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Characterization and screening of urease activity of ureolytic bacteria from landfills soil in Banda Aceh, Indonesia

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Abstract. *Fitri L, Aulia TB, Fauzi A, Kamil GA. 2023. Characterization and screening of urease activity of ureolytic bacteria from landfills soil in Banda Aceh, Indonesia. Biodiversitas 24: 910-915.* Ureolytic bacteria are capable of producing the calcium carbonate precipitating enzyme urease. Ureolytic bacteria degrade urease into ammonia and carbon dioxide. Ureolytic bacteria can be applied in the bio-grouting technique and concrete mixtures. This study aimed to isolate and characterize ureolytic bacteria isolates, then determine the calcium carbonate precipitation potential of ureolytic bacteria isolates from landfill soil at Gampong Jawa, Banda Aceh, Indonesia. This research successfully isolated twenty-four bacterial isolates from the Gampong Jawa landfills, and ten of these isolates were confirmed to produce the urease enzyme positively. Isolates with codes BTPA-3, BTPA-6, BTPA-7, BTPA-8, BTPA-9, BTPA-15, BTPA-20, BTPA-22, BTPA-23, and BTPA-24 were able to precipitate calcium carbonate in the amounts of 1.49, 1.78, 1.71, 1.70, 1.82, 1.80, 1.32, 1.54, and 1.70 g, respectively. BTPA-3, BTPA-6, BTPA-7, BTPA-8, BTPA-9, BTPA-23, and BTPA-24 were identified as members of the genus *Bacillus*; BTPA-20 was as a member of the genus *Staphylococcus*; and BTPA-15 and BTPA-22 were members of the genus *Solibacillus*. This research data is new information about the potential of bacteria from the Gampong Jawa landfill, which can determine the calcium carbonate precipitation. The research also revealed that the ureolytic bacterial isolates produced could be further improved and utilized in concrete mixtures.

Keywords: Calcium carbonate, Gampong Jawa, ureolytic bacteria

INTRODUCTION

Microorganisms can precipitate carbonates in various natural settings, including soils, geological formations, oceans, and saline lakes. This process is known as microbially induced carbonate precipitation (MICP). This bacteria's capacity to precipitate carbonates has been extensively examined. There have been proposals for active and passive mechanisms to explain how microorganisms mediate the precipitation process (Han et al. 2013). Among them, urease hydrolysis by nitrogen-cycle organisms has been the subject of the most research, particularly in terms of potential engineering applications. Although urease activity is ubiquitous in bacteria, the amount and pace of carbonate precipitation varies between species and genera and is influenced by local environmental variables (Kang et al. 2015)

Ureolytic bacteria are microorganisms capable of producing urease and hydrolyzing urea. Ureolytic bacteria are capable of precipitating calcium carbonate ($CaCO_3$). The deposition of calcium carbonate ($CaCO_3$) by bacteria is a biological mineralization process (Rajasekar et al. 2021). The enzyme urease aids in this process. The urease enzyme is responsible for catalyzing the breakdown of urea into carbon dioxide and ammonia. The digestive tracts of several animal tissues and bacteria include the urease enzyme. This urease enzyme elevates the pH by producing ammonia from the hydrolysis of urea and by increasing carbonate concentration. Then it mixes with calcium in the medium to precipitate calcium carbonate (Dhami et al. 2014).

Urea is one of the most prevalent sources of nitrogen that is not derived from proteins. Several variables could explain this disparity. These include the rate of urea hydrolysis concerning the use of urea as an energy source, the alkalinity of the local environment, which affects carbonate speciation and $CaCO_3$ solubility, the affinity of the bacterial cell surfaces for Ca^{2+} ions, which can create micro-scale supersaturation of Ca^{2+} in the vicinity of cells, and possibly leading to nucleation and crystal growth where carbonate is also sufficiently saturated to promote crystal formation (Rajasekar et al. 2018). Based on the ability of ureolytic bacteria to precipitate $CaCO_3$, these microorganisms can be employed for property development involving sand materials, such as reinforcing concrete constructions (Alonso et al. 2018).

Multiple ureolytic bacteria have been successfully isolated by researchers in the past. *Bacillus megaterium*, *B. simplex*, and *Sporosarcina pasteurii* have been successfully isolated by Achal and Pan (2011) from alkaline soil

samples. Ningsih et al. (2018) were able to extract ureolytic bacteria from landfill soil in Riau, as evidenced by the ability of three of thirty recovered isolates to generate calcium carbonate. The characteristics of bacteria obtained from the study were 77% Gram-positive and 23% Gram-negative, and the *Bacillus* bacterium group accounted for the remaining 3%. According to a separate study by Jalilvand et al. (2020), the bacterial isolates collected from samples of calcareous soil displayed similarities to the genus *Bacillus*, with Gram-positive, stem-shaped bacilli, endospores, and catalase-positive features.

Urease generated by ureolytic bacteria works as a catalyst in the hydrolysis of urea to ammonia, producing calcium carbonate when CO_3^{2-} ions bind with Ca^{2+}. Ca^{2+} ions can alter the ability of calcium carbonate ($CaCO_3$) or calcite to form, with more Ca^{2+} causing calcite formation and vice versa (Phang et al. 2018). The electronegativity of the bacterial cell wall facilitates the adsorption of cations, such as calcium ions, hence promoting the deposition of $CaCO_3$ on the cell wall (Gat et al. 2014). Ureolytic bacteria are present in the environment from various sources, including limestone mounds, sediments, cave stalactites, and marine sediments (Ma et al. 2020). Calcium carbonate ($CaCO_3$) formed by ureolytic bacteria and calcite covering the cell wall can be observed using an electron microscope. The more bacteria that produce calcium carbonate, the more biocement can be used to construct and strengthen soil (Gebru et al. 2021).

Landfills are intricate microbiological systems inhabited by microorganisms that repair or decompose harmful substances (Wang et al. 2022). Stimulating carbonate precipitation in indigenous bacteria already acclimated to the biochemically severe environmental conditions of a landfill could be a cost, material, and energy-efficient alternative to geotechnical or geoenvironmental engineering procedures for controlling landfill leachate (Imron et al. 2019). Indigenous bacteria could be utilized for the modulation of groundwater flow, immobilization of contaminants/heavy metals by coprecipitation as substitute ions for calcium, or simple entrapment of contaminants/heavy metals in cemented pore spaces (Hamid et al. 2022). The objective of this study was to isolate and characterize ureolytic bacteria isolates and then estimate the calcium carbonate precipitation capability of ureolytic bacteria isolates from landfills soil in Gampong Jawa, Banda Aceh, Indonesia.

MATERIALS AND METHODS

Study area and soil sampling

The Gampong Jawa landfills, Banda Aceh, Indonesia were sampled at five separate locations (Figure 1). Soil samples from landfills in Gampong Jawa have been collected aseptically. The plastic clip is first sprayed with alcohol and then dried by air. In addition, the soil is excavated to a depth of 5 cm, followed by the collection of suitable soil samples. The soil samples were then transported to the Microbiology Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala in Banda Aceh, Indonesia.

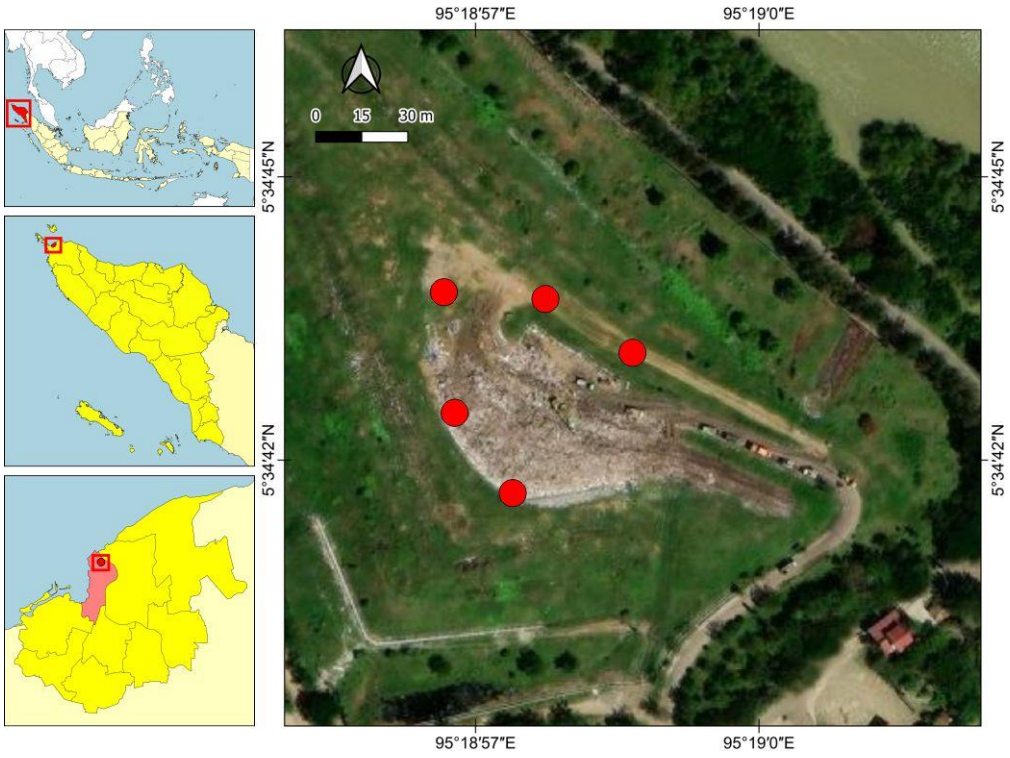

Figure 1. Soil sampling site at Gampong Jawa landfill, Banda Aceh, Indonesia

Isolation of ureolytic bacteria

An Erlenmeyer flask containing 90 mL of sterile NaCl was filled with 10 g of soil. Additional dilution was performed until it reached 10^{-5}. Soil samples 1 mL from dilutions ranging from 10^{-1} to 10^{-5} were spread onto a Petri plate with nutrient agar (NA) medium using the spread plate technique. In addition, the Petri plates were incubated for 24 h at room temperature. Growing bacterial isolates were purified, transferred to slanted agar as stock culture, and kept at 4°C using the quadrant streak plate technique.

Morphological and physiological characterization of bacteria

The morphological properties of bacteria that were seen macroscopically were colony form, elevation, margin, and color. Observations at the microscopic level include cell morphology and Gram staining. The catalase, indole, and Methyl Red-Voges Proskauer (MR-VP), Simmons citrate, and triple sugar iron agar (TSIA) tests were administered to observe physiological characteristics.

Screening of urease-producing bacteria

The creation of urease media was done by adding 5 g of NaCl, 1 g of peptone, 1 g of glucose, 2 g of KH_2PO4, 0.012 g of phenol red, and 15 g of agar were dissolved in 900 mL of distilled water and then sterilized for 15 min in an autoclave at 121°C. After that, 20 g of urea were added to 100 milliliters of distilled water in the preceding medium. In a slanted posture, the medium is poured into the test tube and allowed to solidify. The bacterial isolates were then streaked onto the medium and incubated at 30 °C for four days. A change in color of the medium from yellow to red indicates a positive result (Mekonnen et al. 2021).

Presipitation test of Calcium carbonate

A total of 3 g of nutrient broth, 20 g of urea, and 28.5 g of $CaCl_2$. $2H_2O$ were dissolved in 900 mL of distilled water and mixed it to 1000 mL. Urease positive bacterial isolates were prepared with inoculums with a total population of 10^5 CFU/mL in urea mixed medium. Six hundred microliters of inoculum was inoculated into 30 mL of urea mixed medium and incubated for 7 days at 30°C in a shaker incubator with a speed of 130 rpm. The $CaCO_3$ precipitate was filtered using filter paper (Whatman No. 42) which had been dried in an oven for 3 h at 60°C. The weight of the $CaCO_3$ precipitate (Wc) is the difference between the weight of the filter paper and the precipitate (Wfc) minus the weight of the filter paper (Wf) (Krishnapriya et al. 2015).

Data analysis

The macroscopic, microscopic, and urease activity data of bacteria were qualitatively studied, while the $CaCO_3$ precipitation was quantitatively analyzed.

RESULTS AND DISCUSSION

Characteristic of ureolytic bacteria

A total of 24 bacterial isolates were successfully isolated from landfill soil in the Gampong Jawa area. The results of this investigation contrast with those of Ningsih et al. (2018) at the Riau TPA, who succeeded in getting 30 bacterial isolates. The difference in the results of the isolation carried out is due to the different conditions and sampling locations, so the isolation results obtained are also different. Leeprasert et al. (2022) claimed that one of the elements that influenced the process of colonization was the state of the geographical environment. Observations showed that the isolates collected had various features. Colonies obtained were spherical, uneven, and threadlike. The colonies' borders obtained were smooth, uneven, grooved, and wavy flat with embossed elevation. The color of the colonies varies widely, namely white, cream, orange, milky white, yellow, brownish white, and brownish yellow.

The microscopic characterization was carried out by Gram staining, which attempts to detect the form of the bacterial cells and split them into groups of Gram-positive and Gram-negative bacteria. Gram staining is also an early step to determine the type of bacteria. According to Rohmah et al. (2021), the cell wall of Gram-positive bacteria consists of a thick peptidoglycan layer, while the cell wall of Gram-negative bacteria contains lipids in the form of thick lipopolysaccharide and a thin coating of peptidoglycan. Peptidoglycan is insoluble in non-polar organic solvents, and lipopolysaccharide is soluble in non-polar organic solvents.

Based on the Gram staining that had been carried out, the isolated bacterial isolates were Gram-positive, some were Gram-negative with rod cell morphologies, and two of the cell isolates were spherical. The bacterial isolates obtained were coded BTPA 1, BTPA 2, BTPA 3, and so on until BTPA 24 (Table 1).

Multiple studies on ureolytic bacteria indicate that they are Gram-positive, rod-shaped bacteria. Chahal et al. (2011) noted that ureolytic bacteria are Gram-Positive *Bacillus* bacteria that have the potential to manufacture urease enzymes. Six isolates of Gram-positive ureolytic bacteria with bacilli-shaped cells were identified by Algaifi et al. (2020).

The ability of the isolated bacteria to produce urease enzymes was then evaluated using a urea mixture medium; a change indicated positive results in color from yellow to deep red. Based on the urease activity test, ten isolates with positive results were obtained, namely isolates with the codes BTPA 3, BTPA 6, BTPA 7, BTPA 8, BTPA 9, BTPA 15, BTPA 20, BTPA 22, BTPA 23, and BTPA 24 (Table 2).

The activity of the urease enzyme will cause the medium to change color from yellow to dark red (deep pink) (Figure 2). The medium containing phenol red as an indication of pH color change causes the color change. When bacteria use the enzyme urease to hydrolyze urea in the medium, the originally yellow medium turns a deep red color. Urease is required for the hydrolysis of urea into ammonia and carbon dioxide and the elevation of pH and carbonate in the bacterial environment (Ningsih et al. 2018).

Table 1. Morphological characteristics of Gampong Jawa landfill bacterial isolates

Isolate codes	Form	Margin	Elevation	Color	Gram staining	Cell morphology
BTPA1	Round	Entire	Flat	Cream	Negative	Bacili
BTPA2	Round	Entire	Flat	Cream	Positive	Bacili
BTPA3	Round	Entire	Flat	Tawny	Negative	Bacili
BTPA4	Round	Entire	Raised	Orange	Negative	Coccobacilli
BTPA5	Round	Entire	Raised	Orange	Negative	Coccus
BTPA6	Round	Undulate	Flat	White	Positive	Coccobacilli
BTPA7	Round	Entire	Raised	Cream	Negative	Coccobacilli
BTPA 8	Undulate	Entire	Flat	Orange	Negative	Coccobacilli
BTPA 9	Undulate	entire	Flat	Orange	Negative	Bacili
BTPA 10	Undulate	Erose	Flat	White	Negative	Coccobacilli
BTPA 11	Undulate	Erose	Flat	Cream	Negative	Coccobacilli
BTPA 12	Round	Entire	Flat	White	Positive	Bacili
BTPA 13	Undulate	Erose	Flat	Cream	Positive	Bacili
BTPA 14	Round	Entire	Flat	White	Negative	Bacili
BTPA 15	Round	Entire	Flat	Cream	Positive	Bacili
BTPA 16	Filamentous	Lobate	Flat	White	Negative	Bacili
BTPA 17	Undulate	Entire	Flat	Kuning	Negative	Coccobacilli
BTPA 18	Round	Entire	Flat	Cream	Positive	Bacili
BTPA 19	Round	Entire	Raised	Kuning	Positive	Coccus
BTPA 20	Round	Entire	Raised	White	Positive	Coccus
BTPA 21	Round	Entire	Raised	Cream	Positive	Bacili
BTPA 22	Round	Entire	Raised	Orange	Positive	Bacili
BTPA 23	Round	Entire	Raised	White	Negative	Bacili
BTPA 24	Round	Entire	Raised	White	Positive	Bacili

Table 2. Test of urease activity of bacterial isolates from Gampong Jawa landfill

Isolate codes	Urease activity
BTPA 1	-
BTPA 2	-
BTPA 3	+
BTPA 4	-
BTPA 5	-
BTPA 6	+
BTPA 7	+
BTPA 8	+
BTPA 9	+
BTPA 10	-
BTPA 11	-
BTPA 12	-
BTPA 13	-
BTPA 14	-
BTPA 15	+
BTPA 16	-
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BTPA 20	+
BTPA 21	-
BTPA 22	+
BTPA 23	+
BTPA 24	+

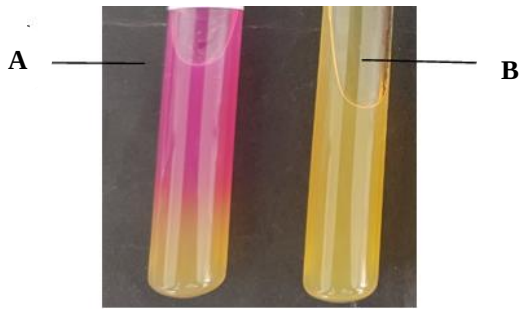

Figure 2. Test of urease activity of bacterial isolates from Gampong Jawa landfill. Note: a. positive urease, b. negative urease

Ten isolates of bacteria capable of producing urease enzymes were subjected to biochemical testing (Table 3). The biochemical test results based on Bergey's Manual of Determinative Bacteria revealed that the positive urease bacterial isolates originated from the genera *Bacillus*, *Staphylococcus*, and *Solibacillus.* BTPA 3, BTPA 6, BTPA 7, BTPA 8, BTPA 9, BTPA 23, and BTPA 24 were identified as members of the genus *Bacillus*; BTPA 20 was as a member of the genus *Staphylococcus*; and BTPA 15 and BTPA 22 were members of the genus *Solibacillus.*

Safitri (2019), in a research, obtained isolates of ureolytic bacteria from several genera, namely *Bacillus*, *Solibacillus, Yersinia*, and *Paenibacillus.* This study obtained bacteria from the genus *Bacillus*, *Solibacillus*, and *Staphylococcus*. Some Gram-negative ureolytic bacteria isolated in this investigation did not have a rod-like form (coccus). In the research, the genera *Yersinia, Klebsiella*, and *Neisseri*a obtained by Safitri (2019) are Gram-negative bacteria that generate the enzyme urease.

Precipitate of $CaCO_3$

On bacterial isolates whose capacity to produce urease enzymes was known, calcite precipitation tests were conducted. There were 10 positive urease isolates from 24 isolated isolates. Carbonate or bicarbonate is produced from the hydrolysis of urea by the enzyme urease in bacteria. The BTPA 15 isolate produced the most $CaCO_3$ (1.82 g) throughout the seven-day incubation period, whereas the BTPA 22 isolate produced the least (1.32 g) (Table 4).

Based on the results, isolate BTPA 15 could precipitate calcium carbonate at a rate of 1.82 g, followed by isolates BTPA 20 (1.80 g) and BTPA 7 (1.78 g). The BTPA 22 isolation yielded the lowest result at 1.32 g. These findings surpass those of Krishnapriya et al. (2015), who isolated ureolytic bacteria capable of precipitating 0.84 g of calcium carbonate from the soil surrounding a cement factory. In addition, the results of this work contrast with those of Ningsih et al. (2018), who isolated ureolytic bacteria from the soil in Riau TPA and demonstrated their ability to isolate species 32 in calcium carbonate precipitation of 0.13 g. Each type of ureolytic bacteria has a unique capacity to produce the enzyme urease, which explains why the yield of calcium carbonate precipitates is variable. The urease enzyme generated impacts the amount of precipitable calcium carbonate (Wen et al. 2020).

Table 3. Biochemical test of positive urease-producers isolate

Isolate code	Catalase	*Simmons citrate*	TSIA	Indole	MR	VP
BTPA 3	-	-	-	-	-	-
BTPA 6	+	+	-	+	-	-
BTPA 7	+	+	-	-	-	-
BTPA 8	+	-	-	+	-	-
BTPA 9	+	-	-	+	-	-
BTPA 15	+	-	-	-	-	-
BTPA 20	+	-	-	-	-	-
BTPA 22	+	-	-	-	-	-
BTPA 23	+	+	-	-	-	-
BTPA 24	+	+	-	-	-	-

Note: +: positive; -:negative

Table 4. Precipitation test results of $CaCO_3$

Isolate codes	Filter paper weight (Wf) (g)	Filter paper weight and $CaCO_3$ precipitate (Wfc) (g)	Precipitate weight of $CaCO_3$ (Wc) (g)
BTPA 3	0.66	2.15	1.49
BTPA 6	0.70	2.41	1.72
BTPA 7	0.68	2.45	1.78
BTPA 8	0.75	2.45	1.71
BTPA 9	0.68	2.39	1.70
BTPA 15	0.75	2.56	1.82
BTPA 20	0.67	2.47	1.80
BTPA 22	0.73	2.05	1.32
BTPA 23	0.65	2.18	1.54
BTPA 24	0.67	2.37	1.70

Note: Wc = Wfc - Wf

At a certain saturation level, the presence of calcium ions in the system causes the accumulation of calcium carbonate. Urease is responsible for calcium carbonate deposition's mechanism. Calcium ions will be attracted to the bacterial cell wall due to their negative charge (Ningsih et al. 2018). After adding urea to the system, microorganisms transform the urea into dissolved inorganic carbon and ammonia, which are then discharged into the environment. Rui and Qian (2022) explained that calcium ions cause saturated conditions that lead to the deposition of calcium carbonate in the cell walls, after which the bacterial cells are coated with calcite deposits. The calcite precipitate is white and located at the urea-mixed medium's bottom.

This study concluded that there were 10 isolates of ureolytic bacteria from TPA Gampong Jawa, and *Solibacillus* sp. strain BTPA 15 had the greatest ability to precipitate calcium carbonate. This study successfully isolated 24 bacterial isolates from the soil of the Gampong Jawa landfill. The produced colonies were round, undulating, and filamentous. Successfully isolated bacteria were Gram-positive and Gram-negative with rod cell forms, while two isolates had spherical cell morphologies. The results of the urease activity test revealed that ten isolates had positive urease results after four days of incubation in the urea-mixed medium. The capacity of the 10 isolates to precipitate calcium carbonate was then evaluated. The research also revealed that the ureolytic bacterial isolates produced could be further improved and utilized in concrete mixtures.

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