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First molecular records of bacteria infecting bagworms in oil palm plantations of South Sumatra, Indonesia

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Abstract. *Anggraini E, Nasution ER, Herlinda S, Irsan C, Muslim A, Suwandi S, Damiri N, Lau WH. 2026. First molecular records of bacteria infecting bagworms in oil palm plantations of South Sumatra, Indonesia. Biodiversitas 27 (1): d270118. https://doi.org/10.13057/biodiv/d270118.* Bagworms such as *Metisa plana*, *Pteroma pendula*, and *Mahasena corbetti* are major defoliating pests in oil palm ecosystems and can cause substantial yield losses during outbreaks. Despite their economic importance, information on bacteria infecting these bagworm species in Indonesia remains limited. This study presents the first molecular identification of bacterial isolates obtained from three dominant bagworm species collected in Banyuasin, South Sumatra, Indonesia. Partial 16S rRNA gene sequences were analyzed using BLASTn and Bayesian phylogenetic inference to determine their taxonomic affiliations. The isolate from *P. pendula* was identified as *Stenotrophomonas maltophilia*, forming a well-supported clade with reference sequences in the Bayesian phylogenetic tree. The isolate from *M. plana* showed high sequence similarity to *Bacillus thuringiensis*, while the isolate from *M. corbetti* was identified as *Mammaliicoccus sciuri* and clustered distinctly from *Staphylococcus* species. All sequences were deposited in GenBank under accession numbers PX487709.1, PX487712.1, and PX487708.1, respectively. These findings represent the first molecular records of bacteria infecting oil palm bagworms in South Sumatra and demonstrate that different bagworm species can be infected by distinct bacterial taxa. A laboratory bioassay using third-instar *Spodoptera litura* larvae was conducted as a preliminary assessment of biological activity, indicating variable larval responses among the bacterial isolates. While *B. thuringiensis* remains the only well-established entomopathogenic species, the responses observed for *S. maltophilia* and *M. sciuri* suggest that their interactions with insect hosts merit further investigation. Overall, this study provides baseline molecular evidence of bacterial infections in oil palm bagworms and contributes to a broader understanding of insect-microbe diversity in plantation ecosystems.

Keywords: Bagworm, biological control, oil palm management, potential entomopathogenic bacteria

INTRODUCTION

The oil palm (*Elaeis guineensis*) is one of Indonesia's most economically important plantation crops, contributing substantially to national development through non-oil export revenue, industrial raw materials, and rural employment opportunities. The sustainability and productivity of oil palm plantations, however, continue to be threatened by a variety of pests and diseases. Among the major insect pests, bagworms (Lepidoptera: Psychidae) are widely regarded as the most destructive defoliators in Southeast Asia (Maidin et al. 2024). Outbreaks of species such as *Metisa plana*, *Mahasena corbetti*, and *Pteroma pendula* can cause severe foliage loss, resulting in marked reductions in photosynthetic efficiency and ultimately decreasing Fresh Fruit Bunch (FFB) production (Wood and Kamarudin 2019a). Periodic and recurrent outbreaks in Indonesia and Malaysia underscore the need for effective and sustainable management approaches.

Chemical insecticides have traditionally been the primary method for bagworm suppression due to their rapid action and ease of application. Nevertheless, heavy and repeated use of broad-spectrum insecticides has generated numerous constraints, including the development of resistance in bagworm populations, resurgence of secondary pests, environmental contamination, and adverse effects on natural enemies and beneficial arthropods (Wood and Kamarudin 2019b). These concerns have strengthened calls for the adoption of Integrated Pest Management (IPM) strategies that emphasize environmentally friendly, biologically based control options. Within this framework, entomopathogenic microbes, particularly bacteria, are increasingly recognized as promising alternatives for reducing dependency on chemical pesticides.

Entomopathogenic bacteria naturally infect and kill insects through multiple modes of action, such as toxin production, enzymatic disruption of host tissues, and interference with host immunity (Vilas-Boas et al. 2024). *Bacillus thuringiensis* (Bt) is the most widely used microbial

insecticide globally, owing to its production of Cry and Cyt toxins that specifically target the midgut epithelial cells of susceptible larvae, causing cell lysis and mortality (Yang et al. 2023a). Bt-based biopesticides are valued for their safety, host specificity, and compatibility with other IPM components. In addition to Bt, other bacterial taxa have been reported in association with diverse insect species. Although their ecological roles remain insufficiently resolved, emerging evidence suggests potential functional interactions, ranging from opportunistic pathogenicity to microbial competition and symbiosis (Kumar et al. 2023; Adeyemo et al. 2024). Despite these developments, the microbial associates of major oil palm bagworm species in Indonesia remain poorly characterized.

A key limitation in understanding insect-microbe interactions in oil palm ecosystems has been the scarcity of studies employing molecular techniques to identify insect-associated bacteria. Traditional morphological or biochemical characterization often lacks the resolution necessary for accurate bacterial identification, particularly among closely related taxa. Recent advances in molecular identification, especially 16S rRNA gene sequencing, have substantially improved the precision and reliability of bacterial classification (Shao et al. 2024). This method has been widely applied to profile bacterial diversity in various insect hosts, enabling researchers to detect entomopathogens, commensals, and environmental associates with greater confidence. Molecular identification is especially relevant for genera with complex taxonomic boundaries.

Despite the expanded use of molecular identification techniques, no published studies have provided molecular evidence of bacteria associated with *M. plana*, *P. pendula*, or *M. corbetti* in Indonesian oil palm plantations. The absence of such information represents a significant knowledge gap that limits the development of microbial-based control strategies in the region. Addressing this gap is necessary not only for advancing scientific understanding of bagworm-microbe interactions but also for supporting practical innovations in sustainable pest management.

This study molecularly identified bacterial species associated with three major bagworm pests, *M. plana, P. pendula*, and *M. corbetti,* from an oil palm plantation in Banyuasin, South Sumatra, Indonesia. Bacterial isolates were characterized using 16S rRNA gene sequencing supported by microscopic observations, representing the first molecular records of bacteria from these bagworm species in South Sumatra. Laboratory bioassays provided preliminary information on the biological activity of the isolates. The findings contribute baseline data on insect-associated bacterial diversity and support future research on insect–microbe interactions and environmentally friendly bagworm management in oil palm ecosystems.

MATERIALS AND METHODS

Sample collection

The sampling of bagworms suspected of being infected by entomopathogenic bacteria was conducted in an oil palm plantation located in Sungai Rengit Village, Talang Kelapa Sub-district, Banyuasin District, South Sumatra, Indonesia (Figure 1). Sample selection was based on the morphological symptoms of infection observed in the bagworms, including body discoloration, softening of the cuticle texture, the presence of fluid exudation from the body, and abnormal positioning of the bagworm cases (Figure 2). The bacterial isolation process was carried out at the Bacteriology Laboratory, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Sriwijaya, Indonesia. Bacterial colonies were isolated from bagworm (*Psychidae* spp.) specimens cultured on Nutrient Agar (NA) medium (1 g meat extract; 2 g yeast extract; 5 g peptone; 5 g sodium chloride and 15 g agar) (Dos Santos Moreira et al. 2024). The resulting entomopathogenic bacterial isolates were incubated at room temperature and maintained for molecular analysis.

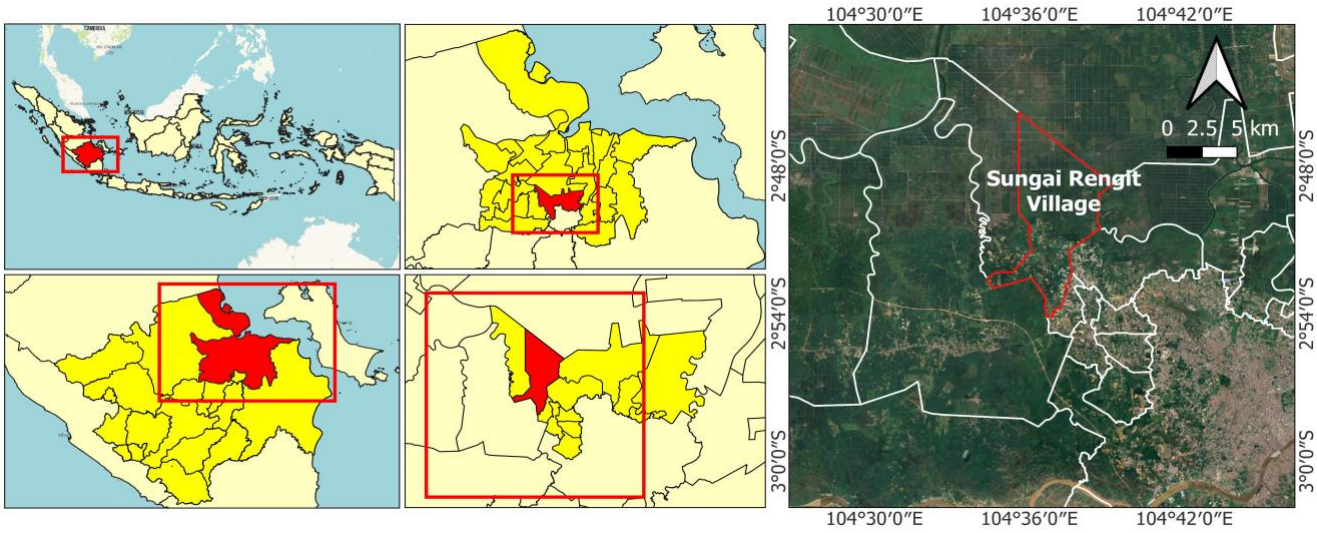

Figure 1. Map of the sampling location in an oil palm plantation in Sungai Rengit Village, Talang Kelapa Sub-district, Banyuasin District, South Sumatra, Indonesia

Figure 2. Bagworm cadaver suspected of being infected by entomopathogenic bacteria in the field. A. *Mahasena corbetti*, B. *Metisa plana*, C. *Pteroma pendula*

DNA amplification

The 16S rRNA gene was amplified using universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3'). PCR reactions were prepared in a 50 µL volume containing 25 µL MyTaq HS Red Mix, 0.5 µM of each primer, 5 µL template DNA (approximately 10 ng), and nuclease-free water. Amplification was performed in a BIO-RAD T100™ Thermal Cycler with the following program: initial denaturation at 95°C for 5 min; 35 cycles of 94°C for 30 s, 55-60°C for 30 s (annealing), and 72°C for 1 min; followed by a final extension at 72°C for 10 min. PCR products were separated on a 1% agarose gel to confirm successful amplification.

Gel electrophoresis

Gel electrophoresis was carried out using 1× TAE buffer and a 1% agarose gel stained with GelRed. A 1 kb DNA ladder and loading dye were used as molecular markers. The gel was placed in a horizontal electrophoresis chamber connected to a power supply. Upon completion of electrophoresis, the gel was visualized under a UV transilluminator to evaluate DNA quality and verify the presence of the target DNA fragment.

GenBank submission

The isolates obtained in this study were deposited in the GenBank database under the following accession numbers:

PX487709.1 - *Stenotrophomonas maltophilia* strain PP (isolated from *Pteroma pendula* (de Joannis, 1929))

PX487712.1 - *Bacillus thuringiensis* strain MP (isolated from *Metisa plana* (Walker, 1883))

PX487708.1 - *Mammaliicoccus sciuri* strain MC (isolated from *Mahasena corbetti* (Tams, 1928))

Scanning Electron Microscope (SEM) observation

The morphological characteristics of the bacterial isolates were examined using a Scanning Electron Microscope (SEM). Each bacterial isolate (*S. maltophilia* isolated from *P. pendula*, *B. thuringiensis* isolated from *M. plana*, *M. sciuri* isolated from *M. corbetti*) was cultured on nutrient agar and incubated for 48 hours at 30°C to obtain fresh colonies. Scanning Electron Microscopy (SEM) analysis was performed to observe the morphology of the bacterial cells. Sample preparation followed Naskar et al. (2020). The prepared specimens were observed under a Scanning Electron Microscope (SEM) at an accelerating voltage of 10-15 kV with magnifications of 10,000×, and 20,000×.

Evaluation of entomopathogenic bacterial potential using *Spodoptera litura* larvae as test insects

The bioassay was conducted using the leaf-dipping method. Bacterial cultures were grown in 150 mL of nutrient broth until reaching a concentration of 10^8 CFU/mL. Water spinach (*Ipomoea aquatica*) leaves were dipped into each 72-hour bacterial culture for 10 minutes and then air-dried. Each bacterial treatment consisted of four replicates, with ten larvae per replicate. The treated leaves were placed into experimental cups, each containing one individual third-instar *S. litura* larva. Leaves dipped in sterile distilled water served as the control. Fresh leaves were provided daily. Larval survival was observed for seven days. This method was adapted from Krishanti et al. (2017).

Data analysis

Multiple sequence alignments were generated using MAFFT v7 (Katoh and Standley 2013), integrated in Geneious Prime 2025.2.2, with default iterative refinement settings. The resulting alignments were then trimmed using trimAl v1.5 (Capella-Gutiérrez et al. 2009) under the "-automated1" option to remove ambiguously aligned positions. Phylogenetic analyses were performed using Bayesian Inference (BI) implemented in MrBayes v3.2.6 (Ronquist et al. 2012) through the Geneious Prime 2025.2.2 interface. The HKY85 nucleotide substitution model (Hasegawa et al. 1985) with gamma-distributed rate variation (Γ) across four categories was applied. A strict molecular clock with uniform branch lengths was specified according to the MrBayes prior settings. Two independent MCMC runs were conducted for 100,000 generations, each consisting of four chains with a heated chain temperature of 0.2. Trees were sampled every 10,000 generations, and the first 1,000 trees were discarded as burn-in. Convergence was assessed based on the standard deviation of split frequencies and inspection of trace plots. A 50% majority-rule consensus tree was generated, and posterior probabilities were used to evaluate clade support.

The observation of larval mortality began one day after application and continued for 24 days. The percentage of larval mortality was calculated using the formula:

$$P = \frac{a}{b} \times 100\%$$

Where, *P*: The mortality percentage of *Spodoptera litura*, *a*: The number of dead larvae, *b*: The total number of larvae observed (Abbott 1925)

The mortality data were analyzed descriptively and presented in graphical form. Prior to further analysis, the data were first tested for normality and homogeneity, followed by transformation using the arcsine. The transformed data were then analyzed using ANOVA, and significant differences among treatments were determined using the Tukey HSD test.

RESULTS AND DISCUSSION

Collection and preparation of bacterial samples

A total of three bacterial isolates were collected for this study, consisting of one isolate from the bagworm *Pteroma pendula* (PP), one isolate from *Mahasena corbetti* (MC), and one isolate from *Metisa plana* (MP). Sampling was conducted following the completion of the research proposal seminar. Once all samples were obtained, further experimental procedures were carried out. The bacterial isolate from *P. pendula* was characterized by a yellowish color, circular shape, convex elevation, smooth margins, shiny surface, and mucoid consistency. The *M. corbetti* isolate exhibited a white to pale-yellow color, circular shape, convex elevation, slightly undulate margins, a dull surface, and dry consistency. In contrast, the *M. plana* isolate showed a whitish-yellow color, slightly irregular shape, convex elevation, uneven margins, shiny surface, and mucoid consistency (Figure 3).

Microscopically, the bacterium isolated from *P. pendula* exhibited a bacillus (rod-shaped) morphology with small, elongated cells. Some cells appeared to form short chains, while others were observed as individual cells (Figure 4). The cells stained red, indicating that the bacterium is Gram-negative. The bacterium isolated from *M. corbetti* showed a coccus (spherical) form arranged in clusters, with purple staining, suggesting a Gram-positive bacterium. The *M. plana* isolate displayed an elongated bacillus morphology with slightly rounded ends. Some cells appeared in short chains, while others occurred singly or in pairs. The cells showed a faint purple coloration, indicating that *M. plana* is a Gram-positive bacterium.

Morphological observation using SEM

The morphological characteristics of the bacterial isolates were examined using a Scanning Electron Microscope (SEM) to observe the cell shape and arrangement of each isolate. The SEM observations showed distinct differences among the bacterial isolates obtained from bagworms. The isolate coded as MC exhibited spherical (coccus) cells that appeared in clustered arrangements, indicating typical morphology of *Mammaliicoccus* species. The isolate coded as MP showed elongated rod-shaped (bacillus) cells, which correspond to the morphology of *Bacillus thuringiensis* (Bt). In contrast, the isolate coded as PP displayed short rod to oval-shaped (coccobacillus) cells, consistent with the characteristics of *Stenotrophomonas* species. These observations clearly align with the independently examined SEM micrographs, which further demonstrated that *M. sciuri* forms irregular grape-like clusters, *B. thuringiensis* presents dense arrangements of elongated rods, and *S. maltophilia* appears as dispersed short rods. These results confirm the distinct morphological patterns of the three isolates and support the molecular identification, showing that each genus exhibits characteristic cellular forms observable under SEM (Figures 5-7).

Figure 3. Bacterial isolates collected from: A. *Pteroma pendula*, B. *Mahasena corbetti,* C. *Metisa plana*

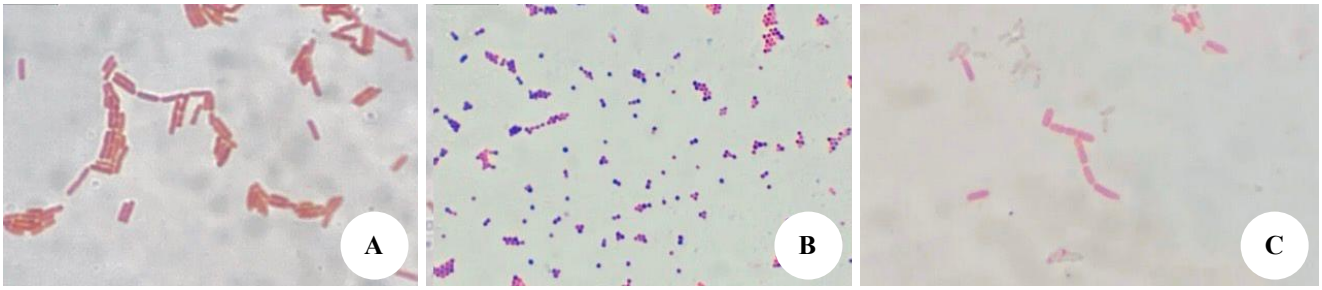

Figure 4. Microscopic morphology of bacteria isolated from: A. *Pteroma pendula*, B. *Mahasena corbetti*, C. *Metisa plana*

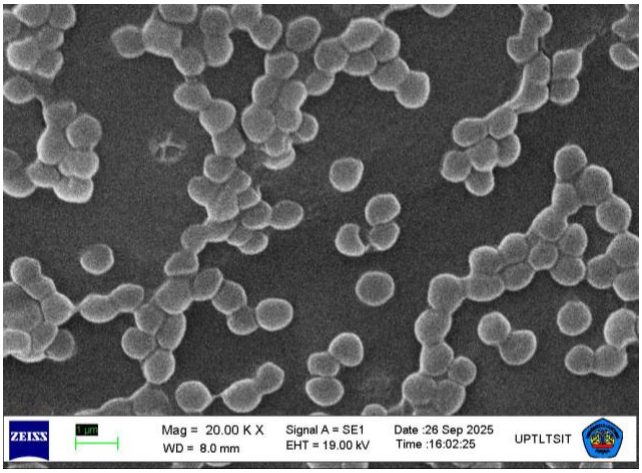

Figure 5. *Mammaliicoccus sciuri* showing spherical cocci (0.5-1.0 µm) observed using a Scanning Electron Microscope (SEM) at magnification 20,000×

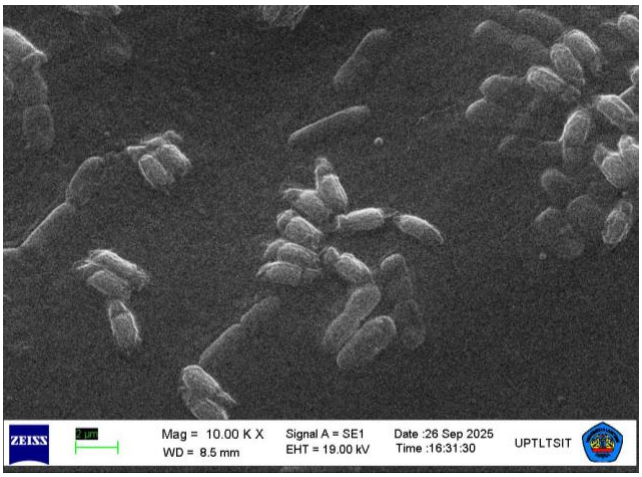

Figure 6. *Bacillus thuringiensis* exhibiting elongated bacilli (1.0-1.2×3-5 µm) observed using a Scanning Electron Microscope (SEM) at magnification 10,000×

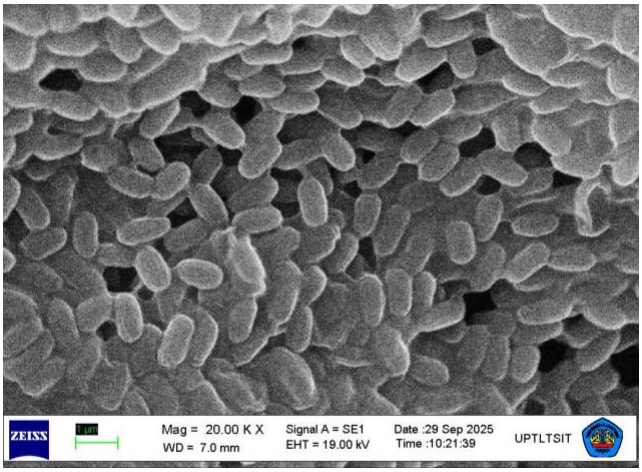

Figure 7. *Stenotrophomonas maltophilia* showing short rods (0.7-1.0×1.5-2.5 µm) observed using a Scanning Electron Microscope (SEM) at magnification 20.00 KX overview (scale bar 1 µm)

DNA concentration and quantitative purity

In the quantitative analysis, variations in DNA concentration were observed among the extracted samples. The *M. plana* (MP) sample showed the lowest DNA concentration at 83.4 ng/µL, while the highest concentration was recorded in the *P. pendula* (PP) sample at 136.4 ng/µL. These results indicate differences in the amount of DNA successfully extracted from each bacterial isolate (Table 1).

Visualization of DNA amplification

The electrophoresis visualization showed distinct DNA bands of approximately 1500 bp, indicating successful amplification of the 16S rRNA target gene from the bagworm-associated bacterial isolates (Figure 8). The presence of clear and single bands confirms that the PCR reactions produced specific amplicons without nonspecific products or primer dimers.

BLASTn analysis of 16S rRNA sequences

The partial 16S rRNA gene sequences were analyzed using the BLASTn program against the NCBI database. The results showed that each isolate exhibited high sequence similarity (97-99%) with known bacterial species, confirming their taxonomic affiliations at the genus and species levels.

Bacteria isolated from Pteroma pendula

The BLASTn analysis showed that the bacterial isolate obtained from *P. pendula* had the highest sequence similarity (98.50%) to *Stenotrophomonas beteli* strain ATCC 19861, followed by matches to *S. maltophilia* and *Stenotrophomonas pavanii* (Table 2). All top hits belonged to the genus *Stenotrophomonas*, indicating that the isolate from *P. pendula* is most closely related to members of this genus based on 16S rRNA gene sequence similarity.

The phylogenetic analysis based on the 16S rRNA gene sequence revealed that *S. maltophilia* strain PP clustered firmly within the *S. maltophilia* clade (Figure 9). The resulting tree showed a clear separation between *S. maltophilia* and the outgroup (*E. coli*), confirming the distinct taxonomic placement of the isolate. Strain PP formed a close association with several *S. maltophilia* strains, including AK2, CGKVJ/16a-2013, GRD, and DZ4, supported by high bootstrap values (up to 1.0). This tight clustering indicated strong genetic similarity and confirmed that strain PP belonged to the *S. maltophilia* species complex. The overall topology of the tree demonstrated consistent branching patterns, further supporting the reliability of the phylogenetic placement of the PP isolate.

Isolate from Metisa plana

The bacterial isolate obtained from *M. plana* showed high sequence similarity to members of the *Bacillus cereus* group (Table 3). The closest BLASTn matches were *B. thuringiensis* strain ATCC 10792 and *B. thuringiensis* strain NBRC 101235, each displaying 98.70% sequence identity. These results indicate that the isolate from *M. plana* is most closely related to *B. thuringiensis* based on 16S rRNA gene analysis.

Based on the phylogenetic tree, the isolate shows a much closer relationship to *B. thuringiensis* than to other members of the *B. cereus* sensu lato group (Figure 10). Although *B. cereus* sensu lato refers to the broader genetic complex that includes *B. cereus*, *B. thuringiensis*, *B. anthracis*, and several related species, the placement of the isolate in the tree indicates a specific affinity toward the *B. thuringiensis* cluster. The isolate forms a tight clade with *B. thuringiensis* strain B07 and falls directly within the *B. thuringiensis* lineage, supported by a strong bootstrap value.

Bacterial isolate from Mahasena corbetti

The bacterial isolate obtained from *M. corbetti* showed the highest similarity to *M. sciuri* strain DSM 20345, with 98.50% sequence identity (Table 4). The next closest matches included *M. lentus*, *M. vitulinus*, and *M. stepanovicii*. These results indicate that the isolate from *M. corbetti* is most closely related to *M. sciuri* based on 16S rRNA gene sequence analysis.

The phylogenetic tree based on the 16S rRNA gene sequence showed that *M. sciuri* strain MC clustered firmly within the *M. sciuri* group (Figure 11). The isolate grouped closely with several reference strains of *M. sciuri*, including strains ZJTR12, HNJZ-2, CDJ17, and 2-38, all supported by strong bootstrap values. In contrast, *Staphylococcus sciuri* strains formed a separate and clearly distinct clade, indicating a clear taxonomic boundary between the two genera. The overall topology confirmed that strain MC exhibited the highest genetic similarity to other *M. sciuri* strains rather than to *Staphylococcus*, supporting its identification as *M. sciuri*.

Table 1. Quantitative analysis of DNA concentration and purity of entomopathogenic bacterial isolates

Bacteria isolate from	DNA concentration (ng/µL)	A260/280	A260/230
Pteroma pendula (PP)	136.4	1.91	2.28
Mahasena corbetti (MC)	104.5	1.82	2.39
Metisa plana (MP)	83.4	1.87	1.44

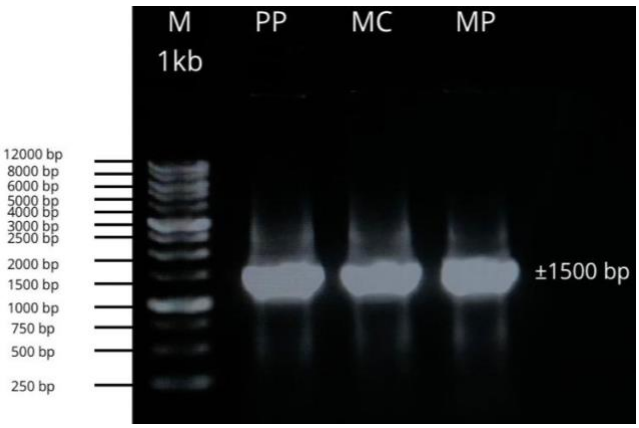

Figure 8. Visualization of PCR amplification of the 16S rRNA target gene (~1500 bp) in bagworm-associated bacterial isolates. M: 1 kb DNA marker, PP: Bacteria isolated from *Pteroma pendula*, MC: Bacteria isolated from *Mahasena corbetti*, MP: Bacteria isolated from *Metisa plana*

Table 2. Results of BLASTn analysis of 16S rRNA gene sequences of bacteria isolated from *Pteroma pendula* compared with National Center for Biotechnology Information (NCBI) reference sequences

Accession	Description	Length	Maximum identity (%)	Query cover	E value
CP040433.1	Complete genome chromosome *Stenotrophomonas maltophilia* strain SKK55	4675446	96.96	98	0
PV359017.1	Partial sequence of 16S ribosomal RNA gene *Stenotrophomonas maltophilia* strain ND6-2	1442	97.04	98	0
ON259665.1	Partial sequence of 16S ribosomal RNA gene *Stenotrophomonas maltophilia* strain B7	1415	97.18	98	0
CP088244.1	Complete genome chromosome *Stenotrophomonas maltophilia* strain 2013-SM4	4575852	96.96	98	0
HM625746.1	Partial sequence of 16S ribosomal RNA gene *Stenotrophomonas maltophilia* strain MHF ENV 20	1448	97.11	98	0
GU170362.1	Partial sequence of 16S ribosomal RNA gene *Stenotrophomonas maltophilia* strain H1137-2	1404	97.17	98	0
MK489413.1	Partial sequence of 16S ribosomal RNA gene *Stenotrophomonas maltophilia* strain DZ 4	1411	97.10	98	0
KC992302.1	Partial sequence of 16S ribosomal RNA gene *Stenotrophomonas maltophilia* strain GRD	1411	97.17	98	0
MK078536.1	Partial sequence of 16S ribosomal RNA gene *Stenotrophomonas maltophilia* strain CGKV/J16a-2013	1421	97.18	98	0
KJ685809.1	Partial sequence of 16S ribosomal RNA gene *Stenotrophomonas maltophilia* strain AK2	1476	97.04	98	0

Note: E value: Expectation value

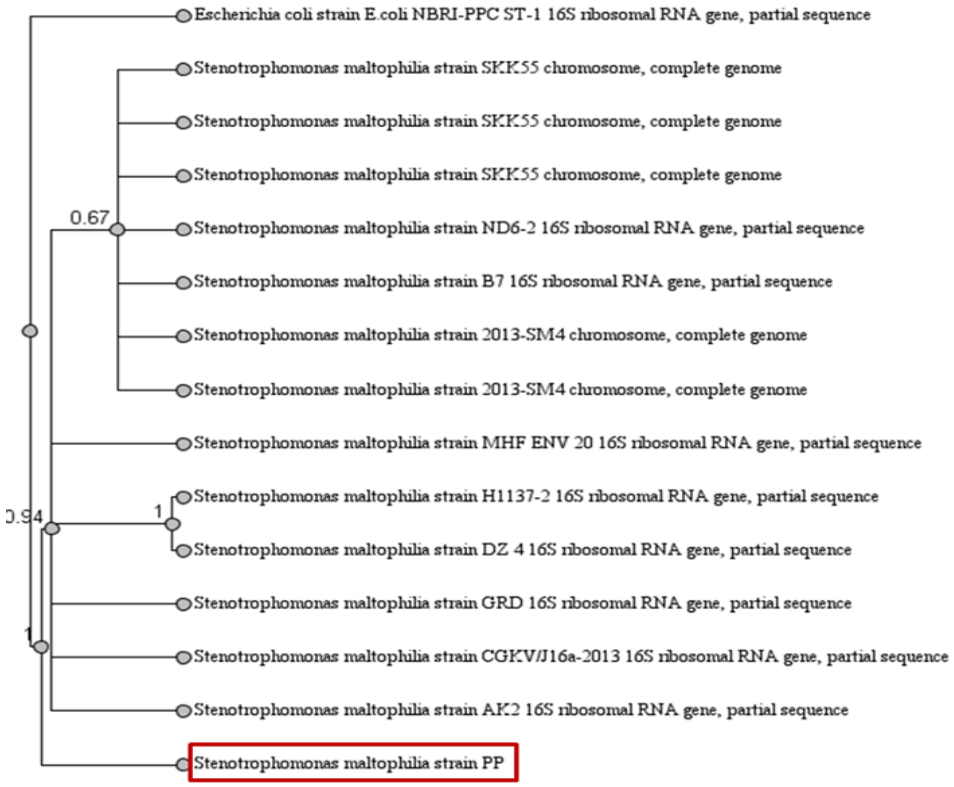

Figure 9. Bayesian inference phylogenetic tree based on 16S rRNA gene sequences showing the placement of *S. maltophilia* strain PP among closely related *S. strains*. Posterior probability values are indicated at the nodes. *E. coli* was used as an outgroup

Preliminary assessment of the biological activity of bacterial isolates using *Spodoptera litura* larvae

The *S. litura* larvae in this study exhibited similar infection symptoms, including reduced mobility, slow response to touch, decreased feeding activity, darkened body coloration, and a soft body texture (Figure 12). Larvae of *S. litura* that died due to bacterial infection displayed blackened bodies, shriveling, foul-smelling fluid exudation, and damaged, rigid cuticles (Figure 13). Larval mortality differed significantly among bacterial treatments. *S. maltophilia* (PP) produced the highest mortality (57.50%), followed by *B. thuringiensis* (MP) at 47.50%, and *M. sciuri* (MC) at 37.50%, while the control showed no mortality (0%) (Table 4). ANOVA revealed a significant difference among treatments at the 1% level (P value: 1.54×10^{-3}). The lowest LT_{50} value was obtained for *S. maltophilia* (4.77 days), indicating faster action, whereas the longest LT_{95} value was recorded for *M. sciuri* (16.27 days), reflecting a slower time required to reach 95% mortality (Table 5). The Tukey HSD test at 1% showed that mortality differences greater than 0.13% among treatments were statistically significant, confirming the distinct effects of each bacterial isolate. Overall, *S. maltophilia* demonstrated the most effective and rapid suppression of larval populations.

The mortality of *S. litura* larvae increased over time following treatment with all three tested bacteria. The treatment with *S. maltophilia* showed the highest effectiveness, with mortality beginning to appear on day 3 and continuing to rise, reaching approximately 57.50% by day 7. Mortality in the *B. thuringiensis* treatment increased to 47.50% by the end of the observation period. Meanwhile, *M. sciuri* showed the lowest effectiveness, reaching around 37.50% on day 7. The absence of mortality in the control treatment indicates that larval death in the bacterial treatments was truly caused by the pathogenic activity of the tested bacteria.

Discussion

The present study confirmed distinct bacterial associations with three bagworm species from oil palm plantations in South Sumatra: *S. maltophilia* from *P. pendula*, *B. thuringiensis* from *M. plana*, and *M. sciuri* from *M. corbetti*. These results indicate that each bagworm species has the potential to harbor specific bacteria formed through host-microbe adaptation in the plantation environment. From three isolates, only *B. thuringiensis* was confirmed as a true entomopathogenic bacterium, found in *M. plana*. Meanwhile, *S. maltophilia* is not a true entomopathogen but has secondary entomopathogenic activity that does not directly kill the host, and *M. sciuri* is an opportunistic bacterium commonly found in soil, animals, and the environment, not an insect pathogen. These findings indicate the potential for local biological control agents against bagworms, especially Bt because it can infect, weaken, or kill insect hosts. Natural sources of entomopathogenic strains can be obtained from infected insect carcasses (Sharma et al. 2020; Dos Santos Moreira et al. 2024).

The combined use of 16S rRNA sequencing and SEM morphological observations increased confidence in taxonomic assignments and is consistent with current practice in insect-microbe studies, where molecular and morphological data are integrated for robust identification (Zhang et al. 2022; Shao et al. 2024). In this study, the sequencing results showed the identification of three different bacterial species from three bagworm hosts. The 16S rRNA gene sequencing method is capable of providing high taxonomic results up to the genus or species level (Johnson et al. 2019). Meanwhile, SEM observations will provide visualization of structural characters, including morphology, surface features, texture, spore damage, germination, and appendages which are often used in bacterial classification (Malyshev et al. 2024). The results of SEM observations show that *S. maltophilia* bacteria have a short to oval rod shape (coccobacilli) in accordance with the findings (Trifonova and Strateva 2018), Bt is in the form of a *Bacillus* with typical spores (Loutfi et al. 2021; Handayani et al. 2024), while *M. sciuri* is in the form of a coccus as reported (Biswas et al. 2022).

Table 3. Results of BLASTn analysis of 16S rrna gene sequences of bacteria isolated from *Metisa plana* compared with NCBI reference sequences

Accession	Description	Length	Maximum identity (%)	Query cover	E value
OQ825034.1	Partial sequence of 16S ribosomal RNA gene *Bacillus wiedmannii* strain ASS-1	1432	98.86	100	0
MG651461.1	Partial sequence of 16S ribosomal RNA gene *Bacillus toyonensis* strain FJAT-46839	1421	98.86	100	0
KJ638984.1	Partial sequence of 16S ribosomal RNA gene *Bacillus thuringiensis* strain ALR-17	940	98.86	100	0
KF863879.1	Partial sequence of 16S ribosomal RNA gene *Bacillus* sp. hb90	1455	98.86	100	0
PP962495.1	Partial sequence of 16S ribosomal RNA gene *Bacillus* sp. (in: firmicutes) strain WRc-1	1453	98.86	100	0
OR831693.1	Partial sequence of 16S ribosomal RNA gene *Bacillus* sp. (in: firmicutes) strain 22STR338	1417	98.86	100	0
MW559247.1	Partial sequence of 16S ribosomal RNA gene *Bacillus cereus* strain T3M5	1444	98.86	100	0
MN485932.1	Partial sequence of 16S ribosomal RNA gene *Bacillus cereus* strain NB40/TK09	1419	98.86	100	0
PP480224.1	Partial sequence of 16S ribosomal RNA gene *Bacillus cereus* strain FD-51	1418	98.86	100	0
OL468256.1	Partial sequence of 16S ribosomal RNA gene *Bacillus cereus* strain DBTD14	1221	98.86	100	0
OP572154.1	Partial sequence of 16S ribosomal RNA gene *Bacillus cereus* strain BS46	1236	98.86	100	0
OQ512008.1	Partial sequence of 16S ribosomal RNA gene *Bacillus thuringiensis* strain B07	1064	99.00	100	0

Note: E value: Expectation value

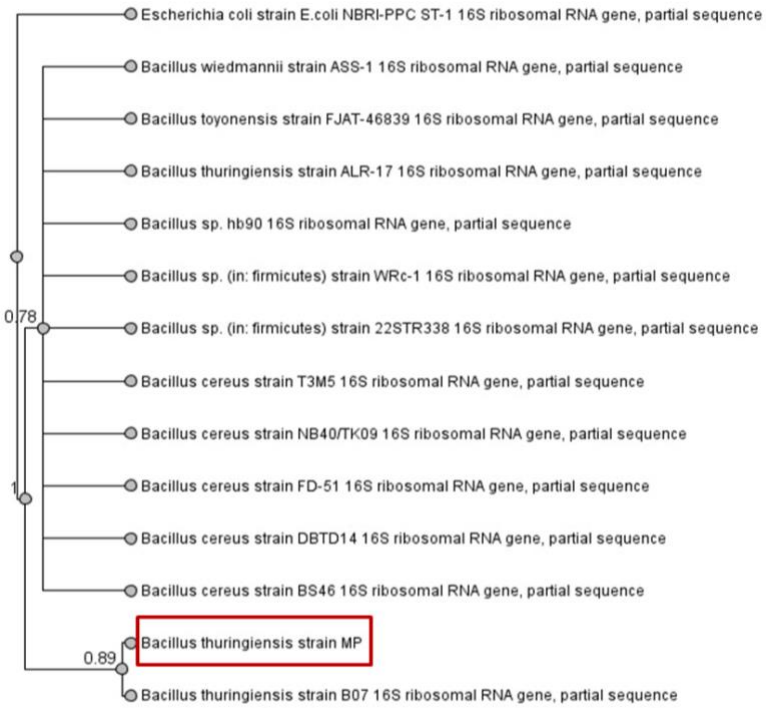

Figure 10. Bayesian inference phylogenetic tree based on 16S rRNA gene sequences showing the placement of *B. thuringiensis* strain MP within the *B. cereus* sensu lato group. Posterior probability values are shown at the nodes. *E. coli* was used as an outgroup

Table 4. Results of BLASTn analysis of 16S rRNA gene sequences of bacteria isolated from *Mahasena corbetti* compared with NCBI reference sequences

Accession	Description	Length	Maximum identity (%)	Query cover	E value
KY316479.1	Partial sequence of 16S ribosomal RNA gene *Staphylococcus sciuri* strain ZLynn1000-43	1463	98.57	100	0
KU240493.1	Partial sequence of 16S ribosomal RNA gene *Staphylococcus sciuri* strain X302	1434	98.51	100	0
KT260766.1	Partial sequence of 16S ribosomal RNA gene *Staphylococcus sciuri* strain RCB554	1412	98.44	100	0
MG722797.1	Partial sequence of 16S ribosomal RNA gene *Staphylococcus sciuri* strain GCF1S2	1420	98.44	100	0
KR067567.1	Partial sequence of 16S ribosomal RNA gene *Staphylococcus sciuri* strain 2218	1420	98.51	100	0
OM319802.1	Partial sequence of 16S ribosomal RNA gene *Mammaliicoccus sciuri* strain ZJTR12	1438	98.57	100	0
OQ772178.1	Partial sequence of 16S ribosomal RNA gene *Mammaliicoccus sciuri* strain HNJZ-2	1486	98.90	100	0
OR282447.1	Partial sequence of 16S ribosomal RNA gene *Mammaliicoccus sciuri* strain CDJ17	1423	98.51	100	0
PP512798.1	Partial sequence of 16S ribosomal RNA gene *Mammaliicoccus sciuri* strain 2-38	1463	98.57	100	0

Note: E Value: Expectation value

Figure 11. Bayesian inference phylogenetic tree based on 16S rRNA gene sequences showing the placement of *Mammaliicoccus sciuri* strain MC among closely related *Mammaliicoccus sciuri* and *Staphylococcus sciuri* strains. Posterior probability values are shown at the nodes. *Escherichia coli* was used as an outgroup

Figure 12. Symptoms of infection in *Spodoptera litura* larvae under each bacterial isolate treatment and the control: A. Control larvae, B. PP isolate treatment, C. MC isolate treatment, and D. MP isolate treatment

Figure 13. Dead *Spodoptera litura* larvae infected by bacteria and healthy control larvae: A. Control, B. PP isolate, C. MC isolate, and D. MP isolate

Table 5. Larval mortality and estimated LT_{50} and LT_{95} (days) following bacterial treatments

Treatment	Mortality (%)±standard deviation	LT 50 (Day) (lower and upper confidence limits)	LT 95 (Day) (lower and upper confidence limits)
Stenotrophomonas maltophilia (PP)	57.50±0.79a	4.77 (0.70-2.96)	6.02 (1.01-3.43)
Bacillus thuringiensis (MP)	47.50±0.75a	5.73 (0.12-5.40)	7.23 (0.33-6.38)
Mammaliicoccus sciuri (MC)	37.50±0.79a	7.92 (0.30-7.15)	16.7 (2.27-10.9)
control	0.00±0.00b	-	-
P value	1.54×10^{-3}	-	-
Tukey HSD at alpha 0.01	0.13	-	-

Note: Mortality (%) is presented as mean±standard deviation (n = 4 replicates). LT_{50} and LT_{95} were estimated using a probit model (95% CL). "-" indicates unavailable data. F-value and P-value were obtained from One-Way ANOVA. Tukey HSD 1% represents the least significant difference at $\alpha = 0.01$

Isolation of Bt from *M. plana* supports extensive literature demonstrating the natural occurrence of Bt in agroecosystems and its efficacy against lepidopteran larvae via Cry/Cyt toxins that disrupt midgut epithelial cells (Heckel 2020; Yang et al. 2023b). In addition, Bt is also known to have toxic activity against insects from the orders Diptera and Coleoptera (Diaz-Mendoza et al. 2012; Loutfi et al. 2021). The detection of indigenous Bt strains in bagworm populations suggests potential for developing local Bt-based biopesticides that align with IPM goals and reduce reliance on synthetic insecticides in oil palm plantations. Bt is an environmentally friendly biopesticide and currently accounts for approximately 90% of the total microbial pesticides available globally (Damalas and Koutroubas 2018).

The presence of *S. maltophilia* in *P. pendula* is noteworthy because members of this genus are increasingly reported in rhizospheric and insect-associated contexts and are known to produce hydrolytic enzymes, secondary metabolites, and volatile compounds that can affect insect physiology or interact synergistically with other entomopathogens (Mason et al. 2022; Kumar et al. 2023). However, it has recently been reported that *S. maltophilia* is capable of causing mortality in *Callosobruchus maculatus* of up to 40% (Soleimani et al. 2023). While *S. maltophilia* is not a canonical entomopathogen like Bt, its ecological versatility means it may act as an opportunistic pathogen or modulator of the insect microbiome; functional assays (bioassays, enzyme/toxin profiling) are required to determine its role (Mason et al. 2022; Kumar et al. 2023). *M. sciuri* recovered from *M. corbetti* likely represents an environmental or opportunistic association rather than a primary entomopathogen, given the species' common detection in animal/soil environments and its recent taxonomic reassignment from *Staphylococcus* (Madhaiyan et al. 2020; Lienen et al. 2022).

The *S. litura* larvae treated with bacteria isolated from bagworm exhibited similar symptoms of infection, including reduced movement, decreased responsiveness to touch, and diminished feeding activity. In addition, the larvae showed body discoloration, turning darker with a softened body texture. These findings align with Rahman et al. (2023), who documented that bacterial infections in larvae typically manifest as reduced mobility, diminished responsiveness to stimuli, decreased feeding activity, and delayed larval development. Meanwhile, dead *S. litura* larvae displayed several distinct characteristics associated with entomopathogenic bacterial infection. Their bodies turned black and showed noticeable shrinkage in size. The dead larvae also released foul-smelling fluids, indicating decomposition and degradation of internal tissues following death. Other physical damage included deterioration of the cuticle, which became fragile and ruptured, as well as a stiffened body due to the loss of turgor and rigidity in the larval muscles and structural tissues. These symptoms are in line with findings by Salaki and Pelealu (2018), who reported that *B. thuringiensis* infection in *S. litura* larvae produces similar pathological signs, such as body darkening, shrinkage, and stiffness, indicating mortality caused by entomopathogenic bacterial activity.

The percentage mortality of *S. litura* represented a critical parameter for evaluating the efficacy of the applied pest management treatments. This measure reflected the extent of larval mortality following exposure to entomopathogenic bacteria. The application of *S. maltophilia*, *M. sciuri*, and *B. thuringiensis* to *S. litura* larvae resulted in a significant and progressive increase in cumulative mortality over time. This trend became particularly evident between day 3 and day 7 post-treatment, indicating the presence of a latency period required for the bacteria to successfully infect the larvae and induce mortality. The *S. maltophilia* treatment demonstrated the highest level of efficacy, yielding the greatest increase in mortality, which reached 57.50% by day 7. This outcome suggested that *S. maltophilia* possessed comparatively stronger pathogenic capabilities or a more rapid infection mechanism than the other bacterial species evaluated. The *B. thuringiensis* treatment produced a mortality rate of 47.50% on day 7, indicating substantial effectiveness, although still lower than that observed for *S. maltophilia*. Conversely, *M. sciuri* exhibited the lowest mortality rate, achieving only 37.50% by day 7. The slower and lower rate of mortality increase may have been attributable to a less aggressive pathogenic mode of action or reduced colonization efficiency within *S. litura* larvae. Despite this, *M. sciuri* remained a potential biological control agent, although its efficacy may require optimization or integration with complementary management strategies. The absence of mortality in the control group provided strong and unequivocal evidence that the larval deaths observed in all treatment groups were the direct result of pathogenic activity exerted by the three bacterial isolates rather than environmental conditions.

In conclusion, this study identified three bacterial species isolated from oil palm bagworms—*S. maltophilia*, *B. thuringiensis*, and *M. sciuri*. The preliminary assessment indicated that all three bacterial isolates caused measurable

mortality in *S. litura* larvae, although the magnitude of the response varied among isolates. *S. maltophilia* resulted in the highest cumulative mortality (57.50%), followed by *B. thuringiensis* (47.50%) and *M. sciuri* (37.50%). While *B. thuringiensis* is the only bacterium widely recognized as an established entomopathogen, the mortality responses observed for *S. maltophilia* and *M. sciuri* suggest that their interactions with insect hosts warrant further investigation. Overall, these findings provide preliminary insights that may inform future research on the ecological relevance and potential role of indigenous microbial isolates in integrated and environmentally considerate pest management strategies.

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