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Isolation and selection of bioflocculant-producing and starch-degrading *Bacillus* spp. from bread production wastewater

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Abstract

In many developing countries, limited wastewater treatment infrastructure can lead to environmental pollution from the food processing industry. Therefore, selecting bacterial strains capable of wastewater treatment and well-adapted to local conditions is essential. The aim of the present study was to isolate and characterize *Bacillus* spp. strains capable of producing amylase and bioflocculants for the treatment of starch-rich wastewater from traditional bread production facilities in Can Tho, Vietnam. From bread production wastewater, 13 *Bacillus* spp. strains were isolated with starch-degrading capability, of which seven strains exhibited the strongest substrate degradation after two days. The CT14 bacterial strain showed the highest starch degradation capability. After 24 h of wastewater treatment application of cultivated CT14 at a 4% concentration, the reduced sugar content reached a high level of 1.36 mg mL^{-1}. Additionally, the strain CD11 exhibited effective bioflocculant production in the form of polysaccharides, achieving 81.4% flocculation efficiency after 5 min of settling. Using 16S rRNA gene sequencing combined with several biochemical tests, two bacterial strains, CT14 and CD11, were identified as *Bacillus amyloliquefaciens*, with a similarity of over 99.7%.

Key words: bioflocculant, enzyme amylase, starch-degrading, wastewater.
Abbreviations: BOD, biological oxygen demand; CFU, colony forming unit; COD, chemical oxygen demand; DNS, dinitrosalicylic acid; OD, optical density; rpm, rotations per minute.

Introduction

Bread was introduced to Vietnam in 1859 in the form of baguettes, with the combination of simple ingredients such as flour, yeast, and some additives carefully made in each stage (Tran 2025). Thanks to its convenience and delicious taste, bread has become a daily meal, promoting the development of production facilities from industrial scale to households. Wastewater in bakery production facilities mainly comes from cleaning activities, including cleaning equipment and washing floors, containing flour, sugar, oil, grease, and yeast as the main components in wastewater (Struk-Sokolowska, Tkaczuk 2018). The pollution source of bread production wastewater is primarily derived from the discharge of starch and easily biodegradable organic matter during the soaking stage after rice immersion. Parameters such as chemical oxygen demand (COD), biological oxygen demand (BOD), total nitrogen content, total phosphorus content etc., exceed the permissible standards. Additionally, bread production wastewater also causes a very unpleasant odour (Abdulla et al. 2023). In 2019, only 26.7% of craft villages had industrial wastewater collection systems, while the country had over 5400 craft villages. Many starch-processing craft villages discharged wastewater rich in starch, organic acids, and solids directly into the environment without treatment, causing serious pollution (Nguyen 2020).

The genus *Bacillus* is composed of Gram-positive, rod-shaped bacteria that are known for their metabolic versatility, adaptability to different environments, and potential for biotechnology. These bacteria are able to form endospores, which allows them to survive in harsh or nutrient-limited conditions. Additionally, their rapid aerobic growth makes them well-suited for industrial and environmental applications, particularly in wastewater treatment (Hassan et al. 2018; Dong et al. 2022). Species such as *Bacillus subtilis, Bacillus megaterium, Bacillus circulans, Bacillus thuringiensis*, and *Bacillus pakistanensis* have been reported to break down complex organic compounds, remove heavy metals (Ag, Cu, Cd), pesticides, and persistent xenobiotic compounds in industrial and agricultural wastewater (Arora et al. 2020; Ali et al. 2023). Particularly in aquaculture, *Bacillus*-based products have been shown to reduce pollution load (COD, BOD, ammonia, nitrite), improve water quality, and simultaneously inhibit biofilm-forming bacteria (Hlordzi et al. 2020; Tran et al. 2024).

Some of the most extensively studied and successfully applied species in this genus include *Bacillus velezensis*, *B. subtilis, B. megaterium, Bacillus licheniformis*, and *Bacillus* *cereus*. These species have been shown to effectively treat various types of industrial wastewater through mechanisms such as the secretion of extracellular enzymes that can break down complex organic compounds (such as starches, proteins, cellulose, and lipids), nitrogen removal, polyphosphate accumulation, and the production of bioflocculants (Sansinenea, Ortiz 2011; Layly et al. 2021; Seo et al. 2022). Among these, *Bacillus amyloliquefaciens* is particularly noteworthy for its ability to produce high-value industrial enzymes, exhibiting thermostability and activity across a broad pH range. *B. amyloliquefaciens* KCP2 has the potential to produce significant amounts of α-amylase using kitchen waste as the sole carbon source, making it an environment-friendly and efficient bioconversion strategy (Bhatt et al. 2020). Similarly, the study by Abd-Elhalim et al. (2015) demonstrated successful production of amylase from agricultural waste through submerged fermentation. Furthermore, Ogunsade et al. (2015) found that *B. amyloliquefaciens* ABL 19 can effectively generate bioflocculants under optimized conditions, using glucose and peptone as carbon and nitrogen sources, respectively, with the addition of $CaCl_2$, at 60 °C and a pH range of 4 to 11. These findings highlight the species' dual role in both enzyme production and enhancing flocculation efficiency during primary wastewater treatment.

Many *Bacillus* strains have been demonstrated to produce bioflocculants with high treatment efficiency in food and starch-rich wastewater. For instance, *Bacillus mucilaginosus* MBFA9 can remove up to 85.5% of suspended solids and 68.5% of COD within just 5 min of treating starch wastewater (Deng et al. 2003). Similarly, *Bacillus* sp. R1, isolated from wheat bran, achieved a flocculation efficiency of up to 91% (Abuduaini et al. 2019), while *B. velezensis* 40B reduced COD and BOD by 78 and 82%, respectively, and turbidity by 90% in brewery wastewater (Agunbiade et al. 2022). *B. subtilis* F9 also showed stable activity under a wide pH range (2 to 8) and temperatures from 10 to 100 °C, demonstrating its strong adaptability to fluctuating wastewater environments (Giri et al. 2015).

Currently, the use of microorganisms in wastewater treatment is considered a safe and environmentally friendly solution (Rani et al. 2019; Bhatt et al. 2020). Among these, the exploitation of bacterial strains capable of starch degradation and bioflocculant production is regarded as a promising research direction, contributing to the foundation for diversifying microbial resources applicable in future wastewater treatment (Chen et al. 2022; Huang et al. 2022). Therefore, this study was conducted to isolate and select Bacillus strains capable of degrading starch and producing bioflocculants from the wastewater of bread factories in Can Tho. The exploitation of indigenous microbial resources not only enhances the efficiency of biological treatment for starch-rich wastewater, but also promotes the development of microbial products tailored to local conditions for food industry wastewater treatment.

Materials and methods

Isolation of bacterial strains from wastewater

Wastewater samples were collected from the discharge points of three bread production factories in Can Tho City, Vietnam. In Binh Thuy District, samples were taken from two locations: location 1 (10°03'05.5"N, 105°43'28.0"E) with a soil pH of 6.43, designated as NK; and location 2 (10°04'04.3"N, 105°45'05.4"E) with a soil pH of 6.37, designated as CD. In Ninh Kieu District, a sample was collected from location (10°02'37.8"N 105°46'25.5"E), where the pH was 6.50, designated as CT. After collection, the wastewater samples were stored in a sample transport box at 4 to 10 °C and transported to the laboratory for subsequent isolation procedures.

Bacterial isolation: The samples were heated in a water bath at 80 °C for 15 min, then diluted with deionized water. The treated solutions (50 μL) were streaked onto starch agar medium (5 g L^{-1} peptone, 1.5 g L^{-1} yeast extract, 5 g L^{-1} sodium chloride, 2 g L^{-1} starch, and 20 g L^{-1} agar at pH 7) (Geetha et al. 2014). After 24 h, the colonial halo was collected and transferred to a nutrient agar medium (5 g L^{-1} peptone, 3 g L^{-1} beef extract, 5 g L^{-1} sodium chloride, and 20 g L^{-1} agar at pH 7) (Shivaji et al. 2006). The bacteria were then stored in test tubes at 4 °C for further experimentation.

Investigation of the starch degradability using the agar well diffusion method

Bacterial strains were cultured in nutrient broth (5 g L^{-1} peptone, 3 g L^{-1} beef extract, and 5 g L^{-1} sodium chloride at pH 7) at 27 ± 3 °C with shaking at 150 rotations per minute (rpm). After 2 days of incubation, the cultures were centrifuged at 16 627 × *g* for 15 min at 4 °C to obtain cell-free supernatants using a centrifuge 5430 R (Eppendorf SE, Hamburg, Germany). Then, 100 μL of cell-free supernatants was added to wells on the starch agar medium (Geetha et al. 2014). After 48 h of incubation, the plates were stained with iodine to visualize the halo zones. Starch degrading ability was determined by subtracting the well diameter from the halo zone diameter (mm).

Determination of reducing sugar content using the dinitrosalicylic acid method

Standard glucose solutions (0 to 1.2 mg mL^{-1}) were prepared by mixing 1 mL of each concentration with 2 mL of dinitrosalicylic acid reagent [10 g 3,5-dinitrosalicylic acid (Macklin, China), 16 g NaOH, 300 g $KNaC_4H_4O_6$ $4H_2O$ per L of deionized water] (Miller 1956), vortexed, boiled for 5 min, cooled, and measured at 540 nm.

For bacterial samples, 1 mL of supernatant (10^7 CFU mL^{-1}) was added to 9 mL of liquid SA medium and incubated at 27 ± 3 °C with shaking at 150 rpm. Samples were collected at 2, 4, 6, and 8 days, centrifuged at 5000 rpm for 15 min, and 1 mL of supernatant was mixed with 2 mL of dinitrosalicylic acid reagent. After boiling for 5 min and cooling for 5 min, the absorbance was measured at 540 nm. Reducing sugar concentration was calculated based on the standard curve equation:

$$y = 0.5304x - 0.0097 \ (R^2 = 0.976),$$

where y is OD_{540} and x is the reducing sugar concentration (mg mL^{-1}).

Experiment on the wastewater treatment capability

The wastewater treatment capability of the bacterial strain selected from the above experiment was evaluated using 100 mL of wastewater collected from a bread production facility in Binh Thuy District (10°04'04.3"N, 105°45'05.4"E), with an initial pH of 6.3. The strain was cultured in nutrient broth medium for 48 h to reach 10^8 CFU mL^{-1}, then added to flasks at 1, 2, 3, and 4% (*v*/*v*). A negative control was nutrient broth medium. After 24 h of incubation, samples were collected to assess starch degradation efficiency based on pH and glucose concentration.

Selection of bacterial strains with bioflocculant-producing ability

The bioflocculant-producing ability of the bacterial strains was assessed by inoculating each isolate onto medium consisting of 10 g glucose, 2 g KH_2PO_4, 5 g K_2HPO_4, 0.2 g $MgSO_4$ $7H_2O$, 0.1 g NaCl, 0.5 g urea, 0.5 g yeast extract, and 20 g agar per L of deionized water, with the initial pH adjusted to 7.0 (Deng et al. 2003). After 48 h of incubation, colony morphology and slime thread formation were observed to determine bioflocculant production. Viscosity was categorized as low (< 2 cm), moderate (2 to 3 cm), and high (> 3 cm) (Le 2020).

To evaluate bioflocculating activity, 1 mL of bacterial suspension (10^8 CFU mL^{-1}) was inoculated into 10 mL of the medium and incubated at 150 rpm for 4 days. After incubation, 100 µL of the bacterial culture was transferred into a 100 mL beaker containing a kaolin suspension (5 g L^{-1}) mixed with a 1% $CaCl_2$ solution at a volume ratio of 9:1, adjusted to pH 7.0. The suspension was stirred vigorously for 30 s and then allowed to stand undisturbed for 5 min to promote flocculation and sedimentation. The supernatant turbidity was measured at 550 nm.

The flocculating activity was calculated according to the formula described by Deng et al. (2003):

$$\text{Flocculating rate (\%)} = [(OD_{blank} - OD_{550}) / OD_{blank}] \times 100.$$

Identification of bacterial strains for wastewater treatment in bread production

The bacterial strain with potential for wastewater treatment was identified using the PCR method combined with 16S rRNA gene sequencing, using primers 1492R (5'–TACGGTTACCTTGTTACGACT–3') and 27F (5'AGAGTTTGATCCTGGCTC–3'). The thermal cycling conditions in the PCR reaction included: denaturation of the DNA template and primers at 95 °C for 5 min, then increasing to 98 °C for 1 min, annealing at 55°C for 30 s, and extension at 72 °C for 5 min.

16S rRNA gene sequencing was performed, followed by BLAST analysis on the NCBI system to compare with similar published sequences. Sequences from two bacterial strains were aligned using Clustal W in BioEdit v7.2.1. Phylogenetic trees were constructed in MEGA X (Kumar et al. 2018) using the Maximum Likelihood method: the General Time Reversible model (GTR + G + I) for strain Y9 and the Kimura 2-parameter model for strain Y11. The tree's reliability was assessed with 1000 bootstrap replicates, using *Wautersiella enshiensis* and *Metabacillus herbersteinensis* as outgroups.

Data analysis

Each experiment was conducted with three independent biological replicates (n = 3). The results are presented as mean values ± SD. The experimental data were analyzed using Minitab 16 software (Minitab Inc., State College, PA, USA) to assess the differences between treatments with a significance level of 5%.

Results

Bacteria isolated from wastewater

Thirteen bacterial strains were isolated from two wastewater samples from two bread production factories in Can Tho City, Vietnam. The isolated bacterial strains exhibited creamy white or opaque white colour, convex or raised elevations, colony morphologies of entire or lobate margins, with bacterial colony sizes ranging from 0.5 to 5 mm. All bacterial strains tested positive for the Gram and catalase tests (Table 1 and Fig. 1).

Starch-degrading ability

The starch-degrading ability of the 13 bacterial strains was determined based on diameter of the halo (Fig. 2). The halo diameters formed by bacterial strains ranged from 14.67 mm to 22.33 mm. Among them, the strains with large halo sizes included CT3, CT16, NK18, CT14, NK16, CT4, and CD9. Notably, strain CT16 produced the largest halo with a diameter of 22.30 mm, while strain CT1 formed the smallest halo at 14.67 mm. Other strains generated halos ranging from 15.67 mm to 22.00 mm. In particular, seven bacterial strains (CT3, CT16, NK18, CT14, NK16, CT4, and CD9) produced significantly larger halos and were selected for quantifying the amount of reducing sugar produced using the dinitrosalicylic acid method.

Reducing sugar content

The reducing sugar concentration produced by the bacterial strains showed an increasing trend from day 2 to day 8 (Table 2). Specifically, on day 2, strain CT14 produced the highest concentration of reducing sugar at 1.34 mg mL^{-1}, while strain NK16 exhibited the lowest value. After 4 days of cultivation in liquid starch agar medium, reducing

Table 1. Colonial and cellular morphological characteristics. Location 1 and Location 2 in Binh Thuy District were designated as NK and CD, respectively; the location in Ninh Kieu District was designated as CT

Bacterial strain	Morphology		Color	Elevation	Margin	Size (mm)
	Cell	Colony				
NK1	Coccus	Round	Creamy white	Convex	Entire	2.0 – 3.0
NK3	Rod-shaped	Round	Opaque white	Convex	Entire	2.5 – 3.5
NK16	Rod-shaped	Round	Opaque white	Convex	Lobate	2.5 – 3.0
NK18	Coccus	Round	Opaque white	Convex	Entire	2.5 – 3.0
CD4	Coccus	Round	Opaque white	Convex	Entire	3.5 – 4.0
CD9	Coccus	Round	Opaque white	Convex	Entire	1.5 – 2.0
CD10	Coccus	Round	Opaque white	Raised	Lobate	3.0 – 3.5
CD11	Rod-shaped	Round	Yellow	Raised	Entire	3.0 – 5.0
CT1	Rod-shaped	Round	Opaque white	Convex	Entire	3.0 – 3.5
CT3	Rod-shaped	Round	Opaque white	Raised	Erose	3.5 – 5.0
CT4	Coccus	Round	Opaque white	Convex	Lobate	3.0 – 3.5
CT14	Rod-shaped	Round	Opaque white	Raised	Erose	3.0 – 4.0
CT16	Coccus	Round	Opaque white	Raised	Lobate	5.0 – 6.0

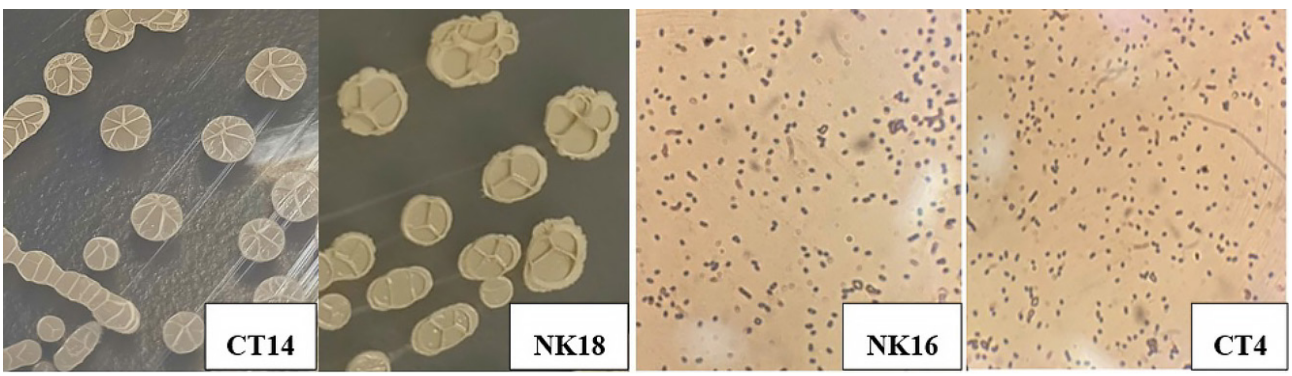

Fig. 1. Colonial and cellular morphological characteristics. Colony morphology on NA medium was observed under a stereo microscope, and cell morphology was examined at 1000× magnification using a light microscope.

sugar levels in all strains increased markedly, with CT14 maintaining the highest level at 2.1 mg mL^{-1}. This difference was statistically significant at the 5% level compared to the other strains, with CT3 showing the lowest concentration at 1.17 mg mL^{-1}. By day 6, a slight decrease in reducing sugar concentration was observed in all strains; however, CT14 still exhibited the highest value. On day 8, reducing sugar levels continued to decrease slightly, but not significantly compared to day 6, with CT14 remaining the strain with the highest concentration at 1.78 mg mL^{-1}.

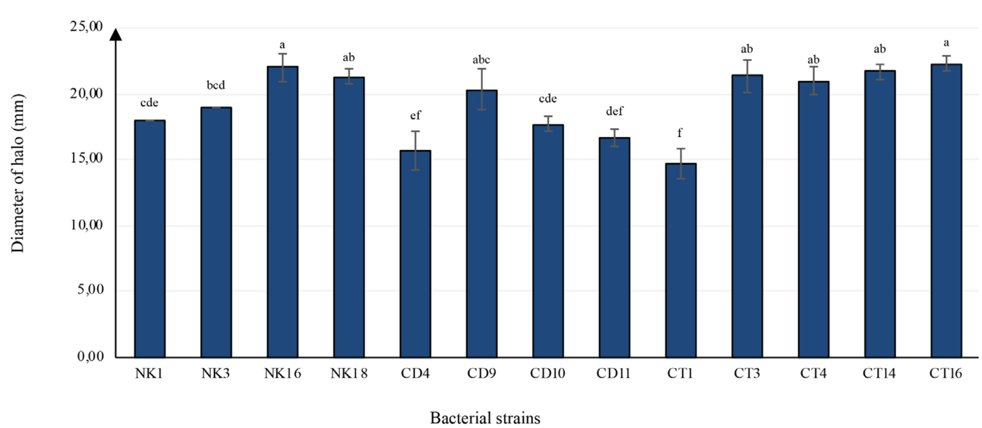

Fig. 2. Starch-degrading ability of bacterial strains based on the halo diameter on starch agar medium. Different letters above the bars indicate statistically significant differences ($p < 0.05$), while the same letters denote no significant difference.

Table 2. Levels of reducing sugar content produced. Mean values in the same column followed by the same letters are not significantly different at the 5% significance level according to Tukey's test

Bacterial strain	Reducing sugar content (mg mL^{-1})			
	2 days	4 days	6 days	8 days
NK16	0.62 ± 0.00 e	1.70 ± 0.01 b	1.55 ± 0.01 d	1.13 ± 0.01 e
NK18	1.25 ± 0.00 b	1.80 ± 0.01 c	1.65 ± 0.01 c	1.38 ± 0.01 c
CD9	1.24 ± 0.00 b	1.74 ± 0.01 d	1.64 ± 0.01 c	1.59 ± 0.01 b
CT3	0.79 ± 0.01 d	1.17 ± 0.02 f	1.12 ± 0.01 e	0.95 ± 0.01 f
CT4	0.69 ± 0.01 e	1.89 ± 0.01 b	1.70 ± 0.01 b	1.63 ± 0.01 b
CT14	1.34 ± 0.01 a	2.10 ± 0.03 a	1.81 ± 0.01 a	1.78 ± 0.01 a
CT16	1.13 ± 0.01 c	1.54 ± 0.01 e	1.50 ± 0.02 d	1.23 ± 0.03 d
Negative control	0.38 ± 0.00 f	0.40 ± 0.00 g	0.40 ± 0.00 f	0.38 ± 0.00 g

These results indicate that reducing sugar concentration varied among strains over the four time points. Throughout the period from day 2 to day 8, strain CT14 consistently produced the highest amount of reducing sugar compared to the other strains. Therefore, CT14 was selected for species-level identification.

Bioflocculant-producing capability

Out of thirteen bacterial strains, six (CD11, CD4, CT14, NK1, CT4, and NK12) were found to be able to grow on the medium used to assess the production of polysaccharide-type bioflocculants. The colonies were generally round, opaque white to ivory in color, with entire margins, raised elevation, and sizes ranging from 0.5 to 2.5 mm. Notably, all strains grown on DBP medium exhibited a filamentous or thread-like appearance on the agar surface, a characteristic commonly associated with bioflocculant synthesis and related to the presence of extracellular slime layers surrounding the cells (Fig. 3A). After selecting the six bacterial strains capable of producing polysaccharide-based bioflocculants, their flocculating activity was quantitatively evaluated using a kaolin solution. All strains achieved flocculating rates above 50%, with strain CD11 showing the highest activity at 80.40%, while strain NK12 had the lowest at 55.375% (Fig. 3B). Based on the results, three bacterial strains, CD11, CD4, and CT14, were selected for further experiments to investigate optimal conditions for bioflocculation efficiency in kaolin medium. Among them, strains CD11 and CT14 were subsequently selected for species-level identification.

Identifying potential bacterial strains for wastewater treatment from bread production processes

The phylogram was rooted with *Metabacillus indicus* (NR 029022.1) and *B. atrophaeus* (NR 112723.1), forming a basal lineage that branched off at a genetic distance of 0.004, approximately 0.004 from the main *B. subtilis* group (Fig. 4). Within this main clade, two well-supported sub-groups emerged. The first (bootstrap 65%) clustered *B. amyloliquefaciens, B. siamensis*, and the study isolates CD11 and CT14, all separated by a minimal 16S rRNA divergence. The second (bootstrap 67%) contained eight closely related taxa, with *B. subtilis, B. velezensis*, and *B. tequilensis* again distinguished by extremely short branches. Overall, the tree indicates that CD11 and CT14 sit firmly within the *B. amyloliquefaciens/siamensis* lineage and that all members

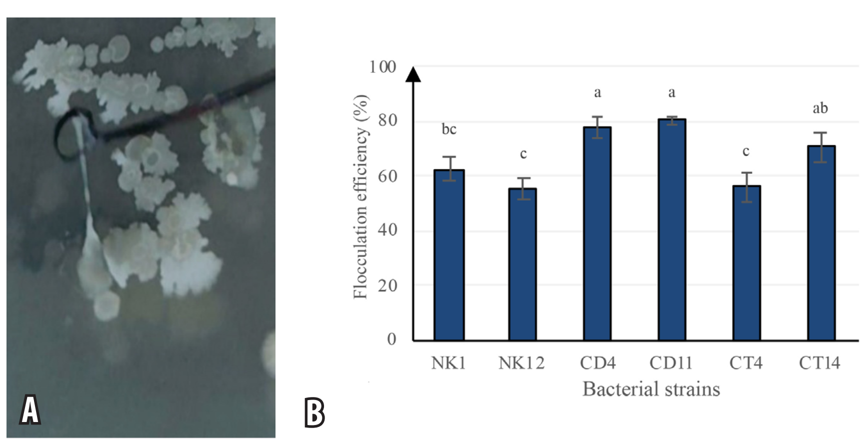

Fig. 3. Mucilage production and flocculation efficiency of flocculant-producing bacteria. Among the 13 bacterial strains, six were identified as capable of producing mucilage on DBP medium (A); the flocculation capacity of six bacterial strains was assessed in kaolin suspension (B). Bars with different letters indicate statistically significant differences at $p < 0.05$ according to Tukey's test.

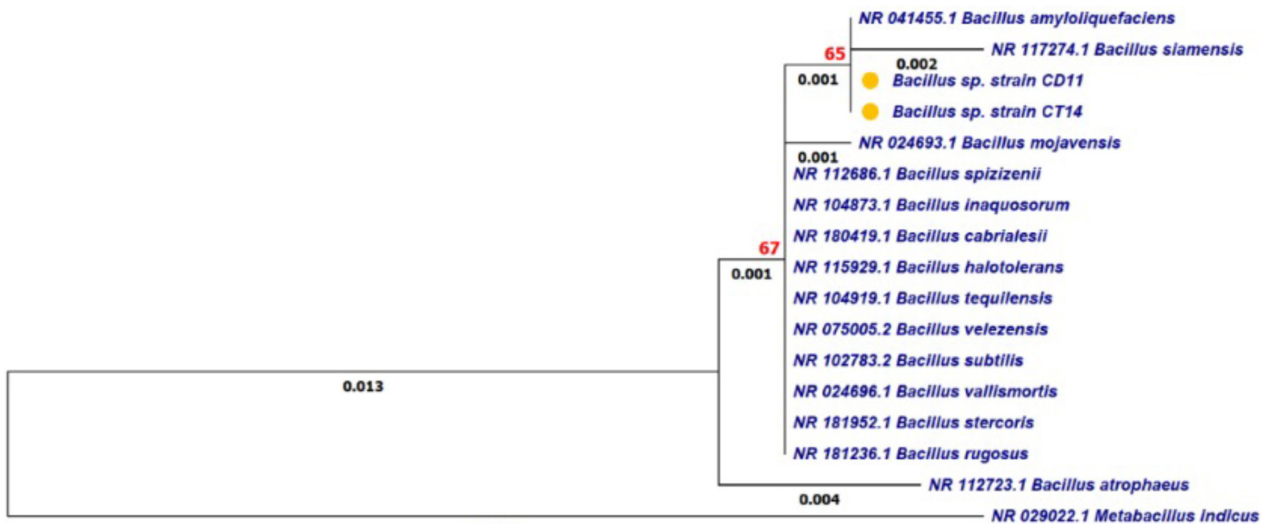

Fig. 4. Phylogenetic tree of the bacterial strain CT14 and CD11 based on the 16S rRNA gene.

of the *B. subtilis* complex are much more similar to each other than to the out-group taxa.

Treatment of wastewater from bread production facilities

The wastewater treatment experiment was conducted using the selected bacterial strain CT14, identified as *B. amyloliquefaciens* (Fig. 4). This is a non-pathogenic strain with high amylase activity, indicating its potential application in treating wastewater containing starch. The bacterial population was increased to 10^8 CFU mL^{-1} before conducting experiments to monitor reducing sugar content and pH over the treatment period (Fig. 5). After 24 h of treatment, the experimental groups with 2, 3, and 4% inoculum concentrations demonstrated significantly different treatment efficiencies at the 5% significance level compared to the control (Fig. 5). Notably, the 4% treatment group achieved a markedly higher reducing sugar concentration of 1.36 mg mL^{-1}, compared to 0.28 mg mL^{-1} in the control. The average pH values of the treatments ranged from 4.22 ± 0.02 to 6.43 ± 0.03. Both reducing sugar content and pH showed an increasing trend, indicating that strain CT14 effectively hydrolyzes starch.

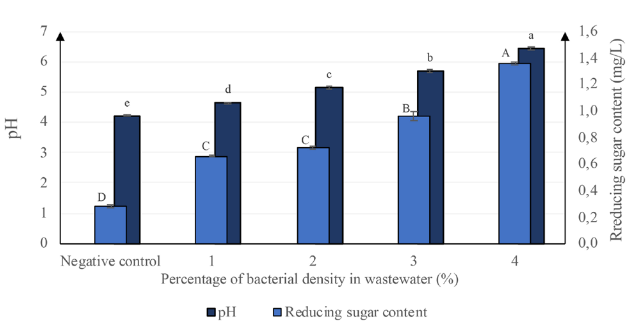

Fig. 5. Effect of bacterial density on wastewater treatment efficiency of strain CT14 after 24 h. The chart shows that bars of the same color with different letters indicate statistically significant differences at the 5% level according to Tukey's test.

Discussion

In this study, two *Bacillus* strains (CT14 and CD11) were selected from the strains isolated from starch-rich wastewater collected from bread production facilities in Can Tho City, Vietnam. These strains possessed two important characteristics: starch degradation and bioflocculant production. Both strains showed strong growth in media containing starch and formed distinct hydrolysis zones, indicating significant extracellular amylase activity. This enzyme is crucial for breaking down complex carbohydrates commonly found in food-processing wastewater. Additionally, the strains secreted mucilaginous substances into the medium, increasing its viscosity and promoting particle aggregation, resulting in improved water clarity. Bacterial extracellular polymeric substances play a crucial role in bioflocculation mechanisms, which are essential for both natural and engineered water treatment systems (Pham et al. 2019). This is supported by the findings of high flocculation efficiency in *Streptomyces platensis* (Agunbiade et al. 2022) and *Bacillus* sp. HQ537128 (Nontembiso et al. 2011).

Other recent studies have highlighted the potential of *Bacillus* strains in degrading organic compounds and producing industrially relevant enzymes. Specifically, *Bacillus* NT1 efficiently degrades xylan, cellulose, starch, and proteins, and reduces COD in wastewater by 80 to 90% (Tran et al. 2018). Similarly, *B. amyloliquefaciens* exhibits strong starch-hydrolyzing activity, with α-amylase production reaching 695.2 U mL^{-1} when cultivated in brown bread waste medium after optimization (Abd-Elhalim et al. 2023). These findings further support the potential of strain CT14 in treating starch-rich wastewater, particularly from bread production facilities.

Phylogenetic analysis based on 16S rRNA gene sequences revealed that CT14 exhibited high genetic similarity to *B. amyloliquefaciens* and *B. velezensis* (bootstrap >90%), both belonging to the *B. subtilis* species complex. This complex is known for its production of extracellular enzymes, secondary metabolites, and antimicrobial compounds (Dunlap et al. 2016; Fan et al. 2017). However, CD11 formed a separate clade, closely related to *B. subtilis* and *B. mojavensis* (bootstrap ~70%). This suggests significant genetic divergence and indicates that CD11 may represent a lesser-characterized lineage within the *B. subtilis* group. To clarify the taxonomic status of CD11, further analysis using housekeeping genes such as *gyrA*, *rpoB*, or *recA*, or multilocus sequence analysis, is warranted (Hong et al. 2022). Additionally, due to the > 99% 16S rRNA sequence similarity between *B. amyloliquefaciens* and *B. velezensis*, misidentification is common when relying solely on this gene (Kim et al. 2014). Therefore, whole-genome sequencing and average nucleotide identity analyses are necessary to accurately differentiate these species, especially considering their distinct ecological niches, metabolic capacities, and industrial relevance (Fan et al. 2018).

This study demonstrates the potential of two indigenous Bacillus strains, CT14 and CD11, in the treatment of carbon-rich wastewater, owing to their capabilities in starch hydrolysis and bioflocculant production. These findings support the development of cost-effective and environmentally sustainable strategies for wastewater management.

Conclusions

From bread production wastewater, thirteen out of fifteen isolates were screened based on their starch-degrading ability, among which six potential strains were demonstrated for effective bioflocculant production. CT14 exhibited outstanding starch hydrolysis on starch agar medium, with the highest reducing sugar concentration at 2.1 mg mL^{-1} after four days of incubation. Meanwhile, strain CD11 showed the highest bioflocculation efficiency, reaching 81.363% in a kaolin suspension, attributed to its production of polysaccharide-based bioflocculants. Both strains CT14 and CD11 were identified as *Bacillus amyloliquefaciens*. The dual functionality of CT14 and CD11 in both starch degradation and floc formation, make them effective in removing organic pollutants and turbidity from wastewater. The flocculating ability of these strains was also confirmed through kaolin clay suspension assays, indicating the presence of extracellular or flocculant-active compounds.

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