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Research article

Cellulolytic activity of bacteria isolated and selected from paddy soil in Vinh Long province, Vietnam

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Abstract

Importance of the work: The cellulose-degrading bacteria studied may be potential candidates to enhance the process of rice straw decomposition in rice cultivation.

Objectives: To isolate and select thermotolerant bacterial isolates with cellulose–degrading activity to supplement the composting process of microbial organic fertilizer from rice straw.

Materials and Methods: The cellulose-degrading ability of the bacterial isolates from the paddy soil samples was determined by staining carboxylmethylcellulose (CMC) plates with Congo red solution. The cellulose–degrading bacterial isolates were identified based on morphological and biochemical characteristics, the API 20E kit and 16S rRNA gene sequencing.

Results: Overall, 28 of the 36 isolates were capable of degrading CMC with halo diameters ranging from 1.0 ± 0.0 to 10.0 ± 0.0 mm. The cellulose-degrading ability of isolate ĐRVL10 was highest at 40°C and pH 7.0. In particular, isolate ĐRVL10 had the ability to decompose cellulose at 45–55°C. The bacterial isolates had the ability to decompose rice straw after 15 d on tryptic soy broth (TSB) medium, with decomposition rates ranging from 23% to 63%, while on nutrient broth medium the range was 26%–53%. Based on the morphological and biochemical characteristics using the API 20E kit and 16S rRNA gene sequencing analysis, two bacterial isolates (ĐRVL7 and ĐRVL10) were identified as two different species in the genus *Bacillus* and *Neobacillus, respectively*.

Main finding: The bacterial isolate had the ability to decompose at high temperature and also to decompose straw strongly under *in vitro* conditions.

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Introduction

Rice (*Oryza sativa* L.) is one of the key crops grown mainly in Vietnam and the Mekong Delta, including Vinh Long province. In 2023, the Department of Crop Production (Ministry of Agriculture and Rural Development) estimated that the country cultivated approximately 7.11 million ha of rice, an increase of nearly 10,000 ha compared to 2022, with an output of more than 43 million t of rice (Nguyen Phuc, 2024). Furthermore, localities in the Mekong Delta region were predicted to cultivate approximately 3,816 million ha of rice in 2023, with an estimated output of nearly 24 million t. In 2023, rice exports were expected to reach 8.2 million t, with an export turnover of USD 4.7 billion, an increase of 14.7% in volume, and an increase of 35.7% in value (Nguyen Phuc, 2024), contributing to Vietnam being one of the top-three rice exporting countries globally (Statista.com, 2018).

Rice straw is considered a by-product after harvesting rice and is usually, the straw is removed during the harvesting process and heaped or spread on the harvested area, depending on whether the harvest is by hand or machine (Gummert et al., 2020). Globally, about 800–1,000 million t of rice straw are produced annually, with the rice-growing countries in Asia producing about 600–800 million t of rice straw annually (Gummert et al., 2020). It is predicted that this amount will continue to increase rapidly due to the shorter turnaround time required for rice intensification (Gummert et al., 2020). Rice straw is an organic source that is extremely beneficial for plants if used properly. However, most of the rice straw buried in the soil is not completely decomposed because the treatment time is too short, leading to sulfide accumulation that adversely affects the growth and development of rice plants (Gao et al., 2004). Currently, the common practice in a locality is to either bury or burn these by-products, which not only wastes valuable resources but also leads to soil and water pollution, increases greenhouse gas emissions and can be a source of pathogens that negatively affect public health (Son et al., 2017; Nguyen and Nguyen, 2018; Phuong et al., 2021). Therefore, it is essential to find advanced solutions to utilize these by-products by converting them into a more useful form.

Cellulose—$(C_6H_{10}O_5)_n$—is a polysaccharide consisting of a linear chain of several hundreds to many thousands of β (1→4) linked D-glucose units, making it durable and difficult to decompose (Heinze, 2016). In nature, the decomposition of cellulose-containing materials occurs due to the activity of microorganisms. These microorganisms secrete the cellulase enzyme system (endoglucanase, exoglucanase and β-glucosidase), which breaks down the molecular structure of cellulose into simpler structures. This biological decomposition process results in the decomposition of cellulose-containing materials, returning nutrients to the soil and allowing them to be used by plants. As a result, cellulose-degrading microorganisms play a crucial role in the carbon cycle in nature (Liu et al., 2021a; Datta, 2024). Studies have shown that these microorganisms are abundant and exist in various environments, including soil, water, decomposed plant residues and even the digestive systems of herbivores and insects (Mandic-Mulec et al., 2015; Vimal et al. 2016). Bacterial isolation studies have identified cellulose-degrading microorganisms as filamentous fungi, actinomycetes, bacteria and (sometimes) yeasts (Chen, 2014). Among these, bacteria have many desirable characteristics such as high enzyme biosynthesis ability, enzyme complex expression, short generation time, high temperature tolerance and the ability to be genetically modified to enhance enzyme biosynthesis (Adebami and Adebayo-Tayo, 2020; Dadwal et al., 2021). However, the ability to synthesize cellulase enzymes and biodegrade cellulose depends on the species, compatibility with the environment and the type of cellulose-containing material (Thapa et al., 2020). In other studies, thermophilic native cellulose-degrading bacterial isolates were isolated from the soil for cellulase enzyme production and organic fertilizer production from agricultural by-products (Khosravi et al., 2022; Kognou et al., 2022). The strong cellulose-degrading bacterial isolates obtained from soil include *Bacillus*, *Brevibacillus* and *Chryseobacterium*. Identifying and utilizing bacterial isolates with strong cellulose-degrading abilities is crucial because they can benefit the environment and economy. Therefore, further research and application of these bacterial isolates could have a substantial impact on the environment and economy of the Mekong Delta region.

Materials and Methods

Sample source for bacterial isolation

Paddy soil was collected in the Long Ho, Mang Thit, Tra On and Vung Liem districts of Vinh Long province, Vietnam (Fig. 1) to isolate cellulose-degrading bacteria. Soil samples were collected at a depth of 0–15 cm from four different locations on the same sampling site. After being transferred to the laboratory, the soil sample was dried, ground finely and then mixed and used to isolate bacteria.

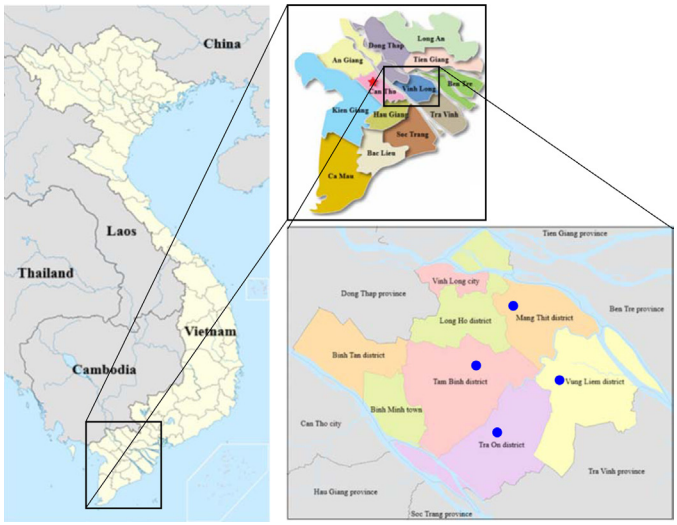

Fig. 1 Soil sample collection sites (blue dots) for cellulose-degrading bacteria isolation, Vinh Long province, Vietnam

Isolation of bacteria

Cellulolytic bacteria were isolated from paddy soil samples based on the method of Shamshitov et al. (2023). First, the soil sample (10 g) was mixed with 90 mL of sterile distilled water on a shaker (OS-350D-C; Digisystem Laboratory; Taiwan) at 120 revolutions per minute (rpm) for 24 hr. Next, the sample was serially diluted to 10^{-6}. An aliquot of 100 µL of sample at each dilution was spread onto a CMC medium (Baharuddin et al., 2010) consisting of: 1 g/L $(NH_4)_2SO_4$, 1 g/L K_2HPO_4, 0.5 g/L $MgSO_4.7H_2O$, 0.001 g/L NaCl, 10 g/L CMC and 18 g/L agar (pH 7.0). Plates were incubated for 2-3 d at 37°C until colonies had grown. Finally, colonies were purified on CMC medium and stored in glycerol (30% volume per volume) at -40°C for further research.

Cellulolytic activity of isolated bacteria

The ability of isolated bacterial isolates to degrade cellulose was determined according to Peristiwati et al. (2018). Bacterial isolates were grown in tryptic soy broth (TSB) medium (Himedia; India) on a shaker at 120 rpm for 24 hr. Then, the bacterial cell solution was centrifuged at 6,000×*g* for 5 min (Centrifuge 5430R; Eppendorf; Germany). The supernatant (80 µL) was added into the wells on CMC medium. Next, the plates were incubated in the refrigerator for 15–30 min and then incubated overnight at 30°C, followed by staining with Congo red solution (1 g/L) for 15 min and washing with 1M NaCl solution. The halo zone (clear zone), appearing around the well indicated that a bacterial isolates was capable of degrading cellulose. The halo diameter was calculated using the formula: Halo diameter = D-d, where D is the halo diameter (in millimeters), including the well diameter, and d is the diameter of the well (6 mm). In this study, the cellulose-degrading activity of each bacterial strain was repeated three times.

Effect of cultivation temperature on cellulose-degrading activity of bacteria

Eleven bacterial isolates (ĐRVL5, ĐRVL6, ĐRVL7, ĐRVL10, ĐRVL11, ĐRVL17, ĐRVL20, ĐRVL23, ĐRVL25, ĐRVL26 and ĐRVL27) with large halo zone mean diameters ± SD of 7.0±0.0, 6.0±0.0, 9.0±1.0, 10.0, 7.23±0.06, 6.4±0.1, 7.13±0.12, 6.47±0.06, 8.23±0.06, 7.17±0.06, and 6.03±0.06 mm, respectively, were selected to evaluate the effect of temperature on cellulose-degrading ability. First, the bacterial isolates were preliminary screened for their ability to grow at different temperatures (35°C, 40°C, 45°C, 50°C, 55°C and 60°C). These isolates were cultured in separate Erlenmeyer flasks containing 100 mL of CMC medium and incubated on a shaker at 120 rpm for 12 hr at one of the above temperatures. The bacterial isolates growing at the highest temperature were selected to evaluate cellulose-degrading activity. Next, the selected bacterial isolate was cultured in TSB medium at temperatures of 35°C, 40°C, 45°C, 50°C, 55°C and 60°C on a shaker at 120 rpm for 24 hr. The cellulose decomposition was performed similarly to the above-presented steps, with each treatment repeated three times.

Effect of pH of medium on cellulose-degrading activity of bacteria

Bacterial isolates capable of degrading cellulose at the highest temperature in the above experiment were selected to evaluate the effect of pH on their ability to decompose cellulose. As above, the bacterial isolates were cultured in TSB at pH values of 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0 on a shaker at 120 rpm for 24 hr. Then, the cellulose-degrading capacity was determined in a similar manner to the above-mentioned steps, with each treatment repeated three times.

Evaluating ability of isolated bacteria to decompose rice straw

Four bacterial isolates (ĐRVL3, ĐRVL5, ĐRVL7 and ĐRVL10) that had the strongest cellulose-degrading activity, were selected to evaluate their ability to decompose rice straw.

First, the bacterial isolates were cultured overnight in TSB and nutrient broth (NB) media (Himedia; India) on a shaker at 110 rpm. Bacterial biomass was collected after centrifuging the bacterial culture broth at 10,000×*g* for 10 min. Then, the bacterial biomass was prepared at 10^8 colony forming units (CFU)/mL by comparison with standard MacFarland 0.5. The rice straw was cut into 1–2 cm pieces and then dried at 105°C to a constant weight. After drying, the rice straw was ground finely, sterilized (121°C for 15 min), and then 1 g was placed into a plastic tube containing 30 mL of TSB and NB media inoculated with 0.3 mL of bacterial density at 1×10^8 CFU/mL. The ability to decompose rice straw was evaluated by weighing the substrate after 15 d of bacterial incubation. The decomposition efficiency was calculated using the formula: H (%) = [(Initial weight of straw - Weight of straw after 15 d of bacterial incubation) / Initial weight of straw] × 100%. Each bacterial strain's capacity to decompose rice straw was tested three times in this investigation.

Identification of cellulose degrading bacteria

Bacterial isolates were identified based on polymerase chain reaction (PCR) and sequencing of the 16S rRNA gene segment with the primer pairs 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-TACGGYTACCTTGTTACGACTT-3' (Heuer et al., 1997). First, bacterial DNA was extracted using an iVAaDNA Extraction Kit P (Thermo Scientific; USA) according to the manufacturer's instructions. Then, the amplified reaction (25 µL) was carried out with the following ingredients: 12.5 µL iStandard iVAPCR Master Mix (Thermo Scientific; USA); 9.5 µL double distilled water; 0.5 µL primer 27F (20 pmol); 0.5 L primer 1492R (20 pmol); and 2 µL sample DNA. Thermal cycling was performed with an initial denaturation at 94°C for 5 min, then 30 cycles including denaturation at 94°C for 1 min, primer annealing at 63°C for 1 min, extension elongation at 72°C for 2 min and final elongation at 72°C for 10 min. The PCR products (1,500 bp) were analyzed based on electrophoresis in 1.5% agarose gel and imaged using an Analytik Jena gel imaging system (Analytik Jena; Germany). Additionally, bacteria were identified using an API 20E kit (BioMerieux; France) combined with characteristics such as colony morphology, cell shape, motility, Gram reaction, spore stain, oxidase and catalase reaction (Zaved et al., 2008; Ahmad and Zargar, 2017).

Data analysis

Descriptive statistical methods were used to determine values as mean ± SD. Data were analyzed using analysis of variance in MiniTab 20 software (Minitab; USA) to investigate the variation in inhibitory activity amongst bacterial isolates (Thi et al., 2023). Mean comparisons were conducted using Tukey test, with a *p*-value of less than 0.05 considered significant. DNA sequences of multiple bacterial isolates were compared to reference *Bacillus* and *Neobacillus* sequences in the Ezbiocloud database (Chalita et al., 2024) using the BLASTn program to evaluate similarity. DNA sequences were multi-aligned using the CLUSTAL W software (Thompson et al., 1997). The MEGA 5.2 software (USA) with a bootstrap value of 1,000 replications and the neighbor-joining algorithm (Saitou and Nei, 1987) were used to construct the phylogenetic tree to illustrate the genetic relationships between bacterial isolates (Tamura et al., 2011).

Results

Bacterial isolation

In total, 36 bacterial isolates were obtained on CMC medium from paddy soil (Fig. 2A) in Vinh Long province (Table 1). Of these, the highest number (12 isolates, 33.33%) were obtained from Vung Liem district, followed by Long Ho district (10 isolates, 27.78%) and Mang Thit district (9 isolates, 25%), with the lowest number in Tra On district (5 isolates, 13.89%).

Morphological and biochemical characteristics of isolated isolates

In general, based on the observed results, most isolated bacterial isolates colonies were opalescent, yellowish, irregular-round shape, having an entire margin, raised elevation, and a colony diameter in the range 2–4 mm (Fig. 2B). In addition, the isolated bacterial isolates were Gram-positive rod-shaped cells (Fig. 2C) that were motile, spore-forming, oxidase and catalase positive (Figs. 2D–2E).

Table 1 Origin of bacterial isolates isolated from rice soil in Vinh Long province, Vietnam

Origin of bacterial isolates	Number of samples	Number of isolates
Vung Liem	8	12
Long Ho	5	10
Mang Thit	4	9
Tra On	3	5
Total	20	36

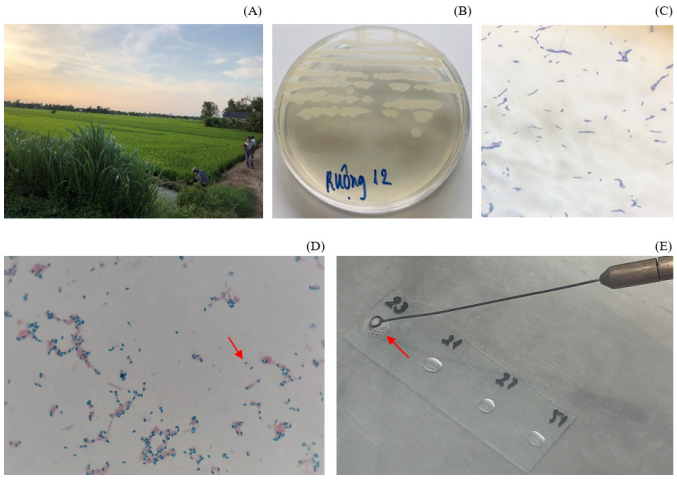

Fig. 2 Cellulolytic bacteria isolated from paddy soil samples on carboxylmethylcellulose (CMC) medium: (A) appearance of rice field where samples were collected; (B) isolate ĐRVL10 cultured on CMC agar medium; (C) Gram-positive staining at 100× magnification; (D) spore staining of isolate ĐRVL10, indicated by a red arrow; (E) positive catalase activity in isolate ĐRVL10, indicated by gas bubbles (red arrow).

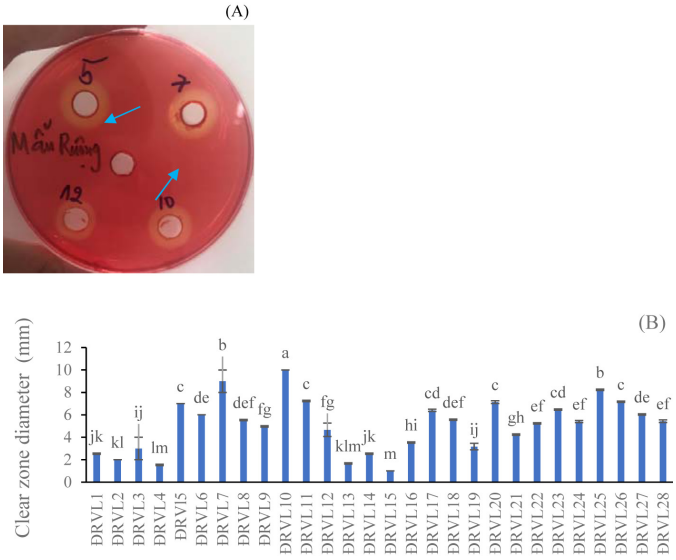

Fig. 3 (A) Cellulose-degrading ability of bacterial isolates determined by Congo red staining, where blue arrows indicate cellulose-degrading zones; (B) cellulolytic halo zone diameters of bacterial isolates, where different letters indicating statistically significant differences ($p < 0.05$) between mean values, and error bars indicate ±SD.

Cellulolytic activity of bacteria

Based on the results of the Congo red staining, 28 out of the 36 bacterial isolates had cellulose-degrading activity (Fig. 3A) based on halo diameters of the bacterial isolates with cellulose-degrading activity in the range 1.0 ± 0.0–10.0 ± 0.0 mm, with isolate ĐRVL10 having the highest diameter (10 ± 0.0 mm), followed by isolate ĐRVL7 (9.0 ± 1.0 mm), isolate ĐRVL25 (8.23 ± 0.06 mm), isolate ĐRVL11 (7.23 ± 0.06 mm) and isolate ĐRVL11 (7.17 ± 0.06 mm), with the lowest being isolate ĐRVL11 with a halo diameter of 1.0 ± 0.0 mm (Fig. 3B).

Effect of temperature on cellulose-degrading activity of bacteria

The chosen bacterial isolates grew well at 35°C and 40°C. However, 10 bacterial isolates were incapable of growing at 45°C, 50°C, 55°C and 60°C. Isolate ĐRVL10 only grew well at 45°C, although it did grow weakly at 50°C and 55°C and could not survive at 60°C (Table 2). Additionally, the strongest cellulose-degrading activity of strain ĐRVL10 occurred with cultivation at 35°C and 40°C with halo diameters of 7.0 ± 1.0 mm and 9.0 ± 1.0 mm, respectively. The cellulose-degrading activity of this strain decreased when cultivated at 45°C, with a halo diameter of 6.83 ± 0.29 mm (Fig. 4A).

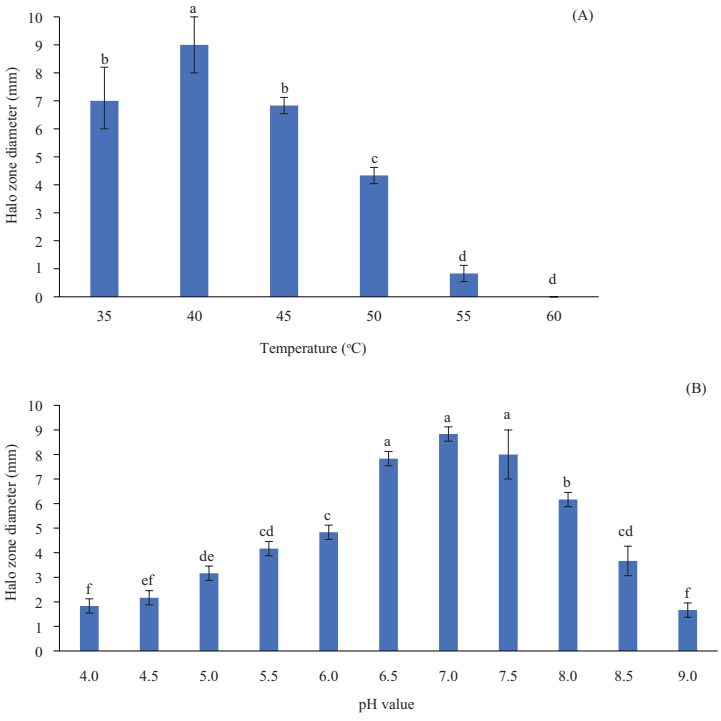

Fig. 4 Effect of (A) temperature and (B) pH on cellulose-degrading activity of isolate ĐRVL10. Different letters indicate statistically significant differences ($p < 0.05$) between mean values, and error bars indicate ±SD.

Table 2 Growth ability of bacterial isolates at different temperatures

Bacterial isolate	Temperature					
	35°C	40°C	45°C	50°C	55°C	60°C
ĐRVL5	+	+	-	-	-	-
ĐRVL6	+	+	-	-	-	-
ĐRVL7	+	+	+	-	-	-
ĐRVL10	+	+	-	-	-	-
ĐRVL11	+	+	-	-	-	-
ĐRVL17	+	+	-	-	-	-
ĐRVL20	+	+	-	-	-	-
ĐRVL23	+	+	-	-	-	-
ĐRVL25	+	+	-	-	-	-
ĐRVL26	+	+	-	-	-	-
ĐRVL27	+	+	-	-	-	-

+ = growth; - = no growth.

Effect of pH on cellulose-degrading activity of bacteria

The pH of the cultivation medium affected the cellulose-degrading activity of strain ĐRVL10; specifically, this isolate had the highest cellulose-degrading activity following cultivated at pH 6.5, 7.0, 7.5 and 8.0 with halo diameters of 7.83 ± 0.29 mm, 8.83 ± 0.29 mm, 8.0 ± 1.0 mm and 6.17 ± 0.29 mm, respectively. However, this isolate had the lowest cellulose-degrading activity at pH 4.0 (1.83 ± 0.29 mm) and pH 9.0 (1.67 ± 0.29 mm), as shown in Fig. 4B.

Rice straw degrading capability of bacterial isolates on TSB, and NB media

On the TSB medium, isolate ĐRVL10 has the highest ability to decompose rice straw (0.63 ± 0.01 g) with a treatment efficiency of 63%, followed by isolates ĐRVL7 and ĐRVL5 with decomposed rice straw weights of 0.44 ± 0.0 g (44%), and 0.33 ± 0.01 g (33%), respectively. Isolate ĐRVL3 had the lowest ability to decompose rice straw (0.24 ± 0.02 g, 24%) and this level was significantly different from the remaining bacterial isolates (Fig. 5A). Similarly, on the NB medium, isolate ĐRVL10 had the highest ability to decompose rice straw (0.55 ± 0.01 g) with a treatment efficiency of 55%, followed by isolates ĐRVL7 (0.46 ± 0.02 g, 46%) and ĐRVL5 (0.33 g ± 0.01, 33%), with isolate ĐRVL3 having the lowest ability to decompose straw (0.13 g, 13%) and this level was significantly different from the remaining bacterial isolates (Fig. 5B).

Identification of cellulose degrading bacteria

Based on the results from using the API 20E kit, two bacterial isolates with the strongest cellulose-degrading activity (ĐRVL7 and ĐRVL10) reacted negatively to orthonitrophenyl galactosidase, lysine, ornithine, H_2S, urease, indole, glucose, mannitol, sorbitol, rhamnose, sucrose, melibiose, amygdalin and arabinose. In total, the bacterial isolates produced only 4 out of 20 positive reactions to arginine, inositol, tryptophane deaminase and gelatin.

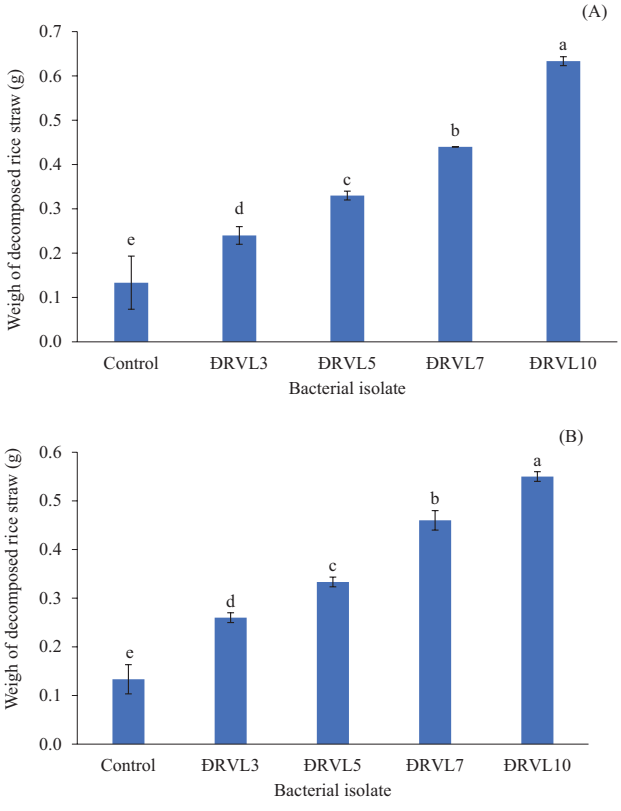

Fig. 5 Rice straw decomposition ability of isolated bacterial isolates on different media: (A) tryptic soy broth; (B) nutrient broth. Different letters indicate statistically significant differences ($p < 0.05$) between mean values, and error bars indicate ±SD.

Based on the PCR results, 11 representative isolates (with the strongest cellulose-degrading activity) were able to amplify the 16S rRNA gene with a PCR product size of 1,500 bp. Of the sequencing results of the 16S rRNA gene of two isolates (ĐRVL7 and ĐRVL10) based on the EzBioCloud database, isolate ĐRVL7 was 98.66% similar to *Bacillus arachidis isolate SY8 (OM062591),* 98.38% homologous to *Bacillus hominis* isolate BML-BC059 (MW674729 and to *Bacillus paramobilis* isolate BML-BC017 (MW674728), as well as being 98.18% homologous to *Bacillus cereus* isolate ATCC 14579 (AE016877), *Bacillus paramycoides* isolate NH24A2 (MAOI01000012), *Bacillus paranthracis* isolate Mn5 (MACE01000012) and *Bacillus nitratireducens* isolate 4049 (KJ812430). The other isolate (ĐRVL10) had 99.56% similarity to *Neobacillus drentensis isolate LMG 21831 (AJ542506),* 98.83% identity to *Neobacillus vireti isolate LMG 21834 (AJ542509)* and 98.62% identity to *Neobacillus soli* isolate NBRC 102451 (BCVI01000121). Based on the phylogenetic tree, these two isolates were divided into two clusters (A and B), as shown in Fig. 6. In cluster A, bacterial isolate ĐRVL7 had a close genetic relationship with *the* reference *Bacillus cereus* isolate ATCC 14579 (AE016877), *Bacillus paramycoides* isolate NH24A2 (MAOI01000012), *Bacillus paranthracis* isolate Mn5 (MACE01000012) and *Bacillus nitratireducens* isolate 4049 (KJ812430) using the EzBioCloud database, while isolate ĐRVL10 had the closest genetic relationship to *Neobacillus vireti LMG 21834 (AJ542509)* in branch B. Therefore, based on the phylogenetic tree in combination with the morphological and biochemical characteristics determined using the API 20E kit, the two bacterial strains belonged to different species in the genera *Bacillus* and *Neobacillus, respectively.*

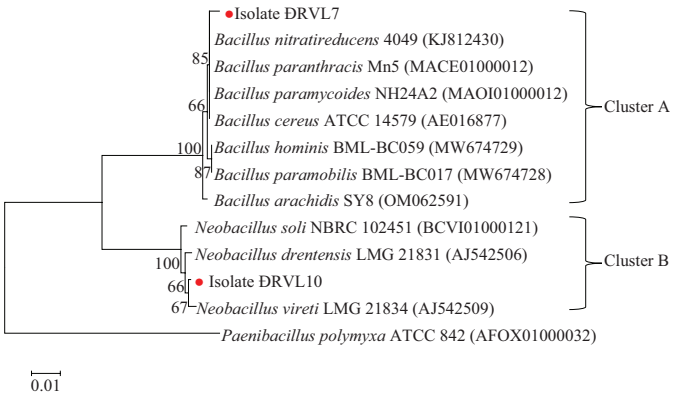

Fig. 6 Phylogenetic tree showing genetic relationships between cellulolytic bacterial isolates and reference *Bacillus* on the Ezbiocloud database, with tree construction using the neighbor-joining algorithm and *Paenibacillus polymyxa* (AFOX01000032) as an outgroup, numbers at left of nodes indicate boostrap values and the scale bar indicates genetic distance of 0.01

Discussion

The study isolated 28 bacterial isolates with cellulose-decomposing activity from paddy soil in Vinh Long province, Vietnam. This result was consistent with the study of Ruttanasutja and Thongdee (2020) that reported isolating cellulolytic bacteria from paddy soil in Thailand. Similarly, Susilowati et al. (2015) obtained cellulolytic bacteria from the rhizosphere of rice (*Oryza sativa* L.) in Indonesia. Additionally, many studies have shown the abundant presence of cellulose-degrading bacteria in nature, such as those isolated from termites (Peristiwati et al., 2018; Phong et al., 2021) and insect guts (Dar et al., 2021), the rumen of ruminants (Guder and Krishna, 2019; Urgessa et al., 2020), and even in harsh environmental conditions (Soares et al., 2012). This could be explained by cellulose being the main structural component of plant cells commonly present everywhere (Maleki et al., 2016). In the current study, two bacterial isolates (ĐRVL7 and ĐRVL10) were identified as *Bacillus* spp. based on morphological and biochemical characteristics, the API 20E kit, and 16S rRNA gene sequence analysis. This result was consistent with Smily et al. (2012), who showed that *Bacillus* spp had the highest frequency of occurrence among paddy field isolates. In addition, *Bacillus* with cellulose-degrading activity were isolated from silkworm excrement (Li et al., 2023) and soil samples (Bhagat and Kokitkar, 2021). The presence of *Bacillus* spp. in paddy soil in the current study supported the potential application of these bacteria in rice cultivation, because they have endospores and thus can adapt to different environmental conditions (Ngalimat et al., 2021).

The halo diameters the 28 bacterial isolates identified as having cellulose-degrading activity in the current study were in the range 1.0 ± 0.0–10.0 ± 0.0 mm, with the four isolates ĐRVL7, ĐRVL10, ĐRVL11 and ĐRVL25, having the largest halo diameters of 10 ± 1.0 mm, 10 ± 1.0 mm, 7.23 ± 0.06 mm, and 8.23 ± 0.06 mm, respectively. These results were consistent with Peristiwati et al. (2018), who reported that the diameter of halo zones from six cellulolytic bacterial isolates obtained from termite (*Cryptotermes* sp.) gut flora varied from 2 mm to 5 mm. However, the halo diameter of the isolated bacterial isolates was lower than in other research (Urgessa et al., 2020; Ghiasi et al., 2024). Li et al. (2023) reported that cellulose-degrading bacteria isolated from silkworm excrement had a cellulolytic index ranging from 2.30 to 3.50. Similarly, Phong et al. (2021) reported that the value of the halo zone ratio formed by the cellulolytic bacterial isolates in the gut of termites was

in the range 1.10–2.83. Another study by Dewiyanti et al. (2022) showed that isolates from mangrove ecosystems in Indonesia had halo zone diameters in the range 1.15 ± 0.86–2.14 ± 0.02 cm. In Bangladesh, Mahmood et al. (2020) reported that cellulose-degrading bacterial isolates from different types of samples, such as sawdust, kitchen-waste, and soil substances, had halo zone diameters in the range 0.2–3.2 cm. On the other hand, Guder and Krishna (2019) revealed that cellulose-degrading bacterial isolates obtained from sheep rumen had diameters of the halo zone on CMC agar in the range 5–26 mm. According to Upadhyaya et al. (2012), the larger the halo diameter, the stronger the ability of the bacterial isolate to decompose cellulose. This variation may be explained by the differences in the components of cellulase enzymes that the various isolates produce (Peristiwati et al., 2018).

All bacterial isolates in the current study grew well at 35°C and 40°C, while isolate ĐRVL10 grew at 45°C. These results were consistent with Bach (2023), who showed that the cellulose-degrading isolate BH01 obtained from sugarcane bagasse could grow at 25–40°C. However, most bacterial isolates cannot grow or grow weakly at temperatures of 45°C, 50°C, 55°C, and 60°C (Bach, 2023). Notably, Liu et al. (2021b) showed that isolate AC-1, obtained from high-temperature compost samples, could decompose lignocellulose at 50–70°C. Based on the results from the current study, the strongest cellulose-degrading activity of bacteria was at 35°C and 40°C. This was consistent with Kognou et al. (2022), who reported on the cellulase activity of cellulose-degrading bacteria isolated from mixed soil samples at their optimum temperature (35–40°C). In particular, in the current study, isolate ĐRVL10 had cellulose-degrading activity at 45°C with a halo zone diameter of 9.0 ± 1.0 mm, which was consistent with Behera et al. (2016), who reported that the isolate CDB-12, obtained from mangrove soils in India, produced maximum cellulase at 45°C. However, this was contrasted by the result from the study by Sethi et al. (2013) showing that cellulase-producing bacteria isolated from soil yielded maximum cellulase production at 40°C. The bacteria in the current study with high-temperature cellulose-degrading activity could have potential for composting microbial organic fertilizers from abundant agricultural by-products in Vietnam such as rice straw.

Based on the results of the current study, the cellulose-degrading activity of the isolate ĐRVL10 was affected by the pH, with its cellulolytic activity being high and stable at pH 6.5–8.0, with the highest cellulose-degrading activity at pH 7.0. This result was consistent with Padilha et al. (2015), who reported that the thermophilic isolate *Bacillus* sp. C1AC5507, obtained from a sugarcane plantation field in Brazil, had an optimum pH of 7.0 for CMCase production, whereas at pH 4.0 and pH 9.0, the cellulose-degrading activity of this isolate was the lowest. Shakoor et al. (2013) showed that *B. megaterium* S3, isolated from vegetable markets in Pakistan, exhibited the optimum temperature (50°C) and pH (8.0) for crude enzyme activity. In China, Liang et al. (2014) reported that the optimum pH and temperature for CMCase activity produced by the isolate ME27-1 from soil samples were 5.5 and 50°C, respectively, and that the enzyme was stable at a wide pH range (5.0–9.5).

The bacterial isolates in the current study could decompose rice straw in both TSB (23–63%) and NB (26–53%) media after 15 d of treatment. Similar results were reported by Dung et al. (2018), where the rice straw decomposition rates of bacterial isolates from soil samples were in the range 18.85–20.99% after 12 d of bacterial inoculation. However, a study by Liu et al. (2021b) showed that isolate AC-1, obtained from high temperature compost samples, had the best degradation efficiency of rice straw at 60°C (78.92%) and of hemicellulose, cellulose, and lignin (82.49%, 97.20% and 20.12%, respectively). However, the cellulose decomposition efficiency of bacterial isolates with the TSB medium was higher than with the NB medium, which may have been due to the influence of cellulase activity from carbon and nitrogen sources (Liang et al., 2014). Therefore, further studies need to be performed to clarify this issue. The rice straw decomposition efficiency of the bacterial isolates in the current study showed their potential for composting microbial organic fertilizers from abundant agricultural by-products such as straw, sugarcane residue, coir dust and other plant residues in Vietnam. However, further studies need to be performed to determine the optimal conditions for utilizing these bacteria effectively.

Conclusion

Of the bacterial isolates capable of degrading cellulose, four (ĐRVL7, ĐRVL10, ĐRVL11 and ĐRVL25) exhibited the strongest cellulose-degrading activity. Based on morphological and biochemical characteristics and 16S rRNA gene sequencing, two of the isolates (ĐRVL7 and ĐRVL10) were highly likely from the genera *Bacillus* and *Neobacillus, respectively.* The cellulose degrading ability of isolate ĐRVL10 was highest at 40°C and pH 7.0. In particular, isolate ĐRVL10 had the ability

to decompose cellulose at 45–55°C. After 15 d, the bacterial isolates could break down rice straw on TSB medium (23–63%) and on NB medium (26–53%). Based on these findings, isolate ĐRVL10 has the potential to compost microbial organic fertilizer from rice straw for application in rice cultivation in the future.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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