary approaches reduces the uncertainty inherent in identification based solely on morphology or biochemical reactions and provides reproducible molecular records through deposition of nucleotide sequences in public databases [21,25,30].

5. Conclusion

The present study identified the major fungal and bacterial microorganisms associated with postharvest deterioration of carrot, beetroot, sweet potato and elephant foot yam from Warangal district, Telangana. Morphological and 18S rRNA analyses confirmed the fungal isolates as *Aspergillus niger* var. *niger*, *Fusarium oxysporum* and *Agroathelia rolfsii*, while biochemical characterization and 16S rRNA sequencing identified the bacterial isolates as *Bacillus cereus*, *Bacillus licheniformis*, *Bacillus pumilus* and *Priestia megaterium*. The corresponding sequences were successfully deposited in GenBank, and phylogenetic analysis strongly supported their taxonomic identities. Overall, the study provides useful molecular baseline information on microorganisms associated with postharvest deterioration of economically important underground vegetables and may support future pathogenicity studies, disease diagnosis and development of effective postharvest management strategies.

Conflict of interest: The authors were declaring no conflict of interest to report regarding this research work.

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