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Research Article

Isolation and identification of bacteria from air coolers in Mosul city, Iraq and study its ability for calcite formation

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Abstract
Evaporative coolers (ECs) are devices used to cool air. They are considered energy-saving and eco-friendly technologies, but eventually precipitate calcite. The present study aimed to isolate calcite-producing bacteria from environmental samples (ECs) and then investigate the influence of environmental parameters, such as pH and temperature, on calcite production. Twenty samples were obtained from sediments on the (ECs) in Mosul city, Iraq. The samples were inoculated on tryptic soy agar. Eleven bacterial isolates were obtained. One of the bacterial isolates exhibited a bush-like growth (complex colony) on tryptic soy agar and was identified using traditional methods. The diagnosis was confirmed by 16S rRNA gene sequencing to be *Paenibacillus dendritiformis*. The ability of the isolate to synthesize calcite was examined by cultivating it on Luria Bertani acetate agar. The effect of environmental parameters on calcite production was studied by culturing the isolate in Luria-Bertani acetate broth at pH 8, 9, and temperatures of 20°C and 30°C. The strain *P. denditiformis* demonstrated the capacity to produce calcite crystals in higher rate at pH 8 and 30ºC. Paenibacillus dendritiformis strain NFH1 was submitted to the National Center for Biotechnology Information (NCBI) Gene Bank database, and was shown to be the first strain isolated from air coolers in Iraq.

Keywords: *Paenibacillus dendritiformis*, 16S rRNA, Evaporative cooler, Calcite precipitation.

INTRODUCTION

In dry, high-temperature settings, evaporative coolers (ECs) are prevalent devices that utilize the process of water evaporation to reduce the air temperature. They supply fresh, humid air and are environmentally sustainable as they do not emit greenhouse gases. Furthermore, evaporative coolers are considered an economically feasible cooling technology due to their minimal installation and operational expenses, as well as their energy efficiency; hence, they are employed in residences, commercial establishments, educational institutions, and shopping centres (Kalsia *et al*., 2023).

ECs are made up of a metal box, a fan that is belt-connected to an electric motor, and three sides with wetted pad doors that are covered in wood wool pads. As heated air is drawn past the wet pads using the fan, the water evaporatively cools the air. Water circulated by a water pump moistens cooling pads. The wood-based pads will cause a variety of sediments to accumulate, including dust, salt, mold, bacteria, and other debris (Bahobail, 2013 and Hashim *et al*., 2022).

Wang and Tionduoli (2009) demonstrated that water used as a coolant in many thermal systems contains dissolved minerals that may precipitate and adhere to surfaces. Inorganic salts, including calcium carbonate, calcium sulfate, calcium phosphate, and iron oxide, are frequently present in cooling water. These deposits play a significant role in determining the ability and effectiveness of many cooling devices.

Microbially Induced Calcite Precipitation (MICP), a biomineralization process in which microorganisms synthesize calcium carbonate, is the term used to describe the precipitation of calcites by certain microbes. The process by which living organisms produce minerals as a result of their metabolic products interacting with their environment is known as biomineralization. Researchers have documented the production of minerals by bacteria, including those that break down urea. Mineral precipitation results from an organism's release of one

or more metabolic products (CO□) that react with environmental ions (Ca^{2}□) (Abdullah *et al*., 2022 and Wilcox *et al.*, 2024).

Ureolytic bacteria can catalyze urea into carbonate and ammonium, increasing pH values later. When calcium ions exist in the solution with carbonate and high pH, calcium carbonate (CaCO□) is precipitated (Burbank *et al*., 2012). MICP uses the ureolytic route through urease activity, which encourages calcite precipitation (Torres-Aravena *et al*., 2018). It has been found that both living and nonliving things can affect the biosynthesis of calcium carbonate. These include the types of bacteria (which affect the structure of the crystal, its size, shape, and the chemistry of CaCO3, the location of the nucleation site, the concentration of bacteria, the concentration of nutrients (calcium, carbonate, and nitrogen source), the temperature, and the pH (Seifan and Berenjain, 2018).

The MICP approach, as an ecologically friendly technology, can address a range of environmental issues, including the sequestration of CO2, bioconsolidation, biocement, and remediation of radioactive elements and heavy metals (Taha *et al*., 2022 and Dawwam *et al*., 2023). The objective of this study was to investigate the problem of calcite precipitation on ECs by bacteria, identify the cause, and determine the environmental factors that increased calcite precipitation.

MATERIALS AND METHODS

Samples collection

Twenty samples from evaporative coolers in Mosul city, Iraq, were collected between May 2022 and August 2022 by scrubbing the sediments from the doors of the ECs using sterile cotton swabs (Khalafallah *et al*., 2016).

Bacterial isolation and identification

Samples were inoculated on tryptic soy agar, followed by a two-day incubation of the plates at 30°C. Subsequently, one isolate of bacteria exhibiting a distinct type of colony was identified using standard microbiology techniques (Cappuccino and Welsh, 2018).

Amplification of the 16S rRNA gene

Molecular identification by 16S rRNA gene sequencing was conducted to identify the bacterial strains, as mentioned elsewhere (Faisal and Younis, 2024). Electrophoresis of DNA was done according to Sambrook *et al.* (1989). The primers used were 27F 5’ AGAGTTTGATCMTGGCTCAG 3’ and 1522R 5’ AAGGAGGTGATCCARCCGCA 3’ and were purchased from IDT (Integrated DNA Technologies company, Canada). Primers were used to amplify the 1495bp fragment of the 16S rRNA gene. A PCR was performed in a 20 µL reaction volume utilizing GoTaq G2 Green Master Mix (2X) provided by Promega (USA). The master mix was used at 1X concentration, and primers at 1µM. The total volume was adjusted to 20 µL with the addition of nuclease-free water.

The conditions employed were an initial denaturation step at 95°C for 3 minutes, followed by 30 amplification cycles. Each cycle included a denaturation step at 95°C for 30 seconds, an annealing step at 55°C for 30 seconds, and an extension step at 72°C for 1 minute. A concluding extension phase was established at 72°C (Khaleel *et al*., 2023). The PCR products were analyzed using a 2% agarose gel that included the Red safe DNA stain from Intron, China. The amplicon's size was determined by aligning the band's position with the bands of the 100 bp DNA ladder (Transgene, Taiwan) (Al-Ani *et al*., 2023; Ibrahim and Faisal, 2024). The sequencing of PCR products was conducted at the Psomagene sequencing company located in Maryland, USA, utilizing the ABI-310 Genetic Analyzer system.

Phylogenetic relationships

The BLASTn program was used to detect the similarity of input sequences to entire sequences available in the NCBI GenBank database (Younis and Faisal, 2024). As by Tamura and others (2021), the phylogenetic tree was constructed by (100X) bootstrap analysis by the software of MEGA-11.

Screening for calcium carbonate precipitation

Bacteria were isolated and inoculated on Luria-Bertani agar with acetate, then incubated at 30°C for one week. After the incubation period, screening for calcite crystals was conducted (Lee, 2003).

Identification of calcite crystals

According to Lee (2003), a loopful of the colonies and suspected calcifications, thought to be calcite crystals, was extracted from the culture on L.B. acetate agar following the incubation period, and the subsequent tests were conducted:

1. Morphological examination: A swab from the colonies exhibiting calcifications on Luria-Bertani agar was placed on a slide, stained with methylene blue, and subsequently analyzed under a magnification power of 1000x using the microscope.

2-The crystals underwent a chemical examination by introducing a few drops of a 10% HCL solution to the suspected calcite calcification, followed by the observation of bubble formation indicative of the presence of calcite (Hamilton *et al*., 1986).

Effect of pH on calcite production

The isolated calcite-producing bacteria were cultured in Luria-Bertani acetate broth at pH 8 and 9 at 30°C for 24 hours. Following incubation, the growth was compared with a McFarland tube of 109 cells/mL. One milliliter of

the culture was introduced into Luria Bertani acetate broth with pH levels of 8 and 9. Following a two-week incubation period, the dry calcite was weighed after the medium was filtered (Lee, 2003).

Effect of temperature on calcite production

A comparison of calcite production was conducted at 20°C and 30°C for the isolate cultured in Luria-Bertani acetate broth over a two-week period. Following the incubation, the resulting calcite crystals were stained with methylene blue and analyzed under a light microscope (Lee, 2003).

RESULTS AND DISCUSSION

Bacterial isolation and identification

The analysis of cultured medium plates from sediment samples on ECs revealed a diverse bacterial community, comprising 11 isolates: 7 gram-positive (63.6%) and 4 gram-negative (36.4%) strains. Anas *et al.* (2016) reported that artificial environments, including evaporative coolers, may be favorable to the growth of bacteria, fungi, mites, and protozoans, which can pose health risks to users. Which was eventually characterized as *Paenibacillus dendritiformis* based on morphological properties and biochemical tests following Goodfellow *et al*. (2012). *Paenibacillus dendritiformis* is a rod-shaped, gram-positive, spore-forming bacterium that develops a bush-like colony with branching patterns as shown in Fig. 1 (Ben-Jacob *et al*., 2000).

The 16S rRNA sequencing of this bacterium showed that it was 98.4% identical to *Paenibacillus dendritiformis* CIP105967 (accession number: AY359885). Phylogenetic analysis of the sequence revealed the most closely related *Paenibacillus* species, with their accession numbers as shown in Table 1 for the strains retrieved from the National Centre for Biotechnology Information (NCBI) database.

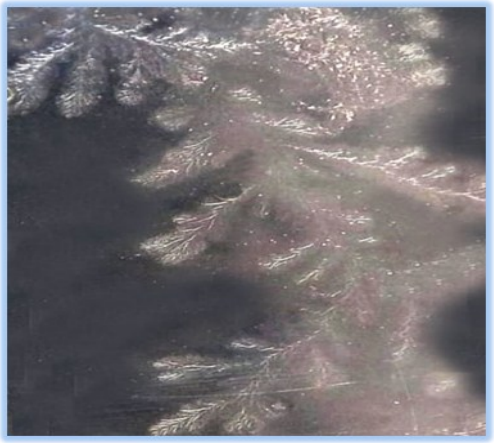

Fig. 1. *Image of bush like branching colony of Paenibacillus dendritiformis*

The neighbour-joining phylogenetic tree (Fig. 2) illustrates the relationship between the strain *Paenibacillus dendritiformis* NHF1 (in a black circle) and related species of *Paenibacillus*, as determined by 16S rRNA sequences using the software MEGA-11 with a scale length of 0.01.

Calcium carbonate precipitation

The findings indicate that the growth culture on Luria-Bertani acetate agar demonstrates that *P. dendritiformis* possesses the capability to produce calcite through calcification on the surface of the colonies within the culture medium. Microscopic examination of the calcite revealed crystals following staining with methylene blue. Additionally, the formation of bubbles was observed upon the addition of a few drops of HCL, a find-

Table 1. Phylogenetic relationships *of Paenibacillus dendritiformis*

Species name	Strain name	Accession No.	Similarity (%)
Paenibacillus dendritiformis	CIP 105967	AY359885	98.40
Paenibacillus thiaminolyticus	NBRC 15656	AB073197	94.89
Paenibacillus popilliae	ATCC 14706	AF071859	93.53
Paenibacillus alvei	DSM 29	AMBZ01000001	93.05
Paenibacillus montaniterrae	MXC2-2	AB295646	92.37
Paenibacillus chungangensis	CAU 9038	GU187432	92.07
Paenibacillus thailandensis	S3-4A	AB265205	91.96
Paenibacillus profundus	SI. 79	AB712351.	91.44
Paenibacillus. Kobensis	DSM. 10249	AB073363	91.42
Paenibacillus xinjiangensis	B538	AY839868	91.15
Paenibacillus gorilla	G1	JX650054	90.91
Paenibacillus algorifonticola	XJ259	GQ383922	90.64
.Paenibacillus macerans.	IAM 12467	.AB073196	90.62
Paenibacillus pinisoli,	NB5	KC415175	90.37
Paenibacillus methanolicus	BL24	HF558628	90.26

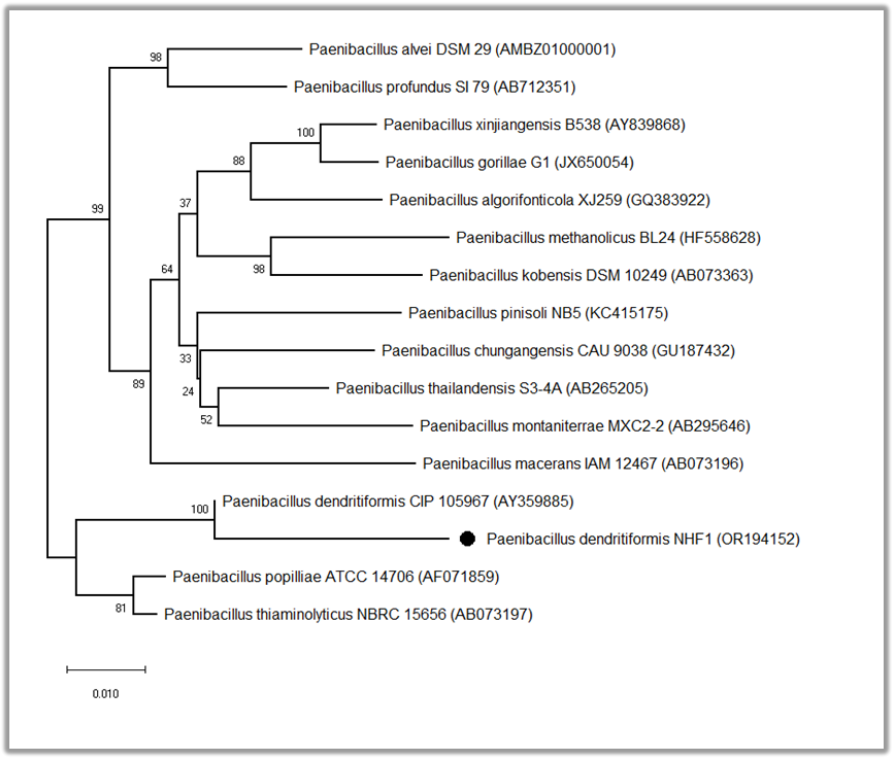

Fig. 2. *Phylogenetic tree of Paenibacillus dendritiformis (NHF1 OR194152) based on 16S rRNA*

ing supported by numerous studies (Vlasceanu *et al*. 2000). Several investigations, such as those conducted by Hamilton *et al*. (1986), demonstrated the capacity of crystals to absorb methylene blue and generate air bubbles upon the addition of acid, attributed to CO2 production as a result of the reaction. These precipitations cause damage and low efficiency to the air coolers and other devices containing microbially induced calcite precipitation MICP (Matter *et al*., 2023).

Factors influencing Microbially Induced Calcite Precipitation (MICP) efficiency

The pH level plays a significant role in the bacterial mineralization of calcium carbonate. Prah et al. (2011) found urease to be active at alkaline pH levels. Agereh *et al.* (2019) indicated that *Paenibacillus dendritiformis* exhibited MICP and demonstrated an enhanced ability to produce urease along with increased levels of carbonate precipitation. The findings indicate that calcite precipitation was most effective at pH 8, yielding a weight of 0.5 g, whereas at pH 9, the recorded weight was 0.2 g, as shown in Table 2. Cheng *et al.* (2013) demonstrated that pH 8 is the most favorable pH for urease production, while levels above this range result in a decline in production.

Temperature significantly influences urease activity; as shown in Table 2, the precipitation rate was elevated at 30°C (0.4 g), whereas it diminished to 0.3 g at 20°C (Table 2). Mitchell *et al.* (2010) demonstrated that urease enzymatic activity rose by 5-10 times with rising temperatures. Sulaiman *et al*. (2024) showed that for most bacteria, the optimum pH and temperature for MICP were (6.5-9.5) and (20-40 °C), respectively.

Because the MICP is influenced by environmental factors, including pH and temperature, we must optimize these factors before conducting the experiments. Temperature and pH are limiting factors when using bacteria in many industrial applications. Stefaniak *et al*., (2025) demonstrated that the treatment of (ECs) water by UV and the modification of (ECs) surfaces to prevent biofilm formation all lead to reducing microbial growth and calcite formation.

Table 2. Factors influencing Microbially Induced Calcite Precipitation (MICP) by *Paenibacillus dendritiformis*

Factors	Weight (grams)
pH - 8	0.5
pH - 9	0.2
Temp. - 20°C	0.3
Temp. - 30°C	0.4

Conclusion

The present investigation revealed the presence of *Paenibacillus dendritiformis* in the sediments found on the ECs. This isolated bacterium reduces the efficiency of ECs by producing calcite on these devices. This process is influenced by various environmental factors such as pH and temperature. On the other hand, *P. dendritiformis* is applicable in Microbially Induced Cal-

cite Precipitation (MICP) techniques and can be utilized in bio-cementation, remediation of heavy metals, CO2 sequestration, enhancement of soil mechanical properties, and numerous other advantageous applications.

Conflict of interest

The authors declare that they have no conflict of interest.

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