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Identification and characterization of calcite producing bacteria isolated from soils in West Java, Indonesia

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Abstract. *Sugata M, Arviana N, Tampubolon L, Widjajakusuma J, Victor H, Jan TT. 2022. Identification and characterization of calcite producing bacteria isolated from soils in West Java, Indonesia. Biodiversitas 23: 3921-3927.* Bio-mediated soil improvement as an interdisciplinary application of geotechnical engineering and microbiology has gained attention due to its environmentally friendly and sustainable properties. This study aimed to isolate indigenous bacteria from soils in Indonesia and evaluate their ability to precipitate calcium carbonate. Fifty-seven colonies were isolated from three soil samples of different locations in Indonesia (Cikarang, Medang, and Karawang). Screening of calcite-producing bacteria was carried out using B4 agar medium incubated for 7-14 days. Only twenty isolates showed the potential ability to form precipitate on B4 agar medium. All the twenty isolates were characterized morphologically and biochemically. For molecular analysis, two isolates, LK3 and NC7, were chosen based on the morphological similarities with *Bacillus*: Gram-positive, spore-forming rod bacteria. According to 16S rRNA gene sequence analysis using the Basic Local Alignment Search Tool (BLAST), LK3 was identified as *Bacillus subtilis* LK3 and NC7 was identified as *Bacillus cereus* NC7. At a temperature of 30 °C, *B. cereus* NC7 showed the highest growth and produced the most calcite precipitates. In addition, pH 9 was the optimum condition for crystal formation by these bacteria. In conclusion, *B. cereus* NC7 as indigenous bacteria might be feasible to be used for local soil improvement.

Keywords: *Bacillus*, biomineralization, calcium carbonate, MICP, precipitation

INTRODUCTION

In recent years, the bio-mediated soil improvement method as an interdisciplinary application between geotechnical engineering and microbiology has gained a lot of attention. One of the focuses of geotechnical engineering is soil stabilization, which is commonly done by the addition of chemical grouting such as cement (Sukumaran and Poulose 2018). However, this conventional method could have a negative impact on the environment due to the toxic components in chemical grouts such as acrylamide, lignosulfonates, and polyurethanes (Achal and Kawasaki 2016). Hence, an environmentally friendly and sustainable method for soil stabilization using microorganisms has been proposed. The application of microorganisms as a prospective catalyst in soil improvement was suggested by Whiffin (2004) and since then, numerous studies have progressed considerably high in this field.

There are a huge number of microorganisms in soil, including bacteria, actinomycetes, and fungi. The metabolic activities of these microorganisms are directly related to geochemical changes. In most cases, the microbial metabolic products react with the surrounding environment and form mineral precipitation (Seifan 2016). Calcium carbonate ($CaCO_3$) is one of the most common minerals widespread on earth with various polymorphs and calcite is the most thermodynamically stable polymorph of $CaCO_3$ (Zafar et al. 2022). Numerous species of bacteria from soil and various extreme environments have been reported for their ability to precipitate calcium carbonate by creating an alkaline condition through different physiological activities (Elmanama and Alhour 2013). The process is known as Microbially Induced Calcite Precipitation (MICP). MICP occurs because microorganisms possess a net negative cell surface charge, which can scavenge divalent cations such as Ca^{2+} and bind them onto their cell surfaces. Therefore, microorganism is an ideal crystal nucleation site. In addition, extracellular polymeric substances (EPS) which are composed of proteins and polysaccharides on bacterial cells also provide nucleation sites for calcium carbonate crystals and modulate the morphology of crystals (Tang et al. 2020). Those crystals act as microbial sealant and have potential in consolidating sand or soil particles, thereby improving soil stabilization (Chahal 2011).

Sporosarcina pasteurii, formerly known as *Bacillus pasteurii* (Yoon et al. 2001), is reported as one of the most appropriate bacteria for biomineralization activity. These bacteria have some distinguished properties, such as high adaptability, less aggregation between cells, and most importantly, they could produce a high yield of calcium carbonate in a certain period of time (Tang et al. 2020). Other bacteria from the genus *Bacillus* have also been reported for their ability to improve soil stabilization. Ng et

al. (2012) treated a residual soil with *B. megaterium* and found that the shear strength ratio of treated to untreated soils was increased. Sharma et al. (2021) showed the feasibility of MICP using *S. pasteurii* ATCC 11859, *B. subtilis* ATCC NCIB 8533, and *B. sphaericus* ATCC 14577 on loose Narmada sand. Oualha et al. (2020) reported the application of MICP using native *B. cereus* from Qatari soils showed improvement in the stability of calcareous soils. Sugata et al. (2020) observed that *B. subtilis* could strengthen expansive soils by decreasing Free Swell Index and increasing the unconfined compression strength as well as cohesion value.

Generally, microorganisms require a specific environmental condition to grow and form precipitate. In many applications, most bacteria fail to adapt to the new environment due to many challenges, including chemical and physical conditions such as pH, osmotic pressure, temperature, and availability of suitable nutrients as well as predation and competition (Rajasekar 2021). Hence, indigenous bacteria isolated from soil are expected to have better adaptability to surrounding conditions and could give a promising soil improvement. In this paper, indigenous bacteria from soils in West Java, Indonesia were isolated and characterized. Furthermore, their ability to precipitate calcium carbonate was also evaluated.

MATERIALS AND METHODS

Isolation and purification of soil bacteria

Three soil samples from different locations in Indonesia (Cikarang, Medang, and Karawang) were collected separately. For isolation, the soil sample (1 g) was suspended in 9 mL of sterile NaCl 0.85%. One mL of the mixture was serially diluted (threefold), and 0.1 mL aliquot of serially diluted samples were spread evenly onto Petri plates containing Nutrient Agar (Sigma, USA). The plates were then incubated under aerobic conditions at 30°C for 24 h. After incubation, representative colonies with distinct morphological characteristics were selected and purified using four-way streak method.

Selection for calcite producing bacteria

Purified isolates were grown on B4 solid precipitation medium with the following components: yeast extract (40.0 g/L, Sigma, USA), dextrose (50.0 g/L, Sigma, USA), calcium acetate (2.5 g/L, Sigma, USA) and agar (14 g/L, Sigma, USA) (Marvasi et al. 2012). The formation of calcite precipitates was monitored on plates incubated at 30°C for 7-14 days.

Phenotypic characterization

Purified isolates with the ability to form precipitate on B4 agar medium were characterized phenotypically according to Bergey's Manual of Systematics of Archaea and Bacteria (Logan and De Vos 2015). Morphology of the isolates was observed microscopically using Gram, endospore, and acid-fast staining. Biochemical activities tested including the ability to produce extracellular (amylase, protease, catalase) and intracellular (gelatinase) enzymes, ferment a variety of sugars to produce acid and/or gas, form acetyl methyl carbinol from sugar degradation (Voges-Proskauer test), and decompose amino acid tryptophan to indole. Oxygen requirement to support the growth of the isolates was also analyzed.

Molecular identification

Genomic DNA of selected isolates was extracted using PrestoTM Mini gDNA Bacteria Kit (Geneaid, Taiwan) and used as a template for PCR reaction. PCR was done using MyTaq HS Red Mix (Bioline, USA) and universal primers, 27F (5′-AGA GTT TGA TCM TGG CTC AG-3′) (IDT 108 Inc., Singapore) and 1492R (5′-GGT TAC CTT GTT ACG ACT T-3′) (IDT Inc., Singapore). PCR products (16S rRNA) were sent to 1st BASE Laboratories Pte. Ltd., Singapore, for sequencing. The 16S rRNA gene partial sequences of selective isolates were processed using Sequence Scanner 2 (Applied Biosystems, USA) and BioEdit software (Ibis Therapeutics, USA). The sequences were then aligned with the NCBI GenBank database using the BLAST algorithm (http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi) for comparison to previously deposited 16S rRNA sequences.

Phylogenetic analysis

The 16S rRNA gene nucleotide sequences of two isolates, *B. subtilis* LK3 (ON358234) and *B. cereus* NC7 (ON358235), were deposited to Genbank. Phylogenetic analysis of both isolates was carried out using Molecular Evolutionary Genetic Analysis (MEGA-X). Prior to analysis, the 16S rRNA gene sequences were processed and aligned in order to know the position of both isolates among related *Bacillus* species. The evolutionary distances were processed using the Maximum Likelihood analysis method. Bootstrap replicates were set to 500. Other sequence data used were obtained from GenBank. The out-group strain was *S. pasteurii.*

Effect of different conditions on bacterial growth dan calcite production

Selected isolates were inoculated to sterile Nutrient Broth (NB; Merck, USA) and incubated at 37°C for 8 h. After incubation, the optical density of the liquid culture was measured at 600 nm. Bacterial liquid cultures were diluted to OD 0.1-0.2. To check the bacterial growth, the liquid culture was inoculated (1%) to sterile B4 liquid medium and incubated at 25, 30, 37°C. The bacterial growth was measured based on OD600 after 6 h incubation, while the quantification of calcite production was done after five-day incubation. To investigate the effect of pH on calcite production, the liquid culture was added (1%) to a sterile B4 liquid medium with pH 3,5, 7, 8 and 9 and incubated at 30°C. After five-day incubation, calcite precipitation from each condition was quantified.

Estimation of calcite production

A modified method of Wei et al. (2015) and Hammad et al. (2013) were adapted for this experiment. For quantitative measurement of calcite precipitation in broth, isolates were inoculated to sterile liquid B4 medium,

followed by incubation at 25, 30 and 37°C for five days. After incubation, the liquid culture was centrifuged at 10,000 rpm for 1 minute. The pellets which contained calcite precipitation and bacterial cells were resuspended using 200 µL TE buffer (10 mM Tris, 1 mM EDTA pH 8.5). Lysozyme (1 mg/mL) was then added, followed by incubation at 37°C for 1 h to break down the cell wall of the bacteria. The mixture was centrifuged at 9,600 × g for 1 min to separate the cell debris from the calcite precipitates. Pellet which contained calcite precipitates were washed using sterile water pH 8.5 and then air-dried at 37°C. The pellets were dissolved using 25 mL distilled water, then two drops of HCl 5 N were added and heated until it boiled. pH of the solution was checked and adjusted to 7. Five milliliters of the solution were put into an Erlenmeyer, then 100 µL of NaOH 1 N and two drops of murexide indicator were added until the color of the solution changed to red. Na-EDTA solution 0.01 M was dropped until the solution changed color to purple. The volume of Na-EDTA used was recorded and the calcium concentration was calculated.

RESULTS AND DISCUSSION

Isolation of calcite producing bacteria from soil

Fifty-seven isolates with different morphologies were obtained from three soil samples: twenty-two isolates originated from Cikarang, twenty-four isolates originated from Karawang, and eleven isolates originated from Medang. All isolates were screened for their ability to grow and precipitate calcium carbonate on B4 agar medium (Figure 1). B4 is the most common medium used to culture microorganisms with the ability to precipitate calcium carbonate (Marvasi et al. 2012). From all isolates, it turned out that only twenty isolates showed the potential ability to form calcite precipitation. The twenty isolates were then further characterized morphologically (Table 1) and biochemically (Table 2). The results were found to be variable: some isolates were able to hydrolyze starch, protein, and gelatin, while others were unable or only hydrolyze certain compounds. Hence, it was hard to confirm the genus of the isolates, therefore molecular analysis was required.

Table 1. Phenotypical properties of calcite-producing isolates

Source	Isolate code	Shape	Gram	Endospore	Acid fast
Cikarang	LC3	Rod	-	-	-
	LC4	Coccus	+	-	-
	LC5	Coccus	+	-	-
	NC3	Rod	+	+	-
	NC7	Rod	+	+	-
	NC8	Rod	+	+	-
Karawang	LK1	Rod	+	+	-
	LK2	Coccus	+	-	-
	LK3	Rod	+	+	-
	LK4	Coccus	-	-	-
	NK1	Coccus	-	-	-
	NK7	Coccus	-	-	-
	NK8	Coccus	-	-	-
Medang	LM3	Rod	-	-	-
	LM4	Coccus	+	-	-
	LM5	Coccus	-	-	-
	LM6	Rod	+	+	-
	NM1	Coccus	-	-	-
	NM4	Rod	+	+	-
	NM6	Rod	-	-	-

Table 2. Biochemical activity of calcite-producing isolates

Isolate	Starch hydrolysis	Protein hydrolysis	Gelatin hydrolysis	Sugar fermentation (acid/gas)			Catalase	Oxygen requirement	Indole production	VP production
				Dextrose	Lactose	Sucrose				
LC3	+	+	+	+/+	+/+	+/+	+	Aerobic	+	-
LC4	-	-	-	+/+	+/+	+/+	+	Aerobic	-	-
LC5	-	+	+	+/+	+/+	+/+	+	Anaerobic facultative	-	-
NC3	+	+	-	+/+	+/+	+/+	+	Aerobic	-	-
NC7	+	+	+	+/+	+/-	+/-	-	Aerobic	-	-
NC8	-	+	-	+/+	+/+	+/-	+	Aerobic	-	-
LK1	+	+	-	+/+	+/+	+/+	-	Aerobic	-	-
LK2	-	-	-	+/+	+/+	+/+	-	Anaerobic facultative	+	-
LK3	+	+	-	+/-	+/+	+/+	+	Aerobic	-	-
LK4	-	-	-	+/+	+/+	+/+	+	Aerobic	-	-
NK1	-	+	-	+/+	+/+	+/+	-	Anaerobic facultative	-	-
NK7	-	+	-	+/+	+/+	+/+	+	Aerobic	-	-
NK8	-	+	+	+/+	+/+	-/-	+	Anaerobic facultative	-	-
LM3	-	-	-	+/+	+/+	+/+	-	Anaerobic facultative	-	-
LM4	-	+	+	+/+	+/+	+/+	+	Aerobic	-	-
LM5	+	+	-	+/+	+/+	+/+	+	Aerobic	-	-
LM6	-	-	-	+/+	+/+	+/-	-	Anaerobic facultative	-	-
NM1	+	-	-	+/+	+/+	+/+	+	Anaerobic facultative	-	-
NM4	-	+	-	+/+	+/-	+/+	+	Aerobic	-	-
NM6	-	+	-	+/+	+/+	+/-	+	Anaerobic facultative	-	-

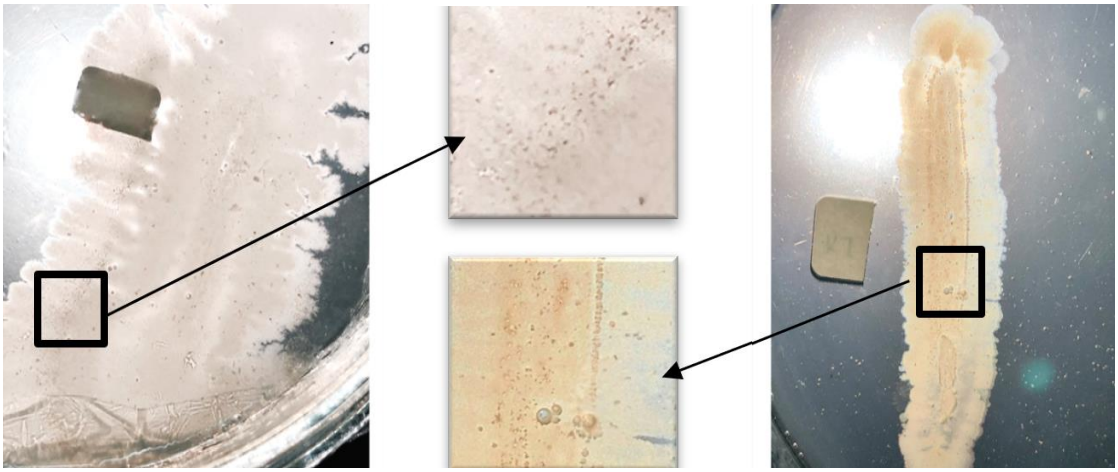

Figure 1. The appearance of calcite precipitates on isolate cultures grown on B4 agar medium after 7 days of incubation at 30°C

Characterization of calcite producing bacteria

Temperature is one of the most important factors for microbial growth and the production of calcium carbonate in MICP process. Kim et al. (2018) reported that microbial growth was slightly better at 30°C than at 20°C and the growth was in line with calcite production. However, no precipitation was found at a temperature of 60°C or higher. In this study, seven isolates from three different soil samples were selected. All selected isolates had similar morphologies with *Bacillus*: Gram-positive, spore-forming rod bacteria. The growth of the selected isolates was tested at different temperatures: 25, 30 and 37°C. All isolates could grow at a temperature 25-37°C, with the slowest growth at 25°C, as shown in figure 2. The optimum temperature for the growth of NC3 and NC7 from Cikarang soil, LK1 from Karawang soil, and NM4 from Medang soil was 30°C. Only LK3 from Karawang soil and LM6 from Medang soil showed the highest growth at 37°C. According to Logan and De Vos (2015), the optimum growth temperature for *B. subtilis* was 28-30°C, with a minimum of 5-20°C and a maximum of 45-55°C. Meanwhile, *B. cereus* had a minimum temperature for growth around 10-20°C, and a maximum 40-45°C, with the optimum about 37°C. However, growth temperature ranges vary considerably between the strains of species, maximum and minimum temperatures may be extended depending on the living environment.

Identification of calcite producing bacteria

For molecular identification NC7 from Cikarang and LK3 from Karawang were selected. Both isolates showed favorable growth at tested temperatures (25, 30 and 37°C). However, their optimum growth temperature was different, which was 30°C for NC7 and 37°C for LK3. Isolates from Medang soil were not selected because the isolates showed very low growth at a temperature of 25°C (Figure 2). A report by Marvasi et al. (2012) stated that *Bacillus* is one of the environmental bacteria that were able to precipitate calcium carbonate in B4 medium. In addition, *Bacillus* has been extensively studied for calcium carbonate precipitation (Anbu 2016). Results from 16S rRNA gene sequencing using Basic Local Alignment Search Tool (BLAST) identified LK3 as *B. subtilis* with 99.93% similarity to *B. subtilis* subsp. *stercoris* strain EGI246, and NC7 was identified as *B. cereus* with 99.93% homology with *B. cereus* strain PJA1.5 (Figure 3). Henceforth, LK3 is referred to as *B. subtilis* LK3, while NC7 is referred to as *B. cereus* NC7. The 16S rRNA gene sequence of both isolates had been deposited in GenBank with the accession number ON358234 for LK3 and ON358235 for NC7. Since *B. cereus* might cause food poisoning, precautions are required in handling these bacteria (Cutting 2011).

The evolutionary distances of both isolates (LK3 and NC7) and other *Bacillus* species based on 16S rRNA sequences is shown in Figure 4. The genetic of isolate LK3 is closer to other *B. subtilis* species, while the genetic of isolate NC7 is closer to other *B. cereus* species. Both isolates are not closely related to *S. pasteurii* (formerly known as *B. pasteurii*) (Yoon et al. 2001), which is widely known as an ecologically sound biological construction material (Sharma et al. 2021). Although noticeably distant from *S. pasteurii*, *B. subtilis* had been reported for their ability to form calcite precipitation and bioaugmentation on Narmada sand sample (Sharma et al. 2021). Meanwhile, *B. cereus* from Qatari soils has been reported to be able to precipitate minerals as effective as *S. pasteurii* (Sohail et al. 2022) and could potentially improve the stability of calcareous soil (Oualha et al. 2020).

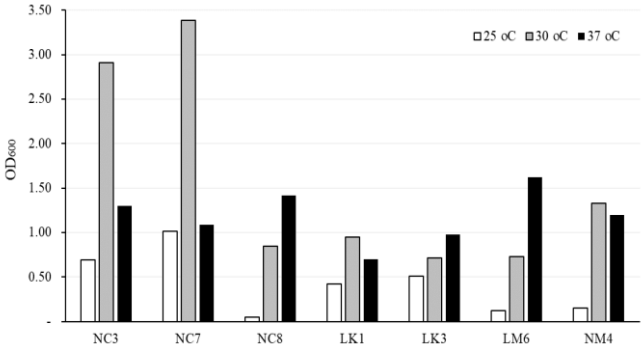

Figure 2. The growth of selected isolates at various temperatures after 6 h incubation

Estimation of calcite production from

Screening of $CaCO_3$ crystals formation on B4 agar medium is a qualitative test. To quantify $CaCO_3$ precipitates, bacterial cells from liquid culture were lysed with lysozyme and the remnants of the lysed cells were removed. The remaining pellets were expected to be $CaCO_3$ crystals which were precipitated by bacteria. In this study, calcite production was estimated using the titration method. The estimated amount of calcite was still useful to determine the optimum temperature and pH needed for the high calcite production.

Ng et al. (2012) suggested to select calcite forming bacteria with optimum growth at soil temperature. Varied soil temperature is influenced by many factors, such as depth of soil, solar radiation, moisture content etc. Alfata and Nurjannah (2020) found that soil temperature in Indonesia at 10 cm depth on August-November ranged between 27.5-28ºC. Hence, as compared to *B. subtilis* LK3, *B. cereus* NC7 might have higher potential for MICP application in Indonesian soils, considering the optimum growth temperature for these bacteria is 30°C (Figure 2). In addition, *B. cereus* NC7 also formed the most calcium precipitates at a temperature of 30ºC (Figure 5). The amount of calcite precipitate might be associated with the number of bacterial cells which act as a nucleation site. The more nucleation site, the more precipitate that can be formed.

The optimum temperature for $CaCO_3$ precipitation by bacteria is in the range of 20-37°C and its activity will increase with increasing temperature. However, at temperatures that are too high, the effectiveness of $CaCO_3$ precipitation will decrease (Anbu et al. 2016). According to Jones and Detwiler (2015), increased temperature could increase the solubility of calcium carbonate crystals. A study by Rodriguez-navarro et al. (2003) revealed that higher temperature caused a higher crystallization rate which produced smaller particle size (not enough for soil strengthening). On the other hand, lower temperatures such as room temperature caused a slow crystallization rate which allow larger calcium carbonate crystals to be formed and distributed uniformly (conducive for strength improvement). According to Tang et al. (2020), large-sized precipitates are suitable for increasing the strength of geotechnical materials and cementing coarse-grained soils, while small-sized crystals are suitable for reducing soil permeability and cementing fine-grained soils.

Aerobic bacteria such as *Bacillus* species release carbon dioxide (CO_2) during cell respiration. The production of CO_2 is in accordance with the pH rise. The rise in local pH makes bacterial cells a potential nucleation site for calcite precipitation (Ng et al. 2012). Figure 5 shows that *B. cereus* strain NC7 produced the most CaCO3 precipitation at pH 9. According to Anbu et al. (2016), most of the $CaCO_3$ precipitation occurs in an alkaline environment, namely pH 8.7 to 9.5 At low pH, carbonate is dissolved, and the crystals are hard to form.

A

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Bacillus subtilis subsp. stercoris strain EGI246 16S ribosomal RNA gene, partial sequence	Bacillus stercoris	2625	2625	100%	0.0	99.93%	1461	MN704488.1
Bacillus subtilis strain soilG2B 16S ribosomal RNA gene, partial sequence	Bacillus subtilis	2619	2619	100%	0.0	99.86%	1467	MT641205.1
Bacillus subtilis strain IB-22 16S ribosomal RNA gene, partial sequence	Bacillus subtilis	2619	2619	100%	0.0	99.86%	1460	MT590663.1
Bacillus subtilis strain JCL16 chromosome, complete genome	Bacillus subtilis	2619	26157	100%	0.0	99.86%	4101682	CP054177.1
Bacillus subtilis strain DK15 16S ribosomal RNA gene, partial sequence	Bacillus subtilis	2619	2619	100%	0.0	99.86%	1453	MT534533.1
Bacillus subtilis strain MDC3500 16S ribosomal RNA gene, partial sequence	Bacillus subtilis	2619	2619	100%	0.0	99.86%	1461	MT534524.1
Bacillus subtilis strain QMA60 16S ribosomal RNA gene, partial sequence	Bacillus subtilis	2619	2619	100%	0.0	99.86%	1457	MT525235.1
Bacillus subtilis strain KA9 16S ribosomal RNA gene, partial sequence	Bacillus subtilis	2619	2619	100%	0.0	99.86%	1550	MT491101.1
Bacillus subtilis strain MPF62 16S ribosomal RNA gene, partial sequence	Bacillus subtilis	2619	2619	100%	0.0	99.86%	1431	MT487693.1
Bacillus subtilis strain 11# 16S ribosomal RNA gene, partial sequence	Bacillus subtilis	2619	2619	100%	0.0	99.86%	1434	MT459200.1

B

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Bacillus sp. (in: Bacteria) strain B5 16S ribosomal RNA gene, partial sequence	Bacillus sp. (in: Bacteria)	2621	2621	100%	0.0	99.93%	1448	OM959314.1
Bacterium strain S137 16S ribosomal RNA gene, partial sequence	bacterium	2621	2621	100%	0.0	99.93%	1454	MZ461724.1
Bacillus cereus strain PJA1.5 16S ribosomal RNA gene, partial sequence	Bacillus cereus	2619	2619	99%	0.0	99.93%	1425	MT337533.1
Bacillus cereus strain DBA1.1 16S ribosomal RNA gene, partial sequence	Bacillus cereus	2619	2619	99%	0.0	99.93%	1443	MT332156.1
Bacillus cereus strain PJS3.10 16S ribosomal RNA gene, partial sequence	Bacillus cereus	2619	2619	99%	0.0	99.93%	1429	MT299636.1
Bacillus cereus strain PJA4.2 16S ribosomal RNA gene, partial sequence	Bacillus cereus	2619	2619	99%	0.0	99.93%	1437	MT275698.1
Bacillus cereus strain LXJ7 16S ribosomal RNA gene, partial sequence	Bacillus cereus	2619	2619	99%	0.0	99.93%	1458	MN746204.1
Bacillus cereus strain LXJ11 16S ribosomal RNA gene, partial sequence	Bacillus cereus	2619	2619	99%	0.0	99.93%	1457	MN746202.1
Bacillus cereus strain LXJ74 16S ribosomal RNA gene, partial sequence	Bacillus cereus	2619	2619	99%	0.0	99.93%	1459	MN746193.1
Bacillus cereus strain LXJ75 16S ribosomal RNA gene, partial sequence	Bacillus cereus	2619	2619	99%	0.0	99.93%	1458	MN746192.1

Figure 3. Results of BLAST analysis of (A) *Bacillus subtilis* LK3 and (B) *Bacillus cereus* NC7

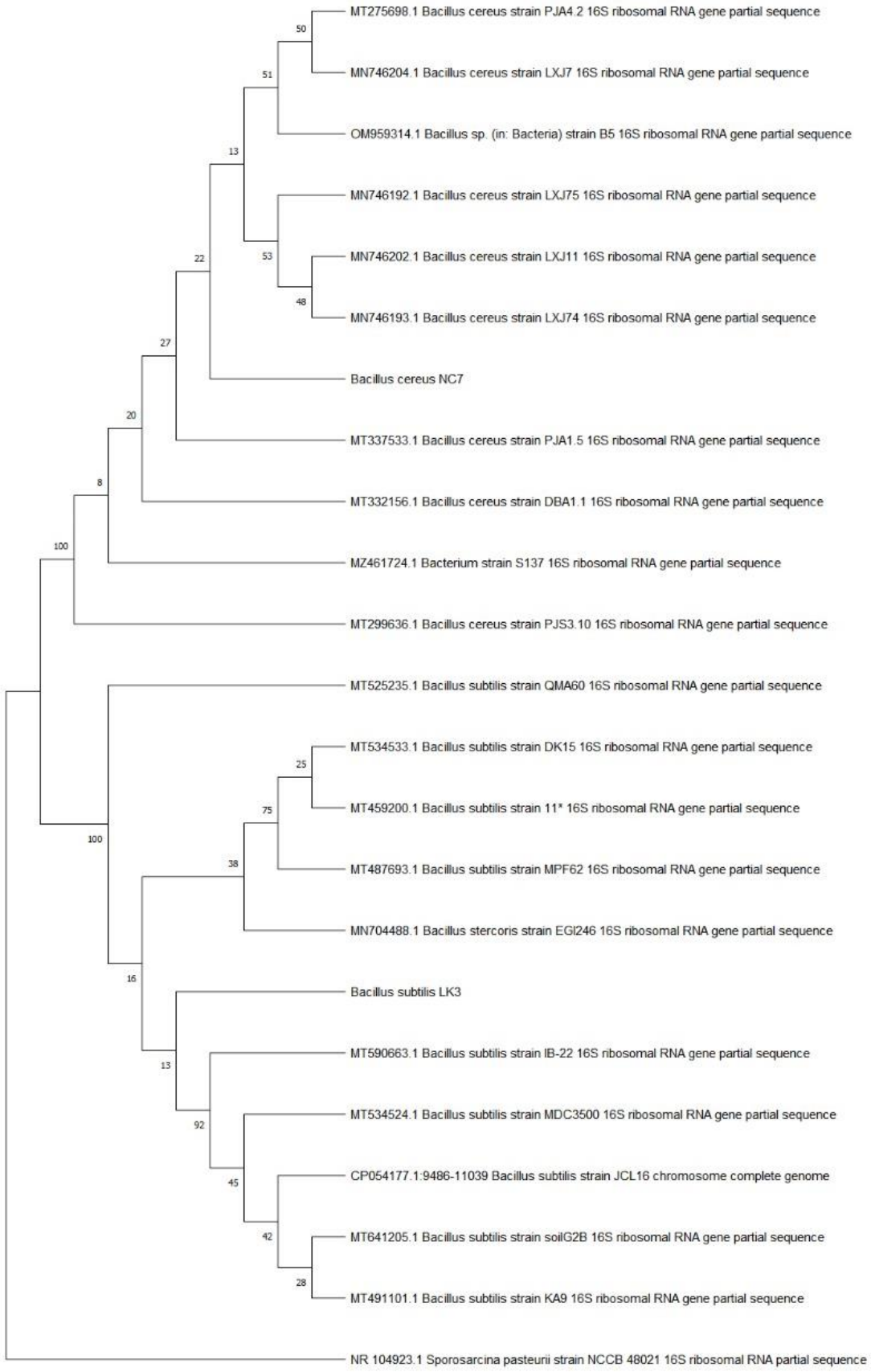

Figure 4. The evolutionary distances of *Bacillus subtilis* LK3 and *Bacillus cereus* NC7 with other *Bacillus* species based on the 16S rRNA gene sequences. Phylogenetic tree was constructed using Maximum Likelihood method with 500 bootstrap replications.

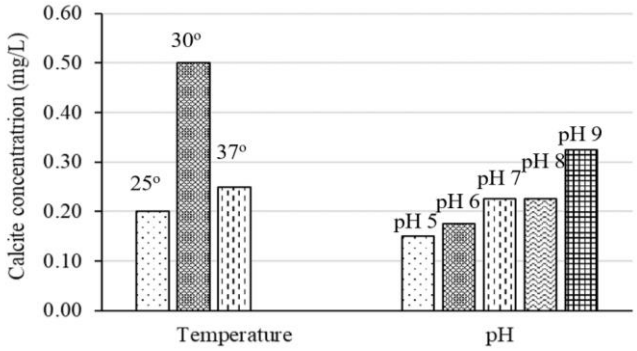

Figure 5. Estimation of calcite concentration precipitated by *Bacillus cereus* NC7 at various temperature and pH after five days of incubation

In summary, *B. cereus* NC7 had been successfully isolated from soil in Cikarang, West Java, Indonesia. These bacteria were capable of forming calcite crystals on mineral precipitation media and showed optimum growth at a temperature of 30°C, which is similar to the average soil temperature in a tropical climate. The most calcite precipitates were produced by these bacteria at a temperature of 30°C and pH 9. Based on the results, indigenous bacteria such as *B. cereus* NC7 showed promising potential for the application of local soil stabilization.

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