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ISOLATION AND CHARACTERIZATION OF POLYHYDROXYALKANOATE PRODUCING BACTERIA FROM LEGUME RHIZOSPHERE SOILS IN ME LINH COMMUNE, HANOI

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ABSTRACT

The increasing demand for sustainable bioplastics has driven the search for polyhydroxyalkanoate (PHA)-producing microorganisms from diverse ecological niches. In this study, we isolated and characterized native soil bacteria with the ability to accumulate PHAs from the rhizosphere of leguminous crops in Me Linh commune, Hanoi. A total of 206 bacterial isolates were obtained from eight soil samples and screened for intracellular PHA granules using Sudan Black B and Nile Blue A staining. Of these, 21 isolates tested positive for PHA accumulation, and five strains (ML53, ML71, ML91, ML113, and ML205) were selected for further analysis. Quantitative fermentation experiments revealed that strains ML113, ML91, and ML71 achieved the highest PHA contents, ranging from 20.7% to 24.8% of dry cell weight (DCW). Fourier - transform infrared (FTIR) spectroscopy confirmed the presence of functional groups characteristic of PHB and PHBV biopolymers. Morphological, biochemical, and molecular analyses - particularly 16S rRNA gene sequencing and phylogenetic tree construction-revealed that the selected isolates belong to three distinct genera: *Pseudomonas* (ML53, closely related to *P. putida*), *Micrococcus* (ML91, closely related to *M. luteus*), and *Priestia* (ML71 and ML205, clustered with *P. megaterium*, and ML113, closely related to *P. aryabhattai*). The results highlight the legume rhizosphere as a rich source of genetically diverse and metabolically competent PHA-producing bacteria with promising potential for future bioplastic development.

Keywords: Bioplastic, polyhydroxyalkanoates (PHAs), *Priestia* spp., *Pseudomonas* spp., soil bacteria.

INTRODUCTION

Polyhydroxyalkanoates (PHAs) are a family of biodegradable polyesters synthesized by various microorganisms as intracellular carbon and energy storage compounds,

particularly under conditions of carbon excess and nutrient limitation. Due to their complete biodegradability, biocompatibility, and thermoplastic properties, PHAs have garnered significant attention as sustainable alternatives to petroleum-based plastics in packaging, agriculture, and biomedical applications (Chen, 2010). Among the diverse sources of PHA-producing microorganisms, soil ecosystems—especially the rhizosphere of leguminous crops such as soybean, mung bean, and peanut—represent a rich and underexplored reservoir of metabolically versatile bacteria.

Numerous soil-derived bacterial genera, including *Bacillus*, *Pseudomonas*, *Cupriavidus*, *Rhizobium*, and *Azotobacter*, have been widely studied for their ability to synthesize PHAs using various low-cost carbon sources such as glucose, glycerol, short-chain fatty acids, and agricultural residues (Getino *et al*., 2024). These bacteria are not only easily isolated and cultivated but also exhibit remarkable adaptability to diverse fermentation conditions, making them promising candidates for large-scale bioplastic production. PHAs produced by soil bacteria can exist in different polymeric forms, including poly(3-hydroxybutyrate) (PHB), poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), and more complex copolymers, depending on the strain and substrate used. Among these, PHBV is particularly attractive due to its enhanced flexibility and processability, attributed to its lower crystallinity and melting point compared to PHB (Arcos-Hernández *et al.,* 2013), thus expanding its applications in packaging, agriculture, and biomedical engineering (Rivera-Briso & Serrano-Aroca, 2018). Soils from legume cultivation areas, especially rhizosphere soils, are recognized as rich reservoirs of PHA-producing microbes. Nitrogen-fixing bacteria such as *Rhizobium*, *Bradyrhizobium*, *Ensifer*, and *Mesorhizobium* contribute to plant nutrition while also possessing the metabolic capacity to accumulate PHA under both free-living and symbiotic conditions. Among these microbial communities, *Pseudomonas* and *Bacillus* are particularly notable for their high yield and versatility. For example, *Pseudomonas stutzeri* isolated from bean rhizosphere soil has been shown to accumulate up to 83% of its dry cell weight (DCW) as PHA when grown on sucrose-based media (Belal, 2013), and it is also capable of synthesizing medium-chain-length PHAs (mcl-PHAs), which exhibit superior elasticity and reduced crystallinity compared to PHB (Chen *et al*., 2006). Similarly, *Bacillus* spp. isolated from groundnut (*Arachis hypogaea*) rhizosphere soils have demonstrated significant PHA accumulation, with *Bacillus* sp. Ti3 producing up to 51.6% DCW as PHB and yielding approximately 0.96 g/L under batch fermentation with glucose and casein hydrolysate (Israni & Shivakumar, 2015), while other *Bacillus* strains have been reported to yield between 1.6 and 6.07 g/L, depending on the cultivation conditions (Shah & Kumar, 2021).

Me Linh, a rural district located approximately 29 km northwest of central Hanoi, has emerged as a major agricultural hub, notably for vegetable and flower cultivation. While widely recognized for its high-tech vegetable zones, it also hosts significant bean production. In this study, we aimed to explore the microbial diversity of legume-cultivated soils in Me Linh commune. We conducted a systematic isolation and screening of PHA-producing bacterial strains from soil samples, with the goal of identifying promising candidates for

bioplastic production. The selected isolates were further evaluated for their PHA accumulation capacity and characterized to assess their potential for future industrial applications.

MATERIALS AND METHODS

Samples

Eight soil samples were collected from Tien Thang, Me Linh commune, located at 21.227492N, 105.656997E. These were stored in a sterilized bag and transported to the Institute of Biology, VAST, in November 2022.

Experimental design

Isolation of bacteria

One gram of soil sample was serially diluted and spread onto Luria–Bertani (LB) agar plates containing the following components (g/L): Bacto Tryptone 10, Yeast Extract 5, NaCl 5. The pH of the medium was adjusted to 7.0–7.2 prior to sterilization. Aliquots from the serial dilutions were plated and incubated at 37°C for 48 hours. Bacterial colonies exhibiting distinct morphologies were counted and subsequently isolated for purification. The number of bacteria present in 1 gram of the sample (N) was calculated using the following formula: $N=\sum C/V*(n_1 + 0.1*n_2)*d$, where: $\sum C$: Total number of colonies counted on all selected plates; V: Volume of inoculum plated on each dish (mL); n_1: Number of plates retained from the first selected dilution; n_2: Number of plates retained from the second selected dilution; d: Dilution factor corresponding to the first selected dilution.

Screening of PHA-producing bacteria

All bacterial isolates were subjected to an initial qualitative screening for PHA production using the Sudan Black B staining method, as described in previous studies (Schlegel *et al*., 1970; Nishida *et al*., 2018). A total of 206 isolates grown on nutrient agar plates were flooded with an ethanolic solution of 0.3% Sudan Black B. After staining, the plates were gently rinsed with 60% ethanol to remove excess, unbound dye. Colonies that retained a bluish-black coloration following the wash were considered presumptive PHA producers, while non-PHA-producing colonies failed to retain the dye. Isolates that tested positive in the Sudan Black B assay were further examined for PHA accumulation using fluorescence microscopy, according to the method described by Spiekermann *et al.* (1999). Cells exhibiting a distinct orange or pinkish fluorescence under the microscope were considered strong PHA producers. Finally, selected positive isolates were analyzed using transmission electron microscopy (TEM) to directly observe the intracellular PHA granules.

Measurement of cell biomass

The pure isolates were first activated in test tubes containing 5 mL of LB broth and incubated at 37°C with shaking at 200 rpm for 24 hours. Subsequently, 5% (v/v) of the preculture was transferred into 1000 mL Erlenmeyer flasks containing 250 mL of HT fermentation medium with the following composition (g/L): glycerol 30, peptone 5, yeast extract 1, $Na_2MoO_4.2H_2O$ 0.005, $MnSO_4$ 0.005, $FeSO_4.7H_2O$ 0.01, $CaCl_2.2H_2O$ 0.02, NaCl 30, $MgSO_4.7H_2O$ 0.2, $(NH_4)_2SO_4$ 1, $KH_2PO_4 \cdot 2H_2O$ 0.2, and K_2HPO_4 1.6. The pH of the medium was adjusted to 7.0 prior to sterilization. Fermentation was carried out at 37°C with shaking at 200 rpm for 72 hours.

After incubation, the bacterial cells were harvested by centrifugation at 6000 rpm for 15 minutes and subsequently dried at 50°C until a constant weight was achieved. The dry cell biomass was then weighed to determine cell yield.

Extraction of PHA

For PHA extraction, the dried cell biomass was treated with sodium hypochlorite (NaClO) and chloroform ($CHCl_3$) at a ratio of 1:100 (w/v). The mixture was incubated at 60°C for 2 hours under gentle agitation at 100 rpm. Following incubation, the upper aqueous layer containing residual biomass and NaClO was carefully removed, while the lower chloroform phase containing the solubilized PHA was collected and transferred into a glass Petri dish. To remove cellular lipids and impurities, methanol and water were added to the extract in a 7:3 (v/v) ratio, and the mixture was centrifuged at 8500 × g for 15 minutes. The supernatant was discarded, and the resulting pellet was washed twice with 95% (v/v) ethanol. The purified PHA was then air-dried overnight at room temperature and weighed. The residual biomass was determined by subtracting the weight of the purified PHA from the initial dry cell weight. The PHA accumulation percentage was calculated as the ratio of PHA content to total DCW. The experiments were performed in triplicate.

PHA structural analysis

Fourier-transform infrared (FTIR) spectroscopy was performed to identify the functional groups present in the extracted PHA. The infrared spectra were acquired using a single-beam spectrometer (Shimadzu IRAffinity-1S, Kyoto, Japan) over a wavenumber range of 4000–500 cm^{-1}.

Characterization of PHA-producing bacteria

Several morphological and biochemical characteristics were analyzed for PHA-producing bacteria. The morphological analysis involved examining colony features on agar plates and observing cellular morphology under the Olympus CH2 light microscope. Gram staining was also performed. In order to further characterize the PHA-producing isolates, key biochemical tests such as the catalase and Voges–Proskauer reactions were conducted. Furthermore, carbohydrate utilization profiling was carried out by assessing the isolates' ability to metabolize a range of sugars, including starch, glucose, sucrose, maltose, and arabinose.

To support the taxonomic identification of PHA-producing bacteria, genomic DNA was extracted from selected strains following the protocol described by Sambrook *et al*. (1989). The 16S rRNA gene was amplified using the universal primer pair 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and 1492R (5'-TAC GGY TAC CTT GTT ACG ACTT-3'). The amplified PCR products were purified using the E.Z.N.A.® Gel Extraction Kit (V-spin) (Omega Bio-tek) and subsequently sequenced on an ABI PRISM 3100 automated sequencer at the Institute of Biology. Sequence data were analyzed using Chromas software, and species-level identification was performed using the NCBI BLAST tool (http://blast.ncbi.nlm.nih.gov/). Phylogenetic trees were generated using MEGA-X software.

Statistical analysis

All data were analyzed using a T-test method on the Microsoft Excel program. A *p*-value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Isolation of bacteria from legume rhizosphere soils in the Me Linh commune

To investigate the microbial diversity in legume-cultivated soils and identify potential PHA - producing bacteria, eight soil samples were collected from bean - growing plots in Me Linh commune, Hanoi. After cultivation on LB agar and incubation at 37°C for 24-72 hours, bacterial population densities ranged from 1.0×10^4 to 8.1×10^6 CFU/g (Table 1). A total of 206 morphologically distinct colonies were isolated, with colony numbers varying independently of CFU counts, suggesting that phenotypic diversity does not necessarily correlate with overall microbial abundance. For instance, Me Linh 2 exhibited the lowest bacterial count but yielded 24 unique isolates, while Me Linh 6 had the highest CFU, yet only 26 isolates. These variations likely reflect differences in microenvironmental conditions and microbial community composition within each soil sample.

Table 1. Bacterial population density and number of isolates obtained from legume-cultivated soils in Me Linh, Hanoi.

Sample	Bacterial density [CFU/g]	Number of isolates	Isolate code
Me Linh 1	3.44×10^6	16	ML1 ÷ ML16
Me Linh 2	1.0×10^4	24	ML17 ÷ ML40
Me Linh 3	1.2×10^5	20	ML41 ÷ ML60
Me Linh 4	4.3×10^5	33	ML61 ÷ ML93
Me Linh 5	2.28×10^6	25	ML94 ÷ ML118
Me Linh 6	8.1×10^6	26	ML119 ÷ ML144
Me Linh 7	3.1×10^6	37	ML145 ÷ ML181
Me Linh 8	6.7×10^6	25	ML182 ÷ ML206
Total		**206**	

The bacterial densities observed in this study are consistent with the typical range reported for legume-associated rhizosphere soils. Bacterial populations in such environments have previously been found to range from 10^5 to over 10^7 CFU/g, depending on soil characteristics, crop type, and cultivation practices (Barillot *et al.,* 2013). Similarly, a study on groundnut rhizosphere soils yielded 21 bacterial isolates, among which only about 20% demonstrated the ability to synthesize PHA (Musa *et al*., 2016).

Screening of PHA-producing bacteria

From the collection of 206 bacterial isolates obtained from legume-cultivated soils in Me Linh, Hanoi, all strains were streaked on LB

agar plates and stained with Sudan Black B, which resulted in 43 isolates showing positive staining (data not shown). However, subsequent confirmation with Nile Blue A fluorescence staining revealed that only 21 of these isolates exhibited fluorescence signals indicative of intracellular PHA granules. Among them, five strains - ML53, ML71, ML91, ML113, and ML205 - displayed particularly strong and distinct fluorescence under UV light, suggesting a higher level of PHA accumulation. These five representative strains were further analyzed using TEM to directly visualize PHA granules within the cells. Both fluorescence micrographs and TEM images of these strains are presented in Figure 1.

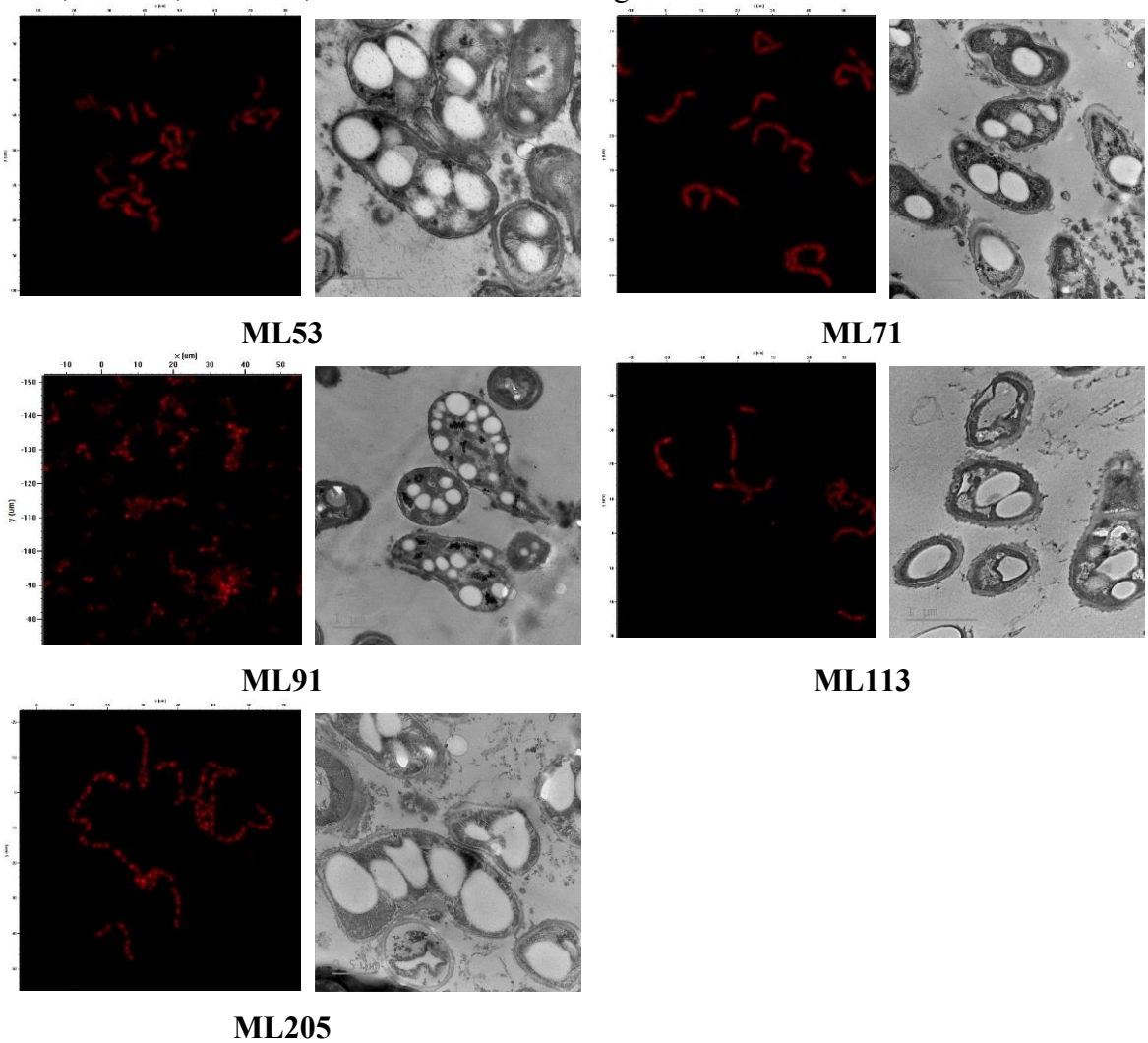

Figure 1. Intracellular PHA accumulation in strains ML53, ML71, ML91, ML113, and ML205. (Left) Cells stained with Nile Blue A and observed under fluorescence microscopy at 460 nm excitation wavelength. (Right) Cells observed under transmission electron microscopy.

The combined application of Sudan Black B, Nile Blue A, and TEM provides a reliable multi-step approach for screening PHA-producing bacteria. Sudan Black B is useful

for rapid, low-cost initial screening, though its low specificity can lead to false positives due to nonspecific staining of other hydrophobic materials (Ostle & Holt, 1982). Nile Blue A improves detection accuracy by specifically binding to PHA granules and producing distinct fluorescence under UV light (Spiekermann *et al*., 1999). To confirm intracellular accumulation, TEM offers direct visualization of PHA inclusions at high resolution and remains the most definitive method for structural confirmation (Koller & Rodríguez-Contreras, 2015). However, to validate and quantify the actual PHA accumulation in bacterial strains, quantitative analytical methods remain essential.

Production of polyhydroxyalkanoates by bacteria isolated from Me Linh, Hanoi

Sixteen bacterial isolates, previously identified as positive for intracellular lipid granules using Sudan Black B and Nile Blue A staining, were cultivated in fermentation medium for 72 hours to assess their PHA production capacity. Quantitative analysis of DCW, PHA concentration, and PHA content (% DCW) is presented in Table 2. The results indicate a broad range of PHA accumulation across isolates, with ML113 achieving the highest PHA content (24.83%), followed by ML53 (22.35%) and ML71 (20.74%). These strains represent strong candidates for further optimization. Conversely, isolates such as ML14 and ML5 exhibited low PHA contents (<11%) despite maintaining moderate biomass levels, suggesting limited carbon flux toward PHA biosynthesis under the tested conditions.

Table 2. PHA production capacity of bacterial isolates obtained from Me Linh, Hanoi.

Isolate	DCW (g/L)	PHA concentration (g/L)	PHA content (%)
ML5	0.925 ± 0.05	0.097 ± 0.001	10.49 ± 0.46
ML14	1.028 ± 0.04	0.102 ± 0.003	9.94 ± 0.09
ML17	0.894 ± 0.03	0.119 ± 0.001	13.31 ± 0.36
ML41	1.316 ± 0.05	0.171 ± 0.002	12.96 ± 0.34
ML53	1.316 ± 0.04	0.245 ± 0.006	18.62 ± 0.11
ML55	1.311 ± 0.04	0.217 ± 0.004	16.55 ± 0.20
ML62	1.193 ± 0.03	0.194 ± 0.003	16.27 ± 0.16
ML71	0.984 ± 0.02	0.204 ± 0.005	20.74 ± 0.09
ML76	0.907 ± 0.03	0.104 ± 0.002	11.44 ± 0.16
ML88	1.258 ± 0.05	0.216 ± 0.005	17.19 ± 0.29
ML89	0.957 ± 0.04	0.099 ± 0.001	10.31 ± 0.33
ML91	1.552 ± 0.05	0.347 ± 0.007	22.35 ± 0.27
ML102	1.323 ± 0.05	0.242 ± 0.004	18.18 ± 0.39
ML113	1.446 ± 0.04	0.359 ± 0.005	24.83 ± 0.34
ML165	1.408 ± 0.04	0.253 ± 0.005	17.97 ± 0.16
ML205	1.175 ± 0.04	0.227 ± 0.005	19.32 ± 0.23

The PHA content observed among the bacterial isolates from Me Linh ranged from 9.94% to 24.83% of DCW, with the top-performing strains – ML53, ML71, and ML113 - accumulating over 20% PHA. Although these values are moderate compared to industrial strains, they fall within the range commonly reported for wild-type organisms cultivated under non-optimized conditions. For instance, *Cupriavidus necator* H16, a widely studied model strain for PHA biosynthesis, is capable of producing up to 80–90% PHA under nitrogen-limited conditions with excess carbon sources (Orita *et al*., 2012; Trakunjae *et al*., 2022). Similarly, strains of *Bacillus megaterium* and *Pseudomonas putida* have been shown to yield 60–65% and 52.2 ± 4.3% PHA content, respectively, depending on substrate type and fermentation parameters (Joyline & Aruna, 2019; Patil *et al.,* 2024; Li *et al*., 2025). Despite the lower PHA yields of the Me Linh isolates compared to these industrial benchmarks, their ability to accumulate PHA without genetic engineering or process optimization is noteworthy. This suggests a strong intrinsic biosynthetic potential that could be further enhanced through medium optimization, nutrient limitation strategies, or adaptive evolution. Strains such as ML91 and ML113, in particular, represent promising candidates for such development.

The quantitative PHA production results obtained for the five selected strains – ML53, ML71, ML91, ML113, and ML205 - further substantiate the qualitative findings derived from initial staining with Sudan Black B and Nile Blue A. The high PHA contents measured in these strains confirm the reliability of the staining-based screening, as they correspond well with the intensity of hydrophobic inclusion visualization observed microscopically. This correlation reinforces the effectiveness of the dual-staining approach as a rapid, cost-efficient method for preliminary identification of potential PHA producers, and demonstrates that qualitative staining can serve as a valid predictive tool when supported by subsequent quantitative analysis.

To investigate the structural characteristics of the PHAs synthesized by the five selected bacterial strains, FTIR spectroscopy was employed (Figure 2). The FTIR spectra of PHA extracted from the five selected bacterial strains (ML53, ML71, ML91, ML113, and ML205) exhibited characteristic absorption bands that confirm the presence of functional groups typical of polyhydroxyalkanoates. A prominent and sharp peak at approximately 1720–1724 cm^{-1}, corresponding to the C=O stretching vibration of ester groups, was consistently observed across all samples. Additionally, strong absorption bands in the range of 2924–2976 cm^{-1} were detected, attributed to the asymmetric and symmetric stretching of $–CH_3$ and $–CH_2$ groups, indicating the aliphatic nature of the polymer backbone. Other notable bands include 1450–1460 cm^{-1} (CH_2 bending), 1380–1385 cm^{-1} (CH_3 bending), and 1170–1270 cm^{-1} (C–O–C stretching), which align well with the PHB and PHBV spectra reported in other publications (Zhang *et al.*, 2022; Râpă *et al*., 2022). Peaks near 1044 cm^{-1} and 1278 cm^{-1}, observed in several samples, suggest the possible incorporation of hydroxyvalerate (HV) monomers, indicative of PHBV-type copolymers. These spectral profiles closely match published FTIR data for microbial PHAs, confirming that the extracted biopolymers are structurally similar to commercial PHB/PHBV (Laycock *et al*., 2013). The consistency of these

characteristic bands across all samples further validates the integrity of the extraction process. It supports the earlier qualitative results obtained via Sudan Black B and Nile Blue A staining. Moreover, the presence of HV-associated bands suggests that certain strains may produce PHBV rather than pure PHB, offering potential advantages in terms of flexibility and thermal stability of the resulting material. These findings underscore the suitability of these native isolates for the future development of biodegradable plastics.

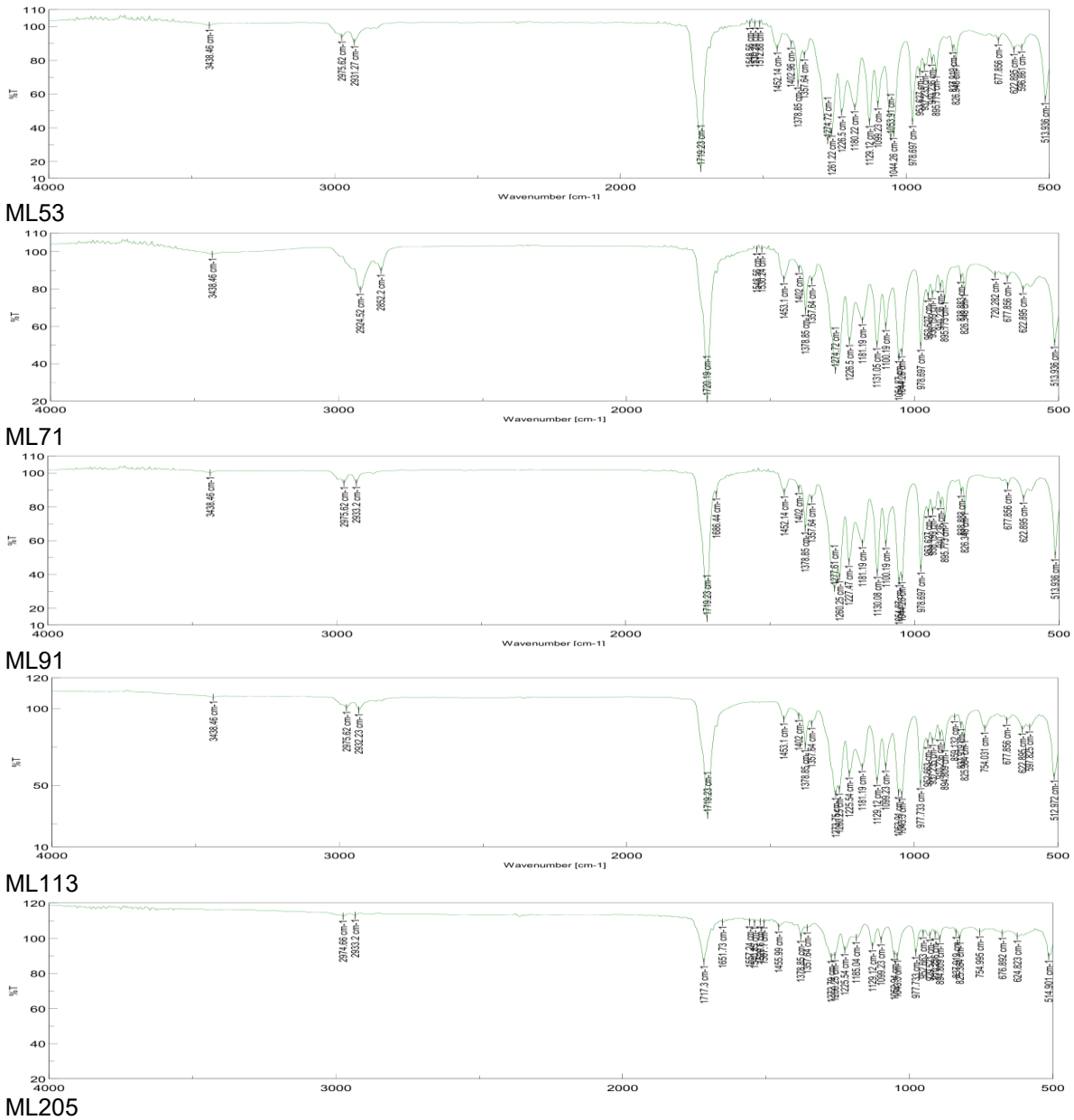

Figure 2. FTIR spectra of PHA extracted from isolates ML53, ML71, ML91, ML113, and ML205. The X-axis represents the wavenumber (cm^{-1}), displaying absorption bands within the mid-infrared range from 4000 to 500 cm^{-1}.

Characterization of PHA-producing bacteria

To further characterize the most promising PHA-producing strains, a series of phenotypic and biochemical tests was conducted. Five bacterial isolates with the highest PHA accumulation capacity were analyzed for colony morphology, cell shape, Gram staining reaction, endospore formation, and selected carbon assimilation traits. These observations provide insight into their taxonomic affiliation and metabolic versatility. The results are presented in Table 3, showing the morphological and biochemical characteristics of five selected PHA-producing bacterial isolates, revealing considerable diversity among them. While ML91 is a Gram-positive coccoid with a yellow colony—traits consistent with the genus *Micrococcus* (Madigan *et al*., 2018) - the remaining four isolates are rod-shaped, predominantly Gram-positive (except ML53), and exhibit features commonly associated with the Bacillaceae family, such as endospore formation (in ML71, ML113, and ML205) and catalase activity (Claus & Berkeley, 1986). All isolates were obligate aerobes and catalase-positive, which aligns with typical profiles of environmental PHA producers (Lee, 1996). ML113 and ML205 demonstrated the highest metabolic versatility, being capable of utilizing a wide range of carbon sources, including glucose, sucrose, maltose, arabinose, and starch. In contrast, ML91 showed limited assimilation ability, suggesting the presence of specialized metabolic pathways or alternative substrate preferences. ML53, the only Gram-negative strain, did not produce spores and exhibited reduced sugar utilization, possibly indicating an affiliation with the genus *Pseudomonas* (Palleroni, 2010).

The phylogenetic tree constructed from 16S rRNA gene sequences provides further insight into the taxonomic identity of the five selected PHA-producing strains and shows good concordance with their morphological and biochemical traits (Figure 3). Isolate ML53 clusters tightly with *P. putida* strains IAM 1236 and NA3, indicating a high degree of similarity and suggesting that it likely belongs to the genus *Pseudomonas*. Similarly, ML91 shows strong genetic affinity to *Micrococcus luteus* strains DSM 20030 and NCTC 2665, supporting its identification as *Micrococcus luteus*. Notably, three isolates - ML71, ML113, and ML205 - form a well-supported clade with reference strains of *Priestia megaterium* and *Priestia aryabhattai*. Among these, ML113 shows the highest similarity to *P. aryabhattai*, while ML71 and ML205 are more closely related to *P. megaterium*, suggesting their classification within the genus *Priestia*. Recent taxonomic revisions within the family *Bacillaceae* have led to the reclassification of several historically recognized *Bacillus* species into new genera based on multilocus sequence analysis and whole-genome phylogenomics. One of the most notable outcomes of this reclassification is the establishment of the genus *Priestia*, which includes species previously assigned to *Bacillus*, such as *B. megaterium* and *B. aryabhattai* (Gupta *et al*., 2020). Members of the genus *Priestia* are characterized as Gram-positive, endospore-forming, rod-shaped bacteria with versatile metabolic capabilities and a high degree of environmental adaptability. Importantly, both *P. megaterium* and *P. aryabhattai* have been reported as efficient PHA producers under diverse fermentation conditions.

These findings are of particular interest, as members of *Priestia* are known PHA producers and may serve as promising candidates for further functional characterization. The overall agreement between phylogenetic placement and biochemical profiles confirms the reliability of the combined approach for bacterial identification.

Table 3. Biological characteristics of selected PHA-producing bacterial strains isolated from legume-cultivated soil in Me Linh, Hanoi.

Characteristic	ML53	ML71	ML91	ML113	ML205
Colony morphology	Circular, slightly raised center, rough, dry, tightly adherent	Circular, raised, and glossy surface, smooth edge	Circular, smooth, raised	Circular, flat, wrinkled surface, round edge	Circular, slightly raised center, wrinkled surface, smooth edge
Colony color	White	Milky white	Yellow	Cream	White
Gram reaction	-	+	+	+	+
Cell morphology	Rods, short chains	Rods, long chains	Cocci, single, pairs, or short chains	Rods, long chains	Rods, long chains
Endospore formation	-	+	-	+	+
Oxygen requirement	+	+	+	+	+
Catalase	+	+	+	+	+
Voges-Proskauer reaction	-	+	-	+	+
Assimilation:					
Glucose	+	+	-	+	+
Sucrose	+	+	-	+	+
Maltose	±	+	-	+	+
Arabinose	-	+	-	+	+
Starch utilization	+	+	-	+	+

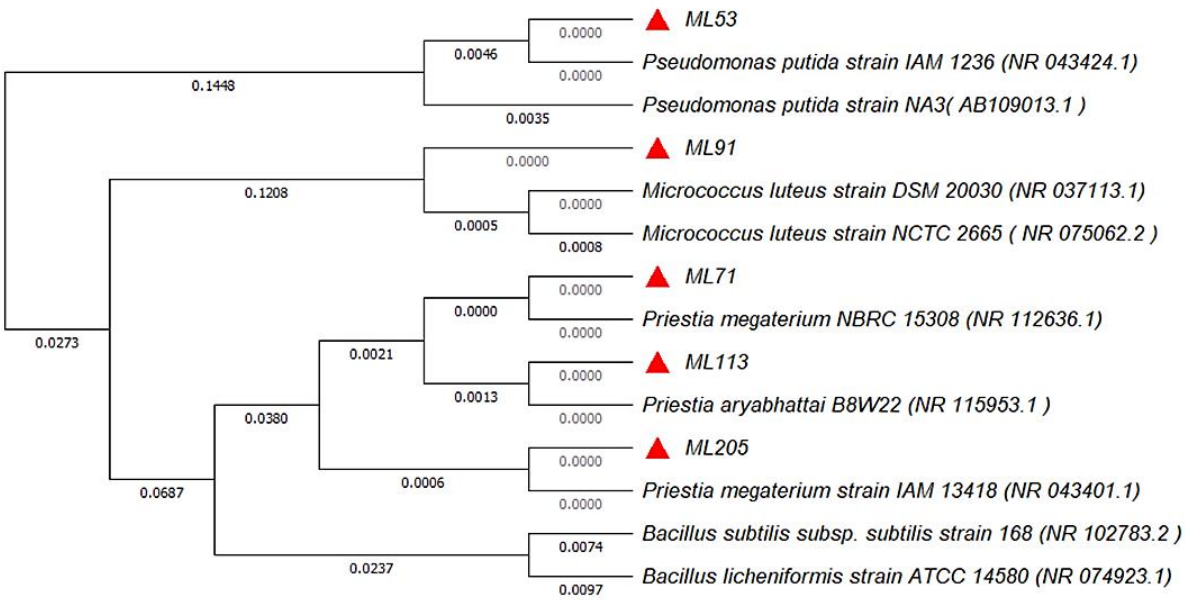

Figure 3. Phylogenetic tree of 5 selected PHA-producing bacteria isolated in Me Linh, Hanoi.

CONCLUSION

Taken together, the results of this study demonstrate the successful isolation and comprehensive characterization of five indigenous bacterial strains capable of accumulating PHAs from legume-cultivated soil in Me Linh, Hanoi. Through a tiered screening approach combining qualitative staining, quantitative fermentation analysis, FTIR spectroscopy, biochemical profiling, and 16S rRNA gene sequencing, the isolates were identified as members of the genera *Priestia*, *Pseudomonas*, and *Micrococcus*. Among them, *Priestia megaterium* (ML71 and ML205) and *Priestia aryabhattai* (ML113) exhibited the highest PHA accumulation along with broad substrate utilization, indicating their potential for scalable bioplastic production. The strong correlation between molecular phylogeny and biochemical traits underscores the effectiveness of integrating classical microbiological techniques with modern molecular tools for identifying robust PHA producers. These findings lay a valuable foundation for the future development, optimization, and application of native microbial resources in sustainable biopolymer manufacturing.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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