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Research Article
Isolation, Screening and Characterization of Ureolytic Bacteria from Cave Ornament

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Abstract

Background and Objective: Ureolytic bacteria are bacteria capable of hydrolyzing urea. In construction, these bacteria are known to help improve soil stability. One of the habitats of ureolytic bacteria is cave ornaments such as gourdam, flowstone, stalagmite and stalactite. This study aims to find isolates and characterization of ureolytic bacteria in cave ornaments. **Materials and Methods:** Urea-$CaCl_2$ was used as the isolation medium and urea agar medium was used as a qualitative urease test for cave ornament bacteria isolate. This study applied a survey method and tested for gram staining, spore staining, mannitol test, catalase test and lactose test for characterization. **Results:** There were 17 isolates positive for urease from 30 isolates from the isolates of cave ornament bacteria. The characteristics of 17 ureolytic bacteria isolates were 2 isolates gram-negative basil with negative lactose test and 1 isolate positive glucose and 1 isolate negative glucose. Total 15 isolates gram-positive basil with spore staining results, 14 isolates spore-positive with 2 isolates positive mannitol and 12 isolates negative mannitol and 1 isolate spore-negative with negative catalase. **Conclusion:** Total 17 ureolytic bacteria isolates were found from cave ornaments. Biochemical characterization showed 1 isolate of *Proteus* spp., 1 isolate of *Pseudomonas* spp, 2 isolates suspected of being *Bacillus megaterium* or *Bacillus subtilis*, 12 isolates of *Bacillus cereus* and 1 isolate of *Lactobacillus* spp.

Key words: Ureolytic bacteria, urease, gourdam, flowstone, stalactite, stalagmite, MICP, soil strength

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Microbially Induced Calcite Precipitation (MICP) is an environmentally friendly technology in the construction industry that involves bacteria as a biocatalyst to induce calcium carbonate precipitation[1]. MICP is becoming popular because it can be a promising alternative in the construction industry[2] such as the binding of soil particles which significantly increases soil strength[3] and repairing cracks in concrete[4]. MICP requires the production of the urease enzyme from bacteria[5]. These urease-producing bacteria are known as ureolytic bacteria.

Ureolytic bacteria are bacteria that hydrolyze urea using the urease enzyme it produces[6]. Hydrolysis of urea by the urease enzyme is one of the reactions that occur biologically to produce carbonate ions. When the urea hydrolysis process occurs in an environment that is rich in calcium carbonate, it will cause the calcium carbonate (calcite) to precipitate to form solid crystals. Where the strength of the crystalline precipitate bond depends on the rate of carbonate formation in the urea hydrolysis process. These crystalline deposits form hard calcite cement[7]. This process contributes to the formation of cave ornaments[8].

The cave ornament (speleothem) is one of the habitats for ureolytic bacteria. Based on existing research, it was found that bacterial strains isolated from limestone caves were able to precipitate calcium carbonate[9]. Other studies have found microbial communities in calcite cave speleothems in nature which are dominated by Firmicutes and Proteobacteria[10]. This is important information regarding the cave ecosystem that holds the potential for diversity of ureolytic bacteria.

This study aims to find and characterize ureolytic bacterial isolates from cave ornaments (gourdam, flowstone stalagmites and stalactites).

MATERIALS AND METHODS

Study area: The study was conducted for 6 months from July, 2020-January, 2021 at the Laboratory of Microbiology, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang. Sampling was carried out at Baba Cave Padang, West Sumatera.

Bacteria isolation: The sample in the litter was diluted to a concentration of 10^{-6} and then diluted with 10^{-4}-10^{-6} concentration. Next, 1 mL of the sample was inoculated on Urea-$CaCl_2$ medium[11] with spread plate technique[12]. Samples that have been inoculated were then incubated for 10 days[13].

Qualitative urease test: The qualitative urease test was carried out using a urea agar (Oxoid) medium. The purified isolates were inoculated into urea agar using the steak plate technique[12]. Isolates that have urease enzyme activity were characterized by a change of the medium's colour to pink[4].

Macroscopic observations: Macroscopic observations were carried out by observing the shape, elevation and margins of the bacterial colonies[12].

Microscopic observations: Microscopic observations consisted of gram staining to determine the isolates group, whether in gram-positive or gram-negative group. It was also to know the cell shape of bacteria[12].

Biochemical tests: Biochemical tests were carried out based on the results of gram staining and cell shape. Gram-positive bacteria isolates in the form of basil, a spore staining would be carried out. A positive spore will be followed by a mannitol test, while a negative spore will be followed by a catalase test. The lactose test was carried out for the gram-negative bacterial isolate in the form of basil. Lactose positive will be followed by an indole test, while lactose negative will be followed by a glucose test[12].

RESULTS AND DISCUSSION

Isolation of bacteria is a way to separate or remove certain microbes from the environment and grow them in an artificial medium to obtain pure culture[14]. The isolation results found 30 isolates of cave ornament bacteria consisted of 8 isolates of gourdam bacteria (GRB 1, GRB 2, GRB 3, GRB 4, GRB 5, GRB 6, GRB 7, GRB 8), 8 flowstone bacterial isolates (FLB 1, FLB 2, FLB 3, FLB 4, FLB 5, FLB 6, FLB 7, FLB 8), 7 isolates of stalagmite bacteria (SMB 1, SMB 2, SMB 3, SMB 4, SMB 5, SMB 6, SMB 7) and 7 isolates of stalactite bacteria (STB 1, STB 2, STB 3, STB 4, STB 5, STB 6, STB 7).

The results of the qualitative urease test showed that from 30 isolates of cave ornament bacteria, 17 isolates were positive for urease, consisted of 6 isolates of gourdam bacteria (GRB 1, GRB 2, GRB 3, GRB 4, GRB 7, GRB 8), 4 isolates of flowstone bacteria (FLB3, FLB 5, FLB 6 and FLB 7), 6 isolates of stalagmite bacteria (SMB 1, SMB 2, SMB 3, SMB 4, SMB 6, SMB 7 and 1 isolate of stalactite bacteria (STB 7).

The results of qualitative urease test bacterial selection can be seen in Fig. 1(a-b). Bacterial isolates that were positive for urease were indicated as ureolytic bacterial isolates characterized by a change in the colour of the urea medium

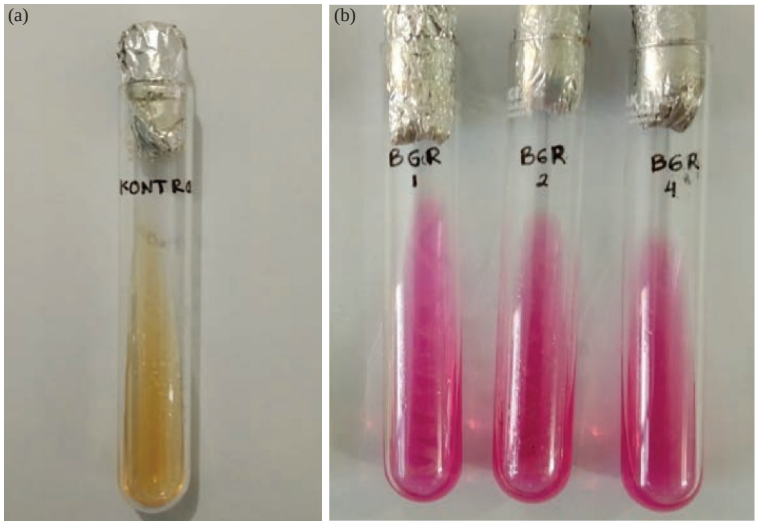

Fig. 1(a-b): Urease qualitative test
(a) Control (yellow) and (b) Positive urease (pink)

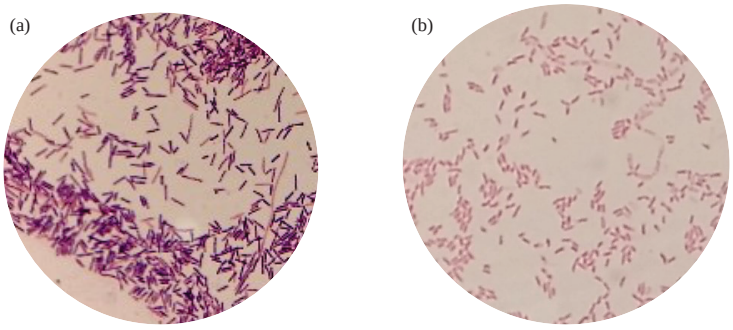

Fig. 2(a-b): Gram staining of ureolytic bacteria isolate
(a) Gram-positive and (b) Gram-negative

from yellow to pink. The result of Fig. 1a is a control in the qualitative urease test, marked in yellow on the medium and Fig. 1b is a positive result in the qualitative urease test, marked with pink on the medium. A change in the colour of the medium indicates a change in pH. The indicator of colour change in agar is phenol red. The nature of phenol red is that it will change from yellow to pink when there is an increase in pH to alkaline[15]. When urea is hydrolyzed by the urease enzyme from microorganisms, ammonia is released and accumulates in the medium which then increases the pH to make it alkaline[16].

Cave ornament ureolytic bacteria isolates have varied characteristics. The results of gram staining of cave ureolytic bacteria isolates are shown in Fig. 2(a-b). The result of Fig. 2a is bacteria isolate gram-positive basil and Fig. 2b is bacteria isolate gram-negative basil. While in Table 1. showed characteristics of the bacterial isolates obtained, 2 gram-negative basil isolates with negative lactose test and 1 isolate with positive glucose and 1 isolate with negative glucose. Fifteen gram-positive basil isolates with spore staining results, 14 isolates spore-positive with 2 isolates positive mannitol and 12 isolates negative mannitol and 1 isolate spore-negative with negative catalase.

For gram-negative basil bacteria was tested for lactose. The lactose test is part of the carbohydrate fermentation test. The test is positive if the colour of the media turns yellow due to the carbohydrate fermentation process. The lactose test of GRB 4 and FLB 5 isolates did not show a change in the colour of the medium. Furthermore, the glucose test is carried out. The glucose test is also part of the carbohydrate fermentation test. The test is positive if the colour of the media turns yellow due to the carbohydrate fermentation process. Positive glucose on GRB 4 isolates indicated *Proteus* spp. while the FLB 5 isolate showed negative glucose indicating *Pseudomonas* spp.[12].

Table 1: Macroscopic, microscopic and biochemical tests of ureolytic bacterial isolates from cave ornament

Isolate code	Macroscopic observation			Microscopic observation		Biochemical tests				
	Form	Margin	Elevation	Gram	Shape of cell	Lactose test	Glucose test	Spore staining	Mannitol test	Catalase test
GRB 1	Irregular	Undulate	Raised	Positive	Basil	-	-	Positive	Negative	-
GRB 2	Irregular	Undulate	Crateriform	Positive	Basil	-	-	Positive	Negative	-
GRB 3	Circular	Entire	Umbonate	Positive	Basil	-	-	Positive	Negative	-
GRB 4	Circular	Entire	Raised	Negative	Basil	Negative	Negative	-	-	-
GRB 7	Irregular	Undulate	Flat	Positive	Basil	-	-	Positive	Negative	-
GRB 8	Circular	Entire	Flat	Positive	Basil	-	-	Positive	Negative	-
FLB 3	Circular	Entire	Umbonate	Positive	Basil	-	-	Positive	Positive	-
FLB 5	Circular	Entire	Flat	Negative	Basil	Negative	Positive	-	-	-
FLB 6	Irregular	Lobate	Flat	Positive	Basil	-	-	Positive	Negative	-
FLB 7	Filamentous	Filiform	Flat	Positive	Basil	-	-	Positive	Positive	-
SMB 1	Circular	Undulate	Umbonate	Positive	Basil	-	-	Positive	Negative	-
SMB 2	Circular	Entire	Flat	Positive	Basil	-	-	Positive	Negative	-
SMB 3	Circular	Entire	Crateriform	Positive	Basil	-	-	Positive	Negative	-
SMB 4	Circular	Entire	Convex	Positive	Basil	-	-	Positive	Negative	-
SMB 6	Irregular	Lobate	Flat	Positive	Basil	-	-	Positive	Negative	-
SMB 7	Irregular	Undulate	Flat	Positive	Basil	-	-	Positive	Negative	-
STB 7	Irregular	Undulate	Flat	Positive	Basil	-	-	Negative	-	Negative

GRB: Gourdam bacteria, FLB: Flowstone bacteria, SMB: Stalagmite bacteria, STB: Stalactite bacteria, -: Not tested

The gram-positive basil bacteria was spore staining. The results showed 14 isolates were spore-positive and 1 isolate was spore-negative. The spore-positive isolates were followed by the mannitol test. The mannitol test is also part of the carbohydrate fermentation test. The test is positive if the colour of the media turns yellow due to the fermentation process. Positive mannitol test on FLB 3 and FLB 7 isolates indicate *Bacillus megaterium* or *Bacillus subtilis* and negative mannitol test on isolates GRB 1, GRB 2, GRB 3, GRB 7, GRB 8, FLB 6, SMB 1, SMB 2, SMB 3, SMB 4, SMB 6 and SMB 7 indicate *Bacillus cereus*[12]. Meanwhile, the spore-negative isolates were continued with the catalase test. A positive catalase test is indicated by the formation of air bubbles during the test. Positive catalase test on STB 7 isolates indicated *Lactobacillus* spp.[12].

To ascertain the type of species from the bacterial isolate, molecular identification is needed by analyzing the 16 s rRNA gene sequence.

CONCLUSION

From this study 17 ureolytic bacterial isolates were found from cave ornament. Biochemical characterization showed 1 isolate of *Proteus* spp., 1 isolate of *Pseudomonas* spp, 2 isolates suspected of being *Bacillus megaterium* or *Bacillus subtilis*, 12 isolates of *Bacillus cereus* and 1 isolate of *Lactobacillus* spp.

SIGNIFICANCE STATEMENT

This research was conducted to obtain an isolate of ureolytic bacteria from cave ornaments and to determine their character. This isolate is expected to be developed in the construction sector to help increase soil strength.

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