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ISOLATION, CHARACTERIZATION, AND IDENTIFICATION OF CELLULOLYTIC BACTERIA FROM HOUSEHOLD COMPOST FOR CELLULASE PRODUCTION

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Using microorganisms for waste treatment is a growing trend that offers numerous economic and environmental benefits. This study aimed to evaluate the characteristics of cellulolytic bacterial strains isolated from compost samples from household waste. Biochemical and molecular tests were conducted to identify highly cellulolytic bacteria. Thirty aerobic strains were isolated, of these three isolates (WS1.7, WS3.1, and WS3.29) demonstrated high extracellular cellulase activity and decomposed 51.34–63.43% of the filter paper mass after 7 days of incubation in a liquid medium. Based on their colony morphology and biochemical properties, these three isolates were predicted to belong to the genus Bacillus. The maximum endoglucanase activity of the crude enzyme produced by Bacillus velezensis WS1.7 was demonstrated. These findings indicate the potential of the B. velezensis WS1.7 isolate for further research and cellulase production.

K e y w o r d s: cellulase production, cellulolytic bacteria, genus Bacillus, endoglucanase activitry, household compost.

In nature, microorganisms play a crucial role in decomposing plant residues and converting them into simple compounds that can be absorbed by plants [1]. The main organic components of plant cells containing carbon are cellulose (38–50%), hemicellulose (23–32%), and lignin (15–25%). These high-molecular-weight compounds are highly stable and require a long decomposition time [2]. Therefore, the treatment of cellulose-rich waste sources is a real challenge for every country. Typically, in the Mekong Delta of Vietnam, which is known as a large agricultural production area of Vietnam and the world [3, 4], the main agricultural byproducts are straw (26.86 million tons), rice husks (5.37 million tons), bagasse (1.33 million tons), fruit peels, etc., with a total output estimated at approximately 88.9 million tons comprising byproducts after harvesting and processing [5]. Traditional waste treatment methods include burning, dumping outdoors, and burying fresh in fields or landfills. However, these methods hurt the lives of people and the environment in the area, including water pollution (surface water and groundwater), soil contamination by organic substances, and air pollution from fires and decomposing landfills [6]. This has also increased the number of diseases in the area. For example, burning agricultural biomasses contributes to air pollution and the risk of respiratory and cardiovascular hospitalization [7]. As a result, local scientists have focused on researching and applying new treatment methods for cellulose-rich agricultural byproducts that are highly effective and environmentally friendly.

This agrees with the development trend of the circular economy, where agricultural byproducts are viewed not as waste but as resources with high reuse value, promoting economic growth [8, 9]. These byproducts can effectively produce biomaterials, biofuels, fertilizers, and can be used for treatment [10]. The common feature of these production processes is the use of biological processes and the short-chain cleavage of cellulose, which is facilitated by an enzyme system known as cellulase [11, 12]. This enzyme system consists of three main enzymes: endoglucanase (EC 3.2.1.4), exoglucanase or cellobiohydrolase (EC 3.2.1.91), and β-glucosidase (EC 3.2.1.21) [13]. Cellulase breaks cellulose chains into simpler sugars such as β-glucose or shorter polysaccharides and oligosaccharides [14]. Thus, the chemical composition and properties of materials containing cellulose are altered. This process is highly economically important because it converts the main component of plant cellulose into easily consumable and usable products [15]. Unlike other chemical processes, this biological degradation does not produce any harmful byproducts. Owing to its versatility, cellulase is one of the most commonly used enzymes and is widely applied in various industries, such as agriculture, environmental treatment, biofuel production, food production, paper production, and garment production [16]. Its use in these industries helps increase processing efficiency in the biological degradation of agricultural waste.

However, research has shown that certain strains of microorganisms can significantly increase the self-decomposition of cellulose-containing materials through their cellulase enzyme systems [16]. Among these microorganisms, bacteria have several advantages, including abundance, fast growth rates, high enzyme production ability, and stability with highly active and durable synthetic enzymes. They can also be genetically modified to increase cellulase synthesis efficiency [17]. The ability of bacterial strains to synthesize cellulase depends on various factors, including origin and environmental conditions. According to Karthika et al. [18], cellulase-producing bacterial strains can be found in various environments, such as waste, decomposed plant residues, compost, soil, and wastewater. These strains are often identified as belonging to the genera *Bacillus*, *Paenibacillus*, *Cellulomonas*, and *Cytophaga* [19].

In terms of culture conditions, factors such as pH, temperature, substrate type, and concentration are of particular research interest, as they strongly influence enzyme biosynthesis efficiency and quality of enzyme biosynthesis [16, 21]. Therefore, isolation studies are often conducted to identify new bacterial strains and establish suitable environmental conditions for their growth both domestically and internationally. Based on these factors, this study established suitable conditions for cellulase synthesis by native cellulolytic bacteria isolated from household waste. This could facilitate the use of these strains in the production of commercial cellulase or in enhancing the composting process for our abundant local agricultural waste.

Materials and Methods

Sample preparation. The household waste samples included the waste of vegetables, fruits, and cut grass. The materials were manually crushed, 10 kg of waste was mixed with 10 kg of dry cow dung, tap water was added, and the mixture was mixed well to achieve a moisture content of approximately 60–65%. The materials were composted naturally in plastic containers with lids. After 60 days, the compost sample was used to isolate the cellulose-degrading bacteria.

Chemicals and culture media. Agar, Congo red reagent, other chemicals (Xilong, China), and distilled water were used. All chemicals used for this work were of high purity (>90%), including CMC-Na, agar, and peptone, and were supplied by Merck (Darmstadt, Germany); other chemicals were obtained from Xilong (Shantou, China).

Isolation of cellulolytic bacteria. The isolated sample was weighed to 10 g and placed in a conical flask containing 90 ml of distilled water. The mixture was then shaken at 150 rpm for 30 min to obtain a dilution of 10^{-1}. The sample was then serially diluted with distilled water to obtain a series of dilutions. From this series, 1 ml of the diluted samples at concentrations of 10^{-3}, 10^{-4}, and 10^{-5} were pipetted and added to a Petri dish containing agar medium with CMC as the sole carbon source (10 g of CMC-Na, 4 g of KH_2PO_4, 4 g of Na_2HPO_4, 2 g of tryptone, 0.2 g of $MgSO_4{\cdot}7H_2O$, 0.004 g of $FeSO_4{\cdot}7H_2O$, 0.001 g of $CaCl_2{\cdot}2H_2O$, 15 g of agar, and the pH was adjusted to 7.0). The medium was evenly spread over the surface and incubated at 37°C for 48–72 h. After incubation, colonies with different characteristics, such as shape, color, and buoyancy, were selected. These colonies were then cultured separately on CMC agar medium supplemented with 0.1% Congo red. Colonies with transparent lysis ring around them were observed and selected for purification by streaking (cultured 2–3 times). The pure bacterial isolates were stored in agar slants at 4–6°C and in 30% glycerol solution at -20°C.

Screening of cellulolytic bacteria

Preparation of the bacterial suspension. To prepare the bacterial suspension, one colony of each tested bacterial isolate was inoculated into 50 ml of liquid LB medium with 1% CMC (10 g of CMC-Na, 10 g of peptone, 5 g of yeast extract, and 10 g of NaCl, and the pH was adjusted to 7.0) and shaken at 150 rpm at 37°C for 12 h. The suspension was then measured at OD_{600nm} and adjusted to 0.8. It was stored at 4–6°C and used within 30 min.

Endoglucanase activity. For the endoglucanase activity test, 2 ml of the bacterial suspension was transferred into 40 ml of liquid medium containing 1% CMC and shaken at 150 rpm and 37°C for 48 h. The mixture was centrifuged at 5,000 rpm at 4°C for 15 min, and the crude enzyme supernatant was collected. 40 µl of the supernatant was injected into the wells of a CMC agar medium Petri dish with a good diameter of d = 6 mm. The Petri dishes were left for 30 minutes and then incubated at 37°C for 48 h. After incubation, the agar plates were stained with 0.1% Congo red to observe the formation of the lysis ring (D). If bacteria produce extracellular enzymes, a transparent ring forms around the well [21]. The enzyme activity (ΔD) was determined by calculating the difference D – d, where D is the diameter of the lysis ring and d is the diameter of the agar well (mm). The results were categorized as follows: weak: ΔD < 10 mm; moderate: 15 mm > ΔD ≥ 10 mm; severe: 20 mm > ΔD ≥ 15 mm; and very severe: ΔD ≥ 20 mm.

Filter paper degradation study (exoglucanase activity). For the exoglucanase activity test, 2 ml of the bacterial suspension was transferred to 40 ml of liquid medium containing 0.1 g of Whatman No. 1 filter paper, which had been cut into small pieces and dried to a constant weight (m1). The mixture was then shaken at 150 rpm and 37°C for 7 days. After incubation, the mixture was filtered using Whatman No. 1 filter paper and washed 2-3 times with a mixture of dilute HCl and HNO_3 solution to remove bacterial biomass. The remaining solid was dried at 80°C until a constant weight (m_2). A control sample was prepared in the same manner as the test sample but without the addition of bacterial colonies [21]. The exoglucanase activity (degradation activity) of the bacterial isolates was determined by calculating the difference in filter paper weight before and after incubation. The decomposition efficiency (H%) was then calculated using the following formula:

$$\mathrm{H}(\%) = \frac{m_1 - m_2}{m_1} \cdot 100\%$$

Identification of bacteria

Morphological and biochemical characteristics. The bacterial isolates were presumptuously characterized based on their morphology, and several biochemical analyses were performed. The parameters investigated included colonial morphology (evaluated for size, pigmentation, form, margin, and elevation), cell shape, spore formation, motility, Gram, catalase, oxidase, indole, H_2S, nitrate reduction, methyl red (MR), Voges–Proskauer (VP), growth under salt conditions (5.0–7.0% NaCl), hydrolysis (starch, urea, gelatin, casein), and sugar fermentation (glucose, fructose, lactose, maltose, sucrose) [22].

DNA extraction and amplification of 16S rRNA gene. The bacteria with the highest cellulase activity were cultured in LB medium enriched at 150 rpm and 37°C for 48 h. The suspension was centrifuged at 5,000 rpm at 4°C for 10 min to harvest the cells for total genomic DNA extraction [23]. The 16S rRNA gene fragment was amplified using the following universal bacterial primers: forward primer 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and reverse primer 1492R (5′-TACGGYTACCTTGTTACGACTT-3′). The polymerase chain reaction (PCR) mixture consisted of a 25 µl mixture containing distilled water, 1X PCR buffer, 2.0 mM $MgCl_2$, 150 µM dNTPs, 10 pmol of each primer, 2.0 IU of Taq DNA polymerase, and 40 ng of DNA sample. The PCR cycle was performed in a thermal cycler (Eppendorf Mastercycler X50s, Germany) with the following thermal conditions: initial denaturation at 95°C for 5 min; 25 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 60 s; and a final extension at 72°C for 12 min. The resulting PCR product was electrophoresed on a 1.5% agarose gel, purified, and sent to Nam Khoa Biotek Company (Ho Chi Minh City, Vietnam) for sequencing. The nucleotide sequences obtained were compared using the BLAST program on the NCBI website (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequence homology analysis of the 16S rRNA gene was conducted using GenBank data. Phylogenetic analysis using the neighbor-joining model was performed using the MEGA version 11 program. The reliability of the branching and clustering patterns was estimated with 1,000 bootstrap replicates. The relationships of the three identified isolates were compared with those of the five closest strains of each species.

Determination of the effects of various culture conditions on cellulolytic activity. Experiments were conducted to investigate the effects of various culture conditions on the cellulolytic activity of the three identified bacterial isolates. The experiments followed a single-factor design, with the fermentation process involving the addition of 2 ml of bacterial suspension to 40 ml of liquid medium containing 1% CMC. The pH of the mixture was adjusted to a range of 6.0–8.0. The mixture was then shaken at 150 rpm and incubated at 37°C for 48 h. After incubation, the crude enzyme was collected to determine endoglucanase activity. Additional experiments were conducted to explore further the impact of other culture conditions on cellulolytic activity. These included varying the cultivation temperature (30–51°C), fermentation period (24–60 h), CMC concentration (0.5–2.5%), inoculum content (1.0–5.0%), and shaking conditions (0–250 rpm). The determination of enzyme production and activity was performed as described above, using the best factors determined in the previous experiments.

Statistical analysis. All experiments were performed in triplicates. All data were entered, calculated, and graphed using Microsoft Excel 2019 software. One-way analysis of variance was performed using Minitab 16 software to determine the significance of differences at $P < 0.05$, and Tukey's test was applied.

Results

Isolation and screening of cellulose-degrading bacteria. In the present study, a total of 30 isolates of cellulolytic bacteria with varying colony morphologies were isolated. These isolates were capable of growing and forming a decomposition ring on an agar medium containing 1% CMC as the sole carbon source stained with 0.1% Congo red (Fig. 1, *A*). The isolates formed circular colonies (76.67%) throughout the entire area (83.33%) at elevation, raised (47.82%), and convex (47.82%) about the margin, displaying diverse colors, such as transparent white, ivory white, milky white, yellow, and orange. After 48 h of incubation, the colonies had diameters ranging from 0.2 to 1.3 mm, with the majority belonging to the group that formed small colonies with diameters <1 mm (56.67%) (Table 1). These results indicate that the naturally composted household waste sample used in this study contains a rich bacterial system capable of decomposing cellulose.

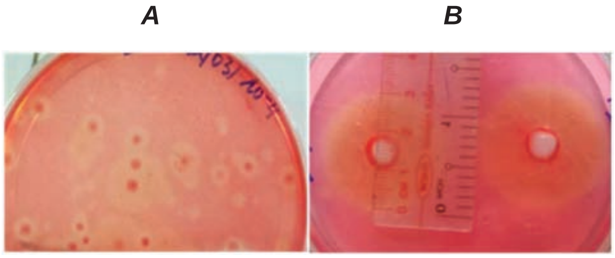

*Fig. 1. CMC hydrolysis zones of colonies (**A**) and of crude enzyme (**B**)*

For exoglucanase activity, the culture medium containing crude enzymes from the 30 selected bacterial strains showed CMC hydrolysis activity (CMCase), with hydrolysis circle diameters ranging from 3.0 to 38.5 mm (Fig. 1, *B*). Among these isolates, three isolates (WS1.7, WS3.1, and WS3.29) exhibited strong CMCase activity, with a $\Delta D \geq 20$ mm, accounting for 13.33% of the total number of selected bacterial strains. Twelve of the 30 selected bacterial strains were able to digest Whatman No. 1 filter paper substrate for growth (exoglucanase), accounting for 40% of the total number of isolates, with the percentage of paper weight degraded ranging from 9.51 to 63.43% after 7 days of shaking culture in liquid medium. Three isolates, WS1.7, WS3.1, and WS3.29, decomposed more than 50% of the filter paper weight after 7 days of fermentation, equivalent to decomposition rates per day of 9.06, 8.39, and 7.33%, respectively. Although CMCase activity did not show a completely positive correlation with exoglucanase activity, it was still closely related to a high Pearson correlation coefficient ($r = 0.8099$). Based on these results, three potential cellulose-degrading bacterial strains (WS1.7, WS3.1, and WS3.29) were selected for further experiments.

Identification of potential cellulose-degrading bacterial strains. Three bacterial isolates with

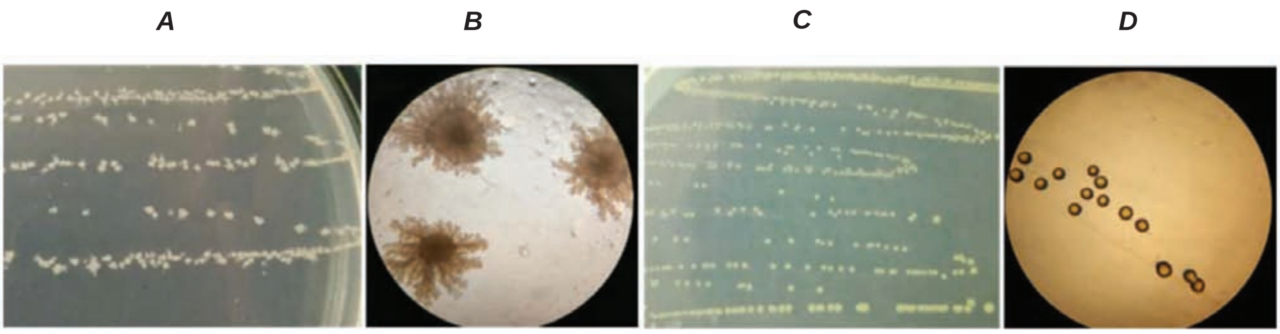

*Fig. 2. WS3.9 (**A**, **B**) and WS1.18 (**C**, **D**) isolate colonies on CMC-agar media*

Table 1. Colony characteristics and screening results of the cellulolytic activity of 30 isolated strains

No.	Isolate	Colony morphology	Colony diameter (mm)	Endoglucanase activity (ΔD mm)		Exoglucanase activity (H%)
				Values	Categorized	
1	WS1.1	Circular, entire, flat, white	0.5	3.0	Weak	-
2	WS1.2	Circular, entire, pulvinate, ivory white	0.3	6.5	Weak	-
3	WS1.7	Irregular, lobate, raised, milk white	0.8	38.5	Very severe	63.43
4	WS1.10	Circular, entire, convex, milk white	0.2	10.5	Moderate	-
5	WS1.11	Circular, entire, raised, light yellow	0.5	8.0	Weak	-
6	WS1.12	Irregular, entire, raised, white	1.3	18.0	Severe	20.56
7	WS1.14	Circular, entire, flat, ivory white	0.8	4.5	Weak	-
8	WS1.18	Circular, entire, raised, yellow	1.0	4.0	Weak	12.93
9	WS1.19	Circular, entire, pulvinate, organge	1.2	17.5	Severe	34.07
10	WS1.20	Circular, entire, raised, white	0.3	9.0	Weak	-
11	WS2.1	Circular, entire, convex, milk white	0.8	10.0	Moderate	12.93
12	WS2.2	Circular, lobate, raised, milk white	1.3	7.5	Weak	-
13	WS2.3	Irregular, lobate, raised, ivory white	1.0	7.5	Weak	26.6
14	WS2.4	Circular, entire, convex, white	0.3	16.0	Severe	-
15	WS2.5	Circular, entire, umbonate, ivory white	0.2	7.5	Weak	27.92
16	WS2.6	Circular, lobate, flat, milk white	0.5	8.0	Weak	-
17	WS2.7	Circular, entire, convex, white	0.8	10.5	Moderate	-
18	WS3.1	Circular, entire, raised, milk white	1.2	33.5	Very severe	58.73
19	WS3.2	Circular, entire, raised, white	1.0	7.0	Weak	-
20	WS3.3	Circular, entire, convex, white	0.8	11.0	Moderate	31.54
21	WS3.4	Filamentous, entire, convex, milk white	1.3	8.5	Weak	-
22	WS3.6	Irregular, entire, pulvinate, ivory white	1.3	9.5	Weak	-
23	WS3.9	Circular, entire, convex, light yellow	1.1	11.0	Moderate	-
24	WS3.10	Circular, entire, convex, white	0.5	5.5	Weak	13.5
25	WS3.12	Circular, entire, umbonate, orange	1.0	9.0	Weak	-
26	WS3.13	Filamentous, lobate, raised, milk white	0.8	16.0	Severe	-
27	WS3.17	Circular, entire, convex, white	0.5	9.0	Weak	-
28	WS3.18	Circular, entire, convex, yellow	0.3	8.0	Weak	9.51
29	WS3.24	Filamentous, entire, convex, yellow	1.0	5.5	Weak	-
30	WS3.29	Circular, entire, raised, white	1.3	31.0	Very severe	51.34

Note: “-” Negative

the highest cellulolytic activity, WS1.7, WS3.1, and WS3.29, were selected for biochemical characterization tests. The results of these tests, shown in Fig. 3 and Table 2, indicate that the three isolates belong to the *Bacillus* genus. Further examination was conducted to identify the isolates, and their 16S rRNA genomes were successfully amplified and sequenced. The PCR products were then electrophoresed on a 1.5% agarose gel, resulting in a band with a size of approximately 1.4 kb (Fig. 4). The sequence lengths were 1433, 1434, and 1436 bp, respectively. The 16S rRNA gene was analyzed using BLAST on NCBI, and a phylogenetic tree was constructed using the MEGA 11 program (Fig. 5). The

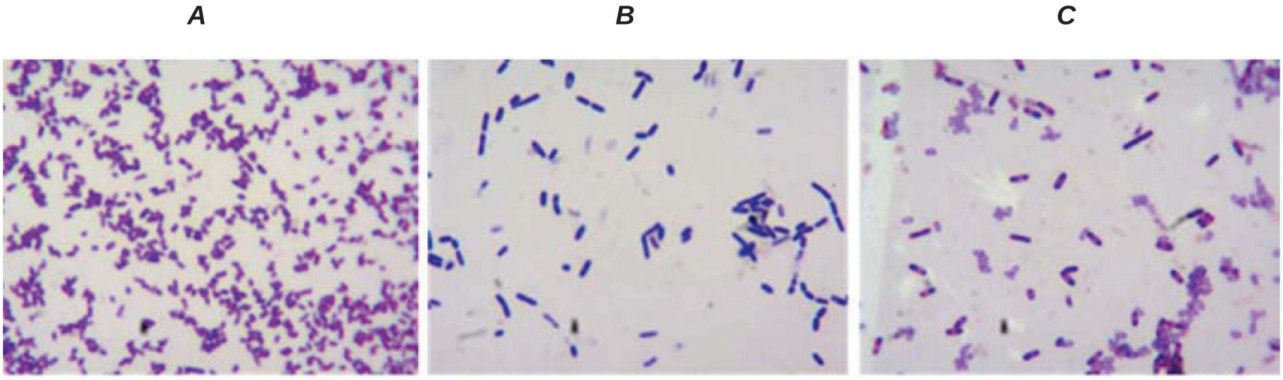

*Fig. 3. Gram test results for the WS1.7 (**A**), WS3.1 (**B**), and WS3.29 (**C**) isolates*

T a b l e 2. Biochemical characteristics of selected cellulolytic bacterial isolates

No.	Test	Results		
		WS1.7 isolate	WS3.1 isolate	WS3.29 isolate
1	Shape	Rod	Rod	Rod
2	Spore formation	+	+	+
3	Motility examination	+	+	+
4	Gram's staining	+	+	+
5	Catalase production	+	+	+
6	Oxylase production	+	-	+
7	Indole production	-	-	-
8	H_2S production	-	+	-
9	Nitrate reduction	+	+	+
10	Methyl red (MR) test	-	-	-
11	Voges-Proskauer (VP) test	-	+	-
12	Growth under salt conditions:			
	5.0% NaCl	+	+	+
	6.0% NaCl	+	+	+
	7.0% NaCl	+	-	-
13	Hydrolysis:			
	Starch	+	+	+
	Urea	+	+	-
	Gelatin	+	-	+
	Casein	+	+	+
14	Sugar fermentation:			
	Glucose	+	+	+
	Fructose	+	+	+
	Lactose	+	+	+
	Maltose	+	+	+
	Sucrose	+	+	+
Preliminary identification		*Bacillus* sp.	*Bacillus* sp.	*Bacillus* sp.

Note: "+" Active reaction, "-" Negative reaction results revealed that the three selected isolates were closely related as they were on the same branch in the phylogenetic tree. Isolate WS1.7 was most closely related to *B. velezensis* SRCM123422 (CP128503), isolate WS3.1 was most closely related to *B. siamensis* B28 (CP066219), and isolate WS3.29 was most closely related to *B. amyloliquefaciens* UMAF6639 (CP006058), with bootstrap coefficients of 32, 25, and 100, respectively. Therefore, the three selected isolates were identified as *B. velezensis* WS1.7, *B. siamensis* WS3.1, and *B. amyloliquefaciens* WS3.29. Based on these results, isolates WS3.1 and WS3.29 can be considered two new bacterial strains compared with existing sequences in GenBank. Finally, the 16S rRNA gene sequences of WS1.7, WS3.1, and WS3.29 isolates were submitted to GenBank as *B. velezensis* WS1.7, *B. siamensis* WS3.1, and *B. amyloliquefaciens* WS3.29, respectively with the accession numbers PQ591715, PQ591747 and PQ591768.

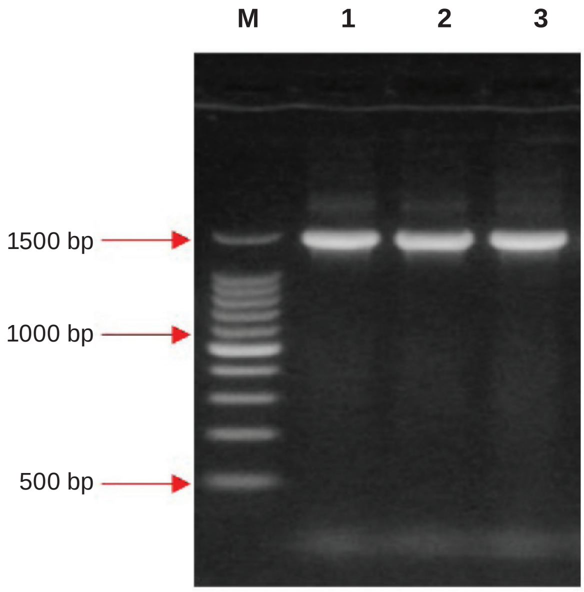

Fig. 4. Agarose gel electrophoresis of PCR amplified products of the 16S rRNA gene. Lane M – Marker; Lane 1 – WS1.7; Lane 2 – WS3.1; Lane 3 – WS3.29

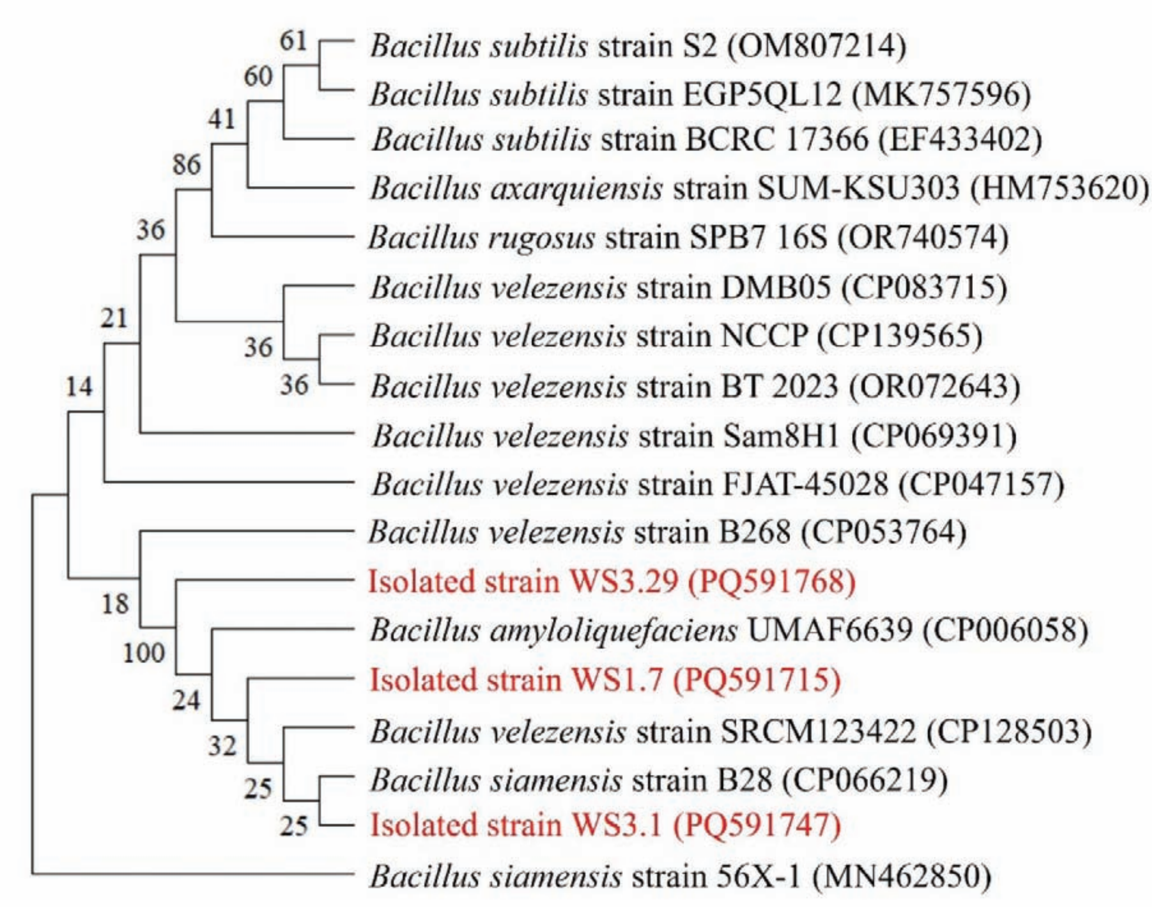

Fig. 5. Phylogenetic analysis of the the WS1.7, WS3.1 and WS3.29 isolates

Determination of endoglucanase production conditions. The results shown in Fig. 6 revealed that cellulase activity was low in all three isolates in acidic media but increased significantly as the pH of the medium reached neutral and slightly alkaline levels. The highest cellulase activitis were observed in WS1.7 and WS3.1 at a pH of 7.5, with values of 38.15 and 38.13 mm, respectively. However, the activity decreased again at a pH of 8.0. Interestingly, the cellulase activities of WS3.1 at pH 7.0 and 7.5 were not significantly different ($P > 0.05$) (Fig. 6, *A*). On the other hand, strain WS3.29 exhibited the highest cellulase activity at a pH of 8.0, with a value of 26.17 mm. The effect of temperature on cellulase activity was also investigated, with a minimum activity observed at 51°C and a maximum activity at 37°C (Fig. 6, *B*). Furthermore, the results revealed that cellulase activity gradually increased for up to 48 h of fermentation in all three strains but sharply decreased thereafter (Fig. 6, *C*). The substrate concentration also affected the ezyme activity, with the highest activity observed at a concentration of 1.5% CMC and a decrease in activity with further increases in concentration (Fig. 6, *D*). However, for WS1.7 and WS3.1, there was no significant difference ($P < 0.05$) in cellulase activity between the 1.5% and 1% CMC treatments (Fig. 5). Similarly, for WS3.29, no significant difference was observed between the CMC concentrations of 1.0, 1.5, and 2.0% CMC. In terms of inoculum content, the highest cellulase activity was observed at a 3% inoculum content and decreased significantly when it increased from 4 to 5% (Fig. 6, *E*), with no significant difference ($P > 0.05$) between 3.0 and 4.0% for WS1.7 and between 2.0, 3.0, and 4.0% for WS3.29. Finally, the effects of different shaking processes on cellulase production were investigated, with shaking at 150–250 rpm significantly increasing enzyme activity in all three bacterial strains compared with static culture and shaking at 100 rpm ($P < 0.05$). However, no significant difference was observed between the different shaking processes at 150–250 rpm (Fig. 6, *F*).

Discussion

Decomposed cellulose-containing materials are considered valuable sources of microorganisms capable of producing cellulase complexes, including bacteria. Over 50 species have been isolated, but their cellulose-degrading abilities vary. Generally, bacteria break down complex lignocellulosic polymers found in cellulose-containing materials (such as dust, straw, and rice husks) into simple sugars, which can be used for biofuel production through fermentation. The growing demand for alternative energy sources has driven scientists to explore biofuels. Many developed countries have already begun producing bioethanol from sources such as compost using cellulose-degrading bacteria. Therefore, our country must use these organisms for biofuel production. In this study, we isolated 30 strains of cellulose-degrading bacteria from naturally composted household waste, including vegetable scraps, sugarcane bagasse, and grass clippings. Among these strains, WS1.7, WS3.1, and WS3.29 exhibited the highest digestion rates of filter paper, with over 50% digestion after 7 days of fermentation. This is equivalent to an average digestion rate of 7.33–9.06% per day. These rates are higher than those reported for bacterial strains (5.16–9.62% per day) and actinomycetes (2.44–8.8% per day) isolated from coffee husks [21]. Additionally, T. F. Egwuatu and O. G. Appeh [24] reported that the weight of filter paper decreased by only 2.5–3.17% per day when fermented by the 5 most promising bacterial strains isolated from the gut of lower termites (*Coptotermes formosanu*). According to M. P. Coughlan and F. Mayer [25], only a few bacteria can synthesize the complete enzyme system necessary for the extensive hydrolysis of crystalline cellulose materials. These bacteria are known as “true cellulolytic bacteria”. Bacterial strains that produce only endoglucanases and β-glucosidases are “pseudocellulolytic bacteria”. However, both types of bacteria play important roles, depending on the intended application [26].

Similar to related studies, bacteria belonging to the genus *Bacillus* are rod-shaped and mostly harmless to humans [27, 28]. These bacterial strains grow rapidly in oxygen-rich conditions and can survive in anaerobic conditions by forming endospores. This ability allows them to survive in harsh environments with limited nutrients for extended periods. Bacillus strains are the dominant bacteria in decomposing plant material, likely due to their robust enzyme secretion system [29, 30]. These bacteria can also be found in various other environments, such as soil and water, and are capable of metabolizing a variety of nutrients. For example, P. Maravi and A. Kumar [31] isolated and identified *B. licheniformis* CD01 from the soil of a fodder storage house, which showed a maximum zone of clearance of 30 mm. In another study, isolated *B. velezensis* GSBZ09 from soil around the roots of healthy grape

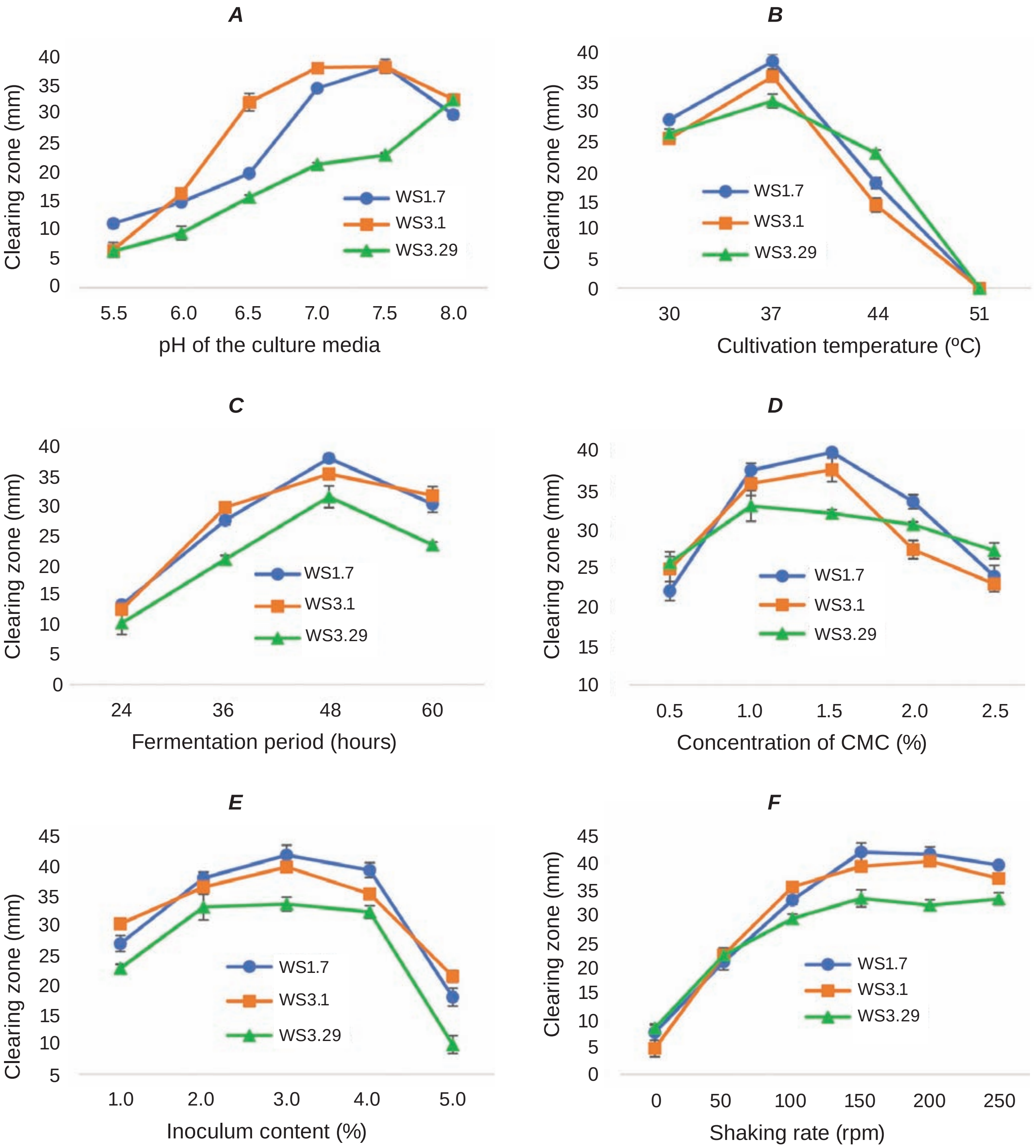

Fig. 6. Effects of different factors on the cellulolytic activity of three selected bacterial strains. ***A*** *– Effect of pH;* ***B*** *– effect of temperature;* ***C*** *– effect of incubation time;* ***D*** *– effect of CMC substrate concentration;* ***E*** *– effect of inoculum content;* ***F*** *– effect of shaking rate*

plants produced cellulase with a diameter of the zone produced on the plate of about 20 mm, it was also found to promote plant growth and suppress plant diseases caused by fungi. It showed that our enzymatic activity had twice this result [32]. In a separate study, *B. siamensis*, was isolated from a termite nest and has been found to effectively produce several important industrial enzymes (protease, pectinase, α-amylase, and cellulase) through solid-state fermentation using different agricultural byproducts [33].

Overall, these results suggest that compost is a good source for the development of potential cellulolytic bacteria. For another species, the peak activity of CMCase in *B. amyloliquefaciens* strain AK9 reached 42 mm [34]. Previous research by L. Luo et al. [35] reported that *B. amyloliquefaciens* is a promising bacterial for use as a biofertilizer and biocontrol agent in agriculture. This is due to its ability to activate soil nutrients, produce hormones and volatile organic compounds that regulate plant growth and root development, and increase plant resistance to biotic and abiotic stresses. Three identified isolates *B. velezensis* WS1.7, *B. siamensis* WS3.1, and *B. amyloliquefaciens* WS3.29 meet safety requirements and can be fully applied in the treatment of organic solid waste containing cellulose for fermentation to produce cellulase or compost organic biofertilizers. Therefore, bacterial strains belonging to the genus *Bacillus* show promise in supporting the treatment of agricultural byproducts containing cellulose.

Cellulose-degrading bacteria have been isolated from various materials and environments, including those with different substrate types, concentrations, temperatures, pH levels, etc. [36, 37]. Previous studies have shown that different strains have varying optimal conditions for enzyme production [38-40]. Therefore, specific culture conditions for each strain are important to ensure optimal growth and enzyme production. In recent years, significant progress has been made in cellulase production and application through research on cellulase composition, production conditions, fermentation methods, and production scale-up. Liquid fermentation is a practical and suitable method for large-scale production, as it allows for high utilization of raw materials, easy control of culture conditions, high yield, and stable quality [41, 42]. In submerged liquid fermentation cultures, cellulase-producing strains require optimal growth conditions to provide nutrients for efficient cellulase production [43, 44]. When designing a bacterial consortium for the fermentation of high-cellulosic materials, it is important to prioritize members with a wide temperature and pH range to ensure that the consortium can perform well throughout the entire fermentation process [16]. Future studies should focus on optimizing conditions for FPase and β-glucanase activity to improve overall cellulase production by the consortium and promote the beneficial use of high-cellulosic agricultural waste.

Conclusion. In our study, we isolated a total of 30 bacterial isolates from compost and screened them for endo- and exoglucanase activity. We identified the three isolates with the highest cellulolytic activity as *B. velezensis* WS1.7, *B. siamensis* 3.1, and *B. amyloliquefaciens* WS3.29. We then performed experiments to determine the parameters for maximum cellulase activity. Our results revealed that *B. velezensis* WS1.7 exhibited the highest enzyme activity (42.07 mm) in a fermentation medium containing 1.5% CMC, with a pH of 7.5, 3% inoculum content, and incubation at 37°C for 48 h with shaking at 150 rpm. Currently, we are conducting further studies to obtain cellulases with higher yields, purities, and stabilities.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ВИДІЛЕННЯ, ХАРАКТЕРИСТИКА ТА ІДЕНТИФІКАЦІЯ ЦЕЛЮЛОЗОЛІТИЧНИХ БАКТЕРІЙ З ПОБУТОВОГО КОМПОСТУ ДЛЯ ВИРОБНИЦТВА ЦЕЛЮЛАЗИ

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Використання мікроорганізмів для переробки відходів є все більш поширеною тенденцією, яка пропонує численні економічні та екологічні переваги. Метою цього дослідження було оцінити характеристики целюлозолітичних штамів бактерій, виділених із зразків компосту з побутових відходів. Для ідентифікації целюлозолітичних бактерій було проведено біохімічний та молекулярний аналіз. Виділено тридцять аеробних штамів, з яких три ізоляти (WS1.7, WS3.1 і WS3.29) продемонстрували високу позаклітинну целюлазну активність і розклали 51,34-63,43% маси фільтрувального паперу після 7 днів інкубації в рідкому середовищі. На підставі морфології колоній та біохімічних власти-

востей визначено, що ці три ізоляти належать до роду *Bacillus*. Максимальну ендоглюканазну активність сирого ензиму, продукованого *Bacillus velezensis* WS1.7, продемонстрував штам *Bacillus velezensis* WS1.7. Отримані результати свідчать про перспективність ізоляту *B. velezensis* WS1.7 для подальших досліджень та виробництва целюлази.

Ключові слова: виробництво целюлази, целюлозолітичні бактерії, рід *Bacillus*, ендоглюканазна активність, компост, побутові відходи.

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