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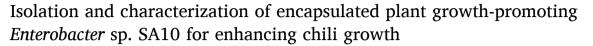
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Full Length Article



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ABSTRACT

Introduction: Plant growth-promoting rhizobacteria (PGPR) can increase plant growth and encapsulation of PGPR with biochar ensures the viability and survival of PGPR. However, this approach is still underexplored. Objectives: The objectives of this study: 1) to isolate and select a potential PGPR from rice rhizosphere based on plant growth-promoting characterization, 2) encapsulate the selected PGPR strain SA-10 using biochar and sodium alginate, and 3) assess the effect of encapsulated and non-encapsulated SA-10 on chili plant growth compared to a non-inoculated SA-10 and control.

Methodology: SA-10 was isolated from rice rhizosphere and characterized for plant growth promoting traits including, the nitrogen fixation, phosphate solubilization, production of siderophore, production of indole acetic acid and production of cellulose degradation enzyme. SA-10 was encapsulated in alginate beads along with biochar. A pot experiment conducted using encapsulated SA-10, non-encapsulated SA-10, and a control group to assess the effects of encapsulated SA-10 application on chili plants using complete randomized design (CRD) experimental design with four replications.

Results: About 28 bacteria isolates were isolated from the rhizosphere and endospheric of rice crops to determine their effects on the growth of chili plants. The isolates were characterized with zinc, phosphate, and potassium solubilization, siderophores production, and cellulose degradation. The findings showed isolate SA-10 solubilized zinc at a rate of 41.6 %, phosphate at 50 %, and potassium at 76.9 %. It also fixed nitrogen, produced siderophores and degraded cellulose. The isolate also produced the highest IAA at 4.007 mg/L. Isolate SA-10 was then encapsulated in a mixture of biochar and sodium alginate. The results showed that plants treated with encapsulated SA-10 had significantly higher leaf area (3.68 cm²), leaf diameter (6.55 cm), and dry weight (0.39 g) compared to those treated with non-encapsulated and the control treatment. Through 16S rRNA gene sequencing, it was identified that SA-10 belongs to the *Enterobacter* genus, closely resembling *Enterobacter chuandaensis*.

Conclusion: The findings suggest that *Enterobacter* sp. SA-10 enhances plant growth, and the use of encapsulated SA-10 with biochar contributes to the growth of chili plants. The encapsulated Enterobacter sp. SA-10 has a promising potential in enhancing crop yield production and promoting sustainable agriculture.

1. Introduction

Chili (*Capsicum annum* L.) is the universal spice widely cultivated crop of the Solanaceae family. There are several types of plant chili cultivated in the world and which are the most distributed in Asia. The

genus Capsicum encompasses five well-known species, namely Capsicum. annuum, Capsicum frutescens, Capsicum pubescens, Capsicum baccatum, Capsicum chinense. Capsicum annuum L. and Capsicum frutescens L. are the most widely cultivated chili peppers in Malaysia (Hassan et al., 2019). In chili plantation, chemical fertilizers are widely used by Malaysian

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farmers to obtain a high chili yield to meet the demand. Sofyan et al. (2019) highlighted the long-term effects of inorganic fertilizer usage, including soil acidification, decreased soil fertility, and pollution of air and water. The negative consequences of inorganic fertilizer usage have prompted the exploration of alternative fertilization methods to mitigate these issues. Plant growth-promoting rhizobacteria (PGPR) has been found widely explored to enhance sustainability plantation as nitrogen fixer, phosphate solubilizer, zinc solubilizer, potassium solubilizer, growth hormone producer and siderophore producer (Othman et al., 2022). In a recent study, Kashyap et al. (2022) highlighted the positive effects of Pseudomonas fluorescens PDS1 and Bacillus subtilis KA9 in terms of chili plant growth and its biocontrol efficiency against Ralstonia solanacearum. Other than that, PGPR also have mechanisms to help host plant through solubilization or release of nutrient elements from sparingly available sources while not causing any harm to their plant host (Boleta et al., 2020). Studies have shown that Azotobacter sp., Bacillus megaterium, Bacillus coagulants, Flavobacterium sp. and Pseudomonas atmuta relatively effective to provide an increased on the growth and seed germination of chili plants from the variety PM999 (V1) and Kiyo F1 (V2), suggesting that they have the potential to enhance crops growth and yield (Syamsuddin et al., 2022; Hu et al., 2023). This finding opens possibilities for targeted application in the future.

Other than chili plant, inoculation of Azospirilum brasilense on wheat plants caused the top part of the plants to have more micronutrients (Boleta et al., 2019; Iqbal et al., 2023a). This inoculation has the potential to reduce reliance on chemical fertilizer while effectively enhancing plant growth and nutrition. Commensalism occurs when bacteria colonize varied parts of plant foliage without inflicting harm or providing considerable benefits to the host plant (Iqbal et al., 2023a; Iqbal et al., 2023b). The presence of unfavorable environmental conditions and abiotic stresses presents a major challenge to the adoption of sustainable agricultural methods for chili (Capsicum annuum L.) (Achari and Kowshik, 2018). Abiotic stresses, such as drought, salinity, and heat, negatively impact crop yields but PGPR can lessen the bad effects of stress as occurred in the Brassicaceae family (Casagrande et al., 2022; Jalal et al., 2021; Jalal et al., 2023). Plant growth-promoting rhizobacteria can help plants grow in several mechanisms, such as breaking down nutrients, making phytohormones, and fixing nitrogen and it also can integrate with zinc nanomaterial (He et al., 2023).

Plant growth-promoting rhizobacteria have many beneficial properties, however, effectively protecting these microorganisms can be challenging due to environmental stress (Adedeji et al., 2020). Encapsulation of PGPR with sodium alginate has emerged as a guaranteed approach to enhance the survival and activity of beneficial bacteria in plants. This method causes the progressive release of encapsulated bacteria into the soil, enhancing the lifespan of bacterial isolates and thus, boosting their potency. (Lee at al. 2021). Martínez-Cano et al. (2022) reviewed alginate-encapsulated plant growth-promoting bacteria for diverse agricultural crops. Microbial encapsulation may safeguard beneficial microbes and improve plant root colonization. Encapsulation might help to protect the heavy metal effects on microbial communities in soil which contribute to optimum condition in nutrient cycling and fertility level in soil (Iqbal et al., 2023b; Iqbal et al., 2024). To develop the encapsulation formulation, other organic mixtures might help to boost plant growth like biochar (Xu et al., 2022). A study showed that biochar treatment on chili production in Bandung, Indonesia (Rematwa et al., 2022). These studies demonstrate the efficacy of using biochar and microbial inoculants for sustainable crop production. However, sodium alginate-biochar encapsulation remains underexplored as biofertilizer production including Malaysia.

Given the advantages of PGPR and encapsulation technique as potential biofertilizer, this study proposed three hypotheses. The hypotheses are as follows: 1) PGPR can be isolated and selected based on its plant growth-promoting traits; 2) encapsulating SA-10 with biochar and sodium alginate protects the PGPR; and 3) determining whether encapsulated SA-10 can be used as a biofertilizer for chili cultivation.

Thus, the objectives of this study are to: 1) isolate, identify and select a potential PGPR from rice rhizosphere based on plant growth-promoting characterization, 2) encapsulate the selected PGPR using biochar and sodium alginate, and 3) assess the effect of encapsulated and non-encapsulated SA-10 on chili plant growth compared to a non-inoculated SA-10 and control.

2. Materials and methods

2.1. Location of study

Rice soil samples were obtained to isolate microorganisms from the non-rhizosphere, rhizosphere, and endospheric environments. The samples were collected from a rice irrigation region and planted using the MR 269 rice variety. The site is in Sungai Udang, North Malacca, Southern Malaysia (coordinates: $2^{\circ}15'45''N$, $102^{\circ}9'9''E$). A soil auger was utilized to collect soil samples from a depth of 15 cm. The samples were obtained using an auger during the rice vegetative period (about 40 days after seeding). It was assumed that the bacteria were active during this period. The samples were chilled to 4 °C to reduce microbial activity.

2.2. Preparation of bacteria isolation from rice cultivation

Root samples and soil were collected from rice plants growing in the field. The sample was grouped into three categories: non-rhizosphere, rhizosphere, and endophytes. Bacteria was isolated from the nonrhizosphere; 10 g of sample soil was weighed, and 90 mL of sterile water was added into the glass bottle. The solution was shaken until homogenized. Then, 0.1 mL of the solution is directly poured and spread using sterilized glass rod onto the nutrient agar (NA) plate. The plate is placed upside down and incubated at 28 $^{\circ}\text{C}$ for 48 h. Bacteria isolate from the rhizosphere, the root part sample was cut and gently washed. Then, one g of root sample was weighed, and 9 mL of sterile water. The solution was shaken until homogenized. The solution is poured and spread on the nutrient agar exactly like the soil sample in the previous step. The isolation of endophytic bacteria method was adopted from Sondang et al. (2019). The root sample was cut and sterilized with ethanol and sodium hypochlorite. One gram of root was scraped with distilled water into test test. A small amount of the solution was spread onto NA and incubated to observe colony formation.

2.3. Characterization of plant growth-promoting bacteria

Six isolates with different colors and morphology were selected for the following test: Nitrogen fixation qualitative test (Dobereiner and Day, 1976), phosphate solubilizing test (National Botanical Reserch Insitute Phosphate (NBIRP), potassium solubilizing test (Aleksandrov et al., 1967), zinc solubilization ability test (Saravanan et al., 2003). qualitative cellulose degrading enzyme production test (Padaria et al., 2014), qualitative siderophore production test (Schwyn and Neilands, 1987) and indole-acetic acid (IAA production test) (Gordon and Weber et al. 1951) and Qualitative Chrome Azurol-S test for siderophore production.

2.4. Encapsulation of plant growth-promoting bacteria

Encapsulation of an inoculant was modified from the method by Bashan (1986). About one loopful of bacteria isolates SA-10 were transferred into a liquid nutrient broth medium and were grown for 24 h at 30 °C. Fifty mililitres of bacterial culture was aseptically mixed with 2 % sodium alginate powder and stirred gently for one hour. Then, the mixtures of bacteria, sodium alginate and biochar were drawn using a syringe into a solution of 0.1 M Calcium dichloride. Beads immediately formed in the CaCl $_2$ solution and beads were washed twice with sterilized tap water and then were dried for one to two hours.

2.5. Non-encapsulated plant growth-promoting bacteria preparation

One loopful of bacteria Isolates SA-10 were transferred into a nutrient broth media. It was incubated for 24 h at 30 $^{\circ}\text{C}$ in incubator. After 24 h, the liquid was centrifuged at 7000 rpm for 10 mins and washed the liquid with phosphate buffered saline (PBS) pH 7.4 three times where the cell pelleted became small. PBS pH 7.4 is prepared following ingredients, 8 g NaCl, 200 mg KCl, 1.44 g Na₂HPO₄, 245 mg KH₂PO₄ to the 800 mL of distilled water. The solution was adjusted to pH 7.4 before applying to the plant.

2.6. Seed, soil, and plant preparation

In this study, chili (Capsicum annum L.) was used as the model plant organism. Chili seeds were imbibed for 24 h in tap water at room temperature. Then, the seed germinated in a tissue towel. The seed that grows was transferred into the seedling tray for 25 days. After 25 days, the plant was transferred to a polybag with the treatment. The soil was collected from the farm site at UiTM Jasin and mixed well at the ratio of 4:1 of topsoil and sand. This soil was sieved to remove gravel and other material. The media was mixed with the following composition for three kg for topsoil, and one kg sand was put into the pot $15' \times 16'$. The experiment was designed as a completely randomized design (CRD) with three treatments and four replications. A total number of 12 pots were used for the experiment. The chili crop was grown as a control, nonencapsulated SA-10, and encapsulated SA-10. For non-encapsulated (liquid bio-fertilizer). Growth parameters such as plant height (shoot length), leaves number per plant, leaves width (leaf area), diameter of leaves, fresh and dry weight were measured.

2.7. 16S rRNA gene sequencing molecular identification of soil microbial isolates

Isolate SA-10 was selected and incubated in sterile nutrient broth for 24 h at 37 $^{\circ}$ C. The DNA genome was extracted using HiYieldTM Genomic DNA Mini Kit (Bacteria)(Real Biotech, Taiwan). 16S rDNA was polymerase chain reaction (PCR) amplified using thermal cycler (peqSTAR, Germany) and universal bacterial primers 27F Forward (5' AGA GTT TGA TCC TGG CTC AG 3') and 1492R Reverse (5' TAC GGT TAC CTT GTT ACG ACT T 3'). The total volume of 50 uL per reaction PCR was prepared: deionised water (31.5 µL); 25 mM magnesium chloride (Lucigen, USA) (3 µL); 10 mM dNTP (Lucigen, USA)(4.0 µL); 10x reaction buffer (5.0 μ L), 100 uM forward universal primers (0.5 μ L); 100 μ M reverse universal primers (0.5 μL); 5 U/mL EconoTaq Polymerase (Lucigen, USA) (0.5 μL) and genomic DNA as template (50 to 200 ng)(5 μL). The PCR thermal cycle setting are: (a) preheating at 94 °C for 2 min; (b) denaturation (35 cycles at 94 °C for 30 s); (c) annealing (52 °C for 30 s); (d) extension (72 °C for 1 min); (d) final elongation (72 °C for 10 min). Purification of PCR product was done using $HiYield^{TM} \; Gel/PCR$ DNA Mini Kit (Real Biotech, Taiwan). Sequence was obtained by Apical Scientific Sdn. Bhd.

The forward and reverse sequences, multiple alignment and phylogenetic tree were constructed using MEGA Software Version 11.0.8 (Tamura et al., 2021). The 16S rRNA sequence of SA 10 and related species were used to generate phylogenetic locality of the strain by comparing SA 10 sequence with published sequences in NCBI Genbank using Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/) and EzBiocloud (EzBioCloud.net). The phylogenetic tree was constructed with the following setting: (a) analysis by statistical neighbour-joining method, (b) maximum composite likelihood model; (c) 1,000 replicates bootstraps.

2.8. Statistical analysis

The experiment was conducted using a fully randomized design with four replications. Data analysis was carried out using one-way ANOVA

with the SPSS statistical program. The Tukey's test was utilized to compare the treatment means at 5 % significance level.

3. Results

3.1. Bacteria isolation from the rice cultivation soil

In this study, about 28 bacteria isolates with different color and morphology were successfully isolated from the rice cultivation soil and plant, providing a foundation for comprehensive screening. Non-rhizosphere, rhizosphere, and endophytes bacteria were observed to fully grown on agar plate only after two days of incubation. However, non-rhizosphere and rhizosphere bacteria showed a high bacteria population with the amount exceeding 300 colony forming unit (CFU) after two days of incubation and only four endophytes CFU per plate after two-day incubation was observed (Supplementary Fig. 1).

3.2. Characterization of PGPR and encapsulation using biochar and sodium alginate

For the zinc solubilization test, isolate SA-8 showed the highest zinc oxide solubility index at 41.66 %. Isolate SA-8, SA-10, and SA-17 showed the highest phosphate solubilization ability at 50 % (Table 1). The highest potassium solubilization activity (90.90 %) was SA-9 (Table 1). All the isolated bacteria showed positive results for nitrogen fixation and siderophores production (Supplementary Fig. 2). In the cellulose degradation, only five isolated bacteria showed positive results, but SA-8 showed negative results. The highest production of indole-acetic acid (IAA) was 4.00 (mg/L) by isolate SA-10 (Table 1). Thus, SA-10 was chosen and successfully encapsulated using biochar and sodium alginate with 2–3 mm size beads as a potential biofertilizer in the plant growth performance test.

3.3. Plant growth performance of chili plant

The treatment with encapsulated bio-fertilizer showed highest of plant height, where the mean value was 18.25 cm per plant. The nonencapsulated bio-fertilizer the mean values was 14.5 cm per plant (Fig. 1). Control treatment showed lower plant height at 7.37 cm. A comparison of the treatment means showed that the encapsulated PGPR was significantly different from the non-encapsulated PGPR and control treatments in terms of plant height (Fig. 1). For number of leaves per plant, there was no significant difference among all the treatments at p < 0.05 (Fig. 1). The chili plant treated with encapsulated biofertilizer showed a significantly high measurement of leaf area (3.68 cm²) compared to non-encapsulated biofertilizer treatment (3.10 cm²) and control (2.13 cm²) at p < 0.05 (Fig. 1). The chili plants treated with encapsulated PGPR showed highest leaf diameter (6.55 cm) compared to non-encapsulated biofertilizer treated plant at 5.10 cm and control treatment (3.5 cm) at p < 0.05 (Fig. 1). However, the fresh weight of all the treatments showed there were no significant difference at p < 0.05for encapsulated biofertilizer plant, non-encapsulated and control (Fig. 2). For dry weight, encapsulated biofertilizer showed significantly highest reading at 0.39 g compared to non-encapsulated biofertilizer treatment (0.28 g) and control (0.18 g) at p < 0.05 (Fig. 2).

3.4. Molecular identification based on 16S rRNA gene sequence

Based on a similarity comparison using BLAST and EzBiocloud, SA 10 was closely related to *Enterobacter ludwigii, Leclercia adecarboxylata, Enterobacter bugandensi, Enterobacter chuandaensis, Enterobacter kobei,* and *Enterobacter sichuanensis* (Fig. 2). All the species showed pairwise sequence similarities up to 99.0 % (Supplementary Table 1).

Table 1Biochemical properties of bacteria isolates.

| Isolates | Solubilization index ZnO (%) | Phosphate solubilisation ability (%) | Potassium solubilisation ability (%) | Nitrogen fixation | Siderophores production | Cellulose degradation | IAA production (mg/L) |
|----------|---------------------------------|--|--|----------------------|----------------------------|--------------------------|--------------------------|
| SA-8 | 41.66.a | 50.00a | 81.81b | + | + | - | 2.559b |
| SA-9 | 19.04.b | 31.57c | 90.90a | + | + | + | 2.099c |
| SA-10 | 13.04.c | 50.00a | 76.92c | + | + | + | 4.007a |
| SA-14 | 20.00.d | 40.00c | 66.66d | + | + | + | 1.901c |
| SA-17 | 31.25.e | 50.00a | 90.00a | + | + | + | 2.822b |
| SA-20 | 21.24.f | 47.61b | 80.00b | + | + | + | 2.23b |

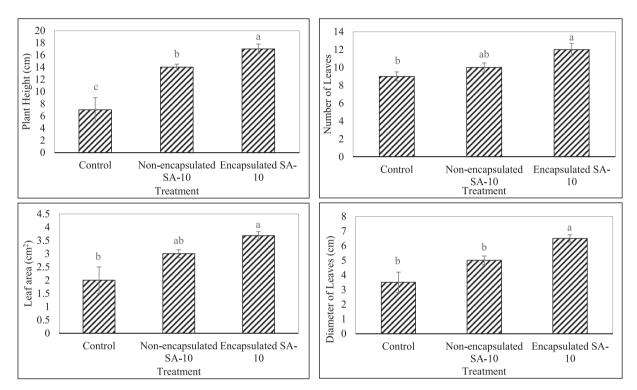


Fig. 1. Mean value for different treatments was observed on plant height, leaf number per plant, leaf area, diameter of leaf at p < 0.05.

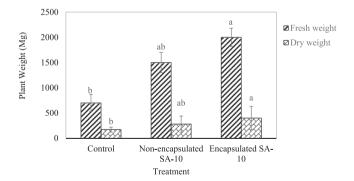


Fig. 2. Mean value for different treatments was observed on plant height, leaf number per plant, leaf area, diameter of leaf at p < 0.05.

4. Discussion

The study successfully isolated 28 bacterial isolates from rice soil samples. Previous studies have explored bacterial isolates associated with rice plants relevant to these findings. Othman et al. (2022) explored that isolated Malaysian rice root rhizosphere and endophytic bacteria, demonstrating their potential biocontrol activity and successful isolation from plant. Similarly, Sharma and Mallubhotla, (2022) found

endophytes showed lower carbohydrate use and stress tolerance compared to rhizosphere and non-rhizosphere population. These isolation studies are consistent with previous findings that explore riceassociated bacteria communities. The current findings that endophytes grew slower than rhizosphere bacteria. This support differences in niche populations which were influenced by carbohydrate utilization. The finding of niche-specific growth rates suggests microbial adaptations deserve deeper study to elucidate colonization mechanisms due to changes in microbial community structure (Nie et al., 2023). Another study also highlights the potential for discovering suitable PGPR among candidates within endophytic habitats. In contrast, a study of sugarcane found a significant number of nitrogen-fixing bacteria in sugarcane roots and leaves, which were influenced by soil salinity levels (Pirhadi, 2018). Approximately 55 saline soil endophytic strains were found in saline soils which are higher than the rhizosphere. While the study focused only on nitrogen fixer, Bacillus sp., the current studdy focus on culturable bacteria population in rice samples. Thus, differences in population might be due to differences in the environment where the bacteria might obtain their needs and also their culturability.

In this study, encapsulated PGPR SA-10, was identified as a potential biofertilizer candidate due to its consistently high activity throughout PGPR characterization test. The hypothesis that SA-10 isolate can promote plant growth via multiple mechanisms and that encapsulation safeguards its viability are supported by the results. The study's findings

on SA-10 bacteria's multifunctional plant growth-promoting features match previous research on PGPR and crop nutrition and yields. Similarly, Bhatt and Maheshwari (2020) identified Bacillus megaterium from cow manure and showed that they promote plant development, including Capsicum annuum L. Indole-3-acetic acid produced by SA-10 bacteria may promote plant development, via specific enzymatic pathways. Plant growth-promoting rhizobacteria use auxin production as one of their main direct ways to benefit plants (Wagi and Ahmed, 2019). This shows that SA-10 bacteria's IAA synthesis directly promotes plant development. Similarly, Srithaworn et al. (2023) examined the effects of Pseudomonas protegens RY2 and Bacillus megaterium on green soybean growth that showed similar increase due to plant growth promoting traits by both bacteria. While, Zahra et al. (2023) found rhizosphere Bacillus sp. predominates in the wheat (Triticum aestivum L.) compared to endospheric like current study, the findings still demonstrate the diverse roles of PGPR in promoting plant growth under stress condition. Through these findings, PGPR show high ability to promote plant growth through nutrient acquisition mechanism such as synthesizing IAA, improving stress tolerance, and acquiring nutrients.

Encapsulated SA-10 with biochar showed encouraging outcomes in enhancing plant growth parameters such as leaf diameter, leaf area, and dry weight which supports the hypothesis that encapsulated SA-10 can be used as a biofertilizer for chili cultivation. Queroz-Sarmiento et al. (2019) encapsulated PGPR which led to a significant 35 % increase in chili seedling growth compared to the control group. Coatings like sodium alginate, can promote bacterial survival and better plant growth in arid soil (Riseh et al., 2021). Zego et al. (2019), found that encapsulated Azospirillium brasilience with alginate-humic acid-maintained shelf-life viability, supporting seedlings growth. In contrast, Beula et al. (2019) found humic acid helps improve encapsulation. stressed on soil and climate can influence plant phenotype. In the current study, biochar helped successfully increase chili cultivation. Similarly, a finding showed that biochar protects encapsulated bacteria by providing habitat and protecting them from stress, maintaining viability which can help for plant growth (Lee et al., 2021). Encapsulation methods like sodium alginate and biochar mixture are highly effective in enhancing the effects of PGPR on plant growth. However, it should be noted that further validation is required in real-life, changeable field situations. More validation and research are required under varied agro-climate (Xue et al., 2023; Xiao et al., 2019).

The findings from current study showed SA-10 was molecularly identified as *Enterobacter chuandesis*. Similarly, *Enterobacter* sp. has also been studied as a biofertilizer in black pepper growth and root disease suppression (Nguyen et al., 2020). Similarly, Lucero et al. (2020) examined the effects of fermentation, endophytic bacteria, and bioactive compounds on plant growth and nutrient concentrations on soybeans, maize and peanut using phosphate-solubilizing *Enterobacter* sp. Endophytic *Enterobacter ludwigi* EB4B has also been researched for biological control and illness suppression in tomato growth (Bendaha and Belaouni, 2020). The discovery of SA-10 as *Enterobacter* sp. links this study to a broader range of research that highlights the ability of this genus to enhance plant growth. Research has shown that *Enterobacter* sp. has the potential to enhance crop yields by promoting nutrient cycling, suppressing diseases, and producing phytohormones in various crop systems (Fig. 3).

5. Conclusion

The results of this study showed 28 bacteria isolates were successfully isolated from rice soi. Bacterial strain SA-10 has several characteristics that promote plant growth in a laboratory environment. SA-10 was able to dissolve zinc at a rate of 41.6 %, phosphate at a rate of 50 %, and potassium at a rate of 76.9 %. Additionally, SA-10 fixed nitrogen positively, produced siderophore, degraded cellulose, and created growth hormone and IAA at a rate of 4.007 mg/L. The 16 s rRNA molecular analysis identified the bacterial strain as *Enterobacter chuandaensis*, which has significant potential for enhancing plant growth. The use of encapsulated SA-10 resulted in better plant growth in various ways. Plants treated with encapsulated SA-10 had significantly higher leaf area (3.68 cm²), diameter (6.55 cm), and dry weight (0.39 g). Encapsulated PGPR SA-10 biofertilizer can enhance soil health in chili farming. It reduces the need for overusing chemical fertilizers, helps in

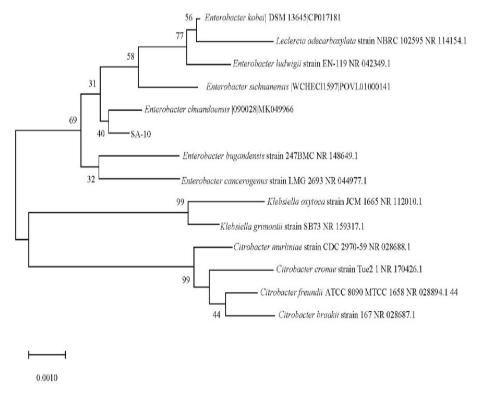


Fig. 3. Neighbor-joining phylogenetic tree of SA 10 based on 16S rRNA sequences analysis.

policy making for sustainable agriculture approach and has the potential to be a global climate-resilient strategy. However, more research is needed to improve formulations, assess field performance, and commercialize innovations.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at $\frac{https:}{doi.}$ org/10.1016/j.jksus.2024.103197.

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