Quagga
Jurnal Pendidikan dan Biologi

p-ISSN 1907-3089, e-ISSN 2615-5869
Vol 18, No. 2, 2026, pp 173 - 183
Available online https://journal.fkip.uniku.ac.id/quagga
DOI: https://doi.org/10.25134/quagga.v18i2.525

Optimization of Antibacterial Production and Molecular Identification of Endophytic Bacteria Isolated from Papaya (*Carica papaya* L.) Seeds

Esti Rizkiana Pratiwi[1*], Nadiah Al Batati[2], Rizka Kusuma Ningrum[3]
[1,2,3*]Microbiology Study Program, Faculty of Health Science, Universitas Maarif Hasyim Latif, Sidoarjo, East Java, Indonesia
*Corresponding Author: esti_rizkiana@dosen.umaha.ac.id

APA Citation: Pratiwi, E. R., Batati, N. A. & Ningrum, R. K. (2026). Optimization of Antibacterial Production and Molecular Identification of Endophytic Bacteria Isolated from Papaya (Carica papaya L.) Seeds. *Quagga: Journal of Education and Biology*, *18(2)*, 173-183. https://doi.org/10.25134/quagga.v18i2.525.

Received: 09-04-2026 Accepted: 02-05-2026 Published: 01-07-2026

Abstract: *Papaya seed endophytic bacteria have been reported to exhibit antibacterial activity against pathogenic microorganisms, including* Staphylococcus aureus *and* Escherichia coli. *However, their antibacterial production remains suboptimal. This study aimed to determine the optimal conditions for antibacterial production and to identify the most potent bacterial isolate through 16S rRNA gene-based molecular analysis. Three endophytic bacterial isolates (CPA2, CPA8, and CPA9) were evaluated. Characterization was performed based on pH variations (5, 7, and 9) and growth media (Nutrient Broth (NB) and Tryptic Soy Broth (TSB)), followed by antibiotic sensitivity testing against ciprofloxacin, amoxicillin, gentamicin, and tetracycline. Antibacterial activity was assessed using the Kirby–Bauer method against* S. aureus *and E. coli. The results showed that all isolates grew optimally in TSB medium at neutral pH (7). Ciprofloxacin and tetracycline exhibited stronger inhibitory effects compared to gentamicin and amoxicillin. Among the isolates, CPA2 in TSB media pH 7 demonstrated the highest antibacterial activity, producing inhibition zones of 30.2 ± 1.77 mm against S. aureus (very strong) and 11.8 ± 0.02 mm against E. coli (strong). Molecular identification revealed that isolate CPA2 was closely related to Bacillus cereus strain JCM 2152 with 99.90% similarity. In conclusion, papaya seed endophytic bacteria, particularly isolate CPA2, show significant potential as a source of antibacterial compounds under optimized culture conditions. These results have the potential as an alternative source of antibacterial agents.*

Keywords: *Antibacterial, Endophytic Bacteria, Identification, Optimization, Papaya Seeds*

1. INTRODUCTION

Endophytic bacteria inhabiting the internal tissues of plants are increasingly recognized as a promising source of bioactive compounds, particularly those with antibacterial properties. These bacteria establish a symbiotic relationship with their host plants and are capable of producing secondary metabolites that contribute to plant defense mechanisms. In recent years, endophytic bacteria have received significant attention due to their potential applications in combating pathogenic microorganisms and addressing the growing global challenge of antimicrobial resistance (Numan et al., 2022; Yunita et al., 2022; Pratiwi et al., 2024).

Papaya (*Carica papaya* L.) is one of the plants reported to possess considerable antibacterial activity. The extracts derived from various parts of the plant, including leaves, fruit peel, and papaya seeds, have been shown to inhibit Gram-positive and Gram-negative pathogenic bacteria (Torar et al., 2017; Ariani et al., 2019; Buang et al., 2019). Ethanol extract of papaya seeds showed higher antibacterial activity compared to leaf extract, indicating that papaya seeds have potential sources of bioactive compounds (Dagne et al., 2021; Gangga Dewanti

Quagga
Jurnal Pendidikan dan Biologi
p-ISSN 1907-3089, e-ISSN 2615-5869
Vol 18, No. 2, 2026, pp 173 - 183
Available online https://journal.fkip.uniku.ac.id/quagga
DOI: https://doi.org/10.25134/quagga.v18i2.525

Gita Maharani et al., 2022). Despite this, research focusing on the microbial communities associated with papaya seeds, especially endophytic bacteria, remains relatively limited. This suggests that, in addition to plant-derived compounds, microbial communities associated with papaya seeds may also contribute to their bioactivity.

These endophytic bacteria from papaya plants can be isolated from various plant tissues, including leaves, stems, roots, and seeds, and have been reported to inhibit the growth of pathogenic bacteria (Faujiah et al., 2023; Irwandi et al., 2023; Pratiwi et al., 2024). Previous studies have demonstrated that endophytic bacteria isolated from papaya seeds exhibit antibacterial activity against important pathogens such as *Staphylococcus aureus* and *Escherichia coli* (Pratiwi et al., 2024). However, these studies were limited to preliminary screening, with limited exploration of factors influencing antibacterial production. In particular, there is a lack of investigation into the optimization of culture conditions, such as nutrient composition and environmental parameters, which are known to significantly influence the biosynthesis of microbial secondary metabolites, including antibacterials.(Al-Ghazali & Omran, 2017; Isleem et al., 2025; Muthma & Supriyono, 2023; Sánchez-clemente et al., 2018; Widjajanti et al., 2022). This gap limits the ability to fully exploit the antibacterial potential of papaya seed endophytic bacteria.

To date, only a few studies have addressed the optimization of antibacterial production from endophytic bacteria associated with papaya. Therefore, more in-depth investigations are needed to enhance antibacterial production and identify bacterial strains with superior bioactivity. In this context, this study offers a novel contribution by optimizing culture conditions to enhance antibacterial production from endophytic bacteria isolated from papaya seeds. Unlike previous studies that primarily focused on screening for antibacterial activity, this study integrates optimization strategies with the identification of the most potent bacterial isolates in producing antibacterials. This study aimed to determine the optimal conditions for antibacterial production by endophytic bacteria from papaya seeds and identify bacterial species with the highest potential as optimal antibacterial producers. The findings of this study are expected to contribute to the development of sustainable microbial-based antibacterial agents and provide new insights into the utilization of endophytic bacteria in agricultural and biomedical applications.

2. RESEARCH METHOD

Time and Place Research

This research was conducted from September 2025 to January 2026. The samples consisted of papaya seed endophytic bacterial isolates that had been previously isolated (CPA-2, CPA-8, CPA-9) (Pratiwi et al., 2024). All bacterial analyses were carried out at the Microbiology Laboratory, Faculty of Health Sciences, Universitas Maarif Hasyim Latif, Sidoarjo.

Research Design

This research uses a laboratory experimental method. The three endophytic bacterial isolates (CPA2, CPA8, CPA9) were found to inhibit *Staphylococcus aureus* & *Escherichia coli*, were further characterized by evaluating the effects of pH variation (5, 7,9) and antibiotic sensitivity (Ciprofloxacin, Amoxcillin, Gentamycin, Tetracycline) with each test repeated three times. Endophytic bacteria were further optimized to enhance antibiotic production against *Staphylococcus aureus* and *Escherichia coli* by the Kirby–Bauer disc diffusion method.

Characteristics of endophytic bacteria based on different pH

The effect of pH on the growth of endophytic bacterial isolates was evaluated using a modified method (Nurmalasari et al., 2020). Nutrient Broth (NB) and Tryptic Soy Broth (TSB) were adjusted to pH 5, 7, and 9. Starter culturses of endophytic bacterial isolates with equal culture age and cell density were inoculated into the respective media and incubated at 30 °C for 24 h. Bacterial growth was monitored by turbidity observation, followed by optical density (OD) measurement and cell density determination to assess the effect of pH variation.

Antibiotic susceptibility Assay

Antibiotic susceptibility of endophytic bacterial isolates was evaluated using the Kirby–Bauer disk diffusion using a modified method

Quagga
Jurnal Pendidikan dan Biologi
p-ISSN 1907-3089, e-ISSN 2615-5869
Vol 18, No. 2, 2026, pp 173 - 183
Available online https://journal.fkip.uniku.ac.id/quagga
DOI: https://doi.org/10.25134/quagga.v18i2.525

(Ashitha et al., 2019; Sharma & Mallubhotla, 2022). Each endophytic bacterial isolate was cultured in NB and TSB and incubated at 30 °C for 24 h. Each cultures with the same age and cell density were inoculated (0.1 mL) onto Mueller–Hinton Agar (MHA) plates. Antibiotic disks (ciprofloxacin, amoxicillin, gentamicin, and tetracycline) were placed to the agar surface, followed by incubation at 37 °C for 24 h. Antibiotic sensitivity was determined by measuring inhibition zones according to sensitivity criteria (Pratiwi et al., 2024).

Optimization of Culture Conditions for Antibacterial Production

Optimization assay were conducted to enhance antibacterial production and improve inhibitory activity against *Staphylococcus aureus* and *Escherichia coli*. Optimization was achieved by varying the pH media and using different fermentation media. Optimization of pH and fermentation media was performed following established (Al-Ghazali & Omran, 2017; Myo et al., 2020). Fermentation was perfomed at pH 5, 7, and 9 using Tryptic Soy Broth (TSB) and Nutrient Broth (NB) media supplemented with 1% glucose. Each isolate was incubated at 30 °C for 24 h. The cultures were centrifuged at 10,000 rpm for 30 min. The supernatants were separated from the pellets and sterilized by filtration through a 0.22 µm membrane filter.

Antibacterial activity was assessed using the Kirby–Bauer disc diffusion method. Sterile blank discs (6 mm) were impregnated with 60 µL of supernatant and dried under laminar air flow. Pathogenic bacterial suspensions (10^8 CFU/mL) were spread (0.1 mL) onto Mueller–Hinton Agar plates, and the dried discs were placed on the agar surface. The plates were incubated at 37 °C for 24 h. Inhibition zones formed around the discs indicated the presence of antibiotic metabolites produced by endophytic bacteria against *Staphylococcus aureus* and *Escherichia coli*. Antibacterial activity was classified as sensitive, intermediate, or resistant based on inhibition zone diameters (Pratiwi et al., 2024).

Identification of 16S rRNA gene

Potential endophytic bacterial isolates were subcultured and subjected to chromosomal DNA extraction using a modified method (Pratiwi et al., 2020). The isolates were grown on Nutrient Agar (NA) at 28 °C for 24 h. Bacterial colonies were suspended in buffer solutions (GT1 and GT2) with proteinase K, followed by incubation at 56 °C. DNA purification was performed using a spin column protocol with sequential washing steps (W1 and W2 buffers), and DNA was eluted in elution buffer and stored at −20 °C. The extracted DNA was verified by 1% agarose gel electrophoresis.

The DNA was then amplified using PCR (Bio-Rad) targeting the 16S rRNA gene with primers 27F and 1492R. The PCR reaction was carried out in a total volume of 30 µL consisting of 15 µL PCR master mix, 2 µL forward primer, 2 µL reverse primer, 2 µL DNA template, and 9 µL nuclease-free water. Amplification conditions included initial denaturation at 95 °C for 2 min, followed by 28 cycles of denaturation (95 °C, 30 s), annealing (50 °C, 30 s), and extension (72 °C, 2 min), with a final extension at 72 °C for 7 min. PCR products were analyzed using 1–1.5% agarose gel electrophoresis with a 1000 bp DNA ladder and visualized using a gel documentation system.

The amplified products were subsequently sent for sequencing to 1st BASE Laboratories Sdn. Bhd. The 16S rRNA gene amplicons were sequenced by First BASE, Malaysia. The obtained sequences were edited using BioEdit, aligned using ClustalW, and compared with reference sequences using BLAST against the NCBI (GenBank) database. Phylogenetic analysis was performed using MEGA 12 based on the Maximum Likelihood method, with bootstrap analysis of 1000 replicates to assess sequence similarity.

3. RESULTS AND DISCUSSION

Characteristics of Endophytic Bacterial Isolates Under Different pH Conditions

The characterization was conducted based on variations in pH and growth media. Qualitative results (Table 1) showed that all bacterial isolates were able to grow within a pH range of 5–9 in both Nutrient Broth (NB) and Tryptic Soy Broth (TSB) media. The change in medium color to turbidity indicated bacterial growth. All isolates exhibited optimal growth at pH 7, consistently in both media types, as indicated by a very high (robust) growth category (+++). The viability of endophytic

Quagga
Jurnal Pendidikan dan Biologi

p-ISSN 1907-3089, e-ISSN 2615-5869
Vol 18, No. 2, 2026, pp 173 - 183
Available online https://journal.fkip.uniku.ac.id/quagga
DOI: https://doi.org/10.25134/quagga.v18i2.525

bacterial cells was further quantified (Table 2) using a spectrophotometer to measure turbidity based on optical density (OD_{600}) and a haemocytometer to determine cell density. The highest OD_{600} values and cell densities were generally observed at pH 7, particularly in TSB medium. Isolate CPA9 grown in TSB at pH 7 exhibited the highest growth, with an OD_{600} value of 1.109 and a cell density of 12.95 × 10^4 CFU mL^{-1}. In contrast, the lowest growth was observed at pH 9, especially in NB medium. Isolate CPA8 in TSB at pH 7 showed a cell density of 10.33 × 10^4 CFU mL^{-1} with an OD_{600} of 0.813, while isolate CPA2 under the same conditions reached 8.28 × 10^4 CFU mL^{-1} with an OD_{600} of 0.711. These results indicate that all endophytic bacterial isolates exhibited optimal growth in TSB medium at pH 7.

Table 1. Characterization of Endophytic Bacterial Isolates under Different pH Conditions of the Growth Medium

Bacterial isolate	Growth medium	pH 5	pH 7	pH 9
CPA2	NB	+	+++	+
	TSB	++	+++	++
CPA8	NB	+	+++	+
	TSB	++	+++	+++
CPA9	NB	+	+++	++
	TSB	++	+++	+++

*Note : +++ Robust Growth ($OD_{600} \geq 0{,}70$)
++ Moderate Growth (OD_{600} 0,40 - 0,70)
+ Poor Growth ($OD_{600} \leq 0{,}40$)

Differences in endophytic bacterial growth across pH variations indicate that medium pH is a critical factor influencing metabolic activity and cell growth rate. Neutral pH (pH 7) is optimal for most mesophilic bacteria because it supports enzyme stability and efficient nutrient transport into the cell. Pada penelitian ini, TSB media consistently produced higher OD_{600} values and cell densities than NB media across all isolates and pH variations. This is likely due to TSB media more complex nutrient content and richer carbon and nitrogen sources, which support optimal cell growth. The relatively decreased growth at pH 9 indicates environmental stress due to alkaline conditions, which can affect cell membrane permeability and intracellular enzyme activity. Nevertheless, the ability of isolates to continue growing at extreme pH demonstrates the potential physiological adaptation of endophytic bacteria to environmental variations.

According to research (Mushtaq et al., 2021), bacterial isolates obtained from plants have an average optimal growth at pH 6-8 for isolate SM-WD6 (Escherichia coli), pH 7-10 for isolate SM-8 (*Staphylococcus scuiri*), pH 6-9 for isolate SM-WD23 (*Bacillus flexus*) and isolate SM-25 (*Bacillus cereus*), and pH 7 for isolate SM-42 (*Brevibacterium borstelensis*). Meanwhile, isolate SM-82 (*Klebsiella pneumoniae*) only grows optimally at pH 9-10. The degree of acidity (pH) of the media plays an important role in influencing the activity of intracellular enzymes, the level of nutrient ionization, and the transport mechanism across the cell membrane, which ultimately determines the rate of bacterial growth and the biosynthesis process of secondary metabolites such as antimicrobials (Al-Ghazali & Omran, 2017). Changes in pH variations are an important factor that influences the production of secondary metabolites. Antibiotic production is influenced by the composition of the media and culture conditions such as pH, temperature, aeration, and agitation, the responses of which differ in each organism. Changes in the pH of the medium can induce the formation of new compounds and affect the rate of antibiotic production (Nurmiati et al., 2024). Changes in pH have been shown to depend on the carbon source used. Cell growth with glucose or glycerol does not affect the extracellular pH, whereas the use of citrate causes an increase in the pH (alkalization) of the medium (Sánchez-clemente et al., 2018).

Quagga
Jurnal Pendidikan dan Biologi

p-ISSN 1907-3089, e-ISSN 2615-5869
Vol 18, No. 2, 2026, pp 173 - 183
Available online https://journal.fkip.uniku.ac.id/quagga
DOI: https://doi.org/10.25134/quagga.v18i2.525

Table 2. Effect of pH variation and growth media on the growth of endophytic bacteria

Bacterial Isolate	Growth Medium	OD_{600} pH5	Cell Density pH 5 (CFU mL^{-1})	OD_{600} pH7	Cell Density pH 7 (CFU mL^{-1})	OD_{600} pH9	Cell Density pH 9 (CFU mL^{-1})
CPA2	NB	0.381	4.15×10^4	0.444	4.90×10^4	0.236	3.16×10^4
	TSB	0.578	5.98×10^4	0.711	8.28×10^4	0.646	6.88×10^4
CPA8	NB	0.273	3.99×10^4	0.572	5.87×10^4	0.233	2.98×10^4
	TSB	0.608	6.01×10^4	0.813	10.33×10^4	0.737	8.43×10^4
CPA9	NB	0.386	4.26×10^4	0.506	5.58×10^4	0.452	4.93×10^4
	TSB	0.869	11.59×10^4	1.109	12.95×10^4	0.864	11.57×10^4

Antibiotic Susceptibility Profiles of Endophytic Bacteria

Antibiotic susceptibility testing showed that all endophytic bacterial isolates (CPA2, CPA8, and CPA9) exhibited different responses to the four antibiotics tested (Table 3). Ciprofloxacin and tetracycline produced larger inhibition zone diameters compared to gentamicin and amoxicillin for all isolates. Isolate CPA9 demonstrated the highest sensitivity to all antibiotics, particularly to ciprofloxacin (25.4 ± 0.85 mm) and tetracycline (24.20 ± 0.21 mm). In contrast, amoxicillin produced the smallest inhibition zones across all isolates, especially in isolate CPA8 (6.45 ± 0.10 mm), indicating a low level of sensitivity to this antibiotic.

Differences in inhibition zone diameters indicate varying levels of sensitivity of endophytic bacteria to the antibiotics tested. Isolate CPA9 exhibited the largest inhibition zone for almost all antibiotics (ciprofloxacin, amoxicillin, gentamicin, and tetracycline), indicating that this isolate was relatively more susceptible to antibiotics, while CPA2 and CPA8 exhibited higher tolerance to certain antibiotics. These results provide preliminary information regarding the antibiotic sensitivity profiles of endophytic bacterial isolates, potentially important for safety considerations and further characterization. The antibiotic ciprofloxacin, a fluoroquinolone, demonstrated the highest effectiveness against all isolates, likely due to its mechanism of action by inhibiting DNA gyrase and topoisomerase IV enzymes, thereby directly disrupting bacterial DNA replication (Tamanna & Ramana, 2016). Tetracycline and gentamicin demonstrated moderate effectiveness, likely related to their mechanism of protein synthesis inhibition via different ribosomal subunits (Graber, 2021). Conversely, low sensitivity to amoxicillin may be due to the ability of endophytic bacteria to produce β-lactamase or modification of the antibiotic target, resulting in reduced β-lactam antibiotic effectiveness.

Table 3. Sensitivity of Endophytic Bacterial Isolates to Antibiotics

Bacterial Isolate	Inhibition Zone Diameters			
	Antibiotics			
	Ciprofloxacin (mm)	Tetracycline (mm)	Gentamicin (mm)	Amoxicillin (mm)
CPA2	21,9 ± 0,37	17,4 ± 0,38	16,4 ± 0,48	10,7 ± 0,19
CPA8	19,5± 0,06	21,2± 0,77	16,2± 0,19	6,4± 0,10
CPA9	25,4± 0,85	24,2± 0,21	20,2± 0,24	18,2± 0,38

Optimization of antibacterial production

Optimization of antibacterial production was carried out on two different fermentation media, using TSB and NB liquid media. Each medium was adjusted at pH variations of 5, 7, and 9 to see the most optimal antibacterial production activity of endophytic bacterial isolates. Based on Table (Table 4), the results of the antibacterial activity against *Staphylococcus aureus* in the form of the diameter of the inhibition zone were affected pH variations and the type of growth media (NB and TSB). In NB media, isolate CPA2 showed the highest activity at pH 7 (25.7 ± 1.77 mm), compared to pH 5 (11.0 ± 0.07 mm) and pH 9

Quagga
Jurnal Pendidikan dan Biologi
p-ISSN 1907-3089, e-ISSN 2615-5869
Vol 18, No. 2, 2026, pp 173 - 183
Available online https://journal.fkip.uniku.ac.id/quagga
DOI: https://doi.org/10.25134/quagga.v18i2.525

(11.6 ± 0.22 mm). A similar pattern was also seen in CPA9, with the largest inhibition zone at pH 7 (19.9 ± 1.03 mm), while CPA8 showed relatively low activity at all pH variations, with the highest value remaining at pH 7 (12.3 ± 0.08 mm). In TSB media, all isolates showed an increase in the diameter of the inhibition zone compared to NB. The highest overall activity was obtained in CPA2 grown in TSB pH 7 (30.2 ± 1.77 mm), followed by CPA9 under the same conditions (22.3 ± 0.23 mm). In TSB media pH 9, activity was still relatively high in CPA2 (22.5 ± 0.37 mm) and CPA9 (16.7 ± 0.54 mm), while CPA8 still showed the lowest activity compared to the other two isolates. In general, pH 7 was the optimum condition for the production of antibacterial compounds in all three isolates, and TSB medium produced higher inhibitory activity than NB (Figure 1). Based on inhibition zone, the CPA2 isolate in TSB media (pH7) has a very sensitive / very strong category to inhibit *S.aureus*.

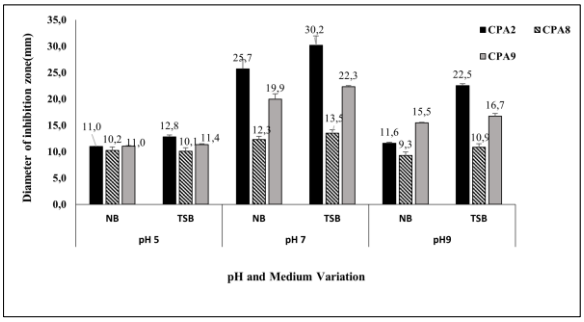

Figure 1. The effect of pH and types of growth media on the antibacterial activity of endophytic bacterial isolates against *Staphylococcus aureus.*

Table 4. Antibacterial activity of endophytic bacterial isolates against *Staphylococcus aureus* based on growth media and pH variations

Bacterial isolate	Inhibition Zone (mm)					
	Staphylococcus aureus					
	NB Medium			TSB Medium		
	pH 5	pH 7	pH9	pH 5	pH 7	pH9
CPA 2	11,0 ± 0,07	25,7 ± 1,77	11,6 ± 0,22	12,8 ± 0,37	30,2 ± 1,77	22,5± 0,37
CPA8	10,2± 0,02	12,3± 0,08	9,3 ± 0,06	10,1 ± 0,29	13,5 ±0,49	10,9 ± 0,17
CPA9	11,0 ± 0,10	19,9 ± 1,03	15,5 ± 0,11	11,4± 0,13	22,3 ±0,23	16,7 ± 0,54

Based on Table 5, the antibacterial activity of endophytic bacterial isolates against *Escherichia coli* showed variations in the diameter of the inhibition zone affected by the type of media and growth pH. In general, all isolates (CPA2, CPA8, and CPA9) showed inhibitory activity in all treatments, although with different intensities. In NB media, the highest activity was obtained at pH 7 for all isolates, with inhibition zone diameters of 10.5 ± 0.10 mm (CPA2), 9.8 ± 0.08 mm (CPA8), and 8.1 ± 0.04 mm (CPA9), respectively. Meanwhile, in TSB media, antibacterial activity also reached a maximum value at pH 7, namely 11.8 ± 0.02 mm (CPA2), 10.2 ± 0.04 mm (CPA8), and 8.7 ± 0.01 mm (CPA9). Overall, the CPA2 isolate showed the highest inhibitory activity compared to other isolates in almost all treatments, especially in TSB pH 7 media. TSB media generally produced a larger inhibition zone diameter than NB media at the same pH (Figure 2). Based on inhibition zone, the CPA2 isolate in TSB media (pH7) has a sensitive / strong category to inhibit *E.coli.*

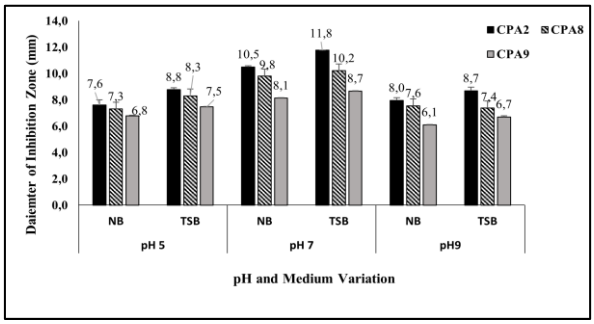

Figure 2. The effect of pH and types of growth media on the antibacterial activity of endophytic bacterial isolates against *Escherichia coli.*

Quagga
Jurnal Pendidikan dan Biologi
p-ISSN 1907-3089, e-ISSN 2615-5869
Vol 18, No. 2, 2026, pp 173 - 183
Available online https://journal.fkip.uniku.ac.id/quagga
DOI: https://doi.org/10.25134/quagga.v18i2.525

Table 5. Antibacterial activity of endophytic bacterial isolates against *Escherichia coli* based on growth media and pH variations

Bacterial isolate	Inhibition Zone (mm)					
	Escherichia coli					
	NB Medium			TSB Medium		
	pH 5	pH 7	pH9	pH 5	pH 7	pH9
CPA 2	7,6± 0,40	10,5 ± 0,10	8,0 ± 0,20	8,8 ± 0,15	11,8 ± 0,02	8,7± 0,28
CPA8	7,3± 0,13	9,8± 0,08	7,6 ± 0,33	8,3 ± 0,02	10,2±0,04	7,4 ± 0,11
CPA9	6,8± 0,04	8,1 ± 0,04	6,1 ± 0,01	7,5± 0,03	8,7 ±0,01	6,7 ± 0,07

The results of the study showed that the antibacterial activity of endophytic bacterial isolates was significantly affected by the pH and composition of the growth medium. The increase in the diameter of the inhibition zone at pH 7 indicated that neutral conditions are the optimum pH for the synthesis of secondary metabolites that act as antibacterial agents. At extreme pHs (acidic or basic), activity tended to decrease, likely due to disruption of enzyme stability and the biosynthesis pathway of active metabolites. The significant differences between the NB and TSB media used in the study indicate that nutrient composition affected the production of bioactive compounds. TSB media, richer in nutrients (especially peptone and complex nitrogen sources), enabled increased cell growth and higher production of secondary metabolites compared to NB media. This is in line with the principle that optimal availability of carbon and nitrogen sources can enhance the expression of genes involved in antimicrobial compound biosynthesis.

The highest overall antibacterial activity was obtained in isolate CPA2 grown in TSB pH 7 (30.2 ± 1.77 mm) followed by CPA9 under the same conditions (22.3 ± 0.23 mm). According to criteria (Pratiwi et al., 2024), both are classified as very strong inhibitors (very sensitive) against *S. aureus* and strong (sensitive) against *E.coli.* Isolate CPA2 showed the highest antibacterial activity in almost all treatments, especially in TSB pH 7 media, which indicates the potential for secondary metabolites in the form of stronger antibacterials compared to isolates CPA8 and CPA9. The differences between these isolates are likely caused by genetic variations, metabolite production capacity, or differences in secondary metabolism regulation. Overall, the combination of TSB and pH 7 media is the optimum condition to maximize the antibacterial activity against *Staphylococcus aureus* and *E.coli*. This is important as a basis for optimizing the production of antibacterial metabolites on a laboratory scale and further development on a larger scale.

The results showed that pH 7 was the optimal condition for the production of antibacterial compounds by the three endophytic bacterial isolates against. This indicates that the production of secondary metabolites that act as antibacterials tends to be optimal at neutral pH conditions. Physiologically, neutral pH supports enzyme stability and bacterial cell metabolic activity, so that the synthesis of bioactive metabolites can take place more efficiently. TSB media produced higher antibacterial activity than NB. This is likely due to the more complex nutrient composition of TSB and its richness in nitrogen sources and growth factors, thus supporting increased biomass and secondary metabolite production. The availability of sufficient nutrients is known to play an important role in regulating the biosynthesis of bioactive compounds in bacteria.

Overall, among the three isolates, isolate CPA2 showed the highest antibacterial activity, indicating that this isolate has greater potential in producing antibacterial compounds against Gram-negative bacteria such as *E. coli*. However, in general, the diameter of the inhibition zone against *E. coli* was smaller than against *Staphylococcus aureus* (Gram-positive), which can be attributed to differences in cell wall structure. Gram-negative bacteria have an outer membrane containing lipopolysaccharide (LPS) that can inhibit the penetration of antibacterial compounds, so their sensitivity tends to be lower. The structure of the bacterial cell wall plays an important role in maintaining cell shape and regulating the transport of water, ions, nutrients, and other molecules, including antibiotics that target cell wall components (Sarkar et al., 2017; Riu et al., 2022). Gram-positive and Gram-

Quagga
Jurnal Pendidikan dan Biologi
p-ISSN 1907-3089, e-ISSN 2615-5869
Vol 18, No. 2, 2026, pp 173 - 183
Available online https://journal.fkip.uniku.ac.id/quagga
DOI: https://doi.org/10.25134/quagga.v18i2.525

negative bacteria have different cell wall structures that affect their level of sensitivity to antibacterial compounds. Gram-positive bacteria are generally more susceptible than Gram-negative bacteria because they have a single-layered cell wall with thick peptidoglycan without an outer membrane, making it easier for antibacterial compounds to enter and reach the target cell (Torar et al., 2017; Pratiwi et al., 2024). In conclusion, in this study, pH and growth medium optimization proved to play a significant role in increasing the antibacterial activity of endophytic bacterial isolates. CPA2 isolates in TSB pH 7 media can be recommended as the best condition for antibacterial production against *E. coli* and *S.aureus.*

The pH of the medium affects cellular enzyme activity, nutrient ionization, and transport processes through cell membranes, thus impacting bacterial growth rate and secondary metabolite biosynthesis. The results of this study align with a previous study (Al-Ghazali & Omran, 2017) which reported that antibiotic production by *Streptomyces* sp. LHR 9 is optimal at neutral or near-neutral pH (around pH 7). This strain is capable of producing antibacterial metabolites with inhibition zone diameters of 18.33±2.52 mm; 19.33±3.21 mm; 24.00±1.73 mm; and 20.00±2.00 mm, respectively, against *E. coli, P. aeruginosa, S. aureus, and S. agalactiae*. Antibacterial metabolite production in bacteria is strongly influenced by pH stability because pH homeostasis plays a crucial role in maintaining protein structure and function, enzymatic reaction kinetics, and the balance of cellular energy metabolism. Bacterial intracellular pH is maintained near neutral to maintain integrity and metabolic capacity, despite changes in environmental pH. Variations in media pH, growth phase, and physiological characteristics of each isolate can influence metabolic activity and secondary metabolite biosynthesis pathways. Furthermore, bacterial metabolic activity itself can alter environmental pH, potentially affecting antibacterial compound production and interactions with other microorganisms in the same niche. Therefore, the neutral pH conditions used in this study likely support optimal enzyme stability and antibacterial metabolite synthesis efficiency.

Identification of endophytic bacterial species

The selected endophytic bacterial isolate (CPA2) that is most optimal in producing antibacterials was identified molecularly based on the 16S rRNA gene. The results of PCR visualization of endophytic bacterial isolates are shown in Figure 3. Next, sequencing was carried out to determine the nucleotide sequence so that the identity of the bacterial species of each bacterial isolate could be known. Based on the results of 16S rRNA gene sequencing (Table 6), the CPA2 isolate showed a very high Query Coverage value (100%), with a similarity level of 99.90% to *Bacillus cereus* strain JCM 2152.

Molecular identification showed that isolate CPA2 had a high similarity to *Bacillus cereus* strain JCM 2152. The *B. cereus* group is a Gram-positive, spore-forming bacterium with wide genomic and phenotypic diversity, including non-pathogenic to potentially pathogenic strains. Some strains are known to have biotechnological value as antibiotic producers, while others can produce enterotoxins (Acevedo et al., 2020). The other *Bacillus* groups such as endophytic bacteria *Bacillus velezensis* Ea73 has inhibitory activity against *Staphylococcus aureus* in culture medium conditions containing 6.55 g/L yeast extract, 6.61 g/L peptone, 20.00 g/L NaCl in broth conditions of 7.95 pH, with 51.04h harvest time, and a temperature of 27.97◦C (Ren et al., 2022). *Bacillus subtilis* strain EP1 as endophytic bacteria with antibacterial activities against *E.coli* (Numan et al., 2022).

Table 6. Identification data of papaya seed endophytic bacterial species

Bacterial Isolate	Nucleotide Lenght	Query Coverage (%)	Species	Similarity (%)
CPA2	1474 bp	100	*Bacillus cereus strain JCM 2152*	99.90

Quagga
Jurnal Pendidikan dan Biologi

p-ISSN 1907-3089, e-ISSN 2615-5869
Vol 18, No. 2, 2026, pp 173 - 183
Available online https://journal.fkip.uniku.ac.id/quagga
DOI: https://doi.org/10.25134/quagga.v18i2.525

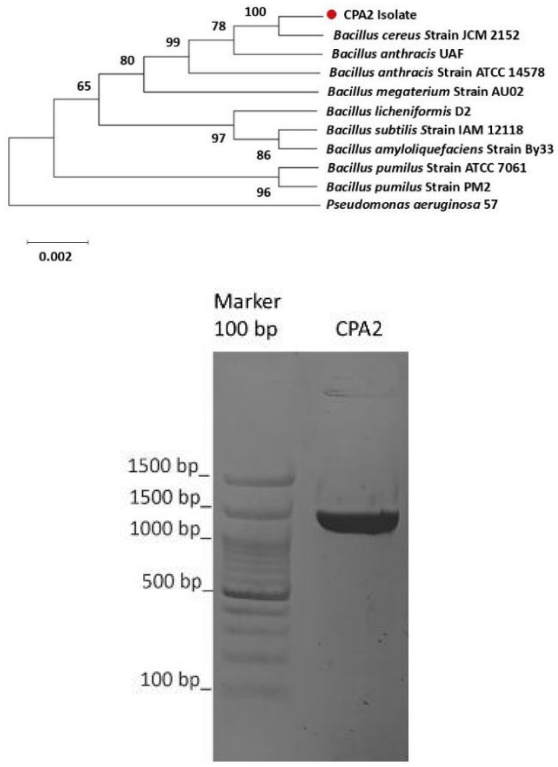

Figure 3. Phylogenetic trees and PCR amplification of endophytic bacteria isolate

4. CONCLUSION

It was concluded that the characteristics of all papaya seed endophytic bacterial isolates (CPA2, CPA8, and CPA9) showed optimum growth in TSB media with neutral pH (7) and sensitivity tests showed that all isolates were more responsive to Ciprofloxacin and Tetracycline antibiotics compared to Gentamicin and Amoxicillin. Optimization of culture conditions showed that TSB media at pH 7 was the best condition for the production of antibacterial compounds. Isolate CPA2 in TSB pH 7 media produced the highest inhibitory activity against Staphylococcus aureus (30.2 ± 1.77 mm) and Escherichia coli (11.8 ± 0.02 mm) compared to other isolates. Molecular analysis identified the most potential isolate (CPA2) as *Bacillus cereus* strain JCM 2152 (99.90%), which has the potential to be developed as a source of new antibacterial candidates. Further studies are required to evaluate the potential for broad-spectrum antibacterial production through testing against a broader range of pathogenic microorganisms.

5. ACKNOWLEDGEMENTS

The authors gratefully acknowledge the support of the research team, laboratory assistants, and colleagues. This work was funded by the LPPM UMAHA and Universitas Maarif Hasyim Latif (UMAHA).

6. REFERENCES

Acevedo, M. M., Carroll, L. M., Mukherjee, M., Mills, E., Xiaoli, L., & Dudley, E. G. (2020). Novel Effective Bacillus cereus Group Species “Bacillus clarus” Is Represented by Antibiotic-Producing Strain ATCC 21929 Isolated from Soil. *American Society For Microbiology*, *5*(6), 1–9.

Al-Ghazali, L. H., & Omran, R. (2017). Optimization production conditions of antibacterial metabolite from Streptomyces SP. *Asian Journal of Pharmaceutical and Clinical Research*, *10*(9), 386–391. https://doi.org/10.22159/ajpcr.2017.v10i9.19243

Ariani, N., Monalisa., & Febrianti, D. R. (2019). Uji Aktivitas Antibakteri Ekstrak Biji Pepaya (Carica papaya L.) Terhadap Pertumbuhan Escherichia coli (Test Activity of Antibacterial Pepaya Seeds (Carica papaya L.) on Growth of Escherichia coli). *Journal of Current Pharmaceutical Sciences*, *2*(2), 2598–2095. journal.umbjm.ac.id/index.php/jcps

Ashitha, A., Midhun, S. J., Sunil, M. A., Nithin, T. U., Radhakrishnan, E. K., & Mathew, J. (2019). Bacterial endophytes from Artemisia nilagirica (Clarke) Pamp., with antibacterial efficacy against human pathogens. *Microbial Pathogenesis*, *135*(July), 103624. https://doi.org/10.1016/j.micpath.2019.103624

Buang, A., Isnaeni, D., & Nurhunaida, E. (2019). Uji Efektivitas Antibakteri Ekstrak Kulit Buah Pepaya (Carica Papaya L.)Terhadap Propioni bacterium acnes Ariani. *Majalah Farmasi Nasional*, *16*(01), 13–20.

Dagne, E., Dobo, B., & Bedewi, Z. (2021). Antibacterial Activity of Papaya (Carica papaya) Leaf and Seed Extracts Against Some Selected Gram-Positive and Gram-Negative Bacteria. *Pharmacognosy Journal*, *13*(6), 1727–1733.

p-ISSN 1907-3089, e-ISSN 2615-5869
Vol 18, No. 2, 2026, pp 173 - 183
Available online https://journal.fkip.uniku.ac.id/quagga
DOI: https://doi.org/10.25134/quagga.v18i2.525

https://doi.org/10.5530/pj.2021.13.223

Faujiah, P., Hijriani, B. I., & Kurniawan, E. (2023). The Inhibition Activity Of Endophytic Bacteria Of Papaya Leaves (Carica papaya L.) Against Staphylococcus aureus. *Journal of Natural Sciences*, *1*(4), 101–106.

Gangga Dewanti Gita Maharani, A., Sukiman, Sukenti, K., Hidayati, E., & Sarkono. (2022). Aktivitas Antibakteri Ekstrak Daun Pepaya (Carica papaya L.) Terhadap Staphylococcus aureus. *Samota Journal of Biological Sciences*, *1*(1), 39–47.

Graber, E. M. (2021). Treating acne with the tetracycline class of antibiotics : A review. *Dermatological Reviews Published*, *2*, 321–330. https://doi.org/10.1002/der2.49

Irwandi, Azyenela, L., Purnama Sari, H., Supri Wardi, E., & Sartika, D. (2023). Isolation and identification with 16s rRNA gene of endophytic bacteria from papaya (Carica papaya L.) and test of its antibacterial activity. *Journal of Pharmaceutical and Sciences*, *6*(3), 1068–1078.

Isleem, R. S., Eid, A. M., Hassan, S. E., Aboshanab, K. M., & El-housseiny, G. S. (2025). Deciphering the nature and statistical optimization of antimicrobial metabolites of two endophytic bacilli. *AMB Express*, *15*(10), 1–16.

Mushtaq, S., Shafiq, M., Ashfaq, M., Ali, M., Hsieh, D., Finan, T., & Haider, M. S. (2021). STUDY OF VARYING pH RANGES ON THE GROWTH RATE OF BACTERIAL STRAINS ISOLATED FROM PLANTS. *Pak. J. Agri. Sci.*, *58*(3), 1051–1057. https://doi.org/10.21162/PAKJAS/21.9711

Muthma, H., & Supriyono, T. (2023). Optimization of Antibacterial Metabolite Production and Antioxidant Activity of Endophytic Fungi Isolate from Kelakai (Stenochlaena palustris). *Formosa Journal of Science and Technology*, *2*(12), 3175–3190.

Myo, E. M., Maung, C. E. H., Mya, K. M., & Khai, A. A. (2020). Characterization of bacterial endophytes from myanmar medicinal plants for antimicrobial activity against human and plant pathogens. *Brazilian Journal of Pharmaceutical Sciences*, *56*, 1–8. https://doi.org/10.1590/s2175-97902019000317705

Numan, M., Shah, M., Asaf, S., Ur Rehman, N., & Al-Harrasi, A. (2022). Bioactive Compounds from Endophytic Bacteria Bacillus subtilis Strain EP1 with Their Antibacterial Activities. *Metabolites*, *12*(12), 1–10. https://doi.org/10.3390/metabo12121228

Nurmalasari, A., Oedjijono, & Lestari, S. (2020). Isolasi dan Uji Resistensi Bakteri Endofit Eceng Gondok (Eichhornia crassipes Mart.) terhadap Krom Secara In-Vitro. *Jurnal Ilmiah Biologi Unsoed*, *2*(2), 266–272.

Nurmiati, N., Putra, W. D., & Periadnadi, P. (2024). Potential and Optimization of Antibiotic Production Isolate Bacteria Antibiosis Bacillus sp . Origin of Salt Liquid Waste of Anchovy (Stolephorus Sp .) Against Bacteria Test. *Jurnal Biologi Tropis*, *24*(3), 385–395.

Pratiwi, E. R., Ardyati, T., & Suharjono, S. (2020). Plant Growth Promoting Endophytic Bacteria of Coffea canephora and Coffea arabica L. in UB Forest. *The Journal of Experimental Life Sciences*, *10*(2), 119–126. https://doi.org/10.21776/ub.jels.2020.010.02.07

Pratiwi, E. R., Batati, N. Al, & Firamadhani, N. A. (2024). Antibacterial of Endophytic Bacteria from Papaya (Carica papaya) Seeds Againts Staphylococcus aureus and Escherichia coli. *Jurnal Biologi Tropis*, *24*(1b), 230–236.

Ren, Z., Xie, L., Okyere, S. K., Wen, J., Ran, Y., & Nong, X. (2022). Antibacterial Activity of Two Metabolites Isolated From Endophytic Bacteria Bacillus velezensis Ea73 in Ageratina adenophora. *Frontiers in Microbiology*, *13*(May), 1–14. https://doi.org/10.3389/fmicb.2022.860009

Riu, F., Ruda, A., Ibba, R., Sestito, S., Lupinu, I., Piras, S., Widmalm, G., & Carta, A. (2022). Antibiotics and Carbohydrate-Containing Drugs Targeting Bacterial Cell Envelopes: An Overview. *Pharmaceuticals*, *15*(8), 1–38. https://doi.org/10.3390/ph15080942

Sánchez-clemente, R., Igeño, M. I., Población, A. G., Guijo, M. I., Merchán, F., & Blasco, R. (2018). Study of pH Changes in Media during Bacterial Growth of Several

p-ISSN 1907-3089, e-ISSN 2615-5869
Vol 18, No. 2, 2026, pp 173 - 183
Available online https://journal.fkip.uniku.ac.id/quagga
DOI: https://doi.org/10.25134/quagga.v18i2.525

Environmental Strains †. *Proceedings*, *2*(1297), 1–5. https://doi.org/10.3390/proceedings2201297

Sarkar, P., Yarlagadda, V., Ghosh, C., & Haldar, J. (2017). A review on cell wall synthesis inhibitors with an emphasis on glycopeptide antibiotics. *MedChemComm*, *8*(3), 516–533. https://doi.org/10.1039/c6md00585c

Sharma, M., & Mallubhotla, S. (2022). Diversity, Antimicrobial Activity, and Antibiotic Susceptibility Pattern of Endophytic Bacteria Sourced From Cordia dichotoma L. *Frontiers in Microbiology*, *13*(May), 1–17. https://doi.org/10.3389/fmicb.2022.879386

Tamanna, & Ramana, J. (2016). Structural Insights into the Fluoroquinolone Resistance Mechanism of Shigella flexneri DNA Gyrase and Topoisomerase IV. *MICROBIAL DRUG RESISTANCE*, *22*(5), 404–411. https://doi.org/10.1089/mdr.2015.0018

Torar, G. M. J., Lolo, W. A., & Citraningtyas, G. (2017). Uji Aktivitas Antibakteri Ekstrak Etanol Biji Pepaya (Carica papaya L.) Terhadap Bakteri Pseudomonas aeruginosa DAN Staphylococcus aureus. *PHARMACONJurnal Ilmiah Farmasi*, *6*(2), 14–22. https://repository.unugiri.ac.id/id/eprint/643/

Widjajanti, H., Nurnawati, E., & Zahwa, E. D. (2022). Optimization of Antibacterial Production of Endophytic Fungi with Various Sources of C , N , and pH using The Response Surface Methodology. *Science and Technology Indonesia*, *7*(2), 150–157.

Yunita, M., Manse, Y., Wulandari, M. C., Sukmawati, S., & Ohiwal, M. (2022). Isolation of endophytic bacteria from nutmeg plant as antibacterial agents against pathogenic bacteria. *JPBIO (Jurnal Pendidikan Biologi)*, *7*(1), 115–122. https://doi.org/10.31932/jpbio.v7i1.1522