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Research Article

# Biomolecular Identification of Bacteria Endophytic From Betel Leaf in Jambi City Forest Park Area as Antibiotic-Producing Potential

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**Abstract:** Background and Objective: Muhammad Sabki City Forest Park in Jambi City has three types of betel plants that contain endophytic bacteria. In 2021, six isolates of endophytic bacteria with the potential for producing antibiotics (halo zone above 10 mm) have been isolated, which can kill Gram-negative (Escherichia coli) and Gram-positive (Staphylococcus aureus) test bacteria. that are 5 (five) isolates derived from red betel were coded: BESKJ-m2, BESHKJ-m3, BESHKJ-m4, BESHKJ-m5, BESHKJ-m6, and 1 (one) isolate came from forest betel, with code BESHKJ-s1. This study aims to determine the species or strains of endophytic bacteria from betel leaf that have the potential to produce antibiotics. Materials and Methods: The research implementation includes rejuvenation and cultivation of endophytic bacterial isolates, characterization of isolates in species determination, bacterial genome DNA isolation, gene amplification with RNA 16s biomolecular, visualization by electrophoresis, sequencing, and phylogenetic tree analysis. Results: Based on the results of the nucleotide blast from the sequencing obtained from the Malaysian first base laboratory and based on the similarity of the sequence arrangement in the NCBI Gene Bank, six species of bacterial isolates were identified, namely, six bacterial isolates belonging to the Bacillus group. isolate BESHKJ M-4 shows a close relationship with Bacillus safeness, BESHKJ M-6 is related to *Bacillus thuringensis*, BESHKJ M-3 has a close relationship with *Bacillus qingshengii*, BESHKJ S-1 has a close relationship with *Achromobacter anxifer*, and isolates BESHKJ M-2 and BESHKJ M-5 are the same isolate as *Bacillus paramcoydes*. Conclusion: This study successfully identified six antibiotic-producing endophytic bacterial isolates from betel leaves in Jambi. These include *Bacillus safensis*, *Bacillus thuringiensis*, *Bacillus qingshengii*, *Bacillus paramycoides* (two isolates), and *Achromobacter anxifer*. These findings contribute to the exploration of natural antimicrobial agents from plant-associated microbiota.

**Keywords:** Antibiotic, Betel-Leaf, Biomolecular, Endophytic-Bacteria, Jambi

## Introduction

Betel (*Piper* sp.) is a medicinal plant that grows naturally and is an alternative antiseptic without side effects. Some components of betel leaf extract have anti-bacterial activity against pathogens in food (Inayatullah, 2012; Suliantari *et al*., 2012). Potential endophytic bacteria that produce antibiotics from betel leaf are secondary metabolites in relatively high amounts, superior because they have a short cycle (Radji, 2005; Prihatiningtias and Sri, 2011). The results of research by Arzita *et al*., (2021) obtained six isolates of endophytic bacteria with the potential to produce antibiotics from betel leaves of Piper betle, *P. aduncum*, *P. sarmentosum*, which live naturally and

wildly in the Jambi City Forest Park area, namely; BESHKJ-M2, BESHKJ-M3, BESHKJ-M4, BESHKJ- M5, BESHKJ-M6 BESHKJ-M7, BESHKJ-S.

The discovery of bacterial endophytes in plants is useful for enriching our collection of microorganisms, identification treatments are useful for studying the physiological properties of life and types/strains, to be applied to a healthy life (Yin *et al*., 2012). To determine the type of species or strain of a bacterium, the Development of microbial identification requires molecular analysis, with the marker of the 16S RNA gene. Some of the things described above serve as the basis for stating the importance of research on the identification of species/strains of antibiotic-producing endophytic bacteria from the City Forest Park. Muhammad. Jambi City Sabki. The functions of betel leaf include; preventing coughing, bad breath, and female internal organs, and providing healing for several diseases caused by bacterial and fungal infections. (Kumar *et al*., 2010). Leaf. this betel contains; eugenol d-germakren, lepidosen, kariopilene, morolen, sellinerol, kadine and cineol (Periyanayagam *et al*., 2011).

Bimolecular-based identification methods are currently being developed very rapidly, because they are relatively fast, and have higher sensitivity and specificity, with rRNA gene sequencing analysis. rRNA coding gene. (ribosomal RNA), which is a highly conserved gene. The proportions of rDNA sequences in various organisms are generally genetically correlated equally. Organisms that have a distance from a certain relative can be aligned, this easier to determine the difference in the sequence as a characteristic of the organism. Ribosomal RNA coding genes are used for taxonomic determination, phylogeny (evolutionary kinship), and estimating the distance of diversity between species (rates of. species divergence) of bacteria (Handoyo and Rudiretna, 2009). This study aims to determine species or strains of endophytic bacteria that have the potential to produce broad-spectrum antibiotics, in the context of developing molecular science and technology in the field of bacteria, as well as enriching microbial biodiversity in Indonesia.

## Materials and Methods

### *Time and Place*

This research has been carried out at the UPT Basic and Central Laboratory, Biomedical Laboratory Faculty of Medicine Andalas University, Padang, and the Faculty of Microbiology Laboratory Saintek, Jambi University, Mendalo Indah Village, Subdistrict Jambi Out of Town, Regency Muaro Jambi from Mei - December 2022.

### *Tools and Materials*

Research using; laminar flow, oven, incubator, autoclave, microscope, microwave, petri dish, test tube, Erlenmeyer, glass beaker, micropipette, glass, loop needle, knife, scalpel, pH meter, thermometer, micrometer, caliper, and analytical balance, ose glass, 1% agarose gel printer, comb, electrophoresis, power supply, UV transilluminator, PCR/thermocycler unit, electrophoresis chamber unit, aluminum foil, refrigerator, camera, and equipment for writing and reports.

The materials used are; medium Nutrien Agar, Bakto Agar, Nutrien Broth, coloring agents, alcohol, logos, distilled water, coffee paper, labels, permanent markers, chemicals for characterization of endophytic bacteria, aluminum foil, betel leaf, isolate endophytic bacteria, Genomic DNA isolation, Genome DNA purification kit, 16s RNA primer, buffer, agarose, aqua dest, PCR master mix, DNA dye, Tris, boric acid, EDTA, NaCl, distilled water, lysis. buffer, Chelex-100 Biorad Rein, ethanol 75% dd H2O, Primer27F&1492 R, Phosphate Buffer Saline, and Marker1500. Bp.

### *Research Implementation*

Rejuvenation of Endophytic Bacteria Isolate Betel Leaf Potential for Producing Antibiotics. Rejuvenation by inoculation of endophytic bacterial isolates in Nutrient Agar (NA) medium by streak plate. Then incubated for 24 hours at 28 0C:

a) Cultivation of Endophytic Bacteria Isolates from Betel Leaf
   One ose of endophytic bacterial isolates was inoculated into a sterile Nutrient Broth (NB) medium then the medium was shaken at 120 rpm for 24 hours at 28 0C, then centrifuged at 10,000 rpm for 10 minutes, and the crude part of the biomass formed was isolated from the DNA of the bacterial genome.
b) Isolation of Endophytic Bacteria Genome DNA from Betel Leaf
   Based on Figure 1. Genomic DNA isolation from bacterial culture with the Genomic DNA Mini Kit (Geneaid) then the procedure for isolating bacterial genomic DNA followed the kit protocol
c) Amplification of the 16s RNA Gene
   The amplification work uses a combi block type PCR. Device from Whatman Biotra, a German product. Amplification of the 16s RNA gene molecularly by analyzing some of its 16S rDNA genes. Primer 9F: 5'-AGRG TTTGATCMTGGCTCAG-3' 1492R: 5'-ACGGYTACCT-TGTT AYGACTT-3' (Melliawati *et al*., 2016)
d) DNA Visualization Treatment and Gel Electrophoresis
   Agarose the amplified isolates were separated by 1% agarose gel electrophoresis. The gel was immersed in a mixture of TrisBorate-EDTA buffer solution and ethidium bromide. Visualization was carried out under UV light on a UV-transilluminator. The detection results are documented (Rau *et al*., 2018).

e) Sequencing:

- Analysis: Analysis of Sequence Data, DNA Chromatogram, or DNA sequenced results are processed using Geneious brand software. The results of DNA sequencing were analyzed using the BLAST program through the NCBI: Online media, to look for the similarity of the nucleotide sequence of the 16S rRNA gene in determining the type of endophytic bacterial isolate from the algae *Halimeda opuntia* which has the greatest antibacterial activity
- Blast Sequence Based on Gene Bank at NCBI: Sequences of potential bacteria that have been contacted were analyzed by BLAST to see the similarity of DNA sequences in the gene bank
- Endophytic Bacteria Phylogenetic Tree: The phylogenetic tree was compiled using the Neighbor-Joining method. Bootstrapping was carried out 1000 times in the MEGA 6.06 program (Melliawati *et al*., 2016)

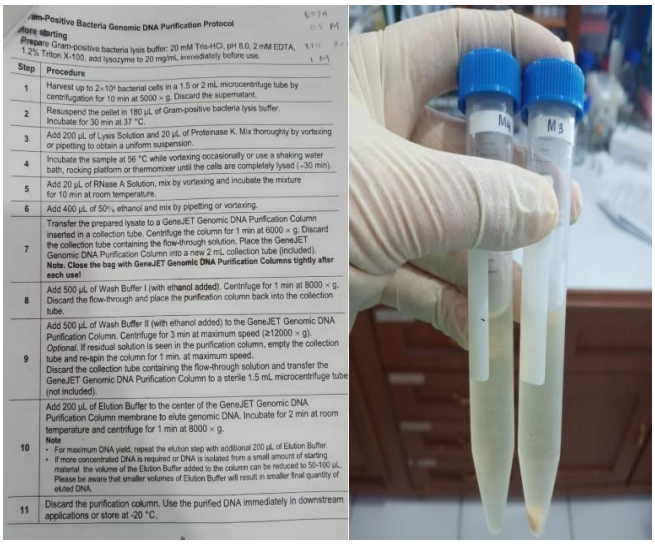


**Fig. 1:** DNA extraction methods and results

## Results and Discussion

### *Isolation DNA Genom Bacteria Endophytic Potential from Betel Leaf*

The results of DNA isolation of endophytic bacteria from betel leaves which have the potential to produce antibiotics, whose concentration was measured using Nanodrop and visualized using electrophoresis are shown in Table 1, and the results of 16s RNA gene amplification showing the PCR conditions are presented in Table 2. Photo of the electropherograms of PCR results visualized using GelDoc Bio-Rad, presented in Fig. 2.

**Table 1:** Results of DNA isolation of endophytic bacteria isolates

| Sample ID | Nucleic Acid Conc | Unit | Volume (µL) |
|---|---|---|---|
| S1 | 40.2 | ng/µl | 1.65 |
| M2 | 54.2 | ng/µl | 1.61 |
| M3 | 6.2 | ng/µl | 2.34 |
| M4 | 30.8 | ng/µl | 1.89 |
| M5 | 8.8 | ng/µl | 1.88 |
| M6 | 15.3 | ng/µl | 1.79 |

**Table 2:** Conditions of PCR-isolated endophytic bacteria

| No. | Phase PCR | Temp (°C) | Duration | Cycle |
|---|---|---|---|---|
| 1 | Pre Denaturation | 95 | 3 minute | 35 |
| 2 | Denaturation extension | 95 | 30 seconds | |
| 3 | Annealing | 50 | 30 second | |
| 4 | Pre Extention | 72 | 1 minute | |
| 5 | Extention | 72 | 5 minute | |

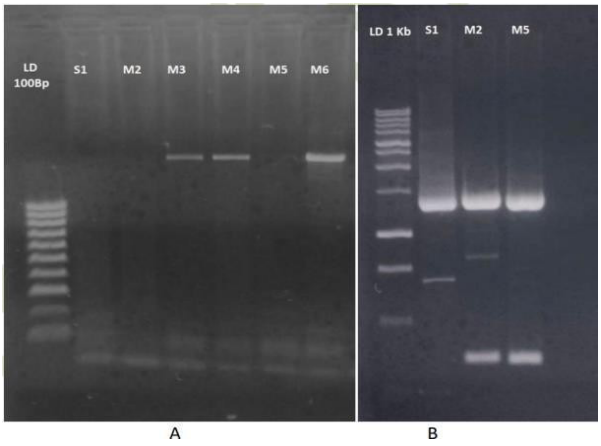


**Fig. 2:** PCR Product Endophytic Bacteria Potential Produce Antibiotic

Note:

A = PCR First time, sample S1, M2, M5 no DNA ribbon

B = Sample re-optimization S1, M2, M5 with KOD Toyobo mastermix obtained DNA tape

Phylogenetic Tree Construction Isolate Endophytic Potential From Pipper Batle Using 16s rRNA Gene Sequences.

Since actinobacteria have been applied in producing different kinds of current medicines, more recent studies are investigating new bacterial sources. This study focused on bacteria, which was found in a large number of the isolated bacteria obtained from Pipper batle plants in Jambi City Park. The potential isolates used in the research are a continuation of previous research, where six isolates of endophytic bacteria were found in betel leaves in the Jambi Cityforest as producers of antibiotics against the pathogenic bacteria Escherichia coli and Staphylococcus aureus. These six isolates are broad- spectrum in nature (Arzita *et al*., 2022).

**Table 3:** Contiq result S1, M2 – M6 (Isolate from red string of sample S1, M2-M6)

| Sample name | Contiq result |
|---|---|
| S2 | GACTTCTCGCTGTGTGGGCGAGTTGGCCAACG GGTGAGTAATGTATCTCGAACGTGCCCAGTAG |
| | CGGGCGATAACTACGCTGAAAGCGTACCTAAT ACCGCATACGCCCTACGGGGGAAAGCAGGGG |
| | ATCGCAAGACCTTGCCCTATTGGAGCGGCCCA TATCGGATTAGCTAGTTGGTGGGGTAACGGCT |
| | CACCAAGGCGACGATCCGTAGCTGGTTTGACA GGACCACCAGCCACACTGGGACTGAGACACG |
| | SCCCAGACTCCTACGGGAGGCAGCAGTGGGG AATTTTGGACAATGGGGGAAACCCTGATCCAG |
| | CCATCCCGCGTGTGCGATGAAGGCCTTCGGGT TGTAAAGCACTTTTGGCAGGAAWGAAACGTC |
| | GCGGGTTAATACCCCGCGGAACTGACGGTACC TGCAGAATAAGCACCGGCTAATTACGTGCCAG |
| | CAGCCGCGGTAATACGTAGGGTGCAAGCGTT |
| | AATCGGAATTACTGGGCGTAAAGCGTGCGCAS GCGGTTCGGAAAGAAAGATGTGACATCCCAG |
| M2 | AGCTTAACTTTGGAACTGCATTTTTAACTACC GAGCTAGAGTGTGTCAGAGGGAGGTGGAATT |
| | CCGCGTGTAGCAGTGAAATGCGTAGATATGCG GAGGAACACCGATGGCGAAGGCAGCCTCCTG |
| | GGATAACACTGACGCTCATGCACGAAAGCGT GGGGAGCAAACAGGATTAGATACCCTGGTAG |
| | TCCACGCCCTAAACGATGTCAACTAGCTGTTG GGGCCTTCGGGCCTTGGTAGCGCAGCTAACGC |
| | GTGAAGTTGACCGCCTGGGGAGTACGGTCGC AAGATTAAAACTCAAAGGAATTGACGGGGAC |
| | CCGCACAAGCGGTGGATGATGTGGATTAATTC GATGCAACGCGAAAAACCTTACCTACCCTTGA |
| | CATGTCTGGAATCCTGAAGAGATTTAGGAGTG CTCGCAAGAGAACCGGAACACAGGTGCTGCA |
| | TGGCTGTCGTCAGCTCGTGTCGTGAGATGTTG GGTTAAGTCCCGCAACGAGCGCAACCCTTGTC |
| | ATTAGTTGCTACGAAAGGGCACTCTAATGAGA CTGCCGGTGACAAACCGGAGGAAGGTGGGGA |
| | TGACGTCAAGTCCTCATGGCCCTTATGGGTAG GGCTTCACACGTCATACAATGGTCGGGACAGA |
| | GGGTCGCCAACCCGCGAGGGGGAGCCAATCC CAGAAACCCGATCGTAGTCCGGATCGCAGTCT |
| | GCAACTCGACTGCGTGAAGTCGGAATCGTTAG TAATCGCGGATCAGCATGTCGCGGTGAATACG |
| | TTCCCGGGTCTTGTACACACCGCCCGTCACAC |
| | CATGGGAGTNGTTTTACCAGAGAGTAGTTAGC CTAACCGCAAGGAGGGGAGCATTACCCACGG |
| | AAAGCCCACGGGCTTAAACTAACGTGCCCAG CCAGCCCGCGGGTAAATAACGTTAGGGTGGG |
| | CAAAGCGGTTTATCCCGGGAAATTTATTGGGG CGTAAAAGCGCGGCGCCAGGGTGGTTTTCTTT |
| | AAGTTCTGAATGTGAAAAGCCCCACGGGCTTC AAACCGTGGGAGGGGTCATTGGAAAACTGGG |
| | GAGAACTTGAGTGCAGAAAGAGGAAAAGTGG AATTCCCATGTGTAGCGGTGAAAATGCGTAGA |
| | GATATGGAGGAACACCCAGTGGCGAAGGCGA |
| | CTTTTCTGGTCTGTAACTGACACTGAGGCGCG AAAGCGTGGGGAGCAAACAGGATTAGATACC |
| | CTGGTAGTCCACGCCGTAAACGATGAGTGCTA AGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGA |
| | AGTTAACGCATTAAGCACTCCGCCTGGGGAGT ACGGCCGCAAGGCTGAAACTCAAAGGAATTG |
| | ACGGGGGCNCCGCACAAGCGGTGGAGCATGT GGTTTAATTCGAAGCAACGCGAAGAACCTTAC |
| | CAGGTCTTGACATCCTCTGAAAACCCTAGAGA TAGGGCTTTCTCCTTCGGGAGCAGAGTGACAG |
| | GTGGTGCATGGTTGTCGTCAGCTCGTGTCGTG AGATGTTGGGTTAAGTCCCGCAACGAGCGCA |
| | ACCCTTGATCTTAGTTGCCATCATTAAGTTAG GCATTTGATCCGTGGACTCGAGCATGAAGACA |
| | GGCCGGCAGGAGTAAATTGATGATTGTCGTAA CCTCTATGCCCACTAAATACTATATGTTTC |
| | ACGCATCAGGATCTATAACAAGTCCGAGGAC |
| | AGGCTAATTCTTCCAGAAGTAATGAATGTCAG AGAACTGTTGACAC |
| M3 | CTGCTCGAGCTCTTTTATGTTCGTAACAACGG TAACCGGCGGACGGGTGAGTAACACGTGGGC |
| | AACCTGCCTGTAAGACTGGGATAACTTCGGGA AACCGAAGCTAATACCGGATAGGATCTTCTCC |
| | TTCATGGGAGATGATTGAAAGATGGTTTCGGC TATCACTTACAGATGGGCCCGCGGTGCATTAG |
| | CTAGTTGGTGAGGTAACGGCTCACCAAGGCA ACGATGCATAGCCGACCTGAGAGGGTGATCG |
| | GCCACACTGGGACTGAGACACGGCCCAGACT CCTACGGGAGGCAGCAGTAGGGAATCTTCCG |
| | CAATGGACGAAAGTCTGACGGAGCAACGCCG CGTGAGTGATGAAGGCTTTCGGGTCGTAAAAC |
| | TCTGTTGTTAGGGAAGAACAAGTACGAGAGT AACTGCTCGTACCTTGACGGTACCTAACCAGA |
| | AAGCCACGGCTAACTACGTGCCAGCAGCCGC GGTAATACGTAGGTGGCAAGCGTTATCCGGA |
| | ATTATTGGGCGTAAAGCGCGCGCAGGCGGTTT CTTAAGTCTGATGTGAAAGCCCACGGCTCAAC |
| | CGTGGAGGGTCATTGGAAACTGGGGAACTTG AGTGCAGAAGAGAAAAGCGGAATTCCACGTG |
| | TAGCGGTGAAATGCGTAGAGATGTGGAGGAA CACCAGTGGCGAAGGCGGCTTTTTGGTCTGTA |
| | ACTGACGCTGAGGCGCGAAAGCGTGGGGAGC AAACAGGATTAGATACCCTGGTAGTCCACGCC |
| | GTAAACGATGAGTGCTAAGTGTTAGAGGGTTT CCGCCCTTTAGTGCTGCAGCTAACGCATTAAG |
| | CACTCCGCCTGGGGAGTACGGTCGCAAGACTG AAACTCAAAGGAATTGACGGGGGCCCGCACA |
| | AGCGGTGGAGCATGTGGTTTAATTCGAAGCAA CGCGAAGAACCTTACCAGGTCTTGACATCCTC |
| | TGACAACTCTAGAGATAGAGCGTTCCCCTTCG GGGGACAGAGTGACAGGTGGTGCATGGTTGT |
| | CGTCAGCTCGTGTCGTGAGATGTTGGGTTAAG TCCCGCAACGAGCGCAACCCTTGATCTTAGTT |
| | GCCAGCATTTAGTTGGGCACTCTAAGGTGACT GCCGGTGACAACCGGAGGAAGGTGGGGATGA |
| | CGTCAAATCATCATGCCCCTTATGACCTGGGC TACACACGTGCTACAATGGATGGTACAAAGG |
| | GCTGCAAGACCGCGAGGTCAAGCCAATCCCA TAAAACCATTCTCAGTTCGGATTGTAGGCTGC |
| | AACTCGCCTACATGAAGCTGGAATCGCTAGTA ATCGCGGATCAGCATGCCGCGGTGAATACGTT |
| | CCCGGGCCTTGTACACACCGCCCGTCACACCA C |
| M4 | TGCAAGTCGAGCGAATGGATTAAGAGCTTGCT CTTATGAAGTTCGCNGAGCAAGACNAACTGA |
| M4 | GTAACACGTGGGTAACCTGCCCATAAGACTGG GATAACTCCGGGAAACCGGGGCTAATACCGG |
| | ATAACATTTTGAACCGCATGGTTCGAAATTGA AAGGCGGCTTCGGCTGTCACTTATGGATGGAC |
| | CCGCGTCGCATTAGCTAGTTGGTGAGGTAACG GCTCACCAAGGCAACGATGCGTAGCCGACCT |
| | GAGAGGGTGATCGGCCACACTGGGACTGAGA CACGGCCCAGACTCCTACGGGAGGCAGCAGT |
| | AGGGAATCTTCCGCAATGGACGAAAGTCTGA CGGAGCAACGCCGCGTGAGTGATGAAGGCTT |
| | TCGGGTCGTAAAACTCTGTTGTTAGGGAAGAA CAAGTGCTAGTTGAATAAGCTGGCACCTTGAC |
| | GGTACCTAACCAGAAAGCCACGGCTAACTAC GTGCCAGCAGCCGCGGTAATACGTAGGTGGC |
| | AAGCGTTATCCGGAATTATTGGGCGTAAAGCG CGCGCAGGTGGTTTCTTAAGTCTGATGTGAAA |

GCCCACGGCTCAACCGTGGAGGGTCATTGGA AACTGGGAGACTTGAGTGCAGAAGAGGAAAG
TGGAATTCCATGTGTAGCGGTGAAATGCGTAG AGATATGGAGGAACACCAGTGGCGAAGGCGA
CTTTCTGGTCTGTAACTGACACTGAGGCGCGA AAGCGTGGGGAGCAAACAGGATTAGATACCC
TGGTAGTCCACGCCGTAAACGATGAGTGCTAA GTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAA
GTTAACGCATTAAGCACTCCGCCTGGGGAGTA CGGCCGCAAGGCTGAAACTCAAAGGAATTGA
CGGGGGCCCGCACAAGCGGTGGAGCATGTGG TTTAATTCGAAGCAACGCGAAGAACCTTACCA
GGTCTTGACATCCTCTGAAAACCCTAGAGATA GGGCTTCTCCTTCGGGAGCAGAGTGACAGGTG
GTGCATGGTTGTCGTCAGCTCGTGTCGTGAGA TGTTGGGTTAAGTCCCGCAACGAGCGCAACCC
TTGATCTTAGTTGCCATCATTAAGTTGGGCAC TCTAAGGTGACTGCCGGTGACAAACCGGAGG
AAGGTGGGGATGACGTCAAATCATCATGCCCC
TTATGACCTGGGCTACACACGTGCTACAATGG ACGGTACAAAGAGCTGCAAGACCGCGAGGTG
GAGCTAATCTCATAAAACCGTTCTCAGTTCGG ATTGTAGGCTGCAACTCGCCTACATGAAGCTG
GAATCGCTAGTAATCGCGGATCAGCATGCCGC GGTGAATACGTTCCCGGGCCTTGTACACAC
M5 TAGCGACTTGGAGGGGATCGGCCCACTGGGA TTGAGACCGGCCCAAATCCTACGGAGGCAGC
AGTAGGGATCTTCCGCAATGACGAAANTCTGA CGAGCAACGCCGCGTGAGNATGAAGGTTTCG
GGTCGTAAAATCTGTTGTTAGGGAAGAACAA GTGCTAGTTGAATAAGCTGGCACCTTGACGGT
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CGTTATCCGGAATTATTGGGCGTAAAGCGCGC GCAGGTGGTTTCTTAAGTCTGATGTGAAAGCC
CACGGCTCAACCGTGGAGGGTCATTGGAAACT GGGAGACTTGAGTGCAGAAGAGGAAAGTGGA
ATTCCATGTGTAGCGGTGAAATGCGTAGAGAT ATGGAGGAACACCAGTGGCGAAGGCGACTTT
CTGGTCTGTAACTGACACTGAGGCGCGAAAGC GTGGGGAGCAAACAGGATTAGATACCCTGGT
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ACGCATTAAGCACTCCGCCTGGGGAGTACGGC CGCAAGGCTGAAACTCAAAGGAATTGACGGG
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TTGACATCCTTTGACAACCCTAGAGATAGGGC TTTTCCTTCGGGAGCAGAGTGACAGGTGGTGC
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ACCGTGAACAGGTACTTCTTCCGGAAGTAATG GGTGGTTGCGAAGTACTGATACGTCTATGTTC
TACACTTTCTTCTACACTASAGAAACCGCGCA AGAATTGTTTTAAATCGAKTCCAGAATA?AYG
AAGCATATCTAATTTCTCTTCTGTTATACCG?C GWCTCTTTCATTTTSTCAATTGTACTGCTTGCT
TGAATWTATGGCGATACCACAATCAGGGTGA GCAACTACGCATACTTCCTCACCGCAGGCTTC
GTAACGGGGTAACCATCATTGGACTATAGCCT GACTGAGCACCGCCGCTTCAGTGATGAAGGTT
TTCGGGCCCTATATCTCTGTTGTTAGGTAAGA TCTACTGCTTTTTTAATAAGTCGGTTCCTTGCC
GGTCCCTCACCATAACGCCACTGTTAACTACG TGCCAGCAGCCGCGGTAATACGTAGGTGGCA
AGCGTTATCCGGAATTATTGGGCGTAAAGCGC GCGCAGGTGGTTTCTTAAGTCTGATGTGAAAG
CCCACGGCTCAACCGTGGAGGGTCATTGGAA ACTGGGAGACTTGAGTGCAGAAGAGGAAAGT
GGAATTCCATGTGTAGCGGTGAAATGCGTAGA GATATGGAGGAACACCAGTGGCGAAGGCGAC
TTTCTGGTCTGTAACTGACACTGAGGCGCGAA AGCGTGGGGAGCAAACAGGATTAGATACCCT
GGTAGTCCACGCCGTAAACGATGAGTGCTAA GTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAA
GTTAACGCATTAAGCACTCCGCCTGGGGAGTA CGGCCGCAAGGCTGAAACTCAAAGGAATTGA
CGGGGGCCCGCACAAGCGGTGGAGCATGTGG TTTAATTCGAAGCAACGCGAAGAACCTTACCA
GGTCTTGACATCCTCTGACAACCCTAGAGATA GGGCTTCTCCTTCGGGAGCAGAATGACAGGTG
GTGCATGGTTGTCGTCAGCTCGTGTCGTGAGA TGTTGGGTTAAGTCCCGCACCGAGCCCAACCC
TTGATCTTAGTTGCCATCATTTAGTTGGGCACT
CTAAGGTGACTGCCGGTGACAAACCGGAAGA AAGGNGGGGATGACGTCAAATCTTCTTGCCCC
TTATGACCTGGGCTA
M6 GACGGGTAAGTAACACGTGGGTAACCTGCCC ATAAGACTGGGATAACTCCGGGAAACCGGGG
CTAATACCGGATAACATTTTGAACCGCATGGT TCGAAATTGAAAGGCGGCTTCGGCTGTCACTT
ATGGATGGACCCGCGTCGCATTAGCTAGTTGG TGAGGTAACGGCTCACCAAGGCAACGATGCG
TAGCCGACCTGAGAGGGTGATCGGCCACACT GGGACTGAGACACGGCCCAGACTCCTACGGG
AGGCAGCAGTAGGGAATCTTCCGCAATGGAC GAAAGTCTGACGGAGCAACGCCGCGTGAGTG
ATGAAGGCTTTCGGGTCGTAAAACTCTGTTGT TAGGGAAGAACAAGTGCTAGTTGAATAAGCT
GGCACCTTGACGGTACCTAACCAGAAAGCCA CGGCTAACTACGTGCCAGCAGCCGCGGTAATA
CGTAGGTGGCAAGCGTTATCCGGAATTATTGG GCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTC
TGATGTGAAAGCCCACGGCTCAACCGTGGAG GGTCATTGGAAACTGGGAGACTTGAGTGCAG
AAGAGGAAAGTGGAATTCCATGTGTAGCGGT GAAATGCGTAGAGATATGGAGGAACACCAGT
GGCGAAGGCGACTTTCTGGTCTGTAACTGACA CTGAGGCGCGAAAGCGTGGGGAGCAAACAGG
ATTAGATACCCTGGTAGTCCACGCCGTAAACG ATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCT
TTAGTGCTGAAGTTAACGCATTAAGCACTCCG CCTGGGGAGTACGGCCGCAAGGCTGAAACTC
AAAGGAATTGACGGGGGCCCGCACAAGCGGT GGAGCATGTGGTTTAATTCGAAGCAACGCGA
ARAACCTTACCAGGTCTTGACATCCTCTGAAA ACCCTAGAGATAGGGCTTCTCCTTCGGGAGCA
GAGTGACAGGTGGTGCATGGTTGTCGTCAGCT CGTGTCGTGAGATGTTGGGTTAAGTCCCGCAA
CGAGCGCAACCCTTGATCTTAGTTGCCATCAT TAAGTTGGGCACTCTAAGGTGACTGCCGGTGA
CAAACCGGAGGAAGGTGGGGATGACGTCAAA TCATCATGCCCCTTATGACCTGGGCTACACAC
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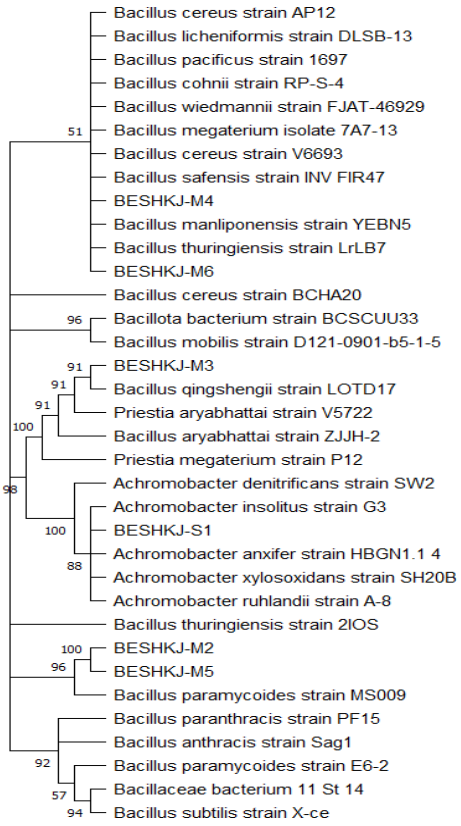


**Fig. 3:** Phylogenetic tree isolates endophytic Pipper battle from forest city park Jambi

Based on Figure 3, six bacterial isolates belong to the Bacillus group. isolate BESHKJ M-4 shows a close relationship with Bacillus safensis, BESHKJ M-6 is related to Bacillus thuringensis, BESHKJ M-3 has a close relationship with Bacillus qingshengii, BESHKJ S-1 has a close relationship with Achromobacter anxifer, isolates BESHKJ M-2 and BESHKJ M-5 is the same isolate as Bacillus paramcoydes.

## Conclusion

Results of the biomolecular identification of six isolates of endophytic bacteria from red betel leaf and forest betel, from the Muhammad Sabki City Forest Park, Jambi City, which have the potential to produce antibiotics can be concluded as follows:

1. The DNA bands obtained were quite clear by analysis of electrophoresis techniques and PCR machines which were visualized using GellDoc BioRad
2. The sequencing process carried out in the Malaysian 1stBase laboratory resulted in 6 different sequences
3. Contic sequencing results and nucleotide blast from sequencing, based on the similarity of sequence arrangement in the NCBI Gene Bank, identified six different species of bacterial isolates, namely; isolate-BESHKJ M-4 shows a close relationship with Bacillus safensis, BESHKJ M-6 is related to Bacillus thuringensis, BESHKJ M-3 has a close relationship with Bacillus qingshengii, BESHKJ S-1 has a close relationship with Achromobacter anxifer, isolates BESHKJ M-2 and BESHKJ M-5 is the same isolate as Bacillus paramcoydes

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## Authors Contribution

**Arzita:** Developed the initial idea, wrote the manuscript, designed the research, and reviewed and approved the manuscript.
**Miranti Sari Fitriani:** Data analysis, experiment development.
**Nyimas Myrna Elsa Fathia:** Designing research, methodology, data analysis, and manuscript writing.
**Addion Nizori:** Materials and equipment involvement, and script writing.
**Mifthahul Jannah:** Materials and equipment involvement, and monitoring research.

## Ethics

The authors acknowledge the novelty of this article, affirming that it is an original contribution that has not been previously published. All participating authors have thoroughly reviewed and endorsed the content, ensuring there are no ethical issues.

The authors declare that the collection and use of *Piper betel* var. Siguramanil 1 (SGM1) leaves for this study complied with all relevant institutional, national, and international guidelines and legislation. The plant material was collected with appropriate permissions from local authorities. Furthermore, the research aligns with the principles of the Convention on Biological Diversity (CBD) and the Nagoya Protocol on Access and Benefit-Sharing (ABS). No endangered or protected species were involved.

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