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Exploration of ureolytic airborne bacteria for biocementation applications from different climate zones in Japan

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The present study investigated the ureolytic airborne bacteria for microbial induced carbonate precipitation (MICP) applications, seeking resilient strains in order to address the problems of bacterial survivability and adaptability in biocementation treatment and to contribute a robust approach that can effectively stabilize diverse soils. Since the airborne bacteria tend to survive in dynamic environments, they are believed to possess remarkable adaptability in harsh conditions, thus holding great potential for engineering applications. Samplings across diverse climatic zones revealed that approximately 10–20% of the isolates were ureolytic bacteria in each sampling site. A series of characterization tests were conducted on selected strains to study the temperature dependency of urease activity. The results revealed that many of these isolates are unique in many aspects. For instance, some trains of *Glutamicibacter* sp. were found to precipitate extra-large calcium carbonate crystals that could be beneficial in the cementation of coarse soils. This study stands out from previous research on standard ureolytic bacteria by focusing on the exploration of airborne bacteria. The findings demonstrate that a significant number of ureolytic airborne bacteria have great potential, suggesting that the air can serve as a bacterial isolation source for MICP applications.

Keywords Airborne bacteria, Microbial induced carbonate precipitation, Urease activity, Bacterial identification, Carbonate precipitation test

With increasing awareness concerning energy and environmental crisis, novel technologies have emerged as pivotal countermeasures to address these global challenges, commanding heightened attention across a vast range of research fields. In the realm of geotechnical engineering, microbial induced carbonate precipitation (MICP) technology employs the urea hydrolysis catalyzed by microbial urease to yield carbonate precipitates, leading to the cementation of loose soil particles. This technology is regarded as a sustainable solution to many environmental issues and a transformative practice for the next generation[1–3]. For soil improvement, numerable studies have examined the factors influencing biocementation effectiveness[4,5]. These factors include cementation media (type of reagents, concentrations)[6], bacterial characteristics (species, urease activity, and cell concentrations)[7], treatment strategies[8,9], mechanical properties of treated soils[10,11], and environmental deteriorations[12–14]. However, in a soil microbiome, life and death is happening every moment in the interactions with local communities, artificially introduced bacteria could die from grazing predation, viral lysis, bacterial predation, chemical warfare, etc.[15]. When considering the utilization of bacteria in large field scale, one of the concerns is whether these bacteria can survive till the desired requirements are achieved[16]. It has been reported that augmented bacteria were eliminated by local communities within a few days of treatment, emphasizing the significant impact of the native bacteria in natural soils on cementation effectiveness[17–19].

Previous researchers have long and continuously studied ureolytic bacteria from soils, sediments, and water bodies in various environments to find potent strains to improve cementation efficiency[20]. Air is generally not considered as a source of isolating bacteria, as the cell concentration is 10 orders of magnitude lower than that in the soil and ocean[21]. This research work has attempted to investigate airborne bacteria to improve the MICP treatment. In a frequently disturbed system such as the atmosphere, bacterial habitat generalists (species that

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inhabit a wide range of environments) tend to dominate due to their metabolic flexibility[22]. Studies have found that many bacteria that remain viable in the air belong to the generalist group, which has a diverse nutrition so that they can utilize different resources for energy production[23]. And it is hypothesized that a fraction of airborne bacteria might be ubiquitously distributed[24]. These bacteria generalists are able to inhabit diverse environments with great adaptability to harsh conditions. Thus, they are considered ideal candidates for borderless applications of MICP technique. In addition, their ubiquity reduces biohazard concerns and increases societal acceptance compared to exogenous bacteria. Considering their characteristics as habitat generalists, they are expected to adapt to various problematic soils, which is an advantageous feature for MICP applications. Furthermore, these bacteria are more likely to be nutritionally versatile, which can contribute to a cost reduction in the culture media if they can efficiently utilize industrial by-products.

This study investigated the prevalence of ureolytic airborne bacteria in an accessible elevation and characterized selected isolates to confirm their effectiveness in biocementation. Samplings of airborne bacteria yielded a considerable number of isolates from different climate zones in Japan. Based on the findings, there are 10–20% ureolytic bacteria in the air, and many ureolytic isolates have shown excellent performance in precipitating carbonate. The study primarily summarizes the findings from samplings in Tsukuba and Okinawa, while concurrently making comparisons with the results of a prior study conducted in Sapporo. The gene analysis of selected isolates indicated that the Sapporo collection exhibited a high degree of similarity in terms of taxonomy with the collections from Okinawa and Tsukuba. Furthermore, the characterization tests indicated that numerous isolates possess distinctive features that could be advantageous for MICP applications. Rather than focusing on standard ureolytic bacteria, this study conducted a more comprehensive examination of the ecological significance of diverse ureolytic bacteria in the air, which contributes to an enhancement of MICP technology by highlighting the pivotal role of employed bacteria. Overall, this study established that there is a substantial proportion of ureolytic bacteria in the air and demonstrated the significant potential for carbonate precipitation induced by some selected isolates, suggesting a substantial biocementation application prospect.

Materials and methodology

Sampling methods and sampling sites

Air samples were collected by an air sampler (MAS-100 Eco®, Merc Millipore). Details of the device can be found in our previous study[25]. The air sampler operates at an airflow rate of 100 L/min (± 4%) for 500 s, collecting airborne particles from 0.83 m^3 of air for each sampling. Prior to bacterial isolation, samples exhibited a dominant growth of fungi were excluded from the isolation process. After 48–72 h incubation, samples with sufficient visible bacterial colonies were subjected to the isolation process.

Samplings of airborne bacteria were conducted in three cities with typical climate features in Japan (see in Fig. 1). Hokkaido is a typical cold region with long, very cold winters. Tsukuba has a moderate climate light snowfall in winter. Okinawa has a subtropical oceanic climate with long summer and mild winters. Sampling locations include parks, campuses, farms, mountain and coastal areas. The first sampling was conducted in Hokkaido, in September of 2022.The findings obtained in this sampling have been reported in a previous publication[25]. The second sampling was conducted in Okinawa. Table 1 shows the details of the sampling sites. This sampling consists of two sampling trials. The initial trial was conducted during the summer season on sunny days (July 18th–21st), and the second trial was conducted on September 27th–28th of 2023. The third sampling site was located in Tsukuba, a city in the southwest region of Ibaraki Prefecture (east of mainland Japan). The sampling was from September 17th to18th of 2023. Table 2 shows the details of the sampling sites.

Culture media and chemicals

Two types of culture media were primarily utilized for sampling and cultivating isolates: NH_4-YE medium and R2A agar medium. In addition to these two media, other culture media were employed for different purposes, such as Zobell medium to capture marine bacteria, carbonate precipitation agar and B4 medium to directly identify microbes capable of precipitating calcium carbonate. The formulations of culture media can be found in Supplementary Table S1 online. In the initial sampling in Okinawa, fungal colonies predominated in most of the samples. To prevent fungal growth, the culture media were modified to enhance screening efficiency. Subsequent to the second sampling trial in Okinawa, a fungicide (Kabicidin, SHIOTANI M.S. CO., LTD) was added to both NH_4-YE and R2A agar. In addition, 0.1 mM nickel chloride (nickel chloride hexahydrate, FUJIFILM Wako Pure Chemical Corporation) was added to the culture media as a nickel source for urease synthesis.

Isolation and screening of ureolytic airborne bacteria

The detailed protocols for isolation and screening procedures have been described in our previous study[25]. The screening procedures were comprised of three sequential steps. Initially, bacterial colonies were isolated and purified. Then, the isolates were tested in a simple urease test that rapidly identifies ureolytic bacteria by observing the color change of the test solution. To further confirm the carbonate precipitation capacity of ureolytic isolates, these isolates were examined in the carbonate precipitation test at 25 °C. The test tube contains 2 mL of bacteria culture (10ˆ7–10ˆ9 CFU/mL) and 8 mL of cementation media (0.5 mol/L of $CaCl_2$ and urea). The precipitation ratio was estimated based on the measurement of calcium ion concentration (Ca^{2+} meter, HORIBA, Ltd., Kyoto, Japan) in the test solution. Among the isolates demonstrating significant calcium carbonate precipitation, those with potential were selected for 16S rRNA gene analysis to ascertain taxonomic classification and biosafety level (BSL).

Characterization of selected airborne bacteria

In order to assess the applicable temperature range, a selection of isolates was cultivated at 15, 20, 25, 30, and 35 °C, and then examined in the urease activity test at the corresponding temperature. The bacterial growth was

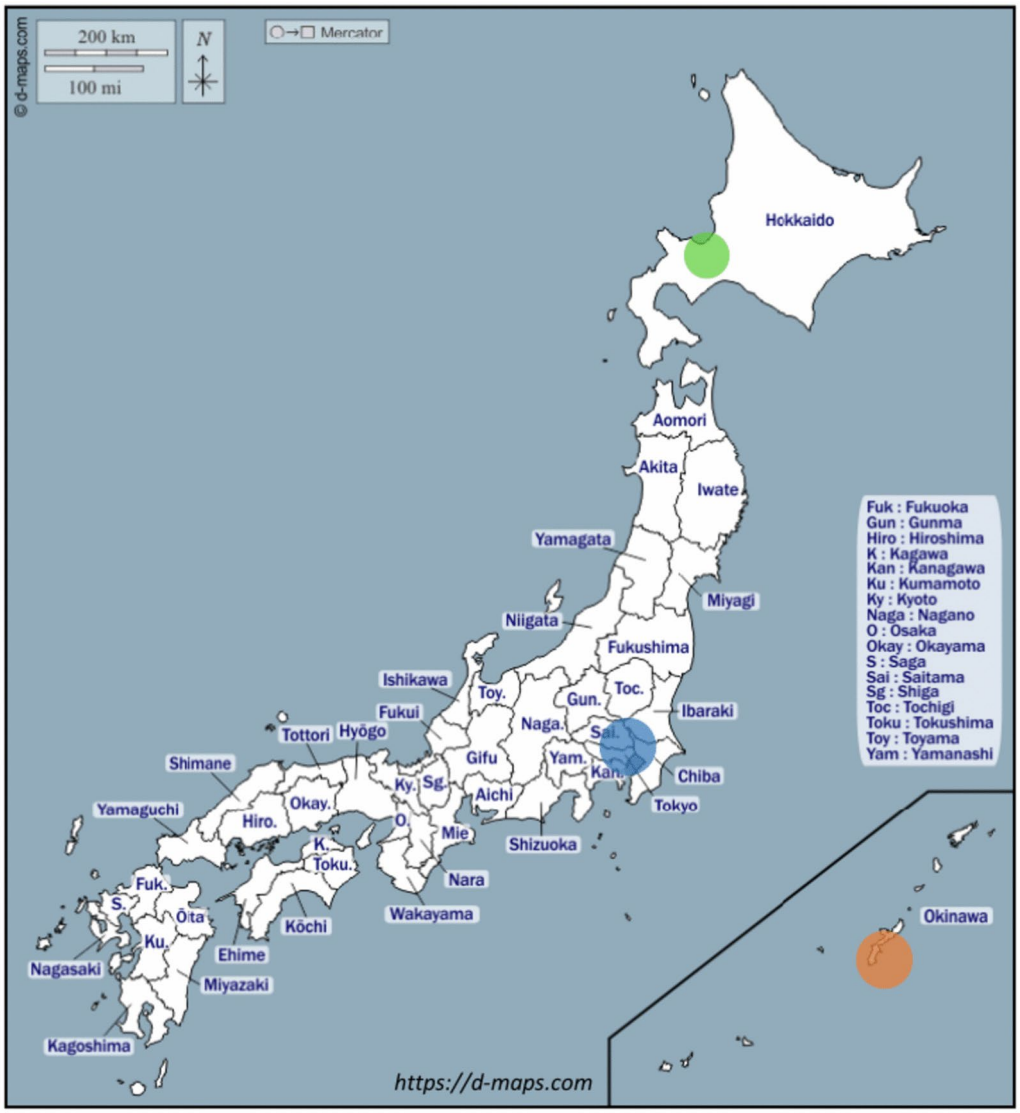

Fig. 1. Three main sampling sites of this study. This figure was created using PowerPoint for Microsoft 365 MSO (version 2412) by modifying a free image available at the following link: https://d-maps.com/carte.php?num_car=29488&lang=en.

Location	Site ID	Latitude (N)	Longitude (E)	Altitude	Note
First trial					
Shurijo	SR	26°13′2"	127°43′2"	120 m	Historical heritage
Kouriohashi	KU	26°40′44"	128°0′47"	0–10 m	Beach
Nakijinjo	NK	26°41′31"	127°55′39"	80 m	Limestone wall
Churaumi	CR	26°41′31"	127°52′39"	40 m	Aquarium
Bise	BS	26°42′13"	127°52′47"	0–10 m	Beach
21C forest Park	21C	26°35′29"	127°58′20"	0–10 m	Coastal park
Kakazu Park	KK	26°15′31"	127°44′13"	100 m	Observation deck
University of the Ryukyus	UR	26°15′4"	127°45′57"	120 m	Forest
Second trial					
Shurijo	SR	26°13′1"–26°13′7"	127°43′2"–127°43′6"	110–130 m	Historical heritage
Kakazu Park	KK	26°15′30"–26°15′32"	127°44′8"–127°44′15"	70–100 m	Observation deck
Urasoe Park	US	26°14′48"–26°14′53"	127°43′54"–127°43′57"	110–130 m	Historical heritage

Table 1. Information on sampling sites in Okinawa.

Location	Site ID	Latitude (N)	Longitude (E)	Altitude	Note
Matsumi Park	TF1, TF2, TF3	36°5′28"–36°5′30"	140°6′24"–140°6′26"	30–75 m	Observation tower, grove
Tsukuba-Agricultural center	TF4, TF5	36°7′6"–36°7′11"	140°5′37"–140°5′43"	30 m	Cow house, farm
Doho Park	TF6, TF7	36°3′34"–36°3′41"	140°7′20"–140°7′25"	30 m	Pond, grove, horse riding
Mt. Tsukuba	TS1, TS2, TS3	36°12′52"–36°13′34"	140°6′1"–140°6′6"	350–800 m	Observation deck, grove
Tsukubasan Shrine	TS4	36°12′48"	140°6′5"	250 m	Stone wall

Table 2. Information on sampling sites in Tsukuba.

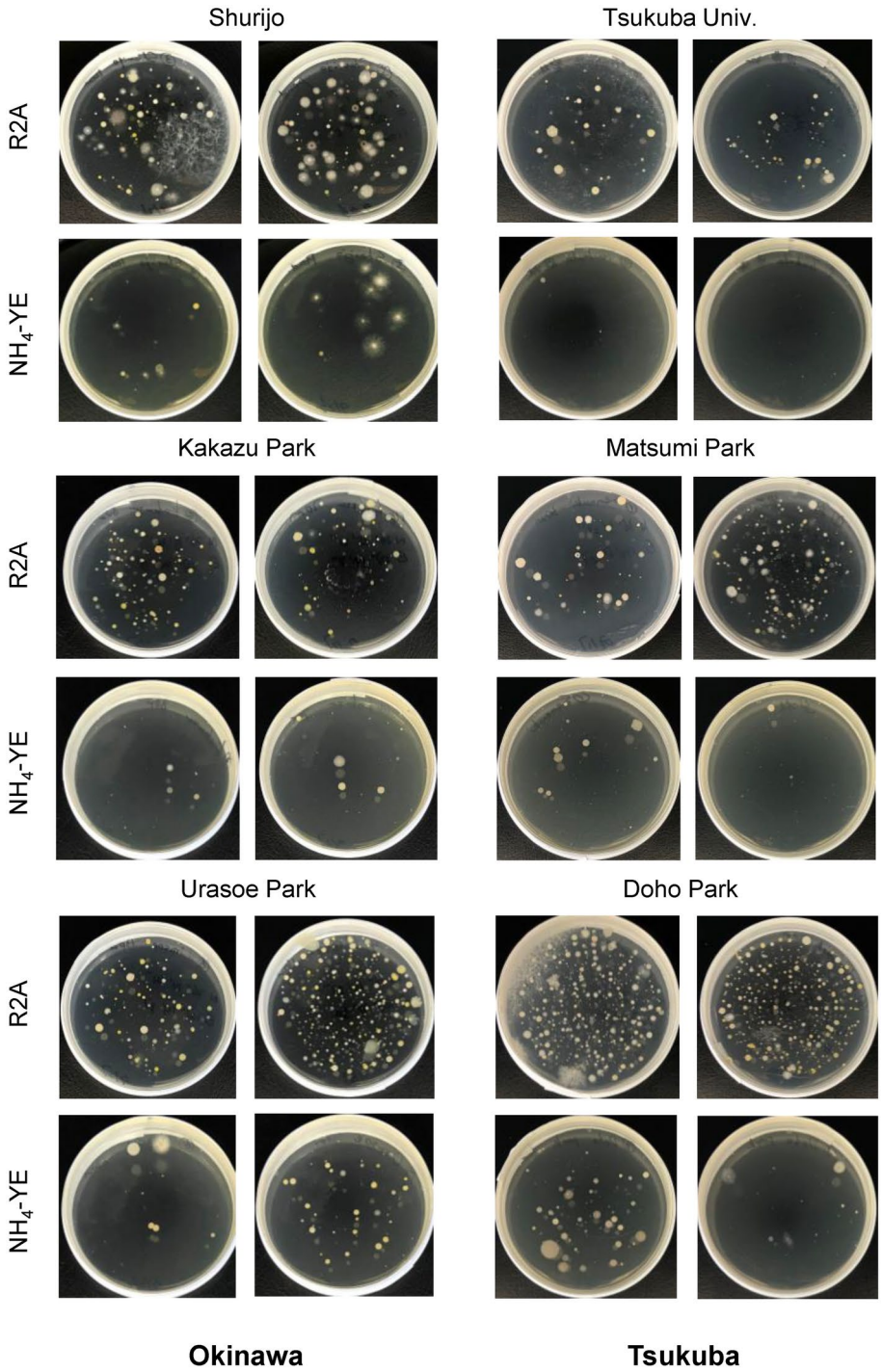

Fig. 2. Representative samples collected from Okinawa and Tsukuba sampling.

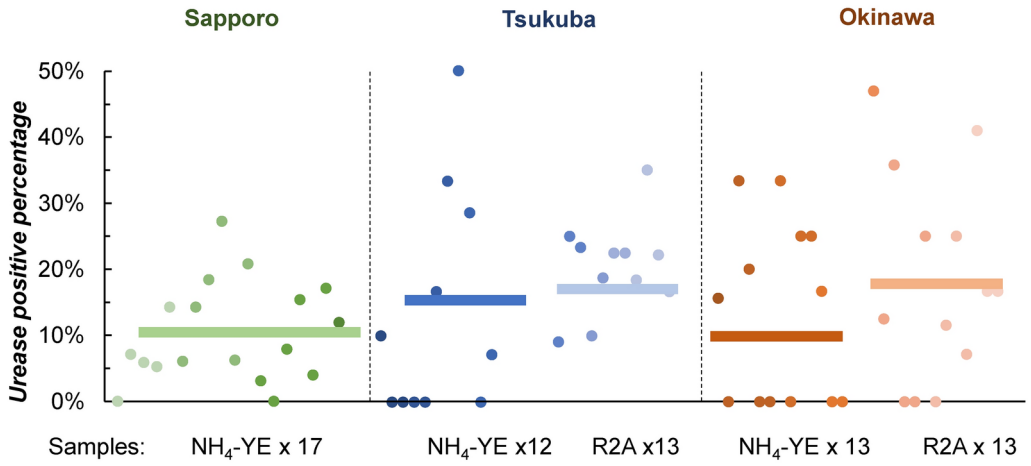

Fig. 3. Comparison of the occurrence of ureolytic bacteria from three sampling sites.

monitored using a spectrophotometer, which measures the turbidity of the bacteria culture by its optical density at a specific wavelength of light (OD_{600nm}). The determination of bacterial urease activity was achieved by monitoring the electrical conductivity changes during urea hydrolysis. The measurement employed a compact EC meter (LAQUAtwin EC-33, HORIBA, Ltd., Kyoto, Japan) with 3-point calibration, ensuring accuracy within ± 2% full scale. The test solution contains 0.5 mL of bacterial culture and 50 mL of 0.1 M urea solution, which is maintained at a specific temperature in a water bath (Thermal Robo TR Series, AS ONE Corporation). Subsequently, a 0.12-mL sample is extracted from the test solution and subjected to conductivity measurement at 5-min intervals (0, 5, 10, and 15 min). The calcium carbonate formed in carbonate precipitation tests was rinsed with distilled water and dried at 60 °C in an oven. The dried precipitates were then observed using a scanning electron microscope (Miniscope TM 3000, Hitachi, Tokyo, Japan).

Results and discussion

Representative pictures of samples

The majority of samples collected from the initial trial in Okinawa were found to be predominantly covered by fungal mycelium, irrespective of the type of culture media utilized for sampling (see Supplementary Fig. S1). One possible explanation for this phenomenon is that the high humidity in the air of subtropical regions promotes the proliferation of fungi and the dispersal of their spores. In this sampling, two carbonate precipitation media, CPA and B4, were employed to directly identify calcifying species by calcium carbonate formation samples. However, these samples did not facilitate rapid identification. It is likely that the formation of calcium carbonate by low activity bacteria on solid media is a time-consuming process and the presence of fungal colonies obscures the visual identification of calcium carbonate. Therefore, a fungicide was incorporated into all the culture media employed for the subsequent samplings. As seen in Fig. 2, the number of fungal colonies on samples was greatly reduced compared to that in the initial sampling. Furthermore, there is a significant difference between the R2A samples and NH_4-YE samples with respect to colony number. This indicates that the NH_4-YE has efficiently enriched a particular group of airborne bacteria capable of thriving in high concentrations of ammonium salts. The Tsukuba sampling exhibited significant variations in the number of bacterial colonies collected from two parks, Matsumi Park and Doho Park, on the same day (see Fig. 2). The dense vegetation of the latter spot might lead to a higher population of airborne bacteria, and the presence of a horse house in the park, where horses provide a urea source, might contribute to a higher population of ureolytic airborne bacteria. A closer observation of the bacterial colonies from Doho Park revealed a striking similarity in their colonial morphology, characterized by irregular shapes, large sizes, and a dull appearance. Based on previous bacterial isolation experience, it is likely that many of these colonies belong to the *Sporosarcina* genus or a related genus within the *Bacillus* family.

Urease-positive percentage by sampling sites

In the initial Okinawa sampling, 31 samples were collected from Naha and Nago. Among the eight sampling spots, Kakazu samples exhibited the highest number of colonies compared to the other sampling locations. Consequently, subsequent analysis was limited to the Kakazu samples. In the second Okinawa sampling, 24 samples (12 samples on NH_4-YE and 12 samples on R2A) were collected from three sampling locations, which found 43 out of 247 isolates that were identified as urease positive. The general medium exhibited a higher prevalence of ureolytic bacteria compared to the NH_4-YE medium. The presence of nickel chloride, added to culture media as a nickel source for urease synthesis, has been hypothesized to have led to an increase in the number of airborne bacteria with identifiable urease activity. The fungicide has been implicated in the promotion of bacterial colony growth, potentially resulting in a variation in the number of colonies observed between the two sampling events in Okinawa. The summary of the percentage of urease-positive isolates in each sampling spot can be found in Supplementary Tables S2 and S3. For the Tsukuba sampling, a total of 25 samples were collected

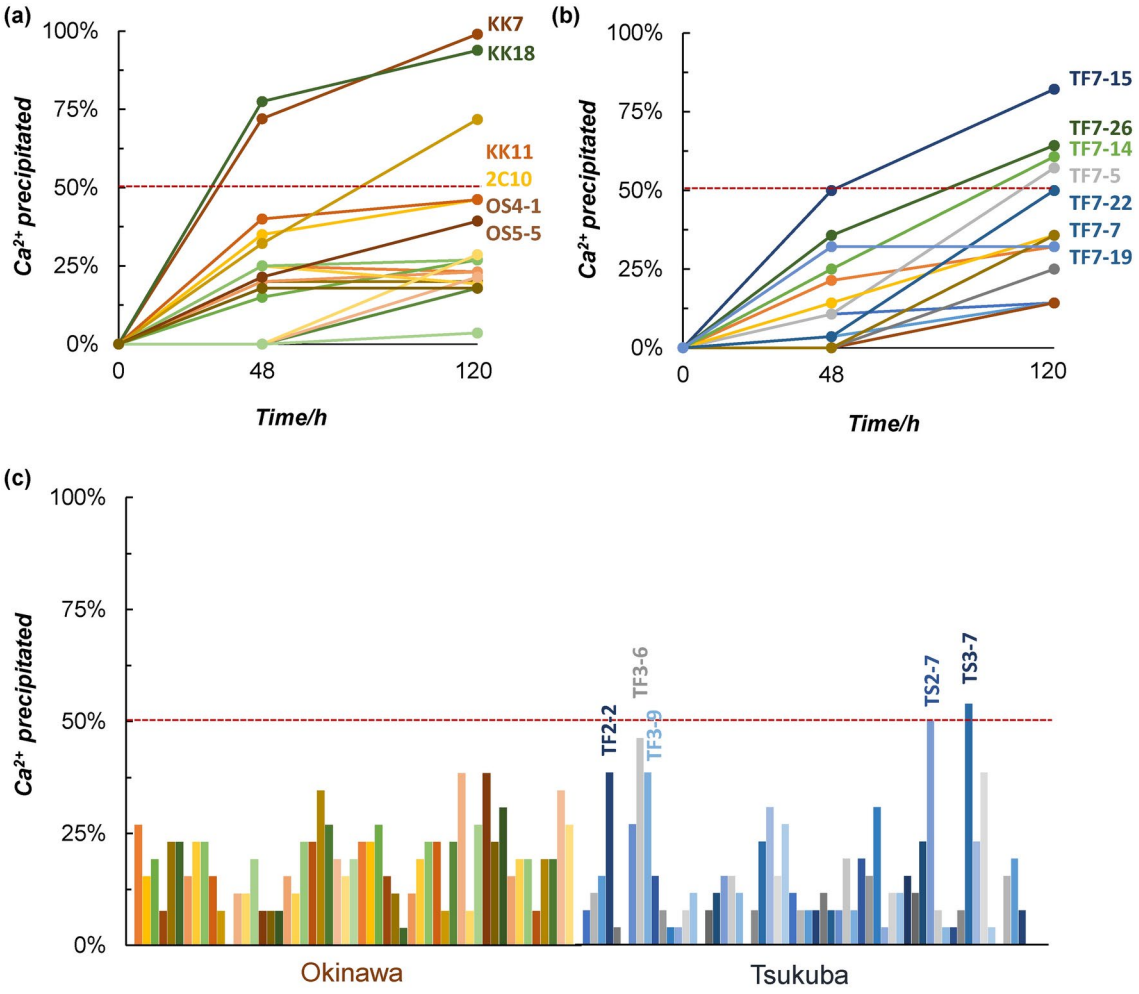

Fig. 4. Calcium carbonate precipitation test. The percentage of calcium ions precipitated after 48 h and 120 h incubation (**a**) 17 isolates from Okinawa- NH4-YE samples; (**b**) 13 isolates from Tsukuba- NH4-YE samples; (**c**) 111 isolates from R2A samples (58 from Tsukuba and 53 from Okinawa) after 144 h incubation.

from five sampling locations. Approximately 20% of the total isolates examined were identified as ureolytic bacteria. Of these isolates, 70% of ureolytic isolates were identified in Doho Park and Tsukuba Mountain. As previously mentioned, the presence of animals that serve as a urea source could potentially contribute to a higher proportion of ureolytic airborne bacteria in these areas. These environments often have dense vegetation, which suggests the presence of a substantial population of bacteria capable of being aerosolized on leaf surfaces[26]. The percentage of urease-positive isolates in each sampling location was summarized in Supplementary Table S4.

A comparison of the occurrence of ureolytic bacteria from three sampling sites is illustrated in Fig. 3. In the Sapporo sampling, NH_4-YE is the only medium used, and on average, approximately 12% of the isolates were found to be ureolytic bacteria. In the subsequent samplings in Okinawa and Tsukuba, due to modifications in the culture media, a higher percentage of urease-positive isolates was observed in Tsukuba and Okinawa compared to Sapporo. However, the proportion of ureolytic isolates did not exhibit a significant difference between the samples from Tsukuba and Okinawa. Ammonium is a nitrogen source that is inexpensive and commonly incorporated into organic compounds[27]. Generally, as a simple organic source of nitrogen, urea can be assimilated by most microorganisms. For this reason, diverse bacteria species can produce urease enzymes to hydrolyze urea and use the products. The simple urease test employed in this study might have identified a limited fraction of ureolytic bacteria in the air. The regulation system of urease in different bacteria varies, and as a result, the requirement that triggers the urease synthesis differs[2]. Therefore, it should be noted that this study only discussed a specific group of ureolytic bacteria that can be quickly identified by a simple urease test. In general, the findings of the prevalence of ureolytic bacteria in the air can provide a basic understanding of their distribution and population and pave a way for further investigation on the engineering potential of airborne bacteria.

Monitoring of calcium carbonate precipitation rate

As previously mentioned, a high percentage of ureolytic bacteria were isolated from the general media. However, it is noteworthy that these isolates demonstrated a relatively weak capacity for calcium carbonate precipitation

Isolate ID	Closest taxonomy				Number of samples in different sources (> 99% sequence identity, Microbe Atlas[32])			
	Genus_Species	Family	Identity	BSL	Aquatic	Soil	Animal	Plant
KK7	*Providencia manganoxydans*	*Morganellaceae*	99.7%	1	–	–	–	–
KK11	*Staphylococcus gallinarum*	*Staphylococcaceae*	99.49%	1	1554	930	23,674	763
KK18	*Lysinibacillus varians*	*Bacillaceae*	99.6%	1	49	72	210	6
2C10	*Staphylococcus nepalensis*	*Staphylococcaceae*	99.67%	2	20	26	679	34
OS4-1	*Staphylococcus saprophyticus*	*Staphylococcaceae*	98.85%	2	1554	930	23,674	763
OS5-5	*Agrobacterium leguminum*	*Rhizobiaceae*	99.28%	1	822	595	3746	731
TF2-2	*Bacillus proteolyticus*	*Bacillaceae*	97.14%	1	446	1920	1524	1079
TF3-6	*Bacillus thuringiensis*	*Bacillaceae*	97.55%	1	446	1920	1524	1079
TF3-9	*Bacillus cereus*	*Bacillaceae*	96.47%	2	446	1920	1524	1079
TF7-5	*Sporosarcina* sp.	*Planococcaceae*	98.28%	1	–	–	–	–
TF7-7	*Sporosarcina soli*	*Planococcaceae*	98.34%	1	–	–	–	–
TF7-14	*Sporosarcina* sp.	*Planococcaceae*	98.57%	1	–	–	–	–
TF7-15	*Lederbergia lenta*	*Bacillaceae*	96.4%	1	15	300	167	12
TF7-19	*Sporosarcina aquimarina*	*Planococcaceae*	97.24%	1	113	383	478	53
TF7-22	*Staphylococcus saprophyticus*	*Staphylococcaceae*	98.66%	2	1554	930	23,674	763
TF7-26	*Sporosarcina ureae*	*Planococcaceae*	99.93%	1	414	999	2247	172
TS2-7	*Kocuria palustris*	*Micrococcaceae*	97.94%	1	8089	5873	47,774	2290
TS3-7	*Micrococcus luteus*	*Micrococcaceae*	100%	1	9695	6060	37,863	3126

Table 3. Summary of closest taxonomy information of selected isolates from Okinawa and Tsukuba samplings.

in comparison to the isolates obtained from the Sapporo sampling, which suggests a weak urease activity. This finding also elucidates the inefficiencies observed in the screening of calcifying bacteria using the CPA and B4 media. Figure 4a illustrates the precipitation of calcium carbonate by 17 isolates from NH_4-YE samples obtained in Okinawa. Of particular notes are KK7 and KK18, which exhibited a relatively high urease activity, leading to a rapid and substantial precipitation of carbonate. In Fig. 4b, the focus is on 13 isolates from NH4-YE samples obtained from Tsukuba, with TF715 and TF7-26 precipitating significant amount of calcium carbonate. Figure 4c demonstrates that 111 isolates from R2A media could only induce a modest amount of precipitation, even after an extended incubation period. In comparison with the findings of the previous study, the current study identified a limited number of isolates with significant potential in carbonate precipitation. As can be seen in the figure, only two strains (TS2-7 and TS3-7) precipitated more than 50% of the calcium ions after 144-h incubation. Collectively, these bacteria cultivated on standard general culture media for heterotrophs may not produce significant levels of urease enzymes, in contrast to the bacterial specialist such as *Sporosarcina pasteurii*, which necessitate urea and ammonium for optimal growth. Consequently, to ensure an adequate number of isolates for taxonomic analysis and comparison between sampling sites, isolates were selected based on their capacity for carbonate precipitation.

Results of bacterial gene analysis

16S rRNA gene analysis was employed to identify 18 isolates from a total of 141 tested isolates obtained from Tsukuba and Okinawa. Table 3 summarizes the results of bacterial gene analysis. The majority of the isolates were found to belong to two phyla, *Firmicutes* and *Actinobacteria*, both of which have been frequently identified in air samples of previous studies[28,29]. Approximately half of the isolates were found to be spore-formers, with the majority belonging to the *Sporosarcina* and *Bacillus* species. As anticipated, many *Sporosarcina* species (TF7-5, 7–7. 7–14, 7- 15, 7–19, 7–22, 7–26) were isolated from Doho Park. Of particular interest, one strain TF7-26 was identified as *Sporosarcina ureae* (similarity 99.93%), a species known for its ability to tolerate high concentrations (up to 8%) of urea[30]. Among the non-spore formers, a significant proportion was from the *Staphylococcus* genus, a prevalent inhabitant of animal skin. And some of them were from the *Micrococcaceae* family. For example, one pigmented strain *Micrococcus luteus* TS3-7, has been found in oligotrophic environments for extended periods of time[31]. Notably, two gram-negative species were identified: one plant-derived species (OS5-5) and one strain (KK7) that was previously isolated in a mining site. The latter was recently classified as a novel species. These strains were selected based on their features and then characterized to assess their applicability for MICP applications.

Figure 5 presents a comparison of the composition of isolates from the Sapporo sampling with those from the Okinawa and Tsukuba sampling. The isolates obtained from the Okinawa and Tsukuba sampling sites were grouped for comparative analysis. As illustrated in Fig. 5a–b, these two collections exhibit a high degree of similarity in terms of taxonomy. Of the isolates from Sapporo, approximately half were found to be *Sporosarcina* and *Bacillus* related strains, while the remaining half were mostly from the *Glutamicibacter* genus, which was reclassified from the *Arthrobacter* genus[33]. The *Arthrobacter* genus has been frequently reported as potential bacteria for various environmental problems due to its versatile features. To date, only a few studies have

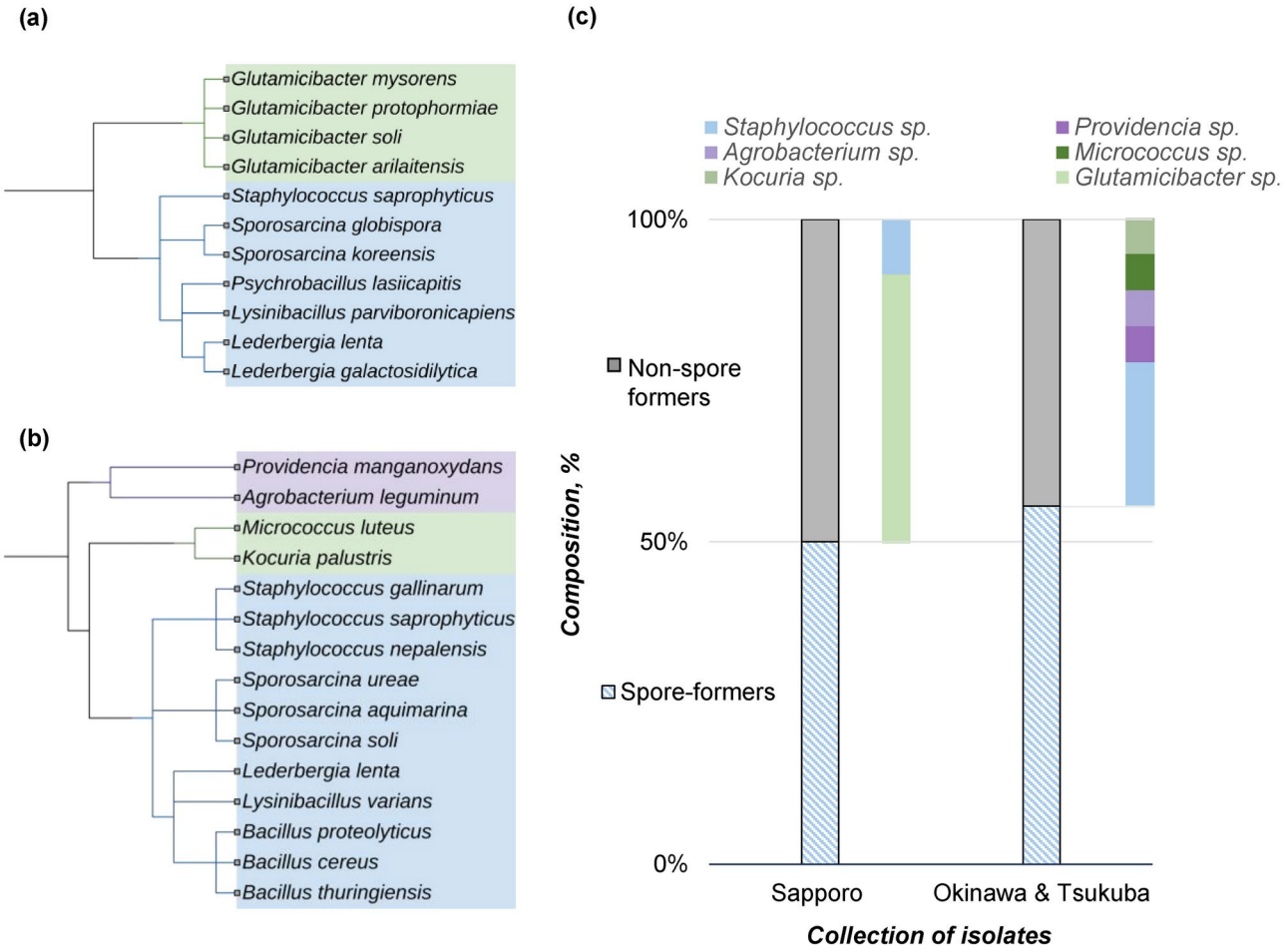

Fig. 5. Comparison of (**a**) Sapporo and (**b**) Okinawa & Tsukuba bacterial collection on closest taxonomy (phylogenetic tree generated by iTOL: Interactive Tree Of Life); (**c**) composition of spore-formers in each collection.

examined the application of *Glutamicibacter* bacteria for MICP applications. In a study by Li et al. (2022), a ureolytic strain from *Glutamicibacter* sp. were found to exhibit a high tolerance for substantial concentrations of heavy metal ions in culture media, contributing to an efficient precipitation of carbonate minerals from Pb, Cd, Cu, and Ni at low temperatures and across a wide pH range[34]. Similarly, the Okinawa-Tsukuba collection consisted of more than half of *Sporosarcina* and *Bacillus* related species, as well as human commensals, such as *Staphylococcus* species and some strains from the *Micrococcaceae* family. The *Micrococcaceae* family is widely distributed due to its ability to inhabit diverse terrestrial and aquatic environments[35]. Figure 5c compares the composition of spore-formers in two collections, revealing that approximately half of them were spore-formers. It is noteworthy that a significant proportion of these spore formers may persist in the atmosphere in a dormant state, where they could reactivate when environmental conditions become conducive to their growth. Given that some of the isolates do not form spores, they are likely to exhibit resilience against adversity, such as desiccation and starvation. Although these bacteria exhibit relatively weak urease activity, their prolonged performance may prove advantageous in the treatment process.

Temperature dependency of selected strains

Four isolates were selected for further characterization based on their ability to precipitate calcium carbonate, including one from *Sporosarcina* sp. (TF7-26), one from *Lederbergia* sp. (TF7-15), one *Lysinibacillus* sp. (KK18), and one Gram-negative strain, *Providencia* sp. (KK7).These isolates were cultivated at temperatures ranging from 15 to 35 °C to examine their temperature dependency in terms of growth and urease activity, As can be seen in Fig. 6, all of the isolates exhibited have a typical growth pattern under varying culture temperatures. The findings indicate that two isolates (TF7-15 and TF7-26) from Tsukuba city, could grow at a wide range of temperatures. Notably, the former demonstrated a remarkable activity. For Okinawa isolates, KK7 showed rapid growth at 20 to 35 °C, which aligns with the findings of a previous study[36]. Notably, it exhibited sustained urease activity over a duration of one week. In contrast, the growth curve of KK18 exhibited substantial growth at temperatures ranging from 25 to 35 °C, but no growth was observed at 15 and 20 °C. Generally speaking, a shared pattern characterizes the urease activity of all isolates, demonstrating optimal activity at elevated temperatures.

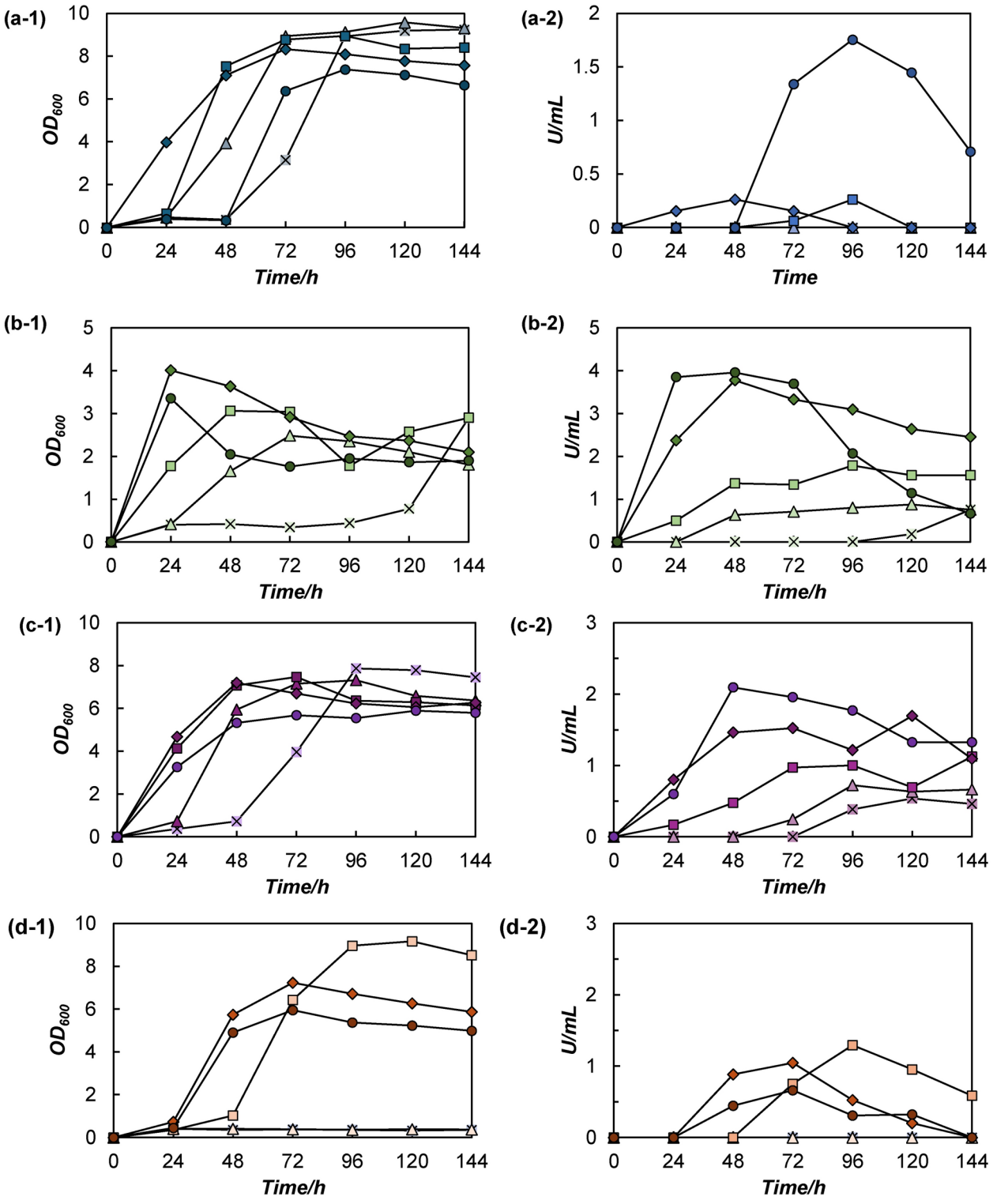

Fig. 6. Temperature dependence of five isolates from Okinawa and Tsukuba samplings. Growth and activity changes with time under × – 15 °C , ▲– 20 °C , ▪– 25 °C , ◆– 30 °C, ●– 35 °C of (**a**) TF7-26; (**b**) TF7-15; (**c**) KK7; (**d**) KK18.

Figure 7 compares the temperature dependency of urease activity of representative isolates from the Sapporo collection and the Okinawa & Tsukuba collection. Most bacteria exhibit growth in a wide range of temperatures, while some isolates exhibit growth in a certain range of temperatures to adapt to the local climate. For instance, the *Sporosarcina* sp. MY2-9 from Sapporo was found to have cold tolerance, enabling it to grow in a 4 °C refrigerator. The psychrophilic feature was also found in a closely related *Sporosarcina globispora* strain

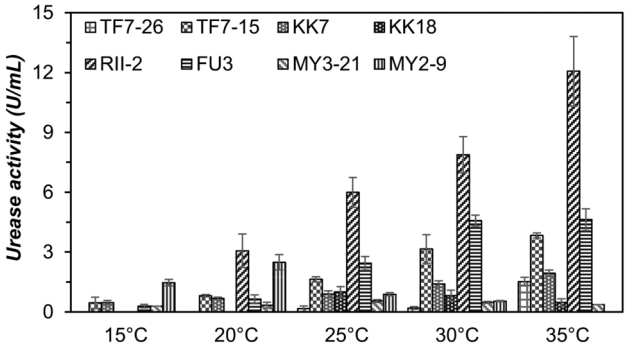

Fig. 7. Temperature dependency of urease activity of four Sapporo isolates, two Okinawa isolates and two Tsukuba isolates.

that was isolated from soil and river water[37]. In contrast, one isolate (KK18) from Okinawa demonstrated growth exclusively at temperatures above 20 °C. With respect of the MICP applicability of these isolates, two isolates with high urease activity were previously examined in sand column solidification tests, revealing a high cementation effectiveness that is comparable to that of standard ureolytic bacteria, *Sporosarcina pasteurii*.[25]. Furthermore, the findings of this study revealed that some of the isolates might precipitate a significant amount of carbonate in carbonate precipitation tests but show relatively low activity in the urease test. This phenomenon was also found in other strains of *Glutamicibacter* sp. from the Sapporo sampling. As previously stated, the regulatory mechanisms of urease vary across different bacterial species, with diverse triggers including urea availability, nitrogen source deficiency, pH changes, etc.[2]. Therefore, these bacteria may produce more urease in a test solution of the precipitation test, while they may not show much urease activity in the activity test when cultivated with sufficient nitrogen sources. From the perspective of urease regulation, these strains are more likely to produce urease when the nitrogen source in the environment is limited, unlike *Sporosarcina pasteurii*, which produces urease regardless of nitrogen availability. The potential of these isolates with relatively weak urease activity should be further evaluated, as some of these isolates possess unique features that could contribute to enhancing the treatment efficiency and cementation effectiveness.

Calcium carbonate induced by different strains

The morphology of calcium carbonate precipitated by eight isolates of four genera was observed to obtain a comprehensive understanding of calcium carbonate morphology induced by various airborne bacteria. Representative SEM images are presented in Fig. 8, which are quite revealing in several ways. Firstly, ureolytic bacteria with high urease activity precipitated small calcium carbonate crystals (see Fig. 8a–d).The calcium carbonate crystals formed by fast precipitators from *Lederbergia* sp. and *Sporosarcina* sp., appear to be a tetrahedral growth form of calcite. On the other hand, low-activity bacteria induced a substantially larger crystal size than the former (see Fig. 8e–g). These observations are essentially consistent with previous findings that investigated the relationship between urease activity and crystal size of calcium carbonate[38,39]. The calcium carbonate formation by KK7 consists of small crystals aggregating into a large "cauliflower"-shaped crystal (see Fig. 8h). It has been documented that this calcium carbonate formation is a calcite phase[40]. In general, crystals formed by strains belonging to the same genus tend to exhibit similarities in the calcium carbonate morphology.

The most notable observation in the figure is the formation of extra-large calcium carbonate crystals by *Glutamicibacter* sp., which typically has glutamic acid functional group in their peptidoglycan interpeptide bridge[33]. The morphology of calcium carbonate crystals is found to be influenced by several microbiological factors, including the bacterial extracellular matrix, thereby leading to strain-specificity in the morphology induced by MICP[10,41–43]. A comprehensive study by Li et al. (2019) revealed that the formation and stabilization of vaterite are promoted by certain amino acids, especially carboxyl-enriched glutamic acid and aspartic acid, through a process of chelating with calcium cions and subsequently incorporating into the vaterite structure[44]. Therefore, the characteristics of the bacterial surface and some metabolites of *Glutamicibacter* sp. may contribute to the precipitation of calcium carbonate in a spherical vaterite structure, as observed in Fig. 8e–g. Previous researchers have found that a low population of high-activity bacteria (a low urease activity) leads to a larger formation of calcium carbonate, which was attributed to fewer nucleation sites and promoted growth of existing crystals[12,45]. In the case of low-activity *Glutamicibacter* sp., the formation of these large crystals was observed to occur in a high biomass concentration, suggesting a potential influence of their unique properties. As a previous member of *Arthrobacter* genus, the strain has been noted to form aggregates in culture media due to its substantial extracellular polymeric substance production (see Supplementary Fig. 2S). This characteristic play a pivotal role in achieving high cementation effectiveness, particularly, for coarse sand solidification, as the relative size of calcium carbonate precipitates should be large enough to support soil particles[46].

Recommendations for further investigation

Microbes have been observed to produce nanobacteria-like structures as nucleation sites for biomineralization, providing a protective barrier against entombment[47]. Bacteria that employ this survival strategy possess a competitive advantage in biocementation applications. In this study, isolates from the *Glutamicibacter* genus

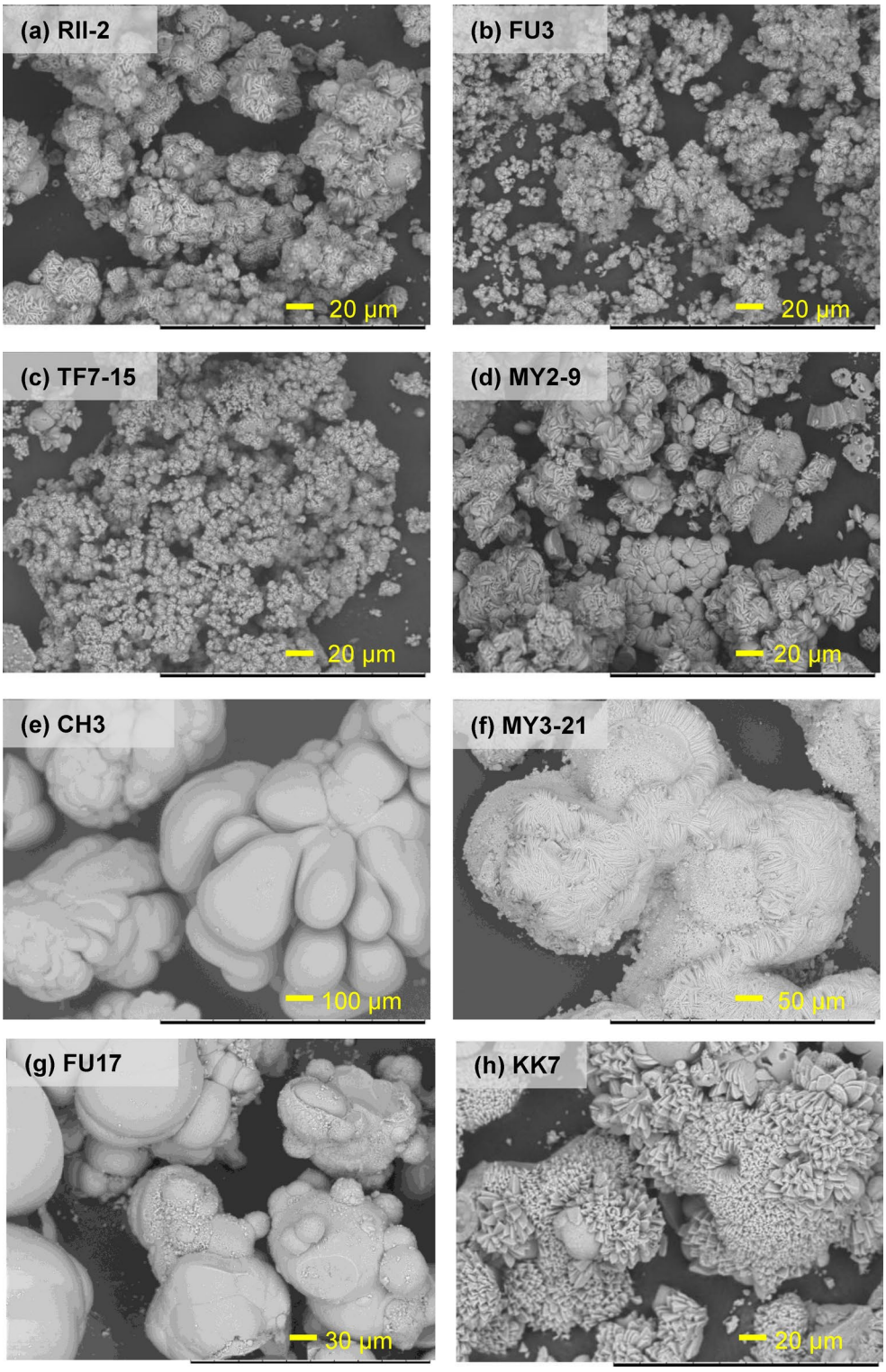

Fig. 8. Calcium carbonate morphology of three *Lederbergia* sp. (**a**) RII-2; (**b**) FU3; (**c**) TF7-15; one *Sporosarcina* sp. (**d**) MY2-9; three *Glutamicibacter* sp. (**e**) CH3; (**f**) MY3-21; (**g**) FU17; one *Providencia* sp. (**h**) KK7.

are very likely to have similar behavior in cementation media. As previously mentioned, these species form aggregations that are relatively robust to environmental stress, particularly with regard to metal toxicity[48]. In this case, employing *Glutamicibacter* species can potentially enhance the bacteria survival rate in cementation process, contributing to a high cementation effectiveness. Additionally, *Glutamicibacter* sp. typically exhibits a rod-coccus growth cycle, with cocci diameters ranging from 0.6 to 1.2 µm[49]. In comparison to rods, which

possess comparatively large cell sizes, bacteria with smaller cell sizes can more efficiently utilize nutrients due to their relatively large specific surface area, which is a key factor in their enhanced nutrient uptake capacity[30]. Furthermore, *Arthrobacter* is a globally distributed, well-known beneficial genus of bacteria that has effective roles in many fields[50]. Similarly, *Glutamicibacter* sp. might share many common characteristics of bacterial generalists that might contribute to the improvement of biocementation treatment. For instance, these bacteria can be cultivated in inexpensive culture media derived from industrial waste, thereby reducing the overall cost of the cultivation process. Their remarkable survivability and adaptability to diverse environments have the potential to enhance the cementation efficiency of complicated soils. To confirm the feasibility of this hypothesis, future investigations should focus on the utilization of *Glutamicibacter* species.

Conclusions

This study collected airborne bacteria from different climate zones in Japan with the objective of identifying potent candidates for MICP application. As the initial study to explore the application of airborne bacteria for biocementation, the present study was exploratory and interpretative in nature. The analysis of air samples collected from three sampling sites identified 10–20% of urease-positive isolates in the tested airborne bacteria. The taxonomic classification of these isolates revealed notable similarities among them, irrespective of their geographical origin. Among the selected isolates, approximately 50% were found to be *Bacillus* related spore-forming species. The characterization of selected isolates revealed that many of them are domesticated by the local climate of origin and exhibited a unique temperature dependency in terms of bacterial growth and urease activity. The calcium carbonate formed by different strains shows a strain-specific feature. Compared to common ureolytic bacteria of *Sporosarcina* or *Bacillus* related species, unique bacteria from *Glutamicibacter* sp. exhibited relatively low urease activity yet produced a substantial quantity of extra-large calcium carbonate crystals, which may have beneficial effect on biocementation. Further investigation is necessary to gain a more comprehensive understanding of their survivability and adaptability under changing conditions.

Data availability

All the experimental data that supports the findings of this study are available from the corresponding author upon reasonable request through email.

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Author contributions

M.C.: Conceptualization, Methodology, Bacteria Sampling, Laboratory experimentation, Writing—Original draft preparation; C.T. & K. N., Supervision, Writing—Reviewing and Editing; S.G.: Reviewing and Editing; S. K.: Supervision, Bacteria Sampling, Writing—Reviewing and Editing.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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