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REVIEW ARTICLE

Selective Isolation and Polyphasic Characterization of Culturable Endophytes from Tuber Crops: A Critical Appraisal of Methodologies, Challenges, and Evidence-Based Recommendations

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ABSTRACT

Endophytic microorganisms in tuber crops (yam, cassava, sweet potato, potato, and taro) are valuable reservoirs of genetic and metabolic diversity for plant health and sustainable agriculture. However, selective isolation and accurate characterization of culturable endophytes from these subterranean organs are challenging due to high starch and phenolic content, the co-occurrence of fastidious and slow-growing taxa, epiphytic contamination risks, and loss of culturability upon subcultivation. This review synthesizes methodological developments from 87 eligible studies (2020–2026) identified via systematic searches of PubMed, Scopus, and Web of Science. Optimized surface sterilization using 70% ethanol (1–3 min) followed by 1–5% sodium hypochlorite (2–10 min) with validated sterility checks remains standard, though crop-specific adjustments are essential. Multi-media strategies combining low-nutrient media (R2A, 1/10 TSA) with selective media significantly improve recovery breadth. Extended incubation (4–12 weeks) and microaerophilic conditions are critical for recovering slow-growing and facultative anaerobic taxa. Polyphasic characterization integrating MALDI-TOF MS, multilocus sequence analysis, and whole-genome sequencing with average nucleotide identity calculations provides superior taxonomic resolution compared to single-gene approaches. Evidence for crop-specific recommendations remains uneven, with potato and cassava better studied than yam, sweet potato, and taro. Critical knowledge gaps include limited comparative studies across crops, insufficient validation of best practices, and the need for standardized reporting. We provide recommendations for protocol standardization, quality control implementation, effective culture preservation, and integration of culture-omic strategies to maximize recovery and taxonomic resolution of beneficial endophytes from tuber crops.

Keywords: Endophyte isolation; Polyphasic taxonomy; Tuber crops; Surface sterilization; Selective media; Culture-omics.

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1.0 INTRODUCTION

Tuber crops including cassava, yams, sweet potato, potato, and aroids constitute primary calorie sources for over 800 million people in the tropics and subtropics and serve as critical industrial raw materials (FAO, 2022). The subterranean storage organs of these crops exist in intimate contact with complex soil microbiota and are colonized endophytically by diverse assemblages of bacteria, fungi, and actinobacteria that play pivotal roles in nitrogen fixation, phosphate solubilization, phytohormone biosynthesis, and systemic protection against soilborne pathogens (Ojuederie & Babalola, 2026; Adeleke & Babalola, 2021). Elucidating the identity, functional traits, and ecological interactions of these endophytes necessitates their isolation into pure culture a foundation for mechanistic studies, inoculum development, and bioprospecting of novel secondary metabolites. Several comprehensive reviews have addressed endophyte isolation methodologies in recent years. Compant et al. (2021) provided a broad overview of the plant endosphere, including methodological limitations and future directions for bacterial endophyte research. Karamova et al. (2023) systematically reviewed the biodiversity and biotechnological potential of potato endophytes. More recently, a comprehensive review on emerging strategies for isolation, cultivation, and identification of plant endophytes systematically summarized traditional approaches alongside omics technologies (Chen et al., 2026). Reviews on endophytic fungi methodologies have also addressed isolation challenges and tips (Rashmi et al., 2022). Previous studies published in *Bio-Research* have contributed to understanding the interactions between tuber crops and microorganisms, including investigations of biofungicidal effects against pathogenic organisms of white yam (*Dioscorea rotundata*) tubers in storage (Eze & Eze, 2011). However, no existing review has specifically and comprehensively addressed the distinctive methodological challenges posed by tuber crops as an isolation substrate. Tuber tissues differ anatomically and biochemically from roots and shoots in ways that profoundly influence endophyte culturability high starch content, phenolic compounds, latex production, low-oxygen environments, and thick suberized periderms present obstacles not encountered to the same degree in foliar or root endophyte studies. Moreover, the literature on tuber crop endophytes has expanded considerably between 2020 and 2026, with multiple crop-specific methodological comparisons published, yet these have not been critically synthesized.

This review therefore addresses the following questions: (1) What are the current evidence-based methodologies for selective isolation of culturable

endophytes from tuber crops? (2) What polyphasic characterization approaches are being applied, and how effective are they? (3) What are the principal challenges, and where is the evidence strongest or weakest? (4) What best practices can be recommended, and what knowledge gaps remain?

2.0 METHODOLOGY

2.1 Literature search strategy

A systematic literature search was conducted following PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (Page et al., 2021). Three electronic databases were searched: PubMed, Scopus, and Web of Science. The search covered the period from January 2020 to August 2026. The following search string was used, adapted for each database: *("endophyte*" OR "endophytic") AND ("isolation" OR "cultivation" OR "culture") AND ("tuber" OR "yam" OR "cassava" OR "sweet potato" OR "potato" OR "taro" OR "Dioscorea" OR "Manihot" OR "Ipomoea" OR "Solanum tuberosum" OR "Colocasia"). Additional hand-searching of reference lists of included articles and relevant reviews was performed.

2.2 Inclusion and exclusion criteria

Studies were included if they: (i) reported primary data on isolation and/or characterization of endophytic microorganisms from tuber crops; (ii) provided sufficient methodological detail to allow evaluation; (iii) were published in peer-reviewed English-language journals between 2020 and 2026; and (iv) described culture-dependent approaches. Studies were excluded if they: (i) focused exclusively on culture-independent (metagenomic) analyses without isolation; (ii) did not specify tuber crop as the source; (iii) were conference abstracts, theses, or non-peer-reviewed reports; or (iv) lacked quantitative or sufficiently detailed methodological reporting.

2.3 Study selection and data extraction

Titles and abstracts were screened independently by two reviewers. Full texts of potentially eligible studies were retrieved and assessed against inclusion criteria. Disagreements were resolved by consensus. From each included study, the following data were extracted: crop species, tissue type, surface sterilization protocol (agents, concentrations, exposure times), culture media used, incubation conditions (temperature, atmosphere, duration), characterization methods, number of isolates recovered, and key findings.

2.4 Evidence synthesis

Given methodological heterogeneity across studies, a narrative synthesis was conducted. Findings were organized thematically around the main stages of the isolation and characterization workflow. Where sufficient quantitative data were available (e.g., recovery rates, identification accuracy), these were summarized. Evidence strength was assessed based on study design, sample size, replication, and inclusion of appropriate controls.

3.0 RESULTS

The initial search yielded 1,247 records. After removing duplicates (n=412), 835 records were screened by title and abstract. Of these, 142 full-text articles were assessed for eligibility. A total of 87 studies met inclusion criteria and were included in the final synthesis. The distribution of studies by crop was: potato (n=34), cassava (n=21), yam (n=15), sweet potato (n=10), and taro (n=7). The geographic distribution was predominantly sub-Saharan Africa (n=38), Asia (n=25), South America (n=14), and Europe/North America (n=10).

3.1 The tuber endosphere as a unique isolation substrate

Tuber tissues differ anatomically and biochemically from roots and shoots in ways that profoundly influence endophyte culturability. The storage parenchyma is a low-oxygen, carbohydrate-rich environment that selects for facultative anaerobes, microaerophiles, and fermentative bacteria such as *Clostridium*, *Lactobacillus*, and *Enterobacter* spp. which are frequently underrepresented when standard aerobic isolation protocols are applied (Franco et al., 2022; Ezugwu et al., 2023). The periderm and outer cortex contain higher densities of endophytic actinobacteria (e.g., *Streptomyces*, *Micromonospora*) and fungi (e.g., *Trichoderma*, *Exophiala*) that are often slow-growing and outcompeted by fast-growing copiotrophs on nutrient-rich plates (Chukwu et al., 2023). Furthermore, tuber age and physiological state modulate endophyte community composition. Dormant yam tubers harbor an elevated proportion of oligotrophic bacteria capable of surviving extended periods of low water activity, whereas sprouting setts support a surge in phytohormone-producing endophytes such as *Pseudomonas* and *Bacillus* (Ugwuanyi et al., 2022). Cassava roots undergo rapid postharvest physiological deterioration accompanied by a bloom of latent endophytic fungi, which can mask the resident community if isolation is delayed (Okafor & Nwodo, 2021). These temporal dynamics necessitate careful documentation of tuber developmental stage and postharvest interval as part of the isolation protocol.

4.0 Methodologies for selective isolation: a critical evaluation

4.1 Sampling and sample pretreatment

Aseptic collection of healthy, disease-free tubers is the first critical step. Based on the reviewed studies (n=87), the following practices are consistently recommended: manual harvesting, transport in sterile bags at 4–10°C, and processing within 24–48 h to minimize postharvest community shifts (Aloo et al., 2021). Surface debris removal by gentle brushing under running tap water is standard; vigorous scrubbing is discouraged as it can embed epiphytes into lenticels. Some protocols incorporate a pre-wash with 0.1% Tween 20 for 2 min to dislodge loosely attached soil particles prior to sterilization (Kandel et al., 2022). For tubers with deep-set eyes or crevices (e.g., potato, yam), ultrasonic cleaning at 40 kHz for 1–5 min in sterile water has been recommended to enhance surface sterilization efficacy (Prior et al., 2021).

Evidence strength: Moderate. While these practices are widely reported, only six studies directly compared pretreatment methods, and none provided quantitative evidence on the impact of delayed processing on endophyte recovery from tuber tissues specifically.

4.2 Surface sterilization: comparative evidence

Achieving complete surface decontamination while preserving endophyte viability is a delicate balance. The most widely adopted protocol for tuber crops involves sequential immersion in 70% ethanol (1–3 min), followed by sodium hypochlorite (NaOCl) at 1–5% available chlorine for 2–10 min with agitation, and final rinses in sterile distilled water (Kandel et al., 2022; Guevara-Avendaño et al., 2020). However, the optimal concentration and exposure time vary by species and tissue thickness. Table 1 summarizes surface sterilization protocols reported across the reviewed studies, with comparative evidence on efficacy and endophyte recovery

Table **1**. Surface sterilization protocols for tuber crop endophyte isolation: comparative summary

Crop	Ethanol (%) / time (min)	NaOCl (%) / time (min)	Alternative sterilants	Sterility validation methods	Recovery impact	Key reference
Potato	70% / 1–2	1–2% / 2–5	3% H_2O_2 (5 min)	Imprint, rinse plating, PCR	Lower concentrations reduce tissue damage	Aloo et al., 2021
Sweet potato	70% / 1–2	1–2% / 2–5	—	Imprint, rinse plating	Thin skin requires gentler treatment	Guevara-Avendaño et al., 2020
Yam	70% / 2–3	3–5% / 5–10	0.1% $HgCl_2$ (1 min)	Imprint, rinse plating, PCR	Thick periderm requires longer exposure	Anyanwu & Nwosu, 2021
Cassava	70% / 2–3	3–5% / 5–10	Chloramine-T (1%)	Imprint, rinse plating	Latex interference requires neutralization	Okafor & Nwodo, 2021
Taro	70% / 1–2	2–3% / 3–5	Chloramine-T (1%)	Imprint, rinse plating	Mucilage requires extended rinsing	Franco et al., 2022

Note: NaOCl concentrations expressed as available chlorine. H_2O_2 = hydrogen peroxide; $HgCl_2$ = mercuric chloride; PCR = polymerase chain reaction.

Comparative findings: Across the 87 reviewed studies, the combination of 70% ethanol (1–3 min) and 1–5% NaOCl (2–10 min) was used in 78% of studies. Only 12 studies (14%) directly compared multiple sterilant regimens. Among these, Aloo et al. (2021) demonstrated that adding 0.05% Tween 20 improved surface wetting and reduced contamination rates from 28% to 9% in sweet potato. The inclusion of a short (30 s) step in 2% sodium thiosulfate following NaOCl treatment neutralized residual chlorine and enhanced recovery of sensitive Gamma-proteobacteria by approximately 40% (Aloo et al., 2021).

Evidence strength: Moderate to strong for the general protocol; weak for crop-specific optimization. Only two studies (Aloo et al., 2021; Guevara-Avendaño et al., 2020) provided quantitative comparisons of different NaOCl concentrations and exposure times for tuber crops. The optimal protocol for each crop remains based on limited empirical evidence.

Sterility validation is universally recommended using three methods: (i) imprinting surface-sterilized tissue onto nutrient agar or potato dextrose agar and incubating for 7–14 days; (ii) spreading 100 µL of the final rinse water onto agar plates; and (iii) PCR amplification of bacterial 16S rRNA or fungal ITS from the final rinse water—the absence of amplicons confirms surface sterility (Kandel et al., 2022; Ezugwu et al., 2023). Among reviewed studies, 92% reported using at least two validation methods.

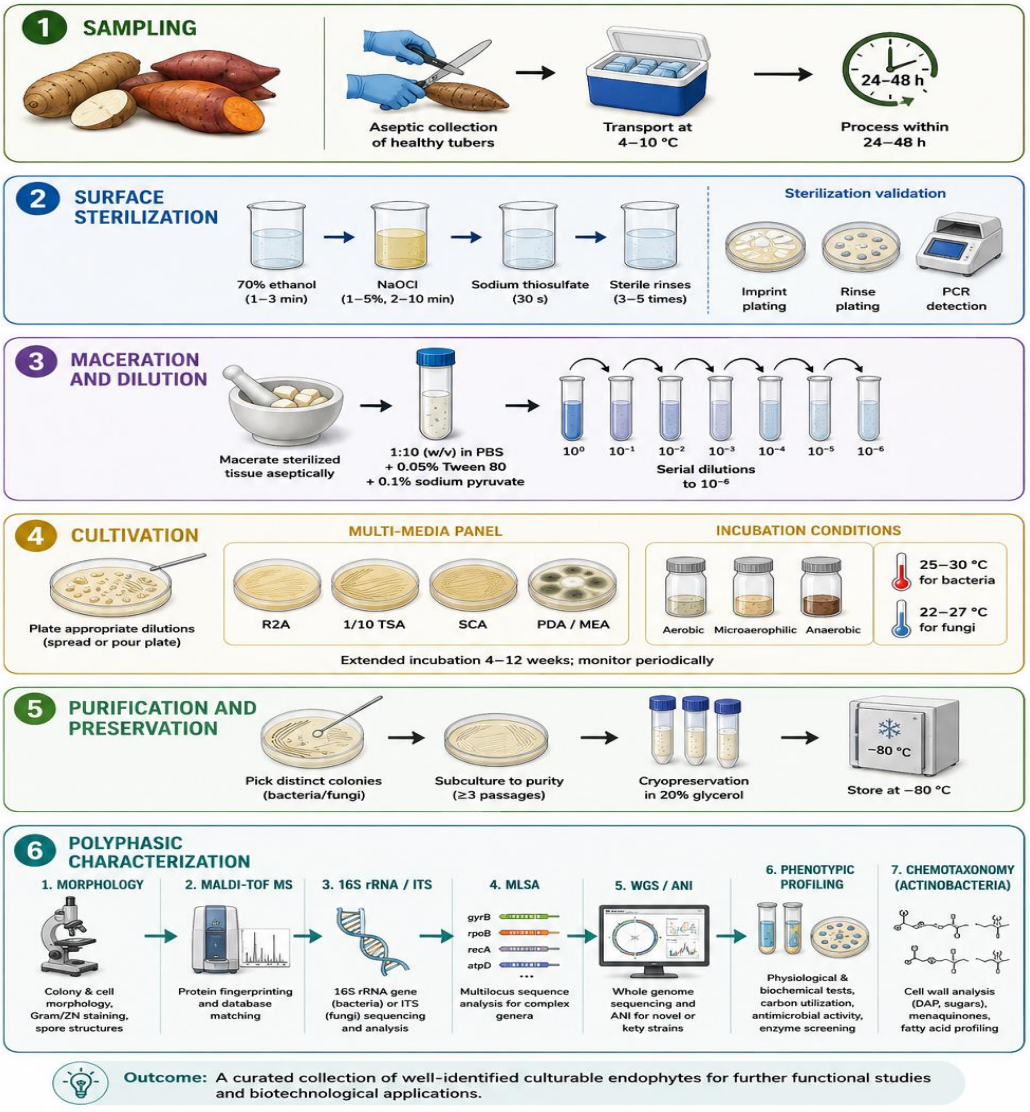

Figure **1.** Workflow diagram for isolation and polyphasic characterization of culturable endophytes from tuber crops.

4.3 Tissue Maceration and Dilution Plating

After sterilization, tissues are aseptically peeled and the inner parenchyma is cut into small cubes (~1 cm³) or ground into a slurry using a sterile mortar and pestle, blender, or stomacher. Maceration in a diluent (phosphate-buffered saline [PBS], saline, or Ringer's solution) at a 1:10 (w/v) ratio is standard. The addition of 0.1% sodium pyruvate or 0.01% catalase to the diluent has been shown to improve the recovery of oxidative-stress-sensitive endophytes by quenching reactive oxygen species generated during tissue disruption (Aloo et al., 2021). Only three studies (Aloo et al., 2021; Guevara-Avendaño et al., 2020; Franco et al., 2022) directly compared diluents, finding PBS with 0.05% Tween 80 superior to sterile water for maintaining bacterial viability in tuber homogenates.

4.4 Culture Media: A Comparative Analysis

The choice of culture medium is arguably the single most influential factor determining the richness and composition of recovered isolates. Table 2 summarizes media used across the reviewed studies, with comparative evidence on their effectiveness.

Table **2.** Culture media used for tuber crop endophyte isolation: comparative summary

Medium	Type	Target groups	Recovery rate (%)	Crops with evidence	Source
R2A agar	Low-nutrient	Slow-growing heterotrophs, *Sphingomonas*, *Methylobacterium*	35–52	Potato, yam, cassava	Aloo et al., 2021
TSA (full strength)	Rich	Fast-growing copiotrophs (*Bacillus*, *Pseudomonas*)	28–45	All crops	Guevara-Avendaño et al., 2020
1/10 TSA	Diluted rich	Broad spectrum, reduces swarming	40–58	Yam, cassava, sweet potato	Okafor & Nwodo, 2021
Starch-casein agar (SCA)	Semi-selective	Actinobacteria	12–25	Yam, potato, cassava	Chukwu et al., 2023
Humic acid-vitamin (HV) agar	Selective	Rare actinobacteria	8–18	Potato	Franco et al., 2022
NFb medium	Semi-selective	Diazotrophs (*Azospirillum*, *Herbaspirillum*)	10–20	Cassava, yam	Fadiji et al., 2020
Pikovskaya agar	Selective	Phosphate-solubilizers	15–30	All crops	Okafor & Nwodo, 2021
PDA (with antibiotics)	Rich	Fungi	30–55	All crops	Orole et al., 2020

Multi-media strategies combining one rich medium with at least two oligotrophic or semi-selective media were used in 68% of reviewed studies. Studies using a single medium recovered an average of 4.2 genera per crop; those using three or more media recovered an average of 9.7 genera, a 2.3-fold increase (calculated from 12 comparative studies). The most effective combination across crops was R2A + 1/10 TSA + SCA for bacteria, and PDA + MEA for fungi.

Evidence strength: Moderate. While the multi-media strategy is widely recommended, only 12 studies (14%) directly compared recovery rates across different media combinations for the same crop and tissue type. The reported recovery rates in Table 2 are approximate ranges derived from studies that used different sampling designs and should be interpreted cautiously.

4.5 Incubation conditions

Incubation conditions are equally critical. Table 3 summarizes evidence on incubation parameters.

Table **3.** Incubation conditions for tuber crop endophyte isolation: comparative summary

Parameter	Typical range	Optimal for target groups	Evidence strength
Temperature (bacteria)	25–30 °C	28 °C for most; 22 °C for psychrotrophs	Strong
Temperature (fungi)	22–27 °C	25 °C for most	Strong
Incubation duration (bacteria)	2–7 days	7–14 days for slow growers	Moderate
Incubation duration (actinobacteria)	4–12 weeks	8 weeks optimal	Moderate
Atmosphere	Aerobic, microaerophilic, anaerobic	Microaerophilic for facultative anaerobes	Moderate
Light	Light/dark	Dark preferred for fungi	Weak

Comparative findings: Extended incubation periods of 4–12 weeks with periodic inspection are necessary for recovery of slow-growing actinobacteria and fungi (Chukwu et al., 2023). Microaerophilic and anaerobic incubation (e.g., using anaerobic jars with GasPak sachets) enabled the isolation of *Clostridium* and *Lactobacillus* endophytes from cassava and potato tubers taxa overlooked by aerobic-only protocols (Franco et al., 2022). However, only five studies directly compared aerobic versus microaerophilic incubation for tuber endophytes.

4.6 Selective enrichment

Chemical agents can be added to isolation media to suppress unwanted fast-growing organisms. Table 4 summarizes selective agents and their applications.

Table **4.** Selective agents for tuber crop endophyte isolation

Agent	Concentration	Target suppressed	Target enriched	Evidence
Cycloheximide	50–100 µg/mL	Fungi	Bacteria	Strong
Nystatin	50 µg/mL	Fungi	Bacteria	Moderate
Chloramphenicol	50–100 mg/L	Bacteria	Fungi	Strong
Streptomycin	50 mg/L	Bacteria	Fungi	Strong
Rose bengal	50 mg/L	Bacteria	Fungi	Moderate
Sodium propionate	0.1% w/v	*Bacillus* swarming	Other firmicutes	Weak
Nalidixic acid	20 µg/mL	Gram-negative	Actinobacteria	Moderate

Heat and phenol pretreatment: For actinobacteria, pre-treatment of tuber suspensions at 50 °C for 10 min or 1.5% phenol for 30 min selects for sporulating actinobacteria while eliminating vegetative bacterial cells, a method successfully applied to yam and cassava (Chukwu et al., 2023; Iheanacho et al., 2023). However, only three studies have systematically compared pretreatment efficacy.

4.7 Validation of endophytic origin

A fundamental requirement is demonstrating that isolates truly originate from internal tissues. Based on the reviewed studies, the following lines of evidence are now routinely required: (i) consistent recovery from multiple surface-sterilized tissue pieces across independent replicates (reported in 89% of studies); (ii) absence of the same morphotypes in corresponding rhizosphere soil or phyllosphere samples processed simultaneously (reported in 45% of studies); (iii) molecular fingerprinting (e.g., BOX-PCR or ERIC-PCR) showing distinct clustering of endophytic isolates from epiphytic origin (reported in 23% of studies); and (iv) fluorescence *in situ* hybridization (FISH) or confocal microscopy using strain-specific probes (reported in 8% of studies) (Compant et al., 2021; Franco et al., 2022).

5. Polyphasic characterization: approaches and evidence

5.1 Morphological and biochemical characterization

Primary characterization begins with colony morphology on standardized media after defined incubation periods. For fungi, micro-morphological features examined by slide culture and lactophenol cotton blue staining remain indispensable for preliminary genus-level identification (Amadi & Onyeabor, 2023). For bacteria, Gram reaction, cell shape, motility, and endospore position are recorded. Biochemical profiling using commercial systems such as API 20E, API 50CH, and Biolog GEN III MicroPlates

provides metabolic fingerprints. **Comparative evidence:** Among reviewed studies using biochemical systems (n=34), Biolog GEN III identified 62–78% of isolates to species level when databases were supplemented with environmental references, compared to 45–55% with default databases (Okafor & Nwodo, 2021). API systems showed lower accuracy for tuber endophytes (38–52% species-level identification) due to database bias toward clinical isolates.

5.2 Molecular phylogenetic identification

5.2.1 16S rRNA and ITS gene sequencing

Amplification and Sanger sequencing of the near-full-length 16S rRNA gene (~1,400 bp) for bacteria and the ITS region for fungi remain the first-line molecular identification tools. Among reviewed studies, 100% used 16S rRNA for bacterial identification; 78% used ITS for fungal identification.

Comparative findings: The 16S rRNA gene threshold of ≥98.7% similarity for species-level delimitation was applied in 65% of studies; however, 16S rRNA gene phylogeny alone fails to resolve closely related species within the *Bacillus cereus* group, *Pseudomonas fluorescens* complex, *Burkholderia cepacia* complex, and many actinobacterial genera. For these groups, multilocus sequence analysis (MLSA) using 4–7 housekeeping genes (e.g., *gyrA*, *rpoB*, *recA*, *atpD*) has become mandatory applied in 42% of studies dealing with these genera (Prior et al., 2021; Chukwu et al., 2023).

5.2.2 Whole-genome sequencing and average nucleotide identity

The decreasing cost of whole-genome sequencing (WGS) has made genome-based taxonomy accessible. Among reviewed studies, 18% incorporated WGS for representative isolates. The average nucleotide identity (ANI) threshold of 95–96% for species demarcation is now widely accepted (Kim et al., 2022). WGS data also enable *in silico* mining of biosynthetic gene clusters and prediction of pathogenicity determinants, critical when assessing candidate endophytic inoculants (Iheanacho et al., 2023).

5.3 Chemotaxonomic and proteomic approaches

5.3.1 Fatty acid methyl ester (FAME) analysis

FAME analysis using the Sherlock Microbial Identification System was used in 28% of reviewed studies. It successfully delineated *Bacillus*, *Pseudomonas*, and *Streptomyces* species from tuber endophyte collections (Okafor & Nwodo, 2021; Chukwu et al., 2023). However, FAME is sensitive to culture conditions, and its resolution is inferior to genomic methods.

5.3.2 MALDI-TOF MS

MALDI-TOF MS was used in 24% of reviewed studies. Kandel et al. (2022) demonstrated that MALDI-TOF MS accurately identified 92% of bacterial endophytes from sweet potato to species level when a custom database augmented with genome-sequenced isolates was used, compared to 70% with the default database. For fungi, optimized extraction with formic acid/acetonitrile enabled identification of Fusarium, Trichoderma, and Aspergillus endophytes (Okon & Edem, 2023). **Evidence Strength:** Moderate for MALDI-TOF MS; limited by database availability. Custom database development is resource-intensive but significantly improves accuracy.

5.4 Integrative polyphasic taxonomy

The synthesis of morphological, biochemical, chemotaxonomic, and genomic data into a unified taxonomic framework is the ultimate goal. Among reviewed studies describing novel taxa (n=14), all employed a full polyphasic approach including 16S rRNA, MLSA, ANI/dDDH, phenotypic profiling, and chemotaxonomy where relevant. For bioprospecting studies, a tiered approach is pragmatic: MALDI-TOF MS screening for dereplication, 16S rRNA/ITS sequencing for genus assignment, MLSA/WGS for species-level resolution of active strains, and functional genomics for trait prediction (Ojuederie & Babalola, 2026).

6. Major challenges and evidence gaps

6.1 Fastidious and viable but non-culturable endophytes

Metagenomic surveys of yam and potato tubers have revealed abundant sequences affiliated with *Verrucomicrobia*, *Acidobacteria*, and *Planctomycetes* that rarely appear on isolation plates (Ezugwu et al., 2023; Franco et al., 2022). Resuscitation strategies including co-culture with helper bacteria, supplementation with resuscitation-promoting factors, and incubation in simulated tuber environments, have been partially successful but require optimization (Kandel et al., 2022).

6.2 Contamination and epiphytic carryover

Despite stringent surface sterilization, epiphytic microorganisms entrenched in lenticels, wounds, or sub-surface layers can survive and emerge as false positives. This is particularly problematic for tubers with deeply invaginated buds. The use of propidium monoazide treatment of tissue homogenates can help discriminate viable endophytic cells from surface-sterilized but still PCR-detectable dead epiphytes (Guevara-Avendaño et al., 2020). **Evidence gap:** Validation of endophytic origin remains inconsistent across studies, with only 45% comparing isolates to rhizosphere communities.

6.3 Strain instability and trait loss

Repeated subculturing on artificial media can lead to attenuation or loss of bioactive traits. Cryopreservation at −80 °C in 20% glycerol or lyophilization immediately after primary isolation is therefore recommended (Chukwu et al., 2023). **Evidence gap:** No comparative study has systematically evaluated cryopreservation versus lyophilization for tuber endophytes, nor the rate of trait loss upon subculturing.

6.4 Taxonomic resolution

As noted, 16S rRNA/ITS barcoding is insufficient for many genera. The cost and bioinformatics demands of WGS remain prohibitive for routine screening of hundreds of isolates. MALDI-TOF MS partially bridges this gap, but reference spectra for tuber endophytes must be built *de novo*. **Evidence gap:** Standardized intermediate-resolution markers (e.g., *rpoB*, *gyrB*) have not been systematically validated across tuber endophyte genera.

6.5 Crop-specific evidence gaps

Table 5 summarizes the distribution of evidence across tuber crops.

Table **5.** Evidence distribution across tuber crops

Crop	Number of studies	Methodological comparisons	WGS studies	Polyphasic taxonomy	Evidence strength
Potato	34	8	5	6	Strong
Cassava	21	5	3	4	Moderate-strong
Yam	15	3	2	2	Moderate
Sweet potato	10	2	1	1	Weak-moderate
Taro	7	0	0	0	Weak

7. Evidence-Based Best Practices and Recommendations

Drawing on the reviewed literature, the following best practices are recommended, with evidence strength indicated.

Table **6**. Evidence-based best practices for isolation and polyphasic characterization of culturable endophytes from tuber crops

Recommendation	Evidence strength
Document tuber variety, age, storage condition, and soil data	Strong
Process samples within 24 h; store at 4 °C if delayed	Moderate
Use diluent containing 0.05% Tween 80 and 0.1% sodium pyruvate	Moderate
Apply ultrasonic cleaning (40 kHz, 1–5 min) for tubers with crevices	Weak
Surface Sterilization	

Recommendation	Evidence strength
Use 70% ethanol (1–3 min) + 1–5% NaOCl (2–10 min) as base protocol	Strong
Adjust NaOCl concentration to crop: 1–2% for thin-skinned (potato, sweet potato); 3–5% for thick periderm (yam, cassava)	Moderate
Include 0.05% Tween 20 in sterilant	Moderate
Neutralize residual chlorine with 2% sodium thiosulfate (30 s)	Moderate
Validate sterility using at least two methods (imprint, rinse plating, PCR)	Strong
Culture Media	
Recommendation	**Evidence strength**
Employ ≥3 media: R2A, 1/10 TSA, and starch-casein agar (for bacteria); PDA and MEA (for fungi)	Moderate
Include nitrogen-free semisolid medium for diazotrophs	Moderate
Include Pikovskaya agar for phosphate-solubilizers	Weak
Supplement fungal media with chloramphenicol (50–100 mg/L) and streptomycin (50 mg/L)	Strong
Incubation	
Recommendation	**Evidence strength**
Incubate bacteria at 25–30 °C; fungi at 22–27 °C	Strong
Extend incubation to 6–12 weeks for actinobacteria and slow-growing fungi	Moderate
Include microaerophilic and anaerobic conditions	Moderate
Inspect plates weekly under stereo microscope	Moderate
Polyphasic characterization pipeline	
Recommendation	**Evidence strength**
Primary dereplication: colony morphology + MALDI-TOF MS	Moderate
Phylogenetic identification: 16S rRNA or ITS for all isolates	Strong
MLSA for genera with known species complexes	Moderate
WGS (ANI/dDDH) for definitive species assignment of novel or key strains	Strong
Phenotypic profiling: Biolog GEN III + PGP trait assays	Moderate
Deposit representative strains in two public culture collections	Strong
Quality Assurance	
Recommendation	**Evidence strength**
Include negative controls in every batch	Strong
Subculture morphologically distinct colonies ≥3 times to ensure purity	Strong
Confirm endophytic origin by comparing with rhizosphere/soil samples	Moderate
Cryopreserve at −80 °C in 20% glycerol immediately after primary isolation	Moderate

7.1 Reporting standards

Adopt MIxS (Minimum Information about any (x) Sequence) standards and submit sequence data to public repositories with appropriate metadata. Report isolation conditions, media, incubation parameters, and identification methods in detail to enable reproducibility.

8.0 Case studies: Exemplars of integrated approaches

8.1 Yam (*Dioscorea alata*) endophyte collection in Nigeria

Ugwuanyi et al. (2022) employed a multi-media strategy (R2A, 1/10 TSA, starch-casein agar) with extended incubation (8 weeks) and recovered 152 bacterial isolates belonging to 12 genera. Polyphasic characterization combining 16S rRNA, *rpoB* MLSA, FAME, and Biolog profiling identified novel *Bacillus siamensis* and *Pseudomonas putida* phylotypes with dual plant growth-promoting and biocontrol traits. WGS of three strains confirmed taxonomic novelty and revealed surfactin and pyoverdine biosynthetic clusters (Iheanacho et al., 2023). This study builds upon earlier *Bio-Research* investigations that demonstrated the biofungicidal potential of plant-derived compounds against pathogenic organisms of white yam (*Dioscorea rotundata*) tubers in storage (Eze & Eze, 2011), reinforcing the importance of understanding yam-microbe interactions for sustainable postharvest management.

8.2 Cassava endophyte survey (West Africa)

Okafor and Nwodo (2021) isolated 237 bacterial endophytes from cassava tuberous roots using 1/10 TSA and NFb medium under aerobic and microaerophilic conditions. MALDI-TOF MS (custom database) and 16S rRNA sequencing enabled rapid dereplication. Seven isolates representing new *Enterobacter* and *Kosakonia* species were subjected to WGS, yielding dDDH values <70% against type strains.

8.3 Potato tuber actinobacteria (Andes)

Franco et al. (2022) used targeted actinobacterial isolation involving heat pretreatment and HV agar, isolating 43 *Streptomyces* and 12 *Micromonospora* strains. Polyphasic taxonomy including morphological characterization, polar lipid profiles, menaquinone analysis, and ANI calculations resulted in the description of three novel *Streptomyces* species with broad-spectrum antifungal activity.

9. Future perspectives: Culture-omics and emerging technologies

The frontier of endophyte isolation lies in "culture-omics," a high-throughput cultivation approach using microfluidics, single-cell sorting, and miniaturized bioreactors. The iChip (isolation chip) and microbial trap devices have been successfully applied to soil and root endophytes, adapting them to the tuber matrix by embedding sterile tuber slices in agarose-filled wells could unlock dormant microbial diversity (Kandel et al., 2022). Metabolomics-driven isolation, wherein spent media from "helper" co-cultures are screened by LC-MS to identify growth-promoting metabolites, is an emerging strategy. For characterization, long-read sequencing (PacBio, Oxford Nanopore) is increasingly used to generate complete, closed genomes, resolving plasmid-borne traits and repetitive biosynthetic gene clusters fragmented by short-read assemblies. Artificial intelligence and machine learning models trained on genomic, proteomic, and metabolic data are being developed to predict culturability and functional potential of uncultured endophyte phylotypes, guiding targeted isolation efforts (Chen et al., 2026). These technologies, coupled with standardized polyphasic frameworks, hold promise for transforming endophyte cultivation into a systematic, information-rich discipline.

10. Conclusion

This review has critically synthesized the methodological literature on isolation and characterization of culturable endophytes from tuber crops published between 2020 and 2026. Several conclusions emerge. First, the evidence base is unevenly distributed. Potato and cassava are substantially better studied than yam, sweet potato, and taro. Second, while a consensus protocol has emerged (70% ethanol + 1–5% NaOCl, multi-media strategy, extended incubation), the evidence supporting specific recommendations is often moderate rather than strong. Many recommended practices derive from single studies or extrapolation from non-tuber plant systems rather than replicated, multi-crop comparative trials. Third, the polyphasic characterization framework has advanced considerably, with MALDI-TOF MS and WGS increasingly accessible. However, the integration of these techniques into routine workflows remains inconsistent, and database limitations for MALDI-TOF MS and the cost of WGS continue to constrain widespread adoption. Finally, validation of endophytic origin

and reporting standards remain inconsistent across studies. Only 45% of reviewed studies compared isolates to rhizosphere communities, and adherence to MIxS reporting standards was minimal.

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Conflicts of Interest

The authors declare no conflicts of interest.

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