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Research Article

The Ureolytic Soil Bacteria *Bacillus albus*, a potential Agent for Biocement

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ABSTRACT

Concrete is a common building material and is very vulnerable to cracking caused by unstable temperature/humidity. Concrete crack repair can be done by using microorganism substitution that can produce $CaCO_3$ (calcite) compounds that can be used as an environmentally friendly method in improving structural formation and increasing the strength and durability of concrete, one of which is using ureolytic bacteria. This study aimed to isolate and characterize ureolytic bacteria isolates and then to assess the calcite precipitation potential of ureolytic bacteria isolates from landfills. The ureolytic bacterial isolates were grown on NB-U/Ca and tap water medium. Analysis of Calcite Structure using Fourier Transform Infra-Red (FTIR), and molecular identification using 16S rRNA gene sequences. Bacterial isolate SP. 48 were able to grow and produce calcite in both media, especially in tap water medium. FTIR results showed that the precipitates produced by bacterial isolates on both mediums had strong absorption peaks, which were detected to be calcite. Molecular identification using the 16S rRNA gene sequence showed that the isolate is *Bacillus albus*. *B. albus* is a proteolytic bacterium collected from landfills that was proven to be a calcite-producing bacterium, a new finding in this study. *B. albus* can grow and produce calcite in a tap water medium with low pH. This finding can be used as an alternative to overcome concrete cracks and increase the strength and durability of concrete. This bacterial isolate could be developed as a biocement candidate.

1. Introduction

Concrete is a common material widely used by the public as a building material construction and is very vulnerable to cracking caused by unstable temperature/humidity (Pourfallahi *et al*. 2020). The concrete crack repair can be done by using microorganism substitution that can produce $CaCO_3$ (calcite) compounds that can be used as an environmentally friendly method in improving structural formation, increasing the strength and durability of concrete, one of which is using ureolytic bacteria (Akindahunsi *et al*. 2021).

Ureolytic bacteria are microorganisms that can hydrolyze urea to produce ammonia and precipitate calcium carbonate (calcite) through the activity of the urease enzyme produced, such as *Bacillus megaterium*, *B. flexus*, and *B. licheniformis* (Krishnapriya *et al*. 2015), *B. aerius* (Siddique *et al*. 2016), *B. cereus* (Anitha *et al*. 2018), *B. Subtilis* (Nguyen *et al*. 2019), and *B. paramycoides* (Aliyu *et al*. 2023). Calcite produced by bacteria will cover cracks that occur in concrete so that it does not reduce the strength of the concrete. This microbially induced calcite precipitation is called Microbially Induced Carbonate Precipitation (MICP) (Sharma *et al*. 2016).

Calcite precipitation by microbes can be influenced by factors such as the type of bacteria, bacterial concentration, urea and calcium concentration,

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temperature, and pH (Chaparro-Acuña *et al*. 2018). Appropriate determination of the bacteria's logarithmic phase also optimizes calcite precipitation conditions by ureolytic bacteria (Anbu *et al*. 2016). Calcite production by ureolytic bacteria can be influenced by the type of bacteria, differences in urease activity, exopolysaccharide (EPS) produced, calcium source, and growth medium composition (Anbu *et al*. 2016). The composition of the growth medium used affects the level of saturation, the MICP process, and the morphology of calcite crystals (Aliyu *et al*. 2023).

Tap water containing various minerals, in addition to urea, can be used as a growth medium for ureolytic bacteria to produce calcite. The use of tap water is expected to be a cost-effective alternative approach and provides high potential for its use in self-healing concrete. Anitha *et al*. (2018) reported *B. cereus* KLUVAA using tap water medium as a calcium source with the addition of urea and obtained the highest calcite of 700 mg/500 ml. In addition, Krishnapriya *et al*. (2015) reported that *B. megaterium* BSKAU, *B. licheniformis* BSKNAU, and *B. flexus* BSKNAU, respectively, produced calcite of 0.84 g, 0.82 g, and 0.76 g on 30 ml of NB-U/Ca medium.

Calcite produced by bacteria can be analyzed for its molecular structure/functional groups using FTIR (Fourier Transform Infra-Red) spectroscopy (Putri *et al*. 2017). Dhami *et al.* (2013) reported the FTIR spectrum of calcite formed by isolates of *B. megaterium*, *B. cereus*, and *B. subtilis* showed a strong absorption peak at $\lambda = 713$ cm^{-1}, corresponding to calcite structure. In comparison, the isolates of *B. thuringiensis* and *L. fusiformis* have an absorption peak of 750 cm^{-1}, which corresponds to the form of vaterite grown on nutrient broth medium with the addition of urea (2%), and $CaCl_2$ with 48 hours incubation. The results of research by Li *et al*. (2017), using *B. cereus* with a calcium concentration of 12.5 mmol/L and FTIR results of absorption bands at 708 cm^{-1}, 874 cm^{-1}, and 1420 cm^{-1} which indicate the formation of calcite with 24 hours incubation.

Phenotypic analysis has not been able to group ureolytic bacteria clearly because the diversity of bacteria is different for each location, so molecular identification is carried out with the 16S rRNA gene sequence. Some ureolytic bacteria have been successfully isolated and molecularly identified using universal primers 8F and 1525R, namely bacterial isolate TSB12, which has 99% similarity with *Sporosarcina pasteurii* (Omoregie *et al*. 2016). Exploration of ureolytic bacteria from peat soil with acidic pH obtained two isolates (P9 and P15 have 99% similarity with *Bacillus* genus bacteria using universal primers 27F and 1492R (Phang *et al*. 2018). Based on phenotypic characterization, positive bacteria for the urease test will be tested for urease activity quantitatively and identified by the 16S rRNA gene sequence.

2. Materials and Methods

2.1. Isolation and Characterization of Bacteria

Bacterial isolates were collected from Muara Fajar landfill, Riau, Indonesia. Location 1: N 00°38,738' E 101°26,565' and location 2: N 00°38,731' E 101°26,553'. Macroscopic characterization of bacteria includes color, shape, margins, and elevation of the colony. The microscopic description was carried out using Gram staining, endospore staining, and cell shape.

2.2. Urease Activity Test

Qualitative urea activity tests using Christensen's Urea Agar Base (UAB) are solid media used for the rapid qualification and screening of urease enzymes (Atlas 2010). It is composed of (g/l); urea, 20.0; NaCl, 5.0; peptone, 1.0; glucose, 1.0; KH_2PO_4, 2.0; phenol red, 0.012 and agar, 15.0; (pH 6.5). Pure isolates were examined continually to record the red color (Hammad *et al*. 2013). A quantitative urease activity test was performed using the spectophotometry method. A standard solution of 5 ml ammonia was put into a test tube, and then 5 ml of Tris/HCl solution 100 mM buffer pH 8 containing 300 mM urea solution was reacted for 3 hours. Next, 1 ml was taken, 100µl of Nessler reagent was added, and then responded for 1 minute. After 1 minute, the absorbance of each solution was measured using a spectrophotometer at a wavelength of 425 nm (Phang *et al*. 2018).

Isolates of ureolytic bacteria, each as much as one Ose, were inoculated into 150 ml of Nutrient Broth (NB) and then incubated on a shaker incubator at 140 rpm at 30°C for 18 hours. Each bacterial isolate (10^6 cfu/ml) was put as much as 30 ml into a tube and then centrifuged. The pellet was added with 10 ml of Tris/HCl solution and then centrifuged; the supernatant was discarded, and the Tris/HCl solution was added again and then centrifuged twice.

The pellet of bacterial suspensions in 30 ml Tris/HCl solution containing 300 mM urea was centrifuged. Each supernatant of the isolate that has been reacted

is taken as much as 1 ml and then put into a test tube and added as much as 100 µl of Nessler reagent. Then responded for 1 minute and measured the absorbance using a spectrophotometer with a wavelength of 425 nm (Phang *et al*. 2018).

This study determined urease activity by measuring the product produced from the enzyme reaction with urea substrate, namely ammonia (µg/ml). One unit (U) of urease activity is defined as the amount of product produced (µmol ammonia/ml/min) (Phang *et al*. 2018).

$$\text{Enzyme activity (U/ml)} = \frac{\text{Ammonia concentration (µg/ml)}}{\text{Incubation time (minute) x Mr urea}}$$

2.3. Bacterial Growth Test

A bacterial growth test is seen based on the turbidity of the culture used to indicate bacterial growth. Inoculum of ureolytic bacterial isolates was used as much as 10% (Zhang *et al.* 2017), which was grown on 90 ml of NB-U/Ca and 90 ml of tap water medium and then incubated on a shaker incubator (30°C 150 rpm). Bacterial growth was measured every day until it reached the logarithmic phase (log) on NB-U/Ca medium and for 7 days on tap water medium based on turbidity assay. Optical density (OD) was measured using a UV-Vis spectrophotometer at a wavelength of 600 nm (Omoregie *et al.* 2017).

2.4. Calcite Production

2.4.1. Starter Culture Preparation

Isolates of ureolytic bacteria with the highest activity were tested for calcite production. The bacterial population used in the starter culture was 10^6 CFU/ml. Bacterial isolate was grown on 50 ml of NB-U/Ca medium and incubated for 24 hours on a shaker incubator at 30°C with a speed of 150 rpm.

2.4.2. Calcite Production Test

Starters of ureolytic bacterial isolates with an inoculum concentration of 10% (Zhang *et al*. 2017) were grown into 90 ml of NB-U/Ca medium and 90 ml of tap water medium. The medium was then incubated on a shaker incubator at 30°C with a speed of 150 rpm based on the determination of the logarithmic phase (log) on the measurement of bacterial growth on the NB-U/Ca medium while the tap water medium was incubated for 7 days. Control was made without inoculation of bacteria on the medium. Calcite deposits from isolate cultures were filtered using filter paper (Whatman No. 42), which had previously been dried in an oven at 60°C for 3 hours and then weighed. The weight of calcite precipitate (Wc) is the difference between the weight of filter paper and calcite precipitate (Wfc) minus the weight of filter paper (Wf) (Krishnapriya *et al*. 2015).

2.5. Analysis of Calcite Structure using Fourier Transform Infra-Red (FTIR)

The resulting calcite crystals were further characterized for their molecular structure using FTIR spectroscopy. The dried calcite was mixed and ground with potassium bromide (KBr). FTIR spectra ranged from 400-4000 cm^{-1} to examine the structure of calcite (Vahabi *et al*. 2013; Anitha *et al*. 2018). The peak of pure calcite is very typical and is in the range of 713, 875, 1423 (single), 1794, and 2516 cm^{-1} Daskalakis *et al*. (2013).

2.6. Molecular Identification

DNA was amplified by using 16S rRNA gene primer 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') (Phang *et al*. 2018). The amplified products were then purified by PCR Clean-Up or Gel Extraction depending on visualization results for Single Pass DNA Sequencing.

2.7. Data Analysis

Data on bacterial isolate growth and calcite production results were analyzed quantitatively, while data from FTIR results were analyzed qualitatively. The sequences obtained were then analyzed using the BLASTn (Basic Local Alignment Search Tool nucleotide) program using the sequence database available at the National Center for Biotechnology Information (NCBI). The phylogenetic tree was then created using the MEGA6 application (Molecular Evolutionary Genetics Analysis version 7.0) and the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method with a bootstrap of 1000 replications.

3. Results

3.1. Isolation and Characterization of Ureolytic Bacteria

Based on the isolation, 48 bacterial isolates were obtained, of which 21 were Gram-positive and 27 were Gram-negative. Five isolates that showed a positive urease test were SP. 32, SP. 34, SP. 48, SP. 83, and SP. 84. Uniquely, these five isolates have rod cell shapes (Table 1).

Then, the urease enzyme activity test was carried out from these five isolates, and one isolate was obtained with the urease enzyme (SP. 48). The urease

Table 1. The Characteristic of bacterial isolates from landfill

Isolate code	Colony color	Colony shape	Colony elevation	Colony edge	Gram	Urease	Cell shape	Endropores
SP 32	Milky white	Circular	Flat	Serrate	+	+	Rod	-
SP 33	Milky white	Circular	Flat	Lobate	-	-	Cocci	-
SP 34	White	Circular	Flat	Lobate	+	+	Rod	-
SP 35	Yellowish white	Circular	Flat	Entire	+	-	Rod	-
SP 36	White	Circular	Flat	Entire	-	-	Cocci	-
SP 37	White	Circular	Flat	Undulate	+	-	Rod	-
SP 38	White	Circular	Flat	Entire	+	+	Cocci	-
SP 41	White	Circular	Flat	Entire	+	+	Cocci	-
SP 42	White	Circular	Flat	Lobate	-	-	Cocci	-
SP 43	White	Filamentous	Flat	Serrate	+	-	Rod	-
SP 44	Milky white	Circular	Raised	Entire	+	+	Cocci	-
SP 45	White	Circular	Flat	Entire	+	+	Cocci	-
SP 46	White	Circular	Flat	Entire	-	-	Cocci	-
SP 48	Milky white	Circular	Raised	Serrate	+	+	Rod	+
SP 50	White	Circular	Flat	Entire	-	-	Cocci	-
SP 51	White	Circular	Flat	Entire	-	-	Cocci	-
SP 52	White	Circular	Flat	Entire	-	-	Cocci	-
SP 53	Milky white	Circular	Flat	Entire	-	-	Cocci	-
SP 54	White	Circular	Flat	Entire	-	-	Cocci	-
SP 55	Milky white	Irregular	Raised	Serrate	+	+	Cocci	-
SP 56	White	Circular	Flat	Entire	-	-	Cocci	-
SP 57	Milky white	Circular	Flat	Serrate	-	-	Cocci	-
SP 58	White	Circular	Flat	Entire	+	-	Rod	-
SP 59	Milky white	Circular	Flat	Entire	+	-	Cocci	
SP 60	Milky white	Circular	Flat	Entire	+	-	Cocci	-
SP 61	Milky white	Circular	Flat	Undulate	-	-	Cocci	-
SP 62	Milky white	Circular	Flat	Entire	-	-	Rod	-
SP 64	White	Circular	Flat	Entire	+	-	Rod	-
SP 65	Milky white	Circular	Flat	Undulate	+	+	Cocci	-
SP 66	Milky white	Irregular	Flat	Undulate	-	-	Cocci	-
SP 67	Milky white	Circular	Flat	Serrate	-	-	Cocci	-
SP 68	Milky white	Circular	Flat	Entire	+	-	Cocci	-
SP 69	Milky white	Circular	Flat	Serrate	+	-	Rod	-
SP 70	Milky white	Circular	Flat	Entire	-	-	Cocci	-
SP 71	Milky white	Circular	Flat	Serrate	-	-	Cocci	-
SP 73	Milky white	Circular	Flat	Entire	-	-	Cocci	-
SP 74	Milky white	Circular	Flat	Entire	-	-	Cocci	-
SP 75	Milky white	Circular	Raised	Serrate	-	-	Cocci	-
SP 76	White	Circular	Flat	Serrate	-	-	Cocci	-
SP 77	White	Circular	Flat	Serrate	+	-	Cocci	-
SP 78	Milky white	Circular	Raised	Serrate	-	-	Rod	-
SP 79	Milky white	Circular	Raised	Serrate	-	-	Cocci	-
SP 81	Milky white	Circular	Flat	Entire	-	-	Cocci	-
SP 82	Milky white	Circular	Flat	Entire	-	-	Cocci	-
SP 83	Milky white	Circular	Flat	Entire	+	+	Rod	+
SP 84	Milky white	Circular	Flat	Entire	+	+	Rod	+
SP 85	White	Circular	Flat	Serrate	-	-	Cocci	-
SP 87	White	Circular	Flat	Entire	-	-	Cocci	-

activity of bacterial isolate SP 32 (0.053U/ml) was not significantly different from SP. 84 (0.044 U/ml) and SP. 34 (0.058 U/ml), SP 34 was not significantly different from bacterial isolates SP. 32 and SP. 83 (0.065 U/ml). Bacterial isolate SP. 48 (0.094 U/ml) significantly differed from other isolates (Table 2).

Table 2. Urease enzyme activity by ureolytic bacterial isolates

Isolate	Urease enxyme activity (U/ml)
SP 32	0.053 ± 0.003^{ab}
SP 34	0.058 ± 0.002^{bc}
SP 48	0.094 ± 0.005^{d}
SP 83	0.065 ± 0.002^{c}
SP 84	0.044 ± 0.004^{a}

The urease enzyme hydrolyzes urea when urea substrate is available so that a spectrophotometer can measure the final result in the form of ammonia through changes in the color of the supernatant of bacterial isolates to yellow after adding Nessler reagent (Figure 1). Based on the figure, can be seen that the supernatant of bacterial isolate SP 48 was concentrated yellow and followed by bacterial isolate SP 84 which looks pale yellow. This indicates that the more intense the yellow colour of the bacterial supernatant, the higher the concentration of the final product of urease enzyme activity produced by ureolytic bacteria (Table 2). The intensity of the color produced was directly proportional to the concentration of ammonia produced. The colorless Nessler reagent would turn yellow when it reacts with ammonia contained in the supernatant of ureolytic bacterial isolates in alkaline pH.

3.2. Growth of Bacterial Isolate

The ureolytic bacterial isolates were grown on NB-U/Ca medium containing nutrient broth (NB), urea, and calcium (Ca) with pH 8 and tap water medium containing urea with pH 5.1. The growth of SP.48 bacterial isolates reached the logarithmic phase with an incubation time of 3 days with an absorbance value (OD 1.44). While in the tap water medium, the bacterial isolate reached the logarithmic phase with an incubation time of 5 days (OD 0.324) (Figure 2). Increased absorbance values indicate an increase in a bacterial population characterized by turbidity in the medium. Turbidity in the medium (OD) measured

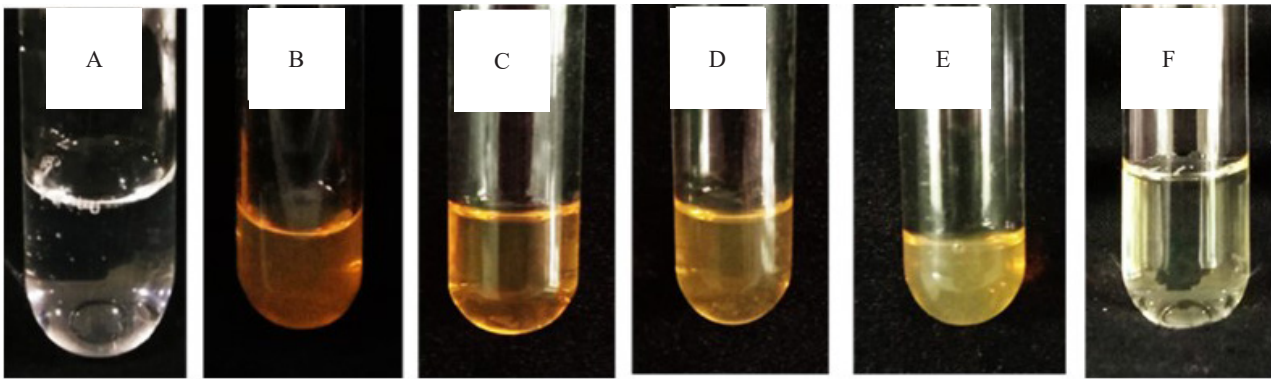

Figure 1. Supernatant of ureolytic bacterial isolate in Tris HCl+urea solution with incubation for 1 min. (A) Control, (B) SP. 48, (C) SP. 83, (D) SP. 34, (E) SP. 32, and (F) SP. 84

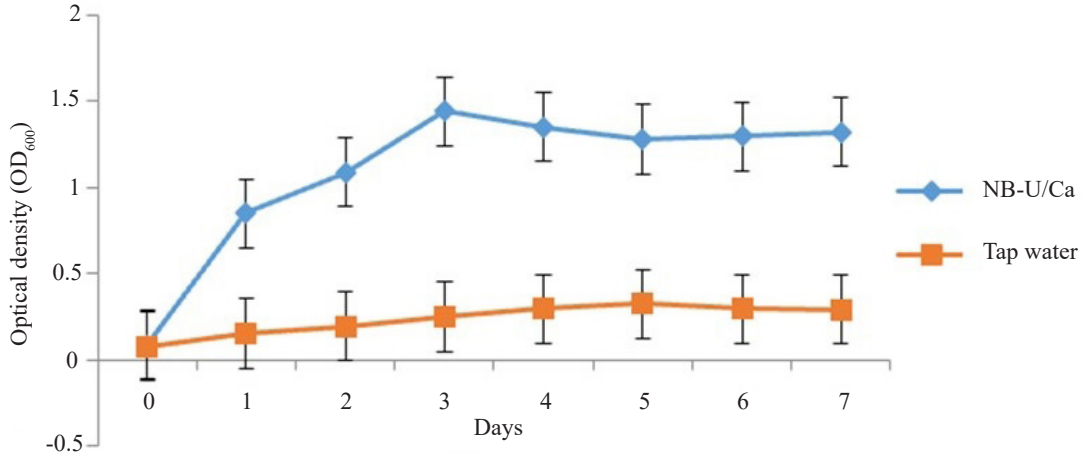

Figure 2. Growth curve of ureolytic bacteria SP. 48 on NB-U/Ca medium and tap water medium

includes live cells and dead cells of bacteria. The growth pattern of SP.48 bacterial isolates showed different growth patterns in the NB-U/Ca medium and tap water medium.

3.3. Calcite Production

Calcite production for isolates on NB-U/Ca medium based on the highest log phase growth time produced. Medium calcite production was determined from bacterial isolates SP. 48 after 7 days on tap water. The calcite produced in the NB-U/Ca medium was higher than in the tap water medium.

Based on the results obtained, isolate SP.48 in the NB-U / Ca medium produced 334 mg of calcite, while in the tap water medium, it was 87 mg (Figure 3) in medium 100 ml. The difference in the amount of calcite precipitate produced by ureolytic bacterial isolates can be influenced by the incubation time and physiological abilities of different bacterial isolates.

3.4. Analysis of Calcite Molecular Structure using Fourier Transform Infra-Red (FTIR)

The residue produced by bacterial isolate SP. 48 on NB-U/Ca medium was analyzed using FTIR spectroscopy (Figure 4). Based on the results of the FTIR spectrum showed that the residue produced by bacterial isolates on both mediums had the same main absorption peak and corresponded to calcite, namely at wavelengths of 712.73, 874.76, 1748.55, 1792.91, and 2510.46 cm^{-1} (Table 3).

3.5. Molecular Identification of Ureolytic Bacteria

Bacterial isolates with the highest ability to produce calcite (SP. 48) were identified using the 16S rRNA gene. The alignment results were analyzed using the Basic Local Alignment Search Tool (BLAST) program through the NCBI Gene Bank site, and the identity of

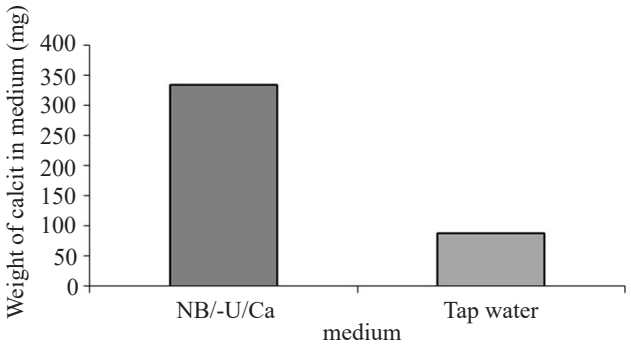

Figure 3. Calcite production by isolate Sp. 48 in different media

Table 3. Functional group analysis by FTIR spectroscopy

Wavelength (cm^{-1})		Functional group
NB-U/Ca	Tap water	
712.73	712.73	C-H (alkenes)
874.76	874.76	
1034.85-1290.43	1085.97-1239.32	C-O (ether)
1748.55	1792.91	C=O (carbonyl)
2510.46	2510.46	O-H (carboxylic acid)
2783.40-2838.37	2875.02-2983.04	C-H (alkanes)

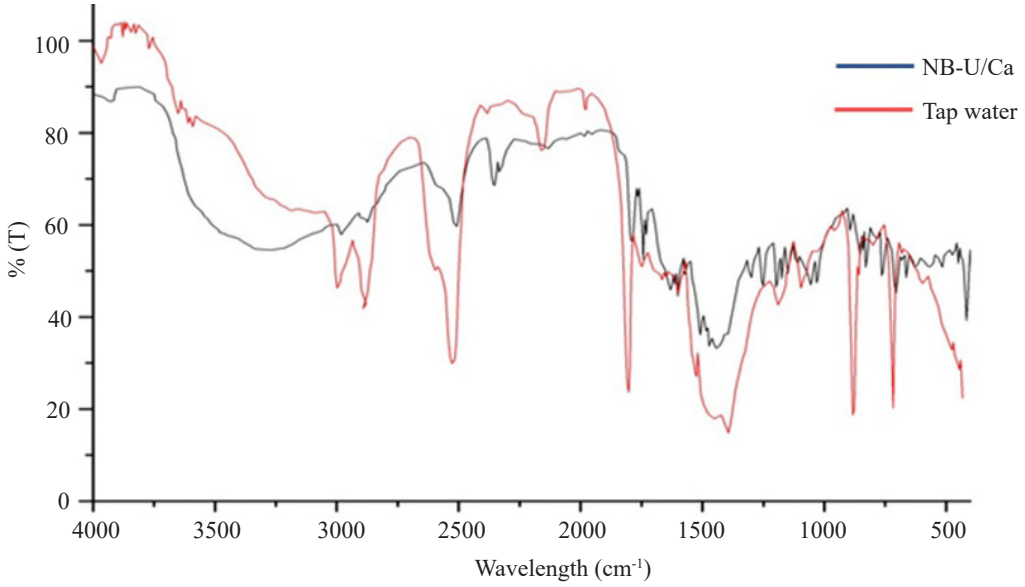

Figure 4. FTIR analysis of calcite produced by bacterial isolate SP. 48 on both mediums

bacterial isolates was obtained. SP. 48 bacterial isolate has similarity (99.93%) and query cover (100%) with *Bacillus albus* strain MCCC 1A02146 (NR157729.1). Isolate SP. 48 is a Gram (+) ureolytic bacterium with rod-shaped cells, producing endospores. The results of the phylogenetic construction analysis of the 16S rRNA gene sequence showed that the bacterial isolate SP. 48 has a kinship with *Bacillus albus* strain MCCC 1A02146 (Figure 5). Based on morphological characters, isolate 48 has a rod-shaped Gram-positive bacterium (Figure 6).

4. Discussion

The kind of nutrients in the culture media, as well as the bacteria, have a significant impact on urease activity and, as a result, microbial calcite precipitation. Several studies have shown that generally, bacteria with rod cell shape can produce the enzyme urease and calcium carbonate (Wong 2015; Seifan *et al*. 2017; Mokhtar *et al*. 2021). *Bacillus* has been shown to optimize the mechanical behavior of cement-based materials (Wong 2015). Some species are *Bacillus sphaericus*, which is used for calcium carbonate biosynthesis (Seifan *et al*. 2017), *Bacillus wiedmannii* strain FSL W8-0169, and *Bacillus paramycoides* strain MCCC 1A04098 an be used to protect water buildings from exposure to frequent cracks (Mokhtar *et al*. 2021).

The difference in incubation time of the resulting isolates in reaching the log phase indicates that the bacterial isolates have different growth on both mediums. This is thought to be due to differences in these bacteria's ability or physiological properties to utilize nutrients in the medium. The diverse

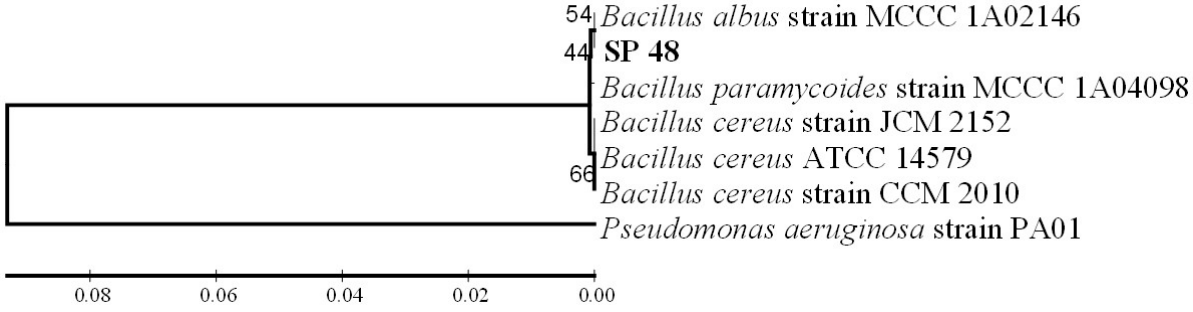

Figure 5. Phylogenetic tree of bacterial isolates SP. 48 based on distance matrix and UPGMA method with 1000 replications bootstrap

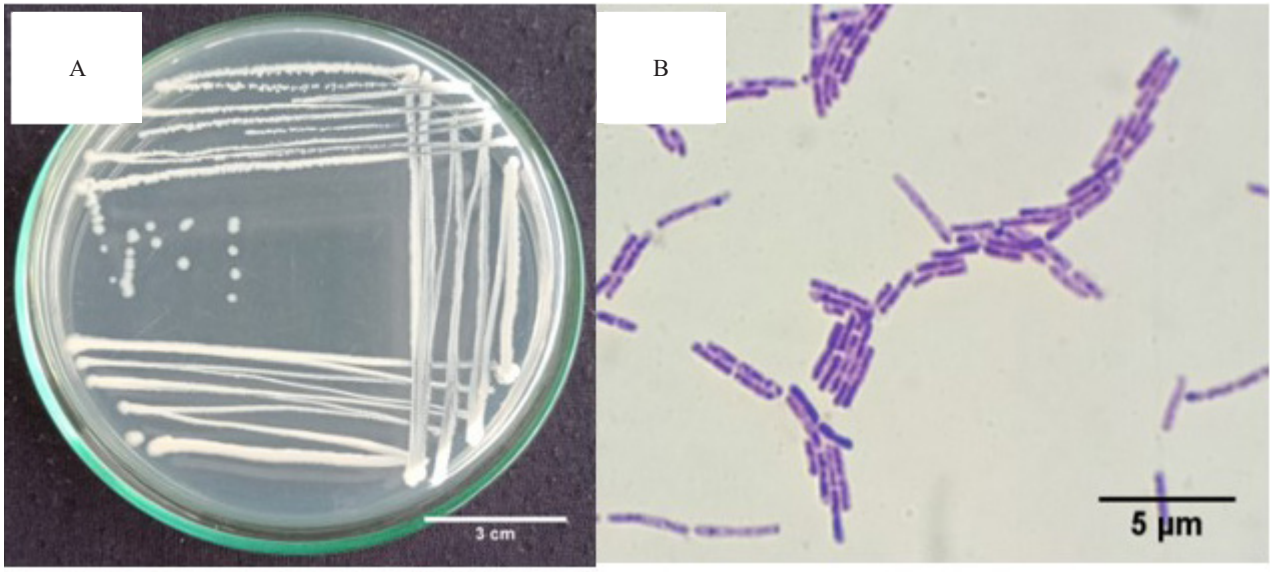

Figure 6. Isolate of ureolytic bacteria SP. 48. (A) colony morphology; (B) cell shape

composition of the NB-U/Ca medium can also be used by bacteria as a source of nutrients and support bacterial growth. So it can be concluded that growth by ureolytic bacterial isolates is influenced by the growth medium used. Research conducted using bacterial isolate *Sporosarcina pasteurii* ATCC 11859 (Okyay 2013), resulted in urease enzyme activity of 17.21 U/ml, and absorbance measurements were made at a wavelength of 560 nm after reacting with Nessler reagent for 1 minute. The urease enzyme activity of bacterial isolate BS-13 was 2.52 U/ml after reaction with urea substrate for 48 hours.

In the results of this study, it can be seen that the ability of each isolate of ureolytic bacteria to hydrolyze urea was different, so the activity of the urease enzyme produced is also different. The isolated species and incubation time can cause these differences. Urease enzyme activity was tested on the supernatant of bacterial isolates under alkaline conditions (pH 8). According to Stocks-Fischer *et al*. (1999), the optimal pH for urease enzyme activity ranges from pH 7.0 to 8.0. The urease enzyme activity of *Bacillus pasteurii* bacterial isolates was optimal at pH 8. The urease enzyme activity contained in ureolytic bacteria also plays a major role in the calcite precipitation process, so the application of ureolytic bacteria was widely used in construction. The ureolytic bacteria of *Sporosarcina pasteurii* has been proven to precipitate calcite in the Calcite Precipitation Agar (CPA) medium (Zoheir *et al*. 2013). The resulting calcite can be used in concrete mixtures to eliminate pores in concrete that cause cracks (Tziviloglou *et al*. 2016; Vashisht *et al*. 2018).

Microbial growth is strongly influenced by the nutrients contained in the growth medium (Aparna *et al*. 2015). *Sporosarcina pasteurii* showed the best growth on a medium containing peptone, beef extract, and sodium chloride with a logarithmic phase within 24 hours of incubation (Liu *et al*. 2020). *Bacillus megaterium* also showed the highest growth after five days of incubation on an NBU medium (nutrient broth, urea, and $CaCl_2$) (Bains *et al*. 2015).

Bacterial isolates produce different OD values when reaching the logarithmic phase. The OD value of isolates on tap water medium is lower than that of NB-U/Ca medium, meaning that the growth of isolates on tap water medium is slower. NB-U/Ca medium contains a more complex composition, while in the tap water medium, ureolytic bacteria only use minerals and urea as a source of nutrients, which greatly affects their growth. Urea added to a tap water medium is used as an inducer of calcite formation by bacteria. The acidic pH of the medium also influences the slower and inconstant growth of bacterial isolates in a tap water medium (pH 5.1). In this study, it can be concluded that the growth medium and pH of the medium greatly affect the growth of bacterial isolates in reaching the logarithmic phase.

The growth of bacterial isolates in the tap water medium is slower than in the NB-U/Ca medium. These bacterial isolates can produce calcite and use minerals and urea in the medium that can be used as an alternative concrete mixture to overcome cracks in concrete/self-healing concrete agents. Anitha *et al*. (2018) reported that tap water added with urea could be used as a cost-effective alternative in inducing calcite precipitation by *B. cereus* KLUVAA bacteria. Tap water medium is used by bacteria as a source of calcium ions in calcite production.

The difference in calcite production can be caused by differences in the amount of bacterial inoculum used, where 10 ml (10%) was used in this study. Krishnapriya *et al*. (2015) reported that *B. megaterium* BSKAU, *B. licheniformis* BSKNAU, and *B. flexus* BSKNAU, respectively, produced calcite of 0.84 g, 0.82 g, and 0.76 g in 30 ml of NB-U/Ca medium with 2% inoculum. The difference in the weight of calcite deposits can occur due to each bacterial species having a different ability to produce the urease on both mediums. This will affect the amount of calcium carbonate formed in the solution. In addition, this can also be caused by the density of bacterial cells on different mediums based on the resulting OD value, where the NB-U/Ca medium has better bacterial isolate growth and produces a higher OD value than the tap water medium. Okwadha & Li (2010) mentioned that higher concentrations of urea, Ca^{2+}, and bacterial cells would increase the amount of $CaCO_3$, sediment, and the rate of urea hydrolysis. Calcite precipitation depends on the type of bacteria, substrate used, urea concentration (Dhami *et al*. 2017), urease activity, Ca^{2+} and $CO3^{2-}$ concentrations in solution (Kang *et al*. 2014b), optimum incubation time, as well as environmental factors such as temperature and pH, and EPS production produced (Bains *et al*. 2015) (Omoreige *et al.* 2017).

Variable calcium carbonate production can be caused by varying medium composition and differences in secondary metabolites produced by bacteria, which will affect the amount of calcite production. Secondary metabolites that can be formed during the calcite precipitation process are exopolysaccharides (EPS)

that can attract Ca^{2+} ions so that the amount of calcite production can be influenced (Bains *et al*. 2015). The composition of the medium used can affect the level of medium saturation, the amount of calcite production, the morphology of calcite crystals produced (Dhami *et al*. 2013), and the strength of concrete (Krishnapriya *et al.* 2015).

When the pH is low, carbonate will tend to dissolve rather than residue. In contrast, a high pH is very important in producing ammonia through urea hydrolysis by the urease enzyme. pH 5.1 is not the optimal pH for urease activity and calcite production. Still, the results show that the ureolytic bacterial isolates used can grow and produce calcite under fairly acidic conditions after the adaptation process. The urease enzyme will only be active at a specific pH for urea hydrolysis, thus affecting the calcite precipitation process. The optimal pH value for the urease enzyme is 8.0. Based on this, it can be concluded that the pH of the medium can affect urease activity and calcite production by ureolytic bacteria.

The increase in pH after the incubation period of bacteria in both mediums where in the NB-U/Ca medium, there was a change in pH from 8 to 8.3, and in the tap water medium, there was a change in pH from 5.1 to 7.3 after the incubation period is thought to be caused by the activity of the urease enzyme produced by bacteria. The urea contained in the medium will be hydrolyzed by bacteria using the urease enzyme, which will produce ammonium, which will increase the pH value of the medium. Changes in pH indicate that the degradation of urea by the urease enzyme produced by ureolytic bacteria can increase the pH of the medium.

In the calcite precipitation process, urea is a source of nitrogen (energy) for bacteria to be hydrolyzed by the enzyme urease and degraded into carbonate and ammonium, consequently increasing the pH value, as well as the concentration of carbonate in the bacterial environment (Ningsih *et al*. 2017). In addition, calcium ions (Ca^{2+}) are essential micronutrients for bacteria (Kang *et al*. 2014b). Calcium ions in the solution will cause calcium carbonate precipitation when a certain saturation level is reached (Ningsih *et al*. 2017). An increase in CO concentration occurs under alkaline conditions when calcium (Ca) and carbonate (CO) ions are abundant (Kang *et al*. 2014a). According to Algafi *et al*. (2020), the negatively charged bacterial cell surface can induce mineral precipitation by providing a nucleation site. Calcium ions can easily attach to the bacterial cell wall because they have a positive charge. As a result, calcium cells are covered by carbonate ions, which then precipitate microbial calcium carbonate surrounding the bacterial cell wall (Liu *et al*. 2017).

Based on the results of the FTIR spectrum showed that the residue produced by bacterial isolates on both mediums had the same main absorption peak and corresponded to calcite, namely at wavelengths of 712.73, 874.76, 1748.55, 1792.91 and 2510.46 cm^{-1}. The absorption peaks at 712.73 and 874.76 cm^{-1} indicate the vibration of the C-H (alkene) bond, and the absorption peaks at 1748.55 and 1792.91 cm^{-1} are caused by the vibration of the carbon-oxygen (CO) double bond in carbonate ions, while the absorption peak at 2510.46 cm^{-1} is caused by the O-H (carboxylic acid) bond (Skoog *et al*. 2018). Abdallah *et al.* (2021) mentioned that the resulting calcite's absorption peak at 874 cm^{-1} is due to the symmetrical bending and bending mode represented by the peak at 713 cm^{-1}. In addition, the absorption peaks at 2510-2512 cm^{-1} were identified as hydrogen bond stretching vibrations of carboxylic acids (Skoog *et al*. 2018). Wang *et al*. (2021) reported that the bands in the range of 1420, 1062, and 874 cm^{-1} are associated with the C-O bond stretching vibrations of CO, which corroborate the formation of $CaCO_3$ (calcite). The absorption spectrum of calcite produced by bacterial isolates in a tap water medium was stronger and sharper than in the NB-U/Ca medium (Figure 4).

FTIR spectrum showed that the residue produced by bacterial isolates on both mediums had the same main absorption peak and corresponded to calcite. Daskalakis *et al*. (2013) mentioned that the peak of pure calcite is very typical and is in the range of 713, 875, 1423 (single), 1794, and and 2516 cm^{-1}. Calcite produced by *B. megaterium, B. subtilis*, and *B. cereus* produced a strong absorption peak at 713 cm^{-1} (Dhami *et al*. 2013). The results of the FTIR spectrum of *Bacillus licheniformis* AK01 showed similarities with standard calcium carbonate with main absorption peaks at 713, 873, and 1425 cm^{-1} (Vahabi *et al*. 2013). In addition, Zaghloul *et al*. (2020) also mentioned that calcite produced by *Staphylococcus epidermidis* has absorption peaks of 706, 18, 874, 33,1420, 23 cm^{-1}.

Bacillus spp. was in higher abundance in the two groups supplemented with urea, indicating it was more responsive to urea. *B. pasteurii, B. lentus*, and *B. cereus* have proven to be ureolytic bacteria (Jin *et al*. 2016). *B. albus* collected from brick kilns, furnaces, and rocks of different areas of Warangal District, Telangana, India, has been shown to grow on calcium carbonate precipitation media (Jyothi & Charya 2020). *B. albus*

is a ureolytic bacterium collected from a landfill that is proven to be a calcite-producing bacterium, a new finding in this study.

Calcite production by ureolytic bacteria can be influenced by the growth medium's composition and the medium's pH. Bacterial isolate SP. 48 (*Bacillus albus*) can grow and produce calcite in both mediums, especially in the tap water medium with a low pH. It can use tap water and urea minerals as the only carbon source for growth and calcite production. As a growth medium for ureolytic bacteria in inducing calcite precipitation, the tap water medium can be used as an alternative approach to the field and as a self-healing agent that resolves concrete cracks, increases concrete strength and durability, and other bioremediation applications.

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