El-Hayah

**JURNAL BIOLOGI**
Journal Homepage: http://ejournal.uin-malang.ac.id/index.php/bio/index
e-ISSN: 2460-7207, p-ISSN: 2086-0064

*Original research article*

# *Characterization of Endophytic Bacteria from Organic and Inorganic Lettuce (Lactuca sativa L.) for Cadmium Tolerance and Amylase Activity*

**Caesaria Rohmatul Hayyi'lana[1], Talitha Widiatningrum[2]**

[1]*Master's Program in Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang, Sekaran Campus, Gunungpati, Semarang 50229, Central Java, Indonesia*
[2] *Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang, Sekaran Campus, Gunungpati, Semarang 50229, Central Java, Indonesia*

[*]*Corresponding author*
*Email:* talitha_widiatningrum@mail.unnes.ac.id
DOI: 10.18860/elha.v10i5.45219

**Article Info**

*Article history:*
Received 24 July 2026
Received in revised form 25 August 2026
Accepted 16 September 2026

*Key Word:*
*Amylase activity*
*Cadmium tolerance*
*Endophytic bacteria*
*Lettuce*

*Copyright © 2026 by the author(s). Published by Program Study of Biology, State Islamic University of Maulana Malik Ibrahim Malang. This is an open access article under a Creative Commons Attribution ShareAlike 4.0 International License.*

**Abstract**

*Lettuce (Lactuca sativa L.) is widely cultivated under organic and inorganic farming systems, where differences in agricultural inputs may influence cadmium (Cd) exposure and plant-associated microbial communities. Unlike bulk soil bacteria, endophytic bacteria inhabit plant tissues and interact directly with their host, providing a distinct microenvironment for examining responses to heavy metal stress. This study aimed to characterize endophytic bacteria from organic and inorganic lettuce leaves and evaluate their Cd tolerance and amylase activity. Endophytic bacteria were isolated on Nutrient Agar (NA), followed by morphological characterization and Gram staining. Cd tolerance was evaluated at 0, 25, 50, and 100 ppm, while amylase activity was determined using Soluble Starch Agar. Six isolates were obtained, comprising four Gram-negative isolates (LO.1, LO.3, LO.4, and LU.2) and two Gram-positive isolates (LO.2 and LU.1). Four isolates grew at 100 ppm Cd, with LO.3 showing the largest colony diameter (7.847 ± 0.130 mm). LO.1 exhibited the highest amylase activity, with a clear zone diameter of 14.29 ± 0.91 mm. These findings highlight the potential of cadmium-tolerant and amylolytic lettuce endophytic bacteria for applications involving heavy metal stress and organic matter degradation.*

## 1. INTRODUCTION

Lettuce (*Lactuca sativa* L.) is one of the most widely consumed leafy vegetables because of its high nutritional value, including vitamins, minerals, and dietary fiber [1]. The increasing demand for lettuce has encouraged intensive cultivation under both organic and inorganic farming systems. Inorganic cultivation generally relies on synthetic fertilizers and pesticides to improve crop productivity. However, the continuous use of these agricultural inputs may increase cadmium (Cd) accumulation in agricultural soils, posing potential risks to environmental quality, food safety, and human health [2-3]. These challenges highlight the need for biological agents that can tolerate heavy metal stress while also contributing to the sustainable management of agricultural waste.

In addition to heavy metal contamination, lettuce production generates considerable amounts of organic waste, particularly damaged or discarded leaves that are unsuitable for consumption. Sustainable management of this waste is essential to minimize environmental pollution. Microorganisms producing hydrolytic enzymes, such as amylase, can degrade starch into simpler compounds and thereby contribute to the biodegradation of organic materials [4]. Therefore, evaluating amylase activity alongside cadmium tolerance can provide insight into whether endophytic bacteria possess multifunctional characteristics, enabling them to maintain their potential under heavy metal stress while also contributing to the degradation of starch-rich agricultural residues.

Endophytic bacteria inhabit internal plant tissues without causing disease symptoms and establish beneficial associations with their host plants [5]. Besides promoting plant growth and improving plant adaptation to environmental stresses, these bacteria can tolerate heavy metals through biosorption, bioaccumulation, and biotransformation mechanisms [6]. Many endophytic bacteria also produce extracellular hydrolytic enzymes, including amylase, which have potential applications in organic waste degradation, bioremediation, and biotechnology [6-7].

Several studies have investigated the diversity of endophytic bacteria in lettuce as well as the cadmium tolerance or amylase-producing ability of bacterial isolates separately. However, studies evaluating the characteristics of endophytic bacteria isolated from organically and inorganically cultivated lettuce with respect to both cadmium tolerance and amylase activity remain limited. Therefore, this study aimed to characterize endophytic bacteria isolated from organic and inorganic lettuce leaves and to evaluate their cadmium tolerance and amylase activity.

## 2. MATERIALS AND METHODS

### Sample Collection

This study was conducted from January to May 2026. Curly lettuce (*Lactuca sativa* var. *crispa*) leaves were collected from an organic farm at PT Sayur Organik Merbabu (SOM), Getasan District, Semarang Regency, and an inorganic farm in Selo Ngisor Village, Getasan District, Semarang Regency, Indonesia. The organic lettuce samples were collected from a farm practicing organic cultivation, where chemical fertilizers and pesticides were not routinely used. The inorganic lettuce samples were collected from a conventional farming site where synthetic agricultural inputs were used. These cultivation practices were used as the basis for classifying the sampling sites as organic and inorganic systems, respectively. Sampling sites were selected purposively based on differences in cultivation systems. Samples were transported to the laboratory and immediately processed for surface sterilization within approximately 2 hours after harvesting to maintain the viability of endophytic bacteria. Healthy lettuce leaves at approximately 30 days after planting were used as samples. Isolation, bacterial characterization, cadmium tolerance assay,

and amylase activity assay were performed at the Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang.

**Isolation and Characterization of Endophytic Bacteria**

Leaf samples were surface sterilized using 70% ethanol followed by 1–2% sodium hypochlorite (NaOCl) and rinsed with sterile distilled water according to previously described procedures [8-9]. Following surface sterilization, the lettuce leaves were aseptically cut and 1 g of tissue was transferred into a sterile mortar and ground using a sterile pestle until a homogeneous tissue suspension was obtained. The homogenate was then transferred into a test tube containing 9 mL of sterile distilled water and vortexed for approximately 1 min to obtain the $10^{-1}$ dilution. Serial dilution was subsequently performed up to $10^{-4}$ by transferring 1 mL of suspension into 9 mL of sterile distilled water at each dilution step [10]. Plates were incubated at 37°C for 24–48 h. Colonies with distinct morphological characteristics were purified by the streak plate method. Colony morphology, including shape, color, margin, elevation, and surface texture, was recorded. Gram staining was performed using crystal violet, iodine solution, 96% ethanol, and safranin, followed by microscopic observation at 1000× magnification [10].

**Cadmium Tolerance Assay**

Cadmium tolerance was evaluated on Nutrient Agar supplemented with $CdCl_2$ at concentrations of 0, 25, 50, and 100 ppm following a modified procedure described by Jan et al. [11]. Each isolate was inoculated using the spot inoculation method with three replicates and incubated at 28-30°C for 24-48 h. Colony diameter was measured using a digital caliper as an indicator of bacterial growth under Cd stress.

**Amylase Activity Assay**

Amylase activity was determined on Soluble Starch Agar (SSA) using the three-point inoculation method as described by Cappuccino and Welsh [10]. After incubation at 34°C for 48 h, the plates were flooded with Lugol's iodine solution. The formation of a clear zone surrounding the bacterial colonies indicated starch hydrolysis. The diameter of the clear zone was measured using a digital caliper at two perpendicular directions, and the mean value was calculated. The reported amylase activity values represent the clear-zone diameter (mm); no hydrolytic index based on the ratio of clear-zone diameter to colony diameter was calculated.

**Statistical Analysis**

Morphological characteristics and Gram-staining results were analyzed descriptively. Colony diameter data from the cadmium tolerance assay were analyzed using two-way analysis of variance (Two-Way ANOVA), whereas clear-zone diameter data from the amylase activity assay were analyzed using one-way analysis of variance (One-Way ANOVA) at a significance level of 5%. Significant differences among treatments were further analyzed using Tukey's Honestly Significant Difference (HSD) test. Data normality and homogeneity of variance were evaluated prior to statistical analysis.

## 3. RESULTS

Six endophytic bacterial isolates were obtained from organically and inorganically cultivated lettuce leaves. The isolates were characterized based on colony morphology and Gram-staining characteristics, followed by evaluation of their cadmium (Cd) tolerance and amylase activity.

**Morphological and Gram Characteristics**

The isolates exhibited diverse colony characteristics, particularly in colony shape,

elevation, margin, color, and surface texture (Table 1). Most isolates formed circular, smooth, and shiny colonies, whereas LO.4 and LU.2 showed irregular colonies with rough and wrinkled surfaces (Figure 1).

Gram-staining analysis showed that four isolates were Gram-negative and two were Gram-positive. The Gram-negative isolates were predominantly coccobacillus, while the Gram-positive isolates exhibited coccus morphology (Table 2; Figure 2).

**Cadmium Tolerance**

The isolates showed different growth responses to increasing Cd concentrations (Figure 3; Table 3). Colony diameter generally decreased in susceptible isolates as Cd concentration increased, whereas LO.1, LO.3, and LU.2 maintained or increased their growth at higher Cd concentrations. LO.2 showed the strongest inhibition and did not grow at 100 ppm Cd.

**Amylase Activity**

Four isolates exhibited amylolytic activity, as indicated by clear zones surrounding the colonies on Soluble Starch Agar after iodine staining (Figures 5 and 6). The intensity of starch hydrolysis varied among the isolates.

**Table 1.** Macroscopic characteristics of endophytic bacterial isolates from organic and inorganic lettuce leaves

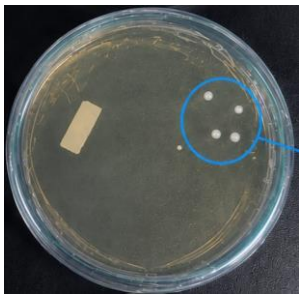
| Isolate | Colony Shape | Elevation | Margin | Color | Surface Texture |
|---|---|---|---|---|---|
| LO.1 | Circular | Convex | Entire | Creamy white | Smooth and shiny |
| LO.2 | Circular | Raised | Entire | Pale creamy white | Smooth and shiny |
| LO.3 | Circular | Convex | Entire | Milky white | Smooth and shiny |
| LO.4 | Irregular | Umbonate | Undulate | Pale cream | Rough, dry, and wrinkled |
| LU.1 | Circular | Convex | Entire | Milky white | Smooth and shiny |
| LU.2 | Irregular | Raised | Undulate | Creamy white | Rough and wrinkled |



**LO.1**



**LO.2**



**LO.3**



**LO.4**



**LU.1**



**LU.2**

**Figure 1.** Colony morphology of purified endophytic bacterial isolates cultured on Nutrient Agar (NA) after 48 h of incubation.

**Table 2.** Gram-staining characteristics of endophytic bacterial isolates observed at 1000× magnification

| Isolate | Cell Shape | Cell Arrangement | Gram Stain Color | Gram Reaction |
|---|---|---|---|---|
| LO.1 | Short bacillus | Diplobacillus | Pink | Gram-negative |
| LO.2 | Coccus | Diplococcus | Purple | Gram-positive |
| LO.3 | Short bacillus | Single | Pink | Gram-negative |
| LO.4 | Short bacillus | Diplobacillus | Pink | Gram-negative |
| LU.1 | Coccus | Staphylococcus | Purple | Gram-positive |
| LU.2 | Short bacillus | Single | Pink | Gram-negative |

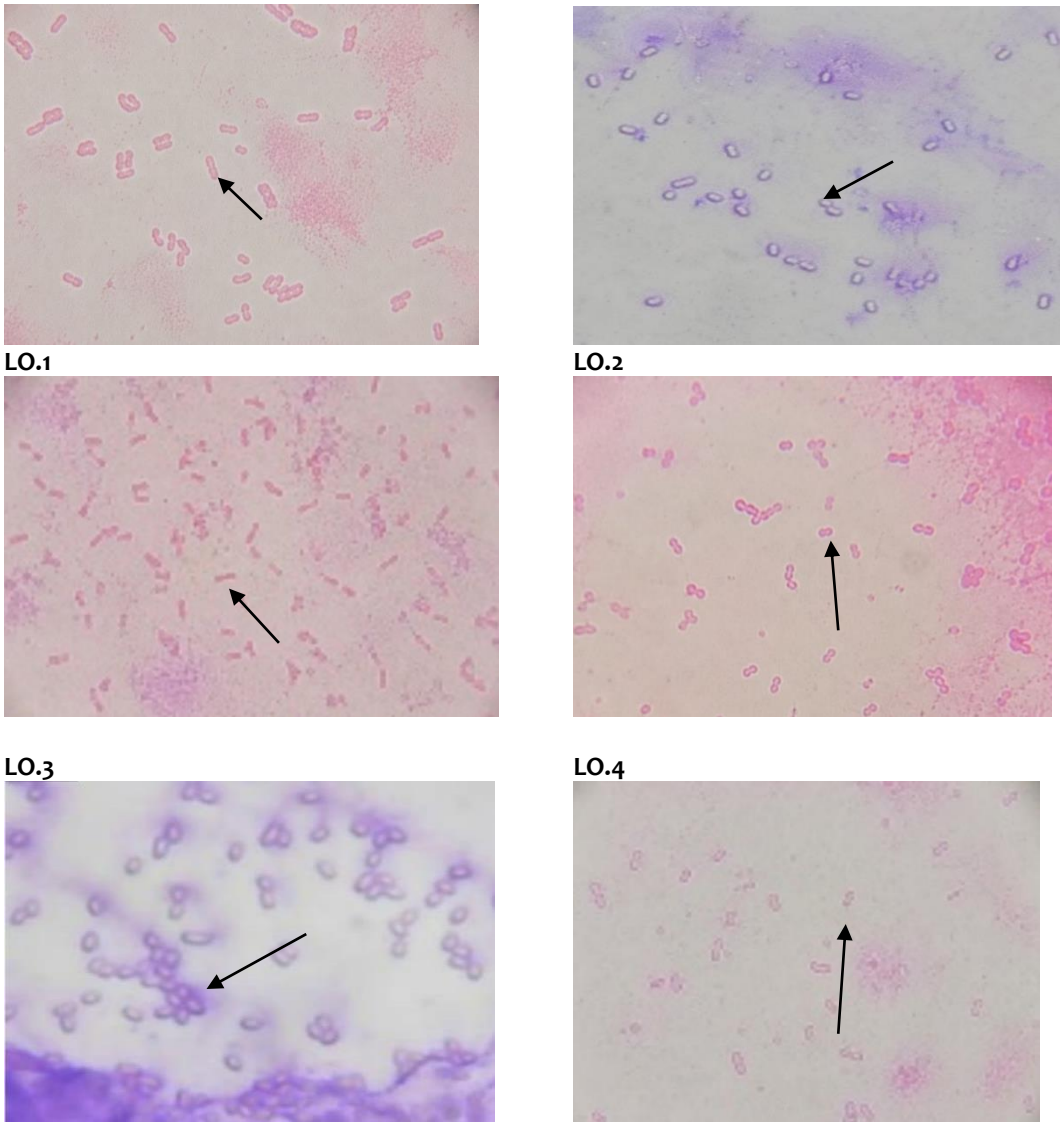


**Figure 2.** Gram-staining characteristics of endophytic bacterial isolates observed under a light microscope at 1000× magnification

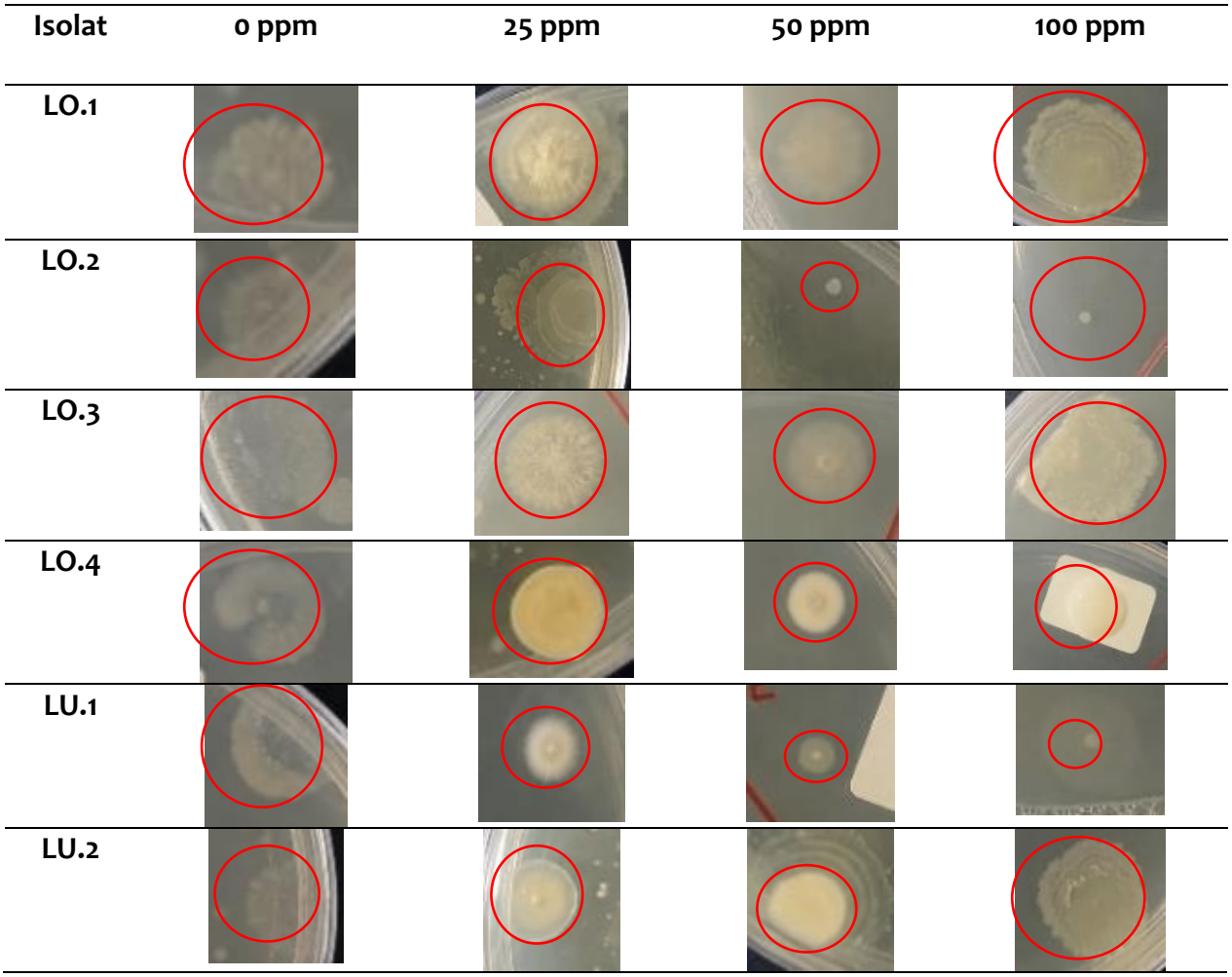



**Figure 3**. Growth of endophytic bacterial isolates on Nutrient Agar supplemented with different cadmium (Cd) concentrations (0, 25, 50, and 100 ppm).

**Table 3.** Mean Colony Diameter (Mean ± SD) of Endophytic Bacterial Isolates at Different Cadmium (Cd) Concentrations Based on Tukey's HSD Test (α = 0.05)

| Isolatate | 0 ppm | 25 ppm | 50 ppm | 100 ppm |
|---|---|---|---|---|
| LO.1 | $4.230 \pm 0.125^{e}$ | $5.220 \pm 0.120^{b}$ | $5.237 \pm 0.074^{b}$ | $6.757 \pm 0.108^{a}$ |
| LO.2 | $2.197 \pm 0.150^{d}$ | $3.307 \pm 0.160^{cd}$ | $1.123 \pm 0.081^{d}$ | $0.000 \pm 0.000^{d}$ |
| LO.3 | $3.267 \pm 0.140^{cd}$ | $5.677 \pm 0.160^{b}$ | $5.240 \pm 0.160^{b}$ | $7.847 \pm 0.130^{a}$ |
| LO.4 | $4.360 \pm 0.111^{e}$ | $4.273 \pm 0.091^{e}$ | $3.277 \pm 0.086^{cd}$ | $5.140 \pm 0.079^{b}$ |
| LU.1 | $3.657 \pm 0.130^{e}$ | $3.240 \pm 0.131^{cd}$ | $1.217 \pm 0.075^{d}$ | $1.457 \pm 0.091^{d}$ |
| LU.2 | $4.107 \pm 0.107^{e}$ | $4.160 \pm 0.085^{e}$ | $5.020 \pm 0.176^{b}$ | $7.820 \pm 0.075^{a}$ |

Note: Values are presented as mean ± SD. Superscript letters indicate the results of Tukey's HSD post-hoc comparisons among all isolate × Cd concentration treatment combinations. Means sharing the same superscript letter are not significantly different, whereas means with different superscript letters differ significantly ($p < 0.05$).

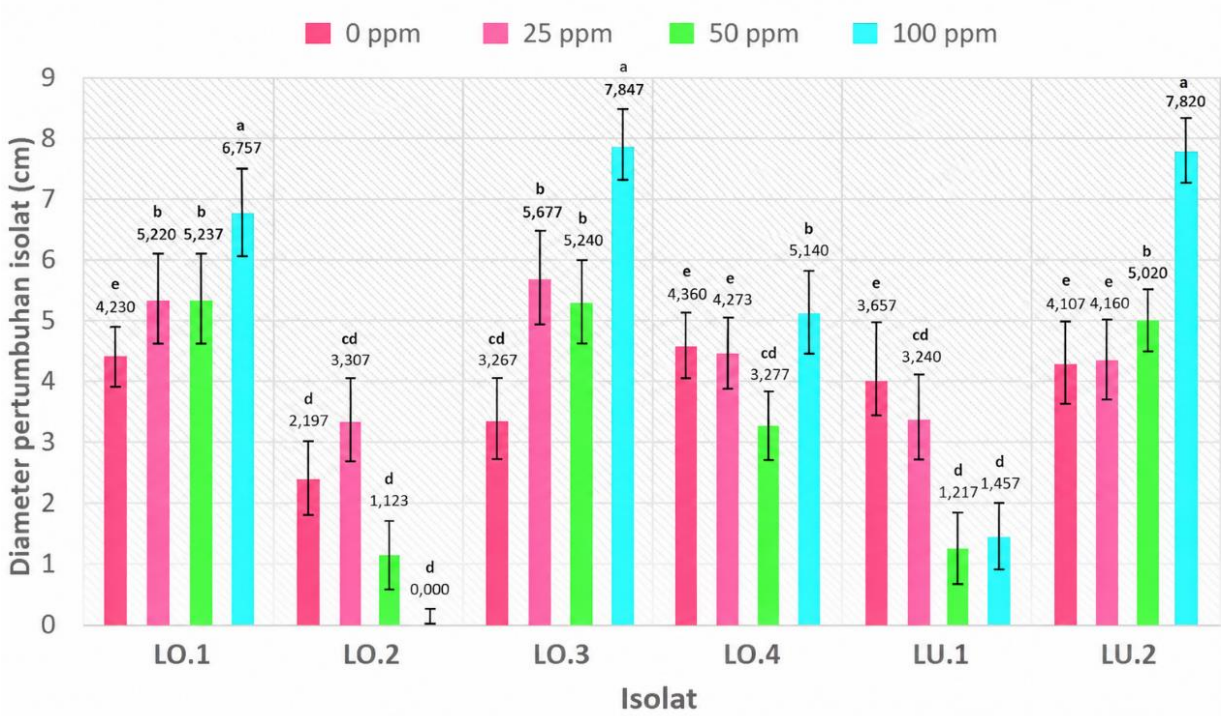
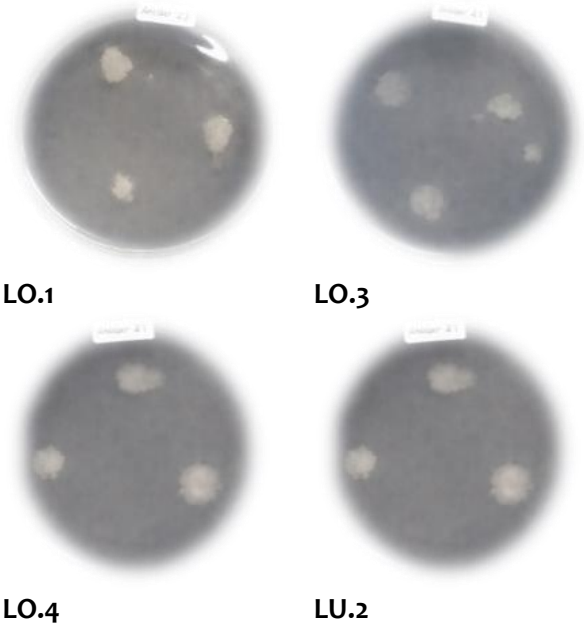



**Figure 4.** Colony diameter of endophytic bacterial isolates at different cadmium (Cd) concentrations (mean ± SD). Tukey's HSD analysis confirmed significant differences in colony diameter among the Cd treatments. At 100 ppm Cd, LO.3 and LU.2 showed the largest colony diameters, whereas LO.2 and LU.1 showed the lowest growth. These differences indicate distinct Cd tolerance responses among the isolates.



**LO.1** **LO.3**

**LO.4** **LU.2**

**Figure 5.** Growth of endophytic bacterial isolates on Soluble Starch Agar (SSA) after 48 h of incubati

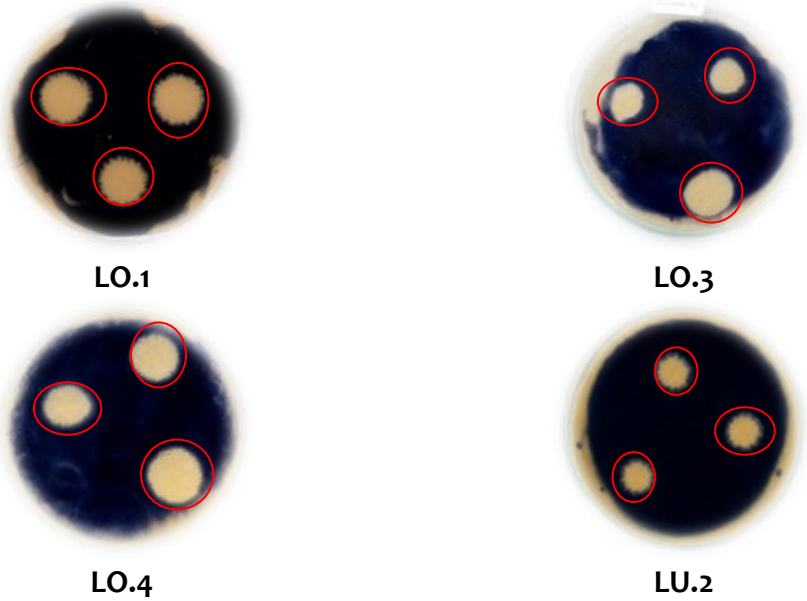



**Figure 6.** Amylolytic activity of endophytic bacterial isolates on Soluble Starch Agar (SSA) after iodine solution staining.

**Table 4.** Mean Clear Zone Diameter (Mean ± SD) of Endophytic Bacterial Isolates Based on Tukey's HSD Test (α = 0.05)

| Isolat | Mean ± SD (mm) |
|---|---|
| LO.1 | 14.29 ± 0.91[a] |
| LO.4 | 13.94 ± 0.75[a] |
| LO.3 | 11.45 ± 1.19[b] |
| LU.2 | 9.42 ± 0.37[c] |

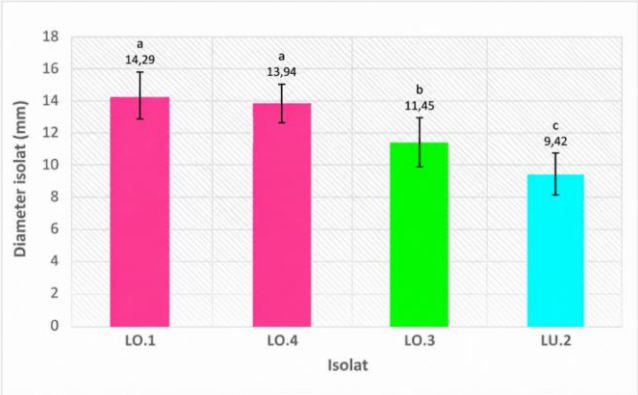



**Figure 7.** Mean clear zone diameter of endophytic bacterial isolates in the amylase activity assay (mean ± SD). LO.1 showed the highest amylolytic activity, followed by LO.4 and LO.3, whereas LU.2

exhibited the lowest activity. Tukey's HSD test showed that LO.1 and LO.4 did not differ significantly, while both showed significantly higher activity than LO.3 and LU.2.

## 4. DISCUSSION

### Morphological and Gram Characteristics of Endophytic Bacterial Isolates

Six endophytic bacterial isolates were successfully obtained from organic and inorganic lettuce leaves. The isolates exhibited distinct macroscopic characteristics, including differences in colony shape, elevation, margin, color, and surface texture (Table 1). Four isolates (LO.1, LO.2, LO.3, and LU.1) formed circular colonies with entire margins, whereas isolates LO.4 and LU.2 exhibited irregular colonies with undulate margins. Colony color ranged from pale creamy white to milky white, while most isolates showed smooth and shiny surfaces. In contrast, isolates LO.4 and LU.2 produced rough and wrinkled colonies. These variations indicate considerable phenotypic diversity among endophytic bacteria inhabiting lettuce tissues.

Morphological diversity is commonly observed among endophytic bacteria because colony characteristics are influenced by genetic composition, extracellular polysaccharide production, nutrient utilization, and adaptation to the internal plant environment [12]. Environmental factors, host plant genotype, and cultivation practices also contribute to differences in the composition and phenotypic characteristics of endophytic bacterial communities [13]. Therefore, the morphological variation observed in the present study likely reflects the heterogeneous nature of endophytic bacteria associated with organically and inorganically cultivated lettuce.

Gram-staining analysis further revealed that four isolates (LO.1, LO.3, LO.4, and LU.2) were Gram-negative, whereas two isolates (LO.2 and LU.1) were Gram positive (Table 2). Among the Gram negative isolates, three exhibited coccobacillus morphology (LO.1, LO.4, and LU.2), while isolate LO.3 was rod-shaped (bacillus). The Gram-positive isolates (LO.2 and LU.1) displayed coccus morphology. The predominance of Gram negative bacteria observed in this study suggests that Gram negative endophytes constitute an important component of the bacterial community associated with lettuce leaves.

The difference between Gram positive and Gram-negative bacteria is mainly determined by cell wall structure. Gram positive bacteria possess a thick peptidoglycan layer that retains the crystal violet iodine complex during Gram staining, resulting in a purple coloration. In contrast, Gram-negative bacteria have a thinner peptidoglycan layer and an outer membrane containing lipopolysaccharides, causing the primary stain to be removed during decolorization and allowing the cells to absorb safranin, which produces a pink appearance [14]. These structural differences are associated with bacterial adaptation to environmental stress and may influence their ecological functions within plant tissues.

The predominance of Gram-negative isolates in the present study may reflect physiological adaptations that facilitate their survival and colonization within plant tissues. The structural characteristics of Gram negative bacteria may contribute to their ability to adapt to the plant endophytic environment and support metabolic versatility under varying environmental conditions. However, Gram staining alone is insufficient to establish taxonomic affiliation at the phylum level, and definitive taxonomic assignments should be based on molecular identification data [15-16]. Gram negative endophytes are also frequently associated with plant growth promotion and tolerance to environmental stresses, including heavy metal contamination [17]. Therefore, the phenotypic diversity observed in this study provides an important foundation for selecting potential endophytic bacteria with functional characteristics, particularly cadmium tolerance and extracellular amylase production, which

were evaluated in the subsequent experiments. When the isolates were considered according to their cultivation system, no consistent pattern was observed in their functional characteristics. Both organic and inorganic lettuce yielded isolates capable of tolerating elevated Cd concentrations, while amylolytic activity was observed among isolates from the organic system. The highest Cd tolerance was observed in isolates from both systems, whereas the strongest amylase activity was exhibited by an organic-derived isolate. Thus, the observed Cd tolerance and amylase activity appear to be more isolate-dependent than directly determined by the cultivation system. These findings suggest that cultivation system may influence the endophytic bacterial community, but the functional traits assessed in the present study cannot be attributed solely to the organic or inorganic system.

**Cadmium Tolerance of Endophytic Bacterial Isolates**

The cadmium tolerance assay demonstrated that all endophytic bacterial isolates were able to grow in media supplemented with Cd, although their growth responses differed depending on isolate and Cd concentration (Table 3). Colony diameter generally decreased in susceptible isolates as Cd concentration increased, whereas several isolates maintained or even increased colony growth under higher Cd concentrations. Based on Tukey's HSD test (Table 4), isolates LO.3 and LU.2 exhibited the highest tolerance to cadmium, producing colony diameters of 7.847 ± 0.130 mm and 7.820 ± 0.075 mm, respectively, at 100 ppm Cd. These values were significantly higher than those of the other isolates ($p < 0.05$). In contrast, isolate LO.2 failed to grow at 100 ppm Cd, while isolate LU.1 showed only limited growth with a colony diameter of 1.457 ± 0.091 mm, indicating lower tolerance to cadmium stress.

Interestingly, the growth responses varied among isolates as Cd concentration increased. Although LO.1, LO.3, and LU.2 showed larger colony diameters at 100 ppm than at 0 ppm, this result should not be interpreted as evidence that Cd promoted bacterial growth. Rather, these isolates appeared able to maintain growth under Cd exposure, whereas LO.2 and LU.1 showed substantial growth inhibition. This variation suggests that Cd tolerance was isolate-dependent and may reflect differences in the physiological mechanisms involved in coping with heavy metal stress. Similar variability has been reported in endophytic bacteria isolated from agricultural crops, where bacterial strains differed markedly in their ability to survive under elevated Cd concentrations depending on their intrinsic resistance mechanisms [18].

Cadmium is a non-essential heavy metal that induces oxidative stress, protein denaturation, membrane damage, and disruption of essential metabolic processes in bacterial cells [6]. To overcome these adverse effects, cadmium-tolerant bacteria have evolved several defense mechanisms, including biosorption of Cd ions to the cell surface, intracellular bioaccumulation, active efflux systems, production of extracellular polymeric substances (EPS), and sequestration of metal ions by metallothioneins or other metal-binding proteins [18]. In the present study, the larger colony diameters observed for LO.1, LO.3, and LU.2 at 100 ppm Cd may not necessarily indicate enhanced cellular proliferation alone. Increased EPS production may contribute to colony expansion by promoting cell aggregation and providing binding sites for Cd ions, potentially reducing Cd bioavailability around bacterial cells. Thus, the observed increase in colony diameter may partly reflect an EPS-associated response to Cd stress rather than an increase in cellular proliferation alone. Furthermore, the stimulation of colony growth at certain Cd concentrations may indicate a possible hormetic response, in which low or moderate exposure to a stressor stimulates physiological activity in tolerant strains before inhibitory

effects become dominant at higher concentrations. However, EPS production, cellular proliferation, and hormetic responses were not directly measured in the present study; therefore, these mechanisms should be considered possible explanations rather than confirmed mechanisms. Further studies measuring EPS production, bacterial biomass, and growth kinetics under different Cd concentrations are needed to verify these responses [7, 8]. These mechanisms reduce the intracellular toxicity of Cd and enable bacterial cells to maintain normal physiological functions under heavy metal stress. The ability of LO.3 and LU.2 to maintain relatively larger colony diameters at 100 ppm Cd suggests greater tolerance to Cd stress than the less tolerant isolates. However, the specific mechanisms underlying this tolerance were not determined in the present study.

The results obtained in this study are comparable, who demonstrated that cadmium-tolerant plant-associated bacteria remained viable at Cd concentrations exceeding 100 ppm because of efficient metal sequestration and antioxidant defense systems [22]. Likewise, reported that heavy metal-tolerant endophytic bacteria exhibited greater adaptability under Cd stress through the production of EPS and activation of metal transport systems, thereby reducing metal toxicity [23]. Although differences in bacterial species, environmental conditions, and experimental design may influence the degree of tolerance, the present findings consistently indicate that endophytic bacteria possess substantial potential for survival in cadmium-contaminated environments.

The remarkable tolerance exhibited by isolates LO.3 and LU.2 highlights their potential application as candidates for bioremediation of cadmium-contaminated agricultural soils. Endophytic bacteria capable of tolerating elevated Cd concentrations may reduce heavy metal toxicity in the rhizosphere and plant tissues through immobilization, transformation, or sequestration of Cd ions, thereby contributing to improved plant growth and environmental sustainability. These findings also provide an important basis for further molecular identification and characterization of genes involved in cadmium resistance.

**Amylase Activity of Endophytic Bacterial Isolates**

The amylase activity assay demonstrated that four endophytic bacterial isolates were capable of hydrolyzing starch, as indicated by the formation of clear zones surrounding bacterial colonies on Soluble Starch Agar (SSA) after iodine staining (Figure 6). The diameter of the clear zone reflects the extracellular amylase activity of each isolate because starch degradation prevents the formation of the characteristic blue-black complex between iodine and starch. Consequently, a larger clear zone indicates greater amylolytic activity.

As presented in Table 5, isolate LO.1 exhibited the highest amylase activity, producing a mean clear zone diameter of 14.29 ± 0.91 mm, followed by isolate LO.4 (13.94 ± 0.75 mm), isolate LO.3 (11.45 ± 1.19 mm), and isolate LU.2 (9.42 ± 0.37 mm). These findings indicate considerable variation in extracellular enzyme production among the endophytic bacterial isolates, suggesting differences in their metabolic capacities and genetic potential for starch degradation.

The results of Tukey's Honestly Significant Difference (HSD) test (Table 6) further confirmed significant differences in amylase activity among the isolates ($p < 0.05$). Isolates LO.1 and LO.4 were assigned to the same statistical group (a), indicating that their amylase activities were not significantly different from each other and represented the highest enzymatic activities observed in this study. In contrast, isolate LO.3 belonged to group (b), demonstrating significantly lower amylase activity than LO.1 and LO.4 but significantly higher activity than LU.2. Isolate LU.2, which formed the smallest clear zone, was classified separately in group (c),

indicating the lowest amylolytic activity among all tested isolates.

The observed variation in amylase activity is likely associated with differences in the ability of each isolate to synthesize and secrete extracellular α-amylase. Amylase is an important hydrolytic enzyme that catalyzes the degradation of starch into maltose, glucose, and other smaller oligosaccharides, enabling bacteria to utilize complex carbohydrates as carbon and energy sources. The production of extracellular amylase is influenced by bacterial species, genetic characteristics, nutrient availability, environmental conditions, and enzyme regulatory mechanisms. Therefore, isolates producing larger clear zones are considered to possess greater enzymatic efficiency and a higher potential for starch degradation [19].

Endophytic bacteria producing extracellular amylase contribute significantly to the decomposition of plant-derived organic matter and nutrient cycling within plant tissues. Beyond their ecological functions, these bacteria have attracted increasing attention because of their potential applications in biotechnology, including organic waste biodegradation, composting, food processing, and industrial enzyme production. Recent studies have reported that microbial amylases remain among the most commercially important hydrolytic enzymes due to their high catalytic efficiency, substrate specificity, and broad industrial applicability [20-21].

Among the isolates evaluated, LO.1 exhibited the greatest amylase-producing potential while also demonstrating relatively high cadmium tolerance by maintaining growth at 100 ppm Cd. The coexistence of cadmium tolerance and extracellular amylase production suggests that LO.1 possesses multifunctional characteristics that may be advantageous for environmental biotechnology. Such characteristics may be relevant to integrated applications involving starch-rich organic waste biodegradation under heavy metal stress. However, the potential industrial application of LO.1 should be interpreted cautiously, as the present study was based on clear-zone measurements and qualitative assessment of Cd tolerance rather than comprehensive quantitative enzymatic assays. Further studies should quantify amylase production and activity, determine enzyme kinetics, and optimize operational parameters such as pH, temperature, substrate concentration, incubation time, and Cd concentration before considering process development or scale-up. Molecular identification and evaluation of cadmium removal efficiency are also required to further validate the biotechnological potential of this isolate [19].

## 5. CONCLUSION

This study demonstrated that endophytic bacteria from organically and inorganically cultivated lettuce exhibited diverse Cd tolerance and amylolytic potential. Endophytes from organically cultivated lettuce showed promising functional potential, with isolate LO.1 exhibiting both relatively high Cd tolerance and the strongest amylolytic activity. These findings highlight the potential of lettuce endophytic bacteria for environmental applications involving heavy metal stress and organic waste degradation. Further molecular identification, quantitative enzymatic assays, Cd removal assessment, and process optimization are required to validate their biotechnological potential.

## 6. REFERENCES

[1] L. Liu, S. Quan, L. Li, G. Lei, S. Li, T. Gong, Z. Zhang, Y. Hu, and W. Yang, "Endophytic bacteria improve bio- and phytoremediation of heavy metals," Microorganisms, vol. 12, no. 11, Art. no. 2137, 2024.

[2] A. Kumar, S. Singh, A. K. Gaurav, S. Srivastava, and J. P. Verma, "Endophytic

bacteria in plant health and sustainable agriculture: Recent advances and future prospects," Microbiological Research, vol. 268, Art. no. 127276, 2023.

[3] Y. Liu, H. Zhang, X. Wang, and S. Chen, "Diversity and characterization of endophytic bacteria associated with leafy vegetables," Frontiers in Microbiology, vol. 14, Art. no. 1189543, 2023.

[4] M. He, J. Wang, H. Yin, and Y. Chen, "Bacterial responses and resistance mechanisms to cadmium stress: A review," Journal of Hazardous Materials, vol. 424, Art. no. 127581, 2022.

[5] Y. Zhou, Z. Xu, Y. Tang, P. Wang, and Y. Li, "Microbial mechanisms of cadmium resistance and remediation in contaminated soils," Frontiers in Microbiology, vol. 13, Art. no. 857349, 2022.

[6] P. A. Patel, D. Dasgupta, A. Ray, and S. K. Suman, "Challenges and prospects of microbial α-amylases for industrial application: A review," World Journal of Microbiology and Biotechnology, vol. 40, no. 2, Art. no. 44, 2024.

[7] R. Singh, J. Kaur, and G. Kaur, "Amylolytic bacteria and their role in biodegradation of organic wastes: A review," Environmental Sustainability, vol. 5, no. 4, pp. 545–557, 2022.

[8] R. H. Wibowo, W. Darwis, Sipriyadi, M. Adfa, E. Silvia, R. Wahyuni, D. A. Sari, and Masrukhin, "Bakteri penghasil amilase yang diisolasi dari ekoenzim limbah buah-buahan," Jurnal Biosilampari, vol. 4, no. 2, pp. 107–117, 2022.

[9] M. Jan, M. Shahid, Z. Shah, M. A. Khan, and M. Naeem, "Cadmium-tolerant plant growth-promoting bacteria improve plant growth and reduce cadmium accumulation under cadmium stress," Environmental Science and Pollution Research, vol. 29, no. 18, pp. 26587–26601, 2022.

[10] I. Afzal, Z. K. Shinwari, S. Sikandar, and S. Shahzad, "Plant beneficial endophytic bacteria: Mechanisms, diversity, host range and genetic determinants," Microbiological Research, vol. 221, pp. 36–49, 2019.

[11] S. Aswani, S. Kumar, and P. Singh, "Functional diversity of endophytic bacteria associated with horticultural crops and their biotechnological applications," Frontiers in Microbiology, vol. 14, Art. no. 1187654, 2023.

[12] Md. Mahmud, Md. S. Islam, and Md. M. Rahman, "Characterization of endophytic bacteria isolated from vegetable crops and their plant growth-promoting traits," Egyptian Journal of Biological Pest Control, vol. 31, Art. no. 118, 2021.

[13] R. Qian, F. Song, J. Xia, and R. Wang, "A glucuronic acid-producing endophyte Pseudomonas sp. MCS15 reduces cadmium uptake in rice by inhibition of ethylene biosynthesis," Frontiers in Plant Science, vol. 13, 2022.

[14] J. G. Cappuccino and C. T. Welsh, Microbiology: A Laboratory Manual, 12th ed. Pearson, 2019.

[15] M. T. Madigan, K. S. Bender, D. H. Buckley, W. M. Sattley, and D. A. Stahl, Brock Biology of Microorganisms, 16th ed. Pearson Education, 2021.

[16] M. J. Kim, Y. Moon, J. C. Tou, B. Mou, and N. L. Waterland, "Nutritional value, bioactive compounds and health benefits of lettuce (Lactuca sativa L.)," Journal of Food Composition and Analysis, vol. 49, pp. 19–34, 2016.

[17] H. Zhang, K. Wang, X. Liu, L. Yao, Z. Chen, and H. Han, "Exopolysaccharide-producing bacteria regulate soil aggregates and bacterial communities to inhibit the uptake of cadmium and lead by lettuce," Microorganisms, vol. 12, no. 11, Art. no. 2112, 2024, doi: 10.3390/microorganisms12112112.

[18] Y. Bai, K. Song, M. Gao, J. Ma, Y. Zhou, H. Liu, H. Zeng, J. Wang, and X. Zheng, "Using multi-omics to explore the effect of Bacillus velezensis SAAS-63 on resisting

nutrient stress in lettuce," Applied Microbiology and Biotechnology, vol. 108, no. 1, Art. no. 313, 2024, doi: 10.1007/s00253-024-13153-y.

[19] D. Pandey, S. Negi, and C. R. Soccol, "Current advances in microbial amylase production and industrial applications," Bioresource Technology Reports, vol. 15, Art. no. 100748, 2021.