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SCREENING OF BACTERIA IN YARIK SINKHOLE, ANTALYA, TURKEY FOR CARBONATE DISSOLUTION, BIOMINERALIZATION AND BIOTECHNOLOGICAL POTENTIALS

PREGLED BAKTERIJ V VRTAČI YARIK, ANTALJA, TURČIJA, V ZVEZI Z RAZTAPLJANJEM KARBONATA, BIOMINERALIZACIJO IN BIOTEHNOLOŠKIM POTENCIALI

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Abstract UDC 582.23:551.435.82:549.742.111(560)

Elif Özlem Arslan- Aydoğdu, Yağmur Avci, Nahdhoit Ahamada Rachid, Batu Çolak & Nihal Doğruöz-Güngör: Screening of bacteria in Yarık Sinkhole, Antalya, Turkey for carbonate dissolution, biomineralization and biotechnological potentials

Abiotic and biotic factors, especially microorganisms, play a role in the development of cave formations and the existence of unique characteristics of each cave. Due to the ecological conditions that characterize the cave environments, highly specialized microorganisms that are the main source of diverse bioactive compounds, inhabit these environments. The aim of this study is to determine the role and biotechnological potential of the bacteria isolated from Yarık Sinkhole located in Antalya (Turkey) by screening their ability to induce the $CaCO_3$ precipitation, to hydrolyze urea, to induce calcite dissolution, and screening their possession of NRPS/PKS gene clusters. The most prevalent phylum is the Bacillota (synonym Firmicutes) (75.7 %), while the dominant species is Bacillus pumilus (33 %). All the isolates showed crystal formation on B4 agar medium, and the Energy dispersive X-Ray spectroscopy (EDS) analyses showed that the crystals are predominately composed of calcium, carbon and oxygen. Ninety-six (96 %) of our iso-

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Elif Özlem Arslan- Aydoğdu, Yağmur Avci, Nahdhoit Ahamada Rachid, Batu Çolak & Nihal Doğruöz-Güngör: Pregled bakterij v vrtači Yarık, Antalja, Turčija, v zvezi z raztapljanjem karbonata, biomineralizacijo in biotehnološkim potenciali

Abiotski in biotski dejavniki, zlasti mikroorganizmi, imajo pomembno vlogo pri nastanku jamskih oblik in glede edinstvenih značilnosti vsake jame. Zaradi ekoloških razmer, ki so značilne za jamska okolja, ta okolja naseljujejo visoko specializirani mikroorganizmi, ki so glavni vir raznih bioaktivnih spojin. Cilj te študije je opredeliti vlogo in biotehnološki potencial bakterij, izoliranih iz vrtače Yarık v Antalji (Turčija), s pregledom njihove zmožnosti, da povzročijo obarjanje $CaCO_3$, hidrolizirajo sečnino, povzročijo raztapljanja kalcita, in njihove vsebnosti genskih skupin NRPS in PKS. Najpogostejše deblo je Bacillota (sinonim: Firmicutes) (75,7 %), prevladujoča vrsta pa je Bacillus pumilus (33 %). Pri vseh izolatih se je na agarnem gojišču B4 pojavila tvorba kristalov, analize z energijsko disperzivno rentgensko spektroskopijo (EDS) pa so pokazale, da so kristali sestavljeni pretežno iz kalcija, ogljika in kisika. Šestindevetdeset (96 %) naših izolatov ima negativno ureolitično aktivnost. Glede na ta rezultat in zmožnost,

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lates have negative ureolytic activity. According to this result and having the ability to induce the $CaCO_3$ precipitation, bacteria in this environment use other biosynthesis pathways than urea hydrolysis. $MgCO_3$ and $CaCO_3$ were dissolved by 61 % and 59 % of the isolates, respectively. In addition, 5.9 % and 53.7 % of the isolates showed the possession of PKS and NRPS genes, respectively. This result reveals that our isolates have high industrial and biotechnological potential. They may constitute good candidates for further biotechnological applications such as construction of bio-concretes, bioremediation, soil fertility, and production of biologically active secondary metabolites.
Keywords: cave bacteria, non-ureolytic bacteria, carbonate dissolution, carbonate precipitation, polyketide synthase, non-ribosomal peptide synthetase.

da povzročijo obarjanje $CaCO_3$, bakterije v tem okolju uporabljajo druge načine biosinteze, ne hidrolizo sečnine. $MgCO_3$ in $CaCO_3$ je raztopilo 61 % oziroma 59 % izolatov. Poleg tega je 5,9 % in 53,7 % izolatov imelo gene genskih skupin PKS oziroma NRPS. Ta rezultat kaže, da imajo naši izolati velik industrijski in biotehnološki potencial. Lahko so zelo primerni za nadaljnjo biotehnološko uporabo, na primer za pripravo biobetona, bioremediacijo, rodovitnost tal in proizvodnja biološko aktivnih sekundarnih metabolitov.
Ključne besede: jamske bakterije, neureolitične bakterije, raztapljanje karbonata, obarjanje karbonata, poliketidna sintaza, neribosomalna peptidna sintetaza.

1. INTRODUCTION

Caves are natural underground cavities accessible to humans and are characterized by poor nutrients, low but constant temperature, and high humidity (Gillieson, 1996; Zada et al., 2021). The most common cave type on earth is the karstic cave (Bartolomé et al., 2021). The formation of caves starts with the dissolution of soluble rocks like gypsum and limestone under the chemical action of water. Under mechanical erosion, caves and cave structure are gradually forming (Becker et al., 2020; Ai et al., 2022). Formation and change of cave structure can be affected by the biogeochemical cycles in the cave environment. These cycles are mediated by biotic factors such as microorganisms and abiotic factors like temperature and organic compounds. The composition of cave microbiota is controlled by different factors like the type of carbon sources and the specific minerals present inside the cave (Ai et al., 2022). Previous studies showed that microorganisms can participate in rock corrosion or in shaping cave structures. Mechanisms like biomineralization, dissolution of minerals and degradation of organic compounds, in which microorganisms use enzymes and their extracellular biological compounds, have been reported (Cañaveras *et al.*, 2001; Jones, 2001; Ríos et al., 2011). The oligotrophic characteristic of the cave environment is not an obstacle to colonization and dominance of microbes that constitute the primary producers in these dark, underground ecosystems. It is reported that chemoautotrophic microbes, common in caves, fix carbon, maintain the energy cycle in caves, and sustain the life of many other cave organisms (Wu et al., 2015). Moreover, some chemoautotrophic caves like Movile and Frasassi Caves, investigated for their microbial diversities with biogeochemical reactions, were observed with microorganisms including sulfur oxidizers, CO_2 fixers, methanotrophs, and nitrifying microorganisms (Jones et al., 2008; Jurado et al., 2020; Chiciudean et al., 2022).

Researchers are mostly interested in studying the cave microbiota due to their ability to adapt to underground living conditions, which have poor nutrients and a lack of light. The deposition of nitrate/carbonate, the breakdown/dissolution of speleothems and cave walls, as well as other chemical reactions are observed in these areas (Johnston et al., 2021). The precipitation or dissolution of nitrate and carbonates has been reported to be passive; microorganisms produce enzymes or behave like nucleation sites that mediate the microbial-induced precipitation or dissolution processes (Zada et al., 2021). Calcium carbonate precipitation is a good example of microbially extracellular biomineralization. Microbially induced $CaCO_3$ precipitation (MICP) results from the metabolic interactions between diverse microorganisms and organic and/or inorganic compounds present in the environment (Sarda et al., 2009; Qian et al., 2010). This process is known to occur through various metabolic pathways such as photosynthesis, sulfate reduction, anaerobic sulfur oxidation, and urea hydrolysis (Sarda et al., 2009; Qian et al., 2010). Microorganisms that induce the precipitation of calcium minerals have been isolated from different ecosystems including soil (Boquet et al., 1973), sea sediments (Turchyn et al., 2021), active anaerobic mud (Su & Yang, 2020), and caves (Baskar, 2009; Miller et al., 2018).

MICP is used with great interest in different areas like the remediation and restoration of various building materials (Ortega-Villamagua et al., 2020), construction of bio-concrete (Su et al., 2021), removal of contaminants from groundwater and soil (bioremediation) (Dhami et al., 2013), heavy metal removal from wastewater (Tyagi et al., 2020), bio self-healing dental composite (Seifan et al., 2020), and technology of microbially enhanced petroleum recovery (MEROR) (Saravanan et al., 2020). The most studied bacteria in the MICP are shown in the literature to have the ability to hydrolyze urea. However, bacteria with urease negative activity with the ability to

induce the calcium precipitation process should not be ignored (Türkgenci & Doğruöz Güngör, 2021).

An opposite process of calcium carbonate precipitation in the cave is the weathering of carbonate rock, cave walls, and sediments. The dissolution pits are regularly observed on rock surfaces in caves. These pits are formed by the dissolution and decomposition of rocks. The breakdown of rocks is the result of biological action and inorganic reactions such as condensation, corrosion, and erosion. Microorganisms can produce organic acids which alter the pH of the environment and result in the dissolution of the calcium carbonate (Cañaveras et al., 2001; Jones, 2001; Johnston et al., 2021). On the other hand, microorganisms can produce enzymes that catalyze the reaction of CO_2 and water releasing ions that induce calcite dissolution (Li et al., 2006; Johnston et al., 2021). The cave microorganisms that cause the dissolution of $MgCO_3$ and $CaCO_3$ may have wide use in soil remediation and soil fertility in areas affected by high salt concentrations (Orhan et al., 2017). Previously, it showed that cave microorganisms can act either as carbonate weathering or/and precipitation inducers. In this fact, it is important to study these two features in the same microorganisms to select the most suitable microorganisms to be used for industrial and biotechnological purposes.

In addition to the microbially-mediated carbonate dissolution and precipitation processes, other biological processes are occurred in cave environments. These processes result to the production of biological compounds that could be used for pharmaceutical, food, and other biotechnological and industrial applications. Polyketides (PKs) and non-ribosomal peptides (NRPs) are two bioactive compound groups that have important roles because of their various biological activities. Due to the oligotrophic nature of the caves, PKs and NRPs can give their producers an advantage over competitive microorganisms (Bauer et al., 2018; Bukelskis *et al.,* 2019; Lukoseviciute et al., 2021). PKs synthesized by bacteria are mostly encoded by the Polyketide synthase (PKS) gene cluster or the hybridization of PKS and nonribosomal peptide synthetase (NRPS) gene clusters. These compounds are known for their wide uses in agriculture (plant growth/antimicrobial substances) and medicine (Olishevska et al., 2019). Lipopeptide bio-surfactants synthesized by non-ribosomal peptide synthetases (NRPSs) have various roles such as antimicrobial, antitumoral, antioxidants, iron acquisition, immunosuppressive, insecticidal, nematocidal, and phytotoxic activities (Sukhanova et al., 2018; Bukelskis et al., 2019; Lukoseviciute et al., 2021).

The geochemical characteristics of cave environments vary from one cave to another and make the unique characteristic of each cave. Since the microbial composition of an environment is also dependent on the local physicochemical parameters, the microbial diversity of caves follows the same rule of cave uniqueness. Therefore, our study aimed to investigate the importance of bacteria isolated from Yarık Sinkhole in the formation of caves and cave structures, as well as their potential uses in industrial/biotechnological areas. In this fact, the MICP and $CaCO_3$/$MgCO_3$ weathering abilities of isolates were studied for determining some of their roles in cave formation and changes of cave structure. In addition, the urease activity was screened to determine if the isolates from this sinkhole exhibiting calcium carbonate precipitation are using the urea hydrolysis pathway. Moreover, to reveal the potential of the Yarık Sinkhole's bacteria to synthesize bioactive compounds, PKS and NRPS gene clusters were screened in these isolates. In the last, the isolates suitable for use within the framework of our purposes for future industrial and/or biotechnological applications were stocked.

2. MATERIALS AND METHODS

2.1. CAVE BACKGROUND

Our studying area was the Yarık Sinkhole (GPS coordinate: UTM 448068.47 E 4036006.77 N) which is located on the Sivastı Plateau centred 30 km in the north of Gazipaşa in Antalya-Turkey. Yarık Sinkhole has a total length of 1378 m and 533 m in depth. At the first entrance, the cave was explored up to a depth of -300 m and reached a depth of -533 m in 2016. The watershed of the cave is a closed valley where the main rock is limestone with little sediment in the basin of the valley.

The entrance of the cave is a broad opening and has a vertical type, occasional narrow passages and a stepped descent down and a shallow pond formation at the base. Generally, the Yarık Sinkhole's ceiling is higher, but some of the narrow passages are difficult to pass, especially in a flood situation when these passages will be blocked. Unlike most vertical caves, in the Yarık Sinkhole speleothems such as flowstones and cave pearls are found in the horizontal portion.

2.2. SAMPLING AND ISOLATION OF MICROORGANISMS

Soil and surface samples were taken from the depth -80 m and -320 m during the cave exploration in 2014. About 25 g of each soil samples were taken under aseptic conditions by using sterile disposable sample container with screw cap. The surface samples were taken with sterile swab from the cave walls (10 cm^2 wide area) located near the soil sampling areas. All samples were transported under refrigerated conditions to the laboratory within 24 hours. After reached to laboratory, samples were instantly diluted in a serial dilution range of 10^{-1} -10^{-5} and 100 µl from each dilution was inoculated on Reasoner's 2 Agar (R2A) and TSA (Tryptic Soy Agar – prepared in 1/2 ratio) plates in triplicate and incubated at 28°C for 7 days. Plates were daily checked and colonies with different morphology were selected, Gram-stained, and purified. Purified colonies were stored at -86 °C for subsequent uses.

2.3. GENOMIC DNA ISOLATION, PCR AMPLIFICATION, AND SEQUENCING

The genomic DNA of the isolates was isolated by using the bacterial Exgene™ Cell SV DNA isolation Kit (GeneAll®). The 16S rRNA gene of each isolated DNA was amplified through polymerase chain reaction (PCR) by using the universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). The PCR mixture was prepared with a volume of 50 µL containing 20 nM of each primer, 10 ng of DNA, 2.5 U of Taq DNA polymerase (Takara Bio) and run in a single block thermal cycler (BIORAD). The reaction was performed under the following conditions: 1 min at 95°C for initial DNA denaturation, followed by 35 cycles composed of second DNA denaturation for 15 s at 95°C, annealing step at 55°C for 15 s, and 10 s of extension step at 72°C, then a final extension step performed at 72°C for 10 min. The PCR products were sequenced by the Sanger sorting method and sequences were read by the ABI 3130 Sequencer (Applied Biosystems). The 16S rDNA sequences were deposited in GenBank (NCBI) under the accession numbers MZ646235- MZ646264, MZ646266- MZ646299, MZ420151, MZ821657.

2.4. DETERMINATION OF UREASE ACTIVITY AND $CACO_3$ PRECIPITATION OF CAVE BACTERIA

All the isolates were inoculated in Christensen's broth (1 liter of the medium contains: Peptone (1.0 g); Glucose (1.0 g); Di-sodium hydrogen-phosphate (1.2 g); Potassium dihydrogen phosphate (0.8 g); Sodium chloride (5.0 g); Phenol red (0.004 g) and 10 ml of urea solution) for urease activity analysis. A change in color after 2-3 days of incubation at 28°C was recorded as urease-positive reaction (Omoregie et al., 2018).

The screening of the bacterial isolates for crystal-forming bacteria was performed with calcification promoting agar medium B4 composed of calcium acetate (0.25 g), yeast extract (0.4 g), agar (1.5 g), and, glucose (1.0 g) in 100 ml distilled water with pH 8.0. The isolates

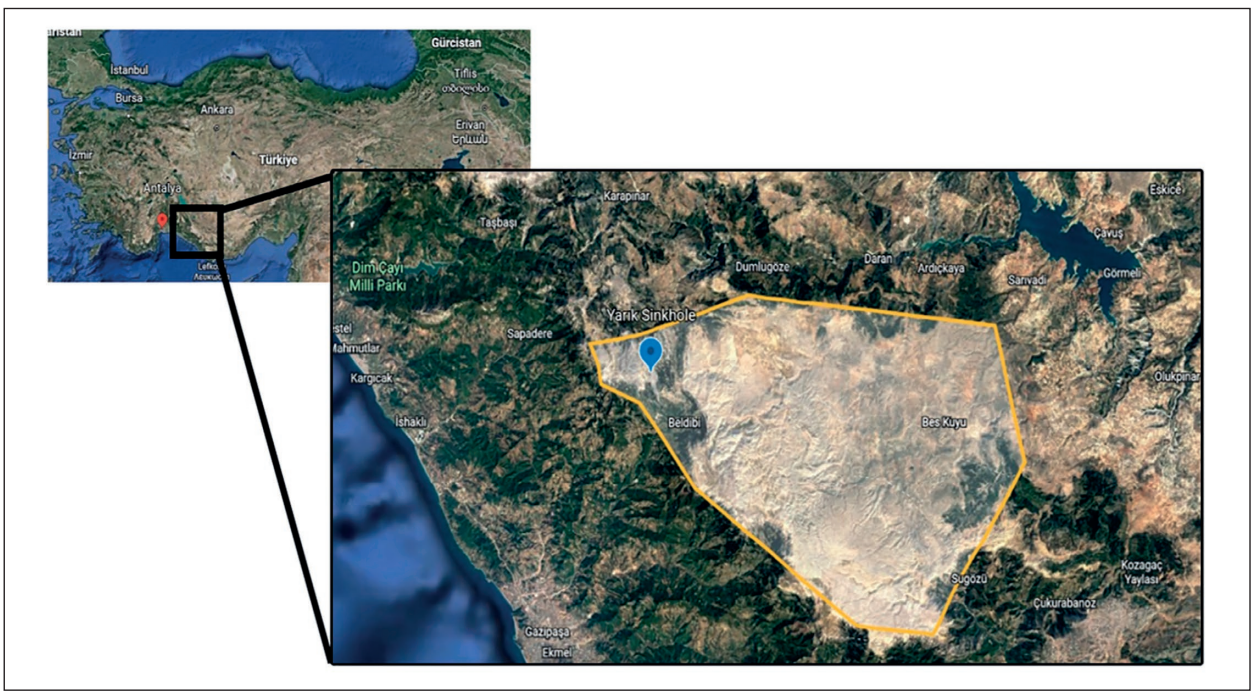

Figure 1: The geographical location of Yarık Sinkhole in Antalya, Turkey (http://www.google.com.tr/maps/place., Reviewed on: May 30, 2021).

were inoculated on the B4 agar medium, incubated at 28°C, and controlled under a light microscope every day for a period of 28 days, then crystal-forming ones were recorded (Boquet et al., 1973). Non-inoculated plates were used as negative controls.

2.5. CRYSTAL ANALYSIS

The crystal morphologies of three isolates selected based on their species were observed using Scanning Electron Microscopy (SEM). Moreover, analysis of elemental composition was performed using energy-dispersive X-ray spectroscopy (EDS). First, the crystals were purified from the B4 agar medium by cutting out agar pieces and transferred to a 50 ml Falcon tube. Then the agar pieces were dissolved using a microwave oven. Both the supernatants and sediments were twice washed with 15 ml sterile distilled water until the agar was removed. As described by Marvasi et al. (2012) with some modifications, the crystals were collected and distributed into sterile empty plates and stored at 28°C for 7 days for drying. SEM-EDS analyses of crystal formations were performed in the physics laboratory of Istanbul University by using an EDAX Octane detector equipped with FEI Versa 3D Dual Beam and Dual Beam. The visual appearance magnifications were ranging from 330 to 12000 times.

2.6. DETERMINATION OF $CACO_3$ AND $MGCO_3$ WEATHERING POTENTIAL OF ISOLATES

Deveze-Bruni's $CaCO_3$ agar (per liter: glucose (20 g); NaCl (10 g); $MgCl_2$ (3 g); $MgSO_4.7H_2O$ (0.5 g); KCl (0.4 g); $(NH_4)_2SO_4$ (0.2 g); agar (15 g) and $CaCO_3$ (10 g) and Deveze-Bruni's $MgCO_3$ agar (per liter: glucose (20 g); NaCl (10 g); $MgCl_2$ (3 g); $MgSO_4.7H_2O$ (0.5 g); KCl (0.4 g); $(NH_4)_2SO_4$ (0.2 g); agar (15 g) and $MgCO_3$ (10 g) were used for the investigation of $CaCO_3$ and $MgCO_3$ dissolution potential of isolates (Cacchio et al., 2004; Orhan et al., 2017). The bacterial isolates were incubated at 30°C for 2–3 weeks on Deveze-Bruni's $CaCO_3$ agar and Deveze-Bruni's $MgCO_3$ agar media. The formation of a clear zone around the bacterial growth indicates the dissolution of $CaCO_3$ or $MgCO_3$ minerals. It should noted that the media used in this experimentation are opaque and become transparant after the dissolution of the minerals ($CaCO_3$ or $MgCO_3$) containing the media.

2.7. SCREENING OF PKS AND NRPS GENE CLUSTERS IN BACTERIAL ISOLATES

For the PCR amplification of PKS and NRPS genes, three sets of primers were used: DegKS2F/DegKS2R for PKS, A3F/A7R and MTF/MTR for NRPS (Table 1).

The components and reaction conditions of the PCR mixture are as follows:

PKS

DegKS2F/DegKS2R (25 µL): 1 µL template, 12.5 µL EmeraldAmp® MAX HS PCR Master Mix (Takara Bio), 5% of DMSO and 2.5 µL each primer (5µM); the PCR conditions were composed of initial denaturation of 5 min at 94°C, followed by 40 cycles of 40 s at 94°C, 40s at 50°C, and 75s at 72°C, and the final extension was at 72°C for 5 min.

NRPS

MT-F/MT-R (25 µL): 1 µL template, 12.5µL EmeraldAmp® MAX HS PCR Master Mix (Takara Bio), 2.5 µL each primer (5µM); initial denaturation was at 94°C for 3 min, followed by 35 cycles of 60 s at 94°C, 60 s at 55°C and 2 min at 72°C and the final extension step was at 72°C for 7 min.

A3F/A7R (25 µL): 1 µL template, 12.5 µL EmeraldAmp® MAX HS PCR Master Mix (Takara Bio), 5% of DMSO, and 2.5 µL each primer (5µM); initial denaturation was 5 min at 95°C, followed by 35 cycles of 30 s at 95°C, 30 s at 59°C and 90 s at 72°C, followed by a 5-min extension at 72°C.

After the polymerase chain reaction for the PKS and NRPS targeting genes, PCR products were run in a 1% agarose gel through electrophoresis.

Table 1: Primers and PCR cycling conditions were used in this study.

Primer	Sequence	Reference
Polyketide synthase		
DegKS2F	5'-GCIATGGAYCCICARCARMGIVT-3'	(Amos et al., 2015)
DegKS2R	5'-GTICCIGTICCRTGISCYTCIAC-3'	(Amos et al., 2015)
Non-ribosomal peptide synthetase		
MT-F	5'-GCNGGYGGYGCNTAYGTNCC-3'	(Tambadou et al., 2014)
MT-R	5'-CCNCGDATYTTNACYTG-3'	(Tambadou et al., 2014)
A3F	5'-GCSTACSYSATSTACACSTCSGG	(Amos et al., 2015)
A7R	5'- SASGTCVCCSGTSCGGTA	(Amos et al., 2015)

3. RESULTS

At the end of the incubation, 70 colonies (33 from soil and 37 from surface samples) growing in R2A and ½ TSA media with different morphological characteristics were isolated. The identified isolates are shown in Figure 2 and Table 2 (supplement). Each amplified fragment of the 16 rRNA gene of each isolate was sequencing, blasted in the NCBI platform. Species in the NCBI platform showing high identity to our isolates were chosen for naming our isolates. When evaluated based on sample diversity, 33% of our isolates were isolated from the soil sample 1S, 24% from the surface sample 1Y, 20% from the soil sample 2S and 23% from the surface sample 2Y. The obtained isolates belong to 11 different genera and 16 different species 75.7 % of the isolates belong to Bacillota (synonym Firmicutes), while 12.9 % and 5.7 % belong to Pseudomonadota (synonym Proteobacteria) and Actinomycetota (synonym Actinobacteria), respectively. The distribution of our isolates was observed with dominance of the strains belonging to *Bacillus* genus (73%) of which the most common species (33%) was observed as *B. pumilus* of which 57% of them were isolated from soil samples (Table 2 (supplement) and Figure 2). The second dominant species is *B. safensis* (19%) of which 62 % of them were observed from the soil samples. It was followed by *Bacillus* sp. (13%) of which 78% were isolated from the soil samples (Table 2 (supplement) and Figure 2). The less common strain of *Bacillus* genus observed in our study is *B. zhangzhouensis* (Figure 2). 8% of isolates belonging to *Bacillus* genus were isolated only from sampling area 2 (-320m) consisting of 1 soil sample and 3 surface samples.

After *Bacillus*, the second most common genus in this study was the *Brevundimonas* with only the species *Brevundimonas vesicularis* was observed (Table 2 in supplement). Most of our isolate belonging to this species are isolated from surface sample Y2. The results presented in Table 2 (supplement) show that these isolates differ from each other in terms of their phenotypic and genotypic characteristics.

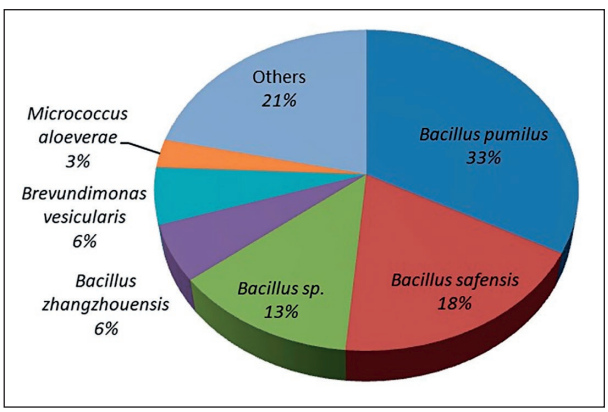

Figure 2: Percentages of culturable heterotrophic aerobic bacteria isolated from the Yarık Sinkhole. The 'others' represent the percentage of non-determined and species represented by less than 2 isolates.

3.1. UREASE ACTIVITY AND CACO3 PRECIPITATION OF CAVE BACTERIA

All isolates except Y112 and Y129 (Table 2 supplement) showed color change of the urea medium from orange to pink color. This observation suggests the production of urease enzyme, which hydrolyzes the urea present in the medium. Under the light microscopy, crystals were observed on all the isolates inoculated on the B4 agar medium (Table 2 supplement). The crystal formation is the result of the precipitation of minerals on the surface of the bacteria. It was observed that 23 %, 6 %, 17 % and 54 % of these isolates formed crystals within 1-3 days, 4-7 days, 8-14 and 15-24 days respectively (Table 2 supplement). Moreover, 75% of the isolates that form crystals in 1-3 days belong to *Bacillus* (Table 2 supplement). The other identified genera observed with crystal formation within 1-3 days are *Sphingomonas, Staphylococcus* and *Stenotrophomonas* (Table 2 supplement). As seen in Table 2 (supplement), the characteristics of the isolates differ although they are the same species. For example, the isolates Y108, Y113, Y124, and other isolates that are identified as *B. pumilus* (Table 2 supplement) shown different features in terms of calcium carbonate precipitation.

3.2. CRYSTAL MORPHOLOGIES AND ELEMENTAL COMPOSITION

Our isolates were observed daily under light microscopy for their potential to induce the formation of crystals on B4 agar medium. Most of the crystals formed are square and rectangular. However, other forms of crystals like spherical, ellipse, hexagonal, and broken rice are also observed in our study (Figure 3). Under the light microscope, crystals formed by most of our isolates (83 %) are away from their colonies (Table 2 in Supplement). No crystal formation was observed on the negative control plates. Crystals observed in some isolates like Y178 (Figure 3 (A)) have regular shape while those that have been observed from isolates like Y 208, Y271 (Figure 3 (B and C)) are of different shapes (spherical, broken rice, rectangular and square shapes).

The crystal formed by three selected isolates (different genera) were purified from the B4 agar medium and were analyzed under electron microscopy for their morphology observation. Spherical and ellipsoidal crystal shapes are noted in (Figures 4, 5, 6). In addition, EDS

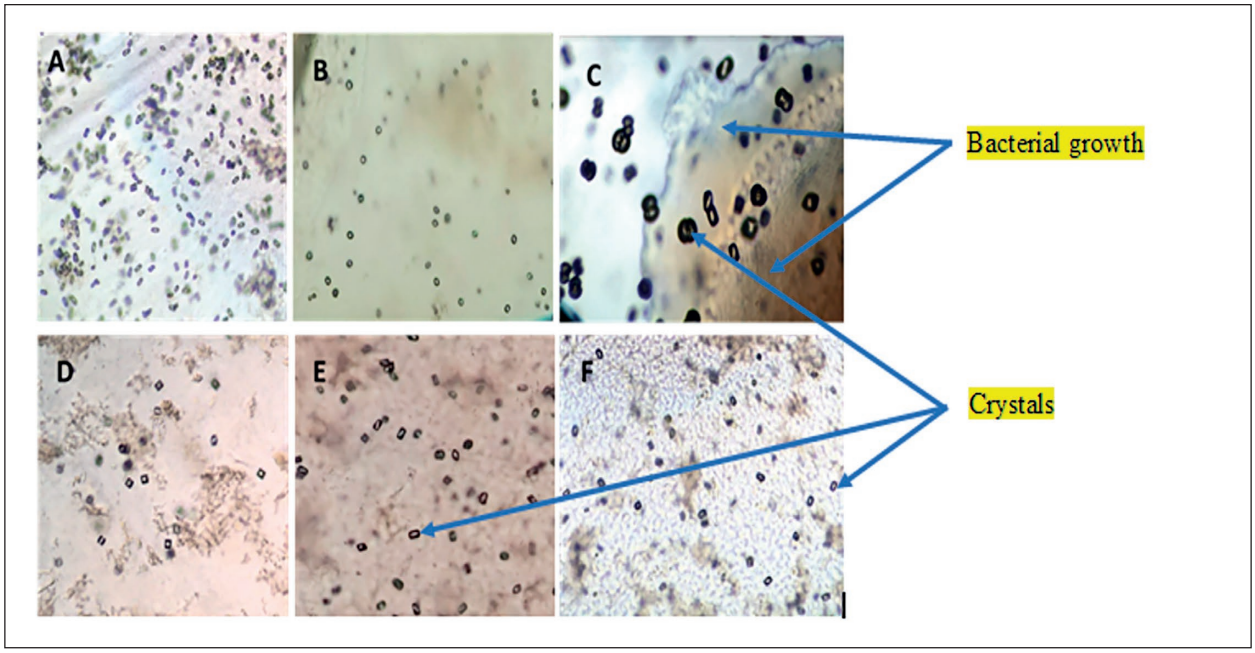

Figure 3: Shapes of crystals produced by different isolates on B4 agar medium under light microscopy (E100- NIKON, ×40 for A, B, D and E; ×100 for C). ***A)*** *Rectangular form of Crystals produced by Sphingomonas mucosissima (Y178) in B4 agar medium.* ***B)*** *Square and spherical forms of crystals produced by Staphylococcus epidermidis (Y208) on B4 agar medium.* ***C)*** *Different shapes of crystals (ice broke, rectangular, irregular) observed on the growth area of Bacillus safensis (Y271) as well as on the other parts of the B4 agar plate.* ***D)*** *Square shape of crystals formed by Brevundimonas vesicularis (Y172) on the B4 agar medium.* ***E)*** *Square and rectangular forms of crystals produced by Bacillus pumilus (Y131) inoculated on B4 agar medium.* ***F)*** *Square and rectangular forms of crystals produced by Micrococcus aloeverae (Y147) inoculated on B4 agar medium.*

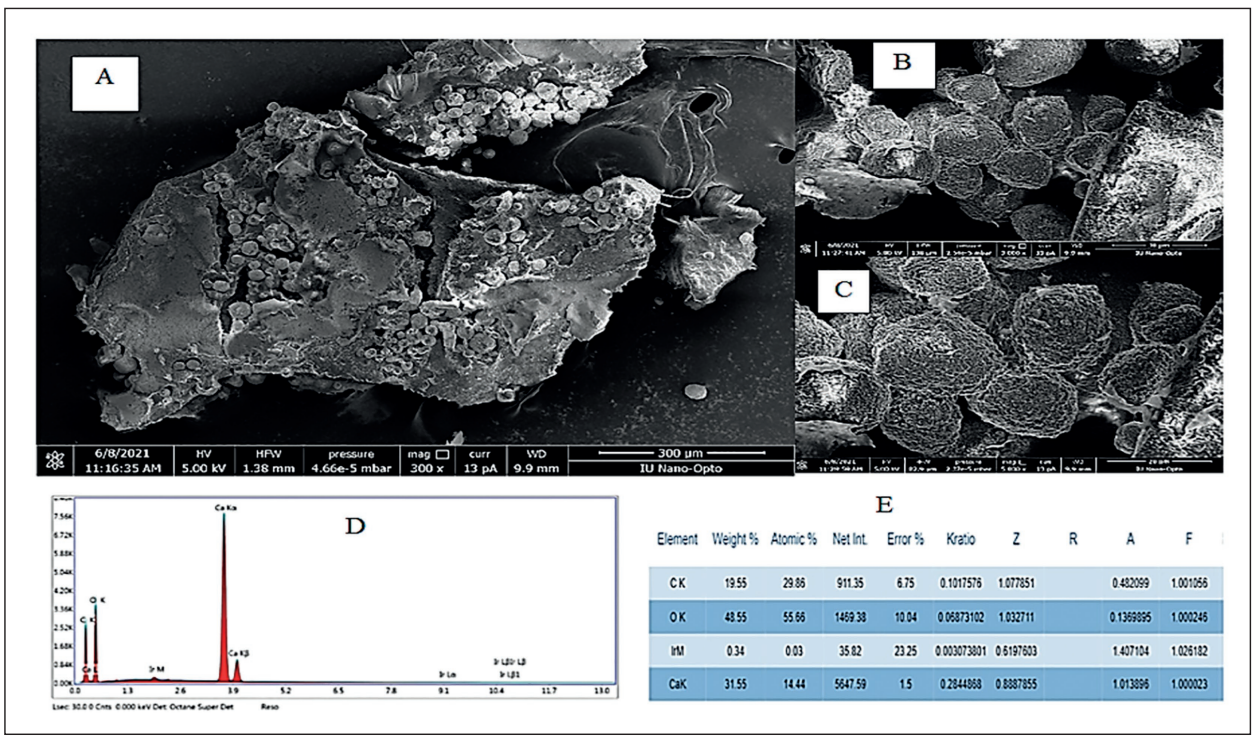

Figure 4. EDS spectrum and SEM analysis images of the calcium carbonate crystals produced by Stenotrophomonas maltophilia (Y116). ***A, B, and C)*** *SEM observation of purified sphere-like shapes crystals formed by the Y116 isolate on the B4 agar medium. D and E) EDS spectrum results show the elemental composition of the calcium carbonate crystals produced by Stenotrophomonas maltophilia (Y116).*

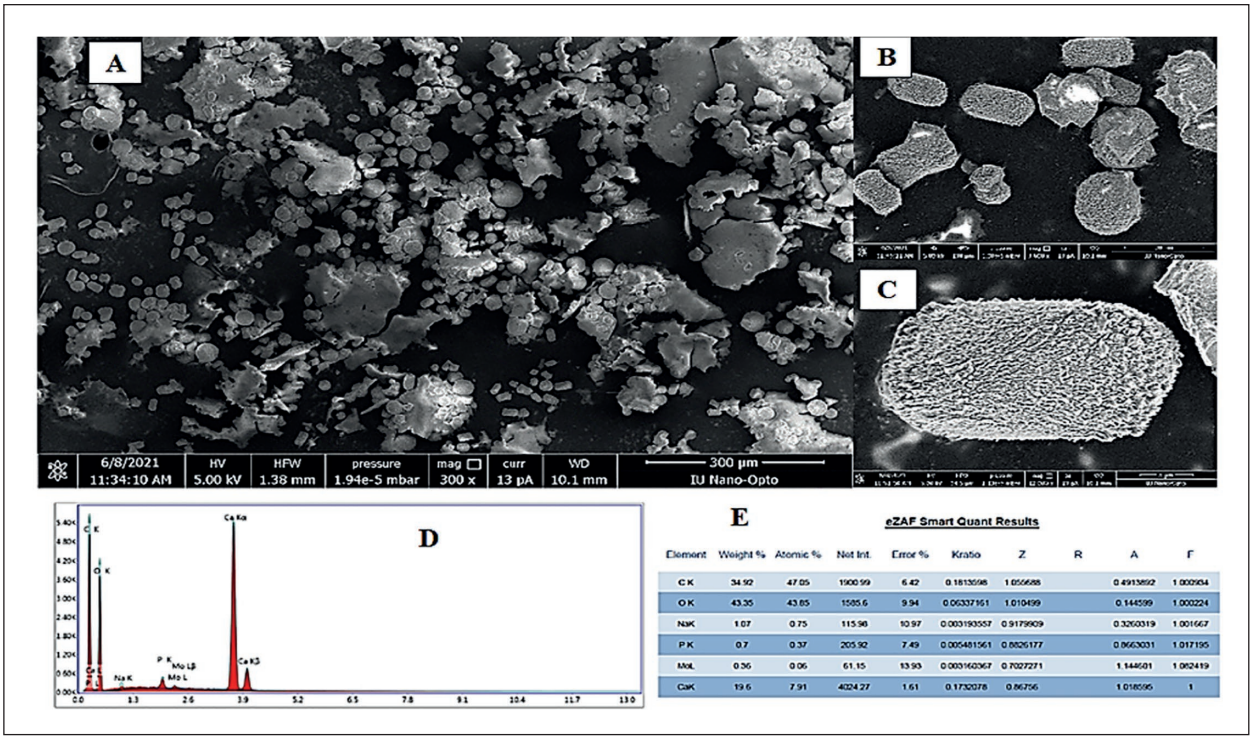

Figure 5: EDS spectrum and SEM images of the calcium crystals produced by Micrococcus luteus (Y154). A and B) SEM observation of purified ellipsoid and spherical-shaped crystals formed by the Y154 isolate on the B4 agar medium. ***C)*** *SEM observation of purified ellipsoid-shaped crystals formed by the same isolate.* ***D and E)*** *EDS spectrum results show the elemental composition of the calcium carbonate crystals produced by Micrococcus luteus (Y154).*

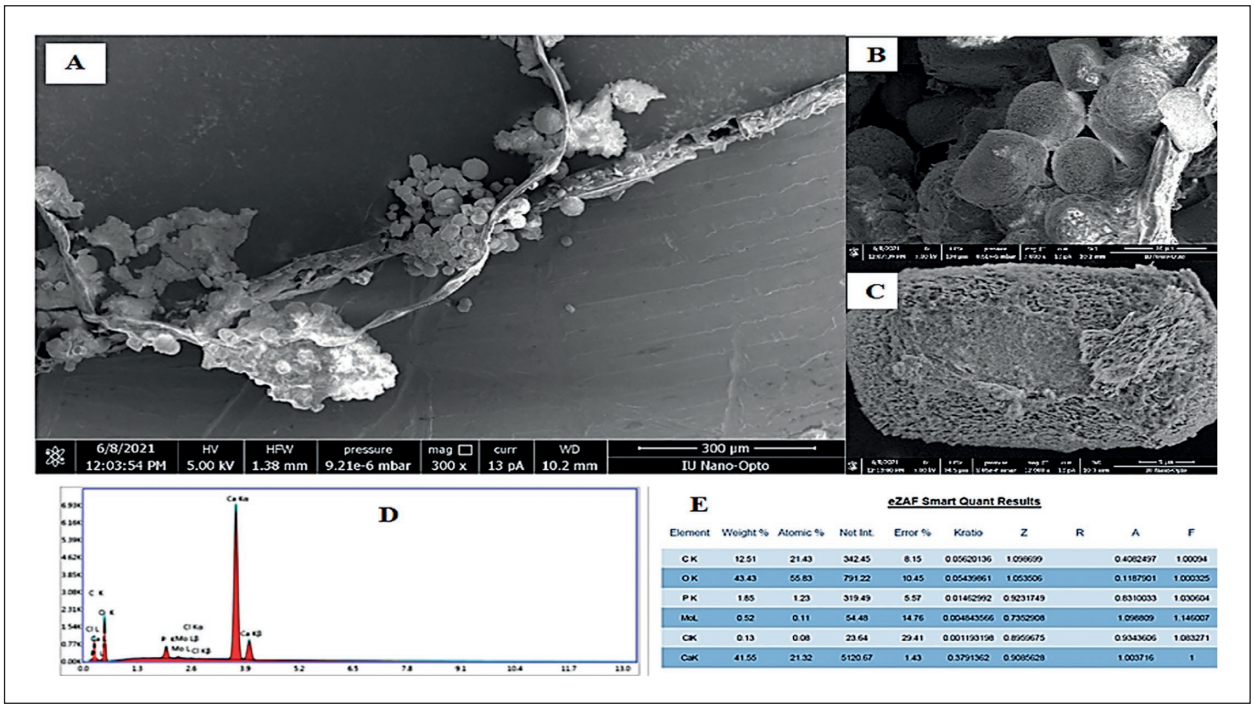

Figure 6 : EDS spectrum and SEM images of the calcium crystals produced by Sphingomonas mucosissima (Y178). A and B) SEM observation of purified ellipsoid and spherical-shaped crystals formed by the Y178 isolate on the B4 agar medium. ***C)*** *SEM observation of purified ellipsoid-shaped crystals formed by the same isolate.* ***D and E)*** *EDS spectrum results show the elemental composition of the calcium carbonate crystals produced by Sphingomonas mucosissima (Y178).*

analyses confirmed that the crystals were composed predominantly of calcium, carbon, and oxygen, suggesting precipitation of calcium carbonate and not of any other compound (Figure 4, 5, 6). The EDS on the isolate Y116 revealed that the atomic percentages of O, C and Ca were 55.66, 29.85, and 14.4%, respectively (Figure 4). When the crystals formed by the Y116 isolate are examined by SEM, it is observed that they have sphere-like shapes with an average length of 30 μm and a diameter of 2412.8 μm² (Figure 4).

The EDS on the isolate Y154 revealed that the atomic percentages of O, C, Ca, Na, Mo, and P were respectively 43.85 %, 47.05 %, 7.91 %, 0.75 %, 0.06 %, and 0.37 % (Figure 5). These results showed that the precipitate is composed of low concentrations of Na, K, Mo and Ca and high concentrations of O and C. Considering the SEM images (Figure 5), the ellipsoid and spherical-shaped crystals formed by Y154 have an average length of 19.10 μm and 21.7 μm, respectively. In addition, the SEM analysis of these crystals showed that the ellipsoid crystals have a surface area of 1074.91 μm² while the spherical-shaped crystals have a surface area of 1478.2 μm²(Figure 5).

According to the EDS results shown in Figure 6, the precipitate formed by *Sphingomonas mucosissima* (Y178) is formed mainly of O, C and Ca with their respective atomic percentages of 55.83 %, 21.43 % and 21.32 %, and a small amount of Mo, K and chlorine. It is seen that the crystals formed by this isolate are also ellipsoid and spherical. According to the SEM images, crystals with ellipsoidal forms have an average length of 37.3 μm and a surface area of 3426.86 μm², while those with spherical shapes have a radius of 20.65 μm and a surface area of 5358.58 μm².

3.3. $MGCO_3$ AND $CACO_3$ DISSOLUTION POTENTIALS OF THE ISOLATES

It was determined that 61 % and 59 % of the total isolates shown clear zones on the Deveze-Bruni's $CaCO_3$ agar and Deveze-Bruni's $MgCO_3$ agar media respectively. These observations show the ability of these isolates to dissolve $MgCO_3$ and $CaCO_3$, respectively. The ratios of bacteria that have $MgCO_3$ and $CaCO_3$ dissolution potential are given in Table 3, according to their isolated areas.

37 % of the 70 strains were observed with a dissolution of both $MgCO_3$ and $CaCO_3$, by showing a transparent zone around their growth, while 17 % did not show clear zones on the two media (Table 2 supplement). Despite the low abundance rate of *Brevundimonas vesicularis* in our study, all of our isolates belonging to this species showed dissolution ability of $MgCO_3$ and 75 % of them dissolved the $CaCO_3$.

In this study, 34 and 29 identified *Bacillus* species were able to dissolve $MgCO_3$ and $CaCO_3$, respectively. In our study, the most dominant isolate *B. pumilus* had $MgCO_3$ and $CaCO_3$ dissolution rates of 45 % and 38 %, respectively, while the $MgCO_3$ and $CaCO_3$ dissolution rates of *B. safensis* were respectively 19 % and 15 % (Table 2 supplement). In addition, the ability of the isolates to dissolve $MgCO_3$ varies according to their isolation depth. Bacteria isolated fron -320 metres showed low $MgCO_3$ dissolution rate than those that are isolated from -80 metres (Table 3).

Table 3: Percentage of isolates able to dissolve carbonate compounds.

Depth	Sample type	$MgCO_3$ (%)	$CaCO_3$ (%)	Both (%)
80 m	Soil	83	91	78
	Surface	76	65	53
320 m	Soil	29	64	7
	Surface	44	69	38

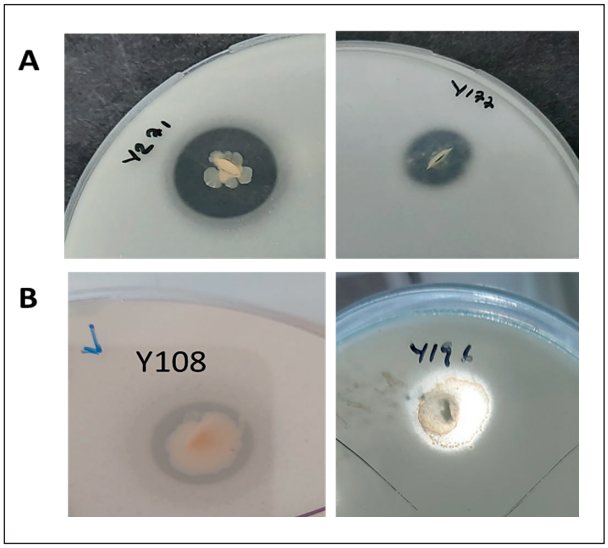

Figure 7: ***A)*** *$CaCO_3$ dissolution potential of isolates: Bacillus safensis (Y271), (Bacillus sp. Y177);* ***B)*** *$MgCO_3$ dissolution potential of isolates: Bacillus pumilus (Y108), Bacillus pumilus (Y196). The clear zones observed around the bacterial growth suggest the dissolution of calcium and magnesium carbonates present in the media.*

3.4. SCREENING OF PKS AND NRPS GENES IN BACTERIAL ISOLATES

In our results, we observed that 64 % of the bacteria screened for their handling of PKS and NRPS gene regions, contain at least one of these gene regions (Table 2 in supplement). We should note that only Y168, Y169, Y184 and Y208 isolates have been observed after electrophoresis with a fragment of 350 base pairs (bp) of the amplified PKS gene fragment. In addition, we observed

amplification of the NRPS gene region in 53.7 % of our isolates. However, most of them are amplified through while the A3R/A7F primer pair amplified fragments of 480 bp observed after electrophoresis. It is interesting to note that the isolate Y184 contains both PKS and NRPS gene amplified fragments (Table 2 supplement). The characteristics of the isolates according to the screened genes are shown in Table 2 (supplement).

4. DISCUSSION

Reducing chemosynthetic materials, such a synthetic cement, hydrocarbons and synthetic fertilizers in the environment may be achieved by replacing them with natural compounds. However, due to the hard laboratory conditions during the discovery and production of such natural compounds from plants or animals, microorganisms become the favorable biological source of most bioactive compounds. In this context, new investigation areas screen for a high probability of new product discovery. One of the least investigated ecosystems in the world is the cave ecosystem. Due to the uncommon conditions that characterize these ecosystems, cave microorganisms, which are considered as successful bioactive compounds producers, are frequently studied to determine their biotechnological and industrial potentials (Hamedi et al., 2019; Teodosiu et al., 2019; Çandıroğlu & Doğruöz Güngör, 2020).

In our study, the most identified isolates belong to Bacillota (synonym Firmicutes) (75.7%), especially the *Bacillus* genus. Similar to these results, the most dominant bacteria isolated from the Kadıini Cave belonged to Bacillota (synonym Firmicutes) (85%) and *Bacillus pumilus* was also the major *Bacillus* species identified in this cave (Doğruöz Güngör et al., 2020). On other hand, Bacillota (synonym Firmicutes) (32.7%), was observed as the second dominant bacterial phylum in the cultured bacteria isolated from Dupnisa Cave (Türkgenci & Doğruöz Güngör, 2021). This bacterial phylum, known for its resistance to stress conditions like desiccation and environmental poor nutrient is frequently identified in extreme environments (Slepecky & Hemphill, 1992). Since each cave is unique in terms of morphological and geochemical features, differences are also shown in its microbial communities. Generally, *Bacillus*, *Streptomcyes*, *Clostridium*, *Kocuria*, *Pseudomonas*, *Microbacterium*, *Paenibacillus*, *Brevibacillus*, *Sphingomonasi,* and *Stahylococcus* are the most identified bacteria in cave environments (Laiz et al., 1999; Canaveras et al., 2001; Ikner et al., 2007; Herzog Velikonja et al., 2014).

In our study, we observed that all selected cave isolates have precipitated calcite on the B4 agar medium on different incubation days. In addition, the EDS spectra (Figures 4, 5, 6) showed Ca, C, and O peaks that could be qualitatively correlated with $CaCO_3$. These results are similar to those observed in the study of Seifan et al. (2016) which determined the EDS spectra of pure calcium carbonate. This result confirms like other studies (Boquet et al., 1973; Cacchio et al., 2003) that many bacteria can form $CaCO_3$ crystals, especially in carbonate-rich environments such as karstic caves. We observe that calcium carbonate precipitation is an important physiological mechanism against bacterial calcium toxicity, which constitutes a vital key, especially for bacteria colonizing high calcium concentrations caves (Banks et al., 2010; Cacchio & Del Gallo, 2019). The key roles of microorganisms in these processes have recently begun to be recognized.

As previously noted, the microbially induced calcium carbonate precipitation (MICP) is achieved through a different pathway. In general, it occurs with the increase in pH of the environment at the end of common metabolic processes of microorganisms such as carbon fixation, ureolysis, denitrification, ammonification, sulfate reduction, and methane oxidation (Fujita et al., 2000; Dupraz et al., 2004; Reeburgh, 2007; Van Paassen et al., 2010). It is also known that microorganisms, apart from these metabolic processes, produce extracellular polymeric substances that bind and condense calcium or other ions (Tourney & Ngwenya 2009; Ercole et al., 2012). Indeed, Ercole et al. (2007) showed that EPS isolated from *B. firmus* and *B. sphaericus* caused calcite precipitation. However, the medium (B4) used for calcium precipitation analysis in our study does not share properties for ureolysis, denitrification, ammonification, sulfate reduction, and methane oxidation. Therefore, it was not possible with our used method to conclude that the isolates are producing calcite precipitation through the EPS production. The results of the urease analysis test performed in our study also showed that the main way taken by most of our isolates for the calcite precipitation is far to be the urea hydrolysis. Lee et al. (2017) suggested that their isolates have used the deamination of the yeast extract present in the medium as a way of the MICP. Even though the B4 medium contains yeast extract which can be the substrate of the deamination, further experimentations will be required to determine the exact metabolic

pathway used by our isolates in the precipitation of the $CaCO_3$. However, the yeast extract in the B4 medium has an important effect as a source of amino acids. In this way, bacteria that can show MICP activity through ureolytic pathway using urea released by arginine decarboxylation can also be monitored in this medium.

Although bacteria using the urea hydrolysis pathway are predominantly selected in microbial calcium carbonate precipitation studies, especially those involved in biotechnology fields, we observed that 96% of our cave isolates precipitate the calcium carbonate without using the ureolytic pathway. In this fact, the use of urease-negative bacteria in these areas should not be ignored. In addition, ureolytic bacteria can cause both environmental and social health problems. Due to the production of ammonia/ammonium and nitrate at the end of the urea hydrolysis, using bacteria which use this way for calcium carbonate production in self-concrete repairing can impact human and animal health (Lee et al., 2017).

The production of self-healing concrete materials is one of the important activities where MICP potential bacteria are used. The most used bacteria in this field are those with positive urease activities. However, these bacteria can also induce the deterioration of the concrete. The ammonium produced as a by-product of urea hydrolysis can act as a mild acid in alkaline environments. It reacts with the hydroxide ions and forms ammonia inside the concrete; the ammonia will be oxidized and produce nitric acid which in turn will react with the precipitated $CaCO_3$ to form a highly soluble component called calcium nitrite. The dissolution of this component may cause the deterioration of the concrete matrix (Ganendra et al., 2014; Farhadi et al., 2020).

Strains of *Bacillus* and *Pseudomonas* were observed through biochemical and biomolecular analyses as the most calcifying strains (Shirakawa et al., 2011; Banerjee & Joshi, 2016). In our previous study, strains belonging to the genus *Bacillus* isolated from Dupnisa Cave were the most calcifying isolates (about 30%) identified in this cave. Rivadeneyra et al. (1994) have previously shown that *Bacillus sp*. plays a major role in the carbonate deposition in natural habitats. Bacteria suggested to be used in the production of bio-concrete (self-healing concrete) should be resistant to harsh conditions such as high pressure during the bio-concrete production. In this context, spore-forming bacteria are the best candidates and most of them belong to the genus *Bacillus* (Reddy et al., 2020). On the other hand, although spore-forming bacteria have shown more advantages, non-spore-forming bacteria have also been shown to crack repairing activities in some previous studies (Bansal et al., 2016; Choi et al., 2021). In our study, both spore-forming and non-spore-forming bacteria have been identified and we suggest that strains like Y108, Y109, Y110, Y122, Y146, Y181, Y193, Y212, and Y213 (Table 2 supplement) which are inducing the $CaCO_3$ precipitation, spore-forming at the same time non-ureolytic bacteria may be good candidates in the production of bio-concrete. In addition, the strains Y116, Y178 which are non-spore-forming bacteria (Table 2 supplement) may have potential uses in the repair of concrete cracks by injecting or spraying them into the opening cracks.

MICP is also considered one of the most effective and efficient methods for the removal of heavy metal pollutants from soil and groundwaters polluted by industrial wastes. Rajasekar et al. (2021) have determined that *B. pumilus*, which precipitates $CaCO_3$, removed high (60-75%) Cr, Zn, and Cu rates from polluted soil. Most of the bacteria used in our study belong to the genus *Bacillus* and precipitates $CaCO_3$. We suggest that they may be easily benefited in these areas. On the other hand, Hammes et al. (2003) used ureolytic bacteria to remove excess calcium from industrial wastes and found that 85-90% of soluble calcium has been precipitated as calcium carbonate in the treatment reactor. Considering this result, they suggested that problems such as clogging of pipes, heat exchangers, and failure of systems caused by the presence of high Ca^{2+} in industrial systems would be prevented (Hammes et al., 2003). Based on our results, we suggest that the $CaCO_3$ precipitating isolates, both ureolytic and non-ureolytic bacteria, may also be used to solve such important problem of pollution

It was determined that the crystal formation period on the B4 medium, of the isolates used in our study differed at the strain level even if the isolates are of the same species. It may be important to pay attention to this parameter in biotechnological or industrial applications of isolates. On the other hand, through the microscopic observation of the isolates inoculated in the B4 medium, it was observed that the precipitation of $CaCO_3$ at 28°C always takes place within and often near the bacterial colony. Meier et al. (2017) suggested that the formation of $CaCO_3$ crystals outside the bacterial colony may be associated with specific proteins. According to our knowledge, the consequence of such crystal formation in the biotechnological uses of these bacteria has not yet been determined. Experimental studies are necessary for more precise conclusions.

The $CaCO_3$ and $MgCO_3$ weathering bacteria of the cave, just like bacteria with $CaCO_3$ precipitation ability, have also a geomicrobiological contribution to the formation of cave structures. Besides, there is an increased potential for using these microorganisms in biotechnological, industrial and environmental fields, which include soil desalination. Soil salinization is the increase of dissolved salts in the salt profile of the soil, and this prob-

lem significantly reduces the crop yield (Parida & Das, 2005). Salt-affected soils contain calcium salts, including $CaCO_3$, $Ca(OH)_2$, $CaSO_4.2H_2O$, and $CaCl_2$. Among the common salts of saline soils, $CaCO_3$ and $MgCO_3$ have the lowest solubility at 0.006 g/l and 0.039 g/l, respectively (Johnson, 2013). Salts with the least solubility have the potential to be used by microorganisms to improve salt-affected soils (Orhan et al., 2017). 37 % of our used isolates showed both $CaCO_3$ and $MgCO_3$ weathering ability (Table 3). These bacteria include species of *Bacillus, Pseudomonas, Microbacterium* and *Serratia* genera. These isolates may constitute good candidates for the improvement of salt-affected soils and soil fertility.

83 % of the *Bacillus pumilus* identified in our study have dissolved the $MgCO_3$ contained in the medium. These species can be good candidates for the dissolution of magnesium ions for soil remediation. On the other hand, *Brevundimonus* species identified in this study have shown high rate of $MgCO_3$ dissolution ability. However, since these species were low identified in this study, it is necessary to test their $MgCO_3$ dissolution ability by multiplying the number of these species in order to determine whether they are good candidates.

NRPS and PKS are two important genes encoding many biologically active secondary metabolites with different biotechnological importance.

These genes were detected in genera like *Bacillus, Peannibacillus,* and *Pseudomonas* from which important bioactive compounds have been secreted (Sukhanova et al., 2018). The NRPS genes in *Bacillus* and *Pseudomonas* have been reported to encode mainly for lipopeptide biosurfactant synthesize. In our study, *B. pumilus*, *B. safensis*, *B. megaterium, B. zhangzhouensis*, *Pseudomonas* species shown either through MT-F/MT-R or A3F/A7R have been successfully screened for the NRPS cluster. Lipopeptide families encoded by the NRPS from species of *Bacillus* including fengycin, pilpastatin, and surfactin have antimicrobial and immunosuppressive functions (Aleti et al., 2015; Sukhanova et al., 2018; Olishevska et al., 2019). In addition, xantholysin, massetolide A, and amphisin are lipopeptides, produced by species of *Pseudomonas*, showing antimicrobial activities against human pathogens and which can be used in biocontrol plant disease (Raaijmakers et al., 2010; Li et al., 2013; Kenawy et al., 2019).

The PKS gene is detected in 5.9 % of our isolates, especially in *Bacillus, Pseudomonas* and *Paenibacillus* genera, by using the degenerate primer DegKS2F/DegKS2R. The presence of this gene cluster in these genera has been observed in previous studies and it was related to the synthesis of a diversity of bioactive compounds. They include the antibiotics bacitracin and bacillaene synthesized by *Bacillus* genus. For those which are produced by *Paenibacillus* species, we can count paenimacrolidin and paenilamicin which have antimicrobial activities against a range of human and plant pathogen bacteria and fungi like MRSA, ampicillin-resistant *Staphylococcus epidermidis, Bacillus* strains and *Saccharomayces cerevisiae* (Olishevska et al., 2019). On other hand, bacteria of the genus *Pseudomonas* are known for their wide synthesized secondary metabolites. Sukhonova et al. (2017) reported that species of *Pseudomonas* produce about 795 secondary metabolites including 610 antibiotics and 185 substances with diverse activities. Some compounds synthesized from the PKS gene clusters of *Pseudomonas* species include mupirocin, pyroles, benzaldehyde, quinolone, and moiramides which are known for their pharmaceutical importance.

In our study, it has also been observed that the bioactive substances production potentials of the bacteria were not related to the isolation of these bacteria from the soil or surface but differ at the sampling points. From this point of view, increasing the sampling points in such studies will increase the chance of obtaining isolates with different characteristics.

5. CONCLUSION

Bacteria used within the scope of this study have been screened for their role in caves and cave structure formations as well as for their potential in biotechnological and industrial areas. Results shown that our isolates have both $CaCO_3$ precipitation and $MgCO_3$ and $CaCO_3$ dissolution abilities. Based on the previous studies in this study field, these observed potentialities suggest that our isolates have a role in the formation and development of the Yarık Sinkhole.

On the other hand, these two main activities (mineral precipitation and dissolution) of our isolates provide ideas of the use of these isolates in diverse fields such as construction and bioremediation. In addition, NRPS and PKS gene clusters have been screened in these isolates and the observed results promise high potential uses of the Yarık Sinkhole's isolates or their products for biotechnological, industrial, and pharmaceutical needs.

Lastly, one of the interesting observations noted in

this study is the crystal formation potential of non-ureolytic bacteria. These bacteria may be suggested to be used alternatively instead of the ureolytic ones due to the environmental and social disadvantages that can be caused by the ureolytic bacteria. Besides, the crystal formation period, under laboratory conditions, as well as the morphologies were diversified even among the isolates of the same species. We suggest that all these observations should be considered in future studies for the selection of the best strains for biotechnological and industrial applications.

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APPENDIX

Table. 2: Biomineralization, dissolution, and some genotypic characteristics of Yarık Sinkhole's isolates.

Isolate code	Sample area	Bacterial division/Accession (Nearest relative)/ % Similarity	$MgCO_3$ dissolution	$CaCO_3$ dissolution	Production of Crystal (Day)	Crystal observed away from colonies	PKS	NRPS	
								MT	A3/ A7
Y108	1S	*Bacillus pumilus* / **JQ773350.1/ 99**	+	-	2	+	-	+	-
Y109	1S	*Bacillus safensis* **MT501806.1/100**	-	-	1	+	-	+	-
Y110	1S	*Bacillus* sp./ **MG553999.1/100**	+	+	2	-	-	+	-
Y112	1S	*Morganella morganii* / **KY938128.1/99**	-	-	24	+	-	+	-
Y113	1S	*Bacillus pumilus* / **JQ773350.1/99**	+	-	17	+	-	+	-
Y116	1S	*Stenotrophomonas maltophilia* / **KF595070.1/99**	+	+	4	+	-	+	-
Y117	1S	Micrococcus aloeverae / **MT733991.1/99**	+	-	16	+	-	-	-
Y118	1S	*Bacillus safensis* / **MT733991.1/99**	+	+	21	+	-	+	-
Y119	1S	*Bacillus* sp. / **KU236475.1/99**	+	-	10	+	-	-	-
Y120	1S	*Bacillus* sp. / **MT598008.1/99**	+	-	15	+	-	+	-
Y121	1S	*Bacillus pumilus* / **HM744710.1/99**	+	-	14	+	-	+	-
Y122	1S	*Bacillus pumilus* / **LT906438.1/99**	+	-	2	+	-	+	-
Y123	1S	*Bacillus simplex* / **MN750767.1/100**	+	-	15	+	-	-	-
Y124	1S	*Bacillus pumilus* / **MN709186.1/95**	+	+	18	+	ND	ND	ND
Y125	1S	*Bacillus pumilus*/ **LT906438.1/100**	+	+	15	+	-	+	-
Y126	1S	*Bacillus megaterium* / **GU122960.1/100**	+	+	9	+	-	+	-
Y129	1S	*Bacillus pumilus* / **LT906438.1/100**	-	+	3	+	-	-	-
Y131	1S	*Bacillus pumilus* / **MN117687.1/99**	+	-	7	+	-	+	-
Y132	1S	*Bacillus safensis* / **MT642941.1/99**	+	-	18	+	-	+	-
Y133	1S	*Bacillus safensis* / **KY820904.1/99**	+	+	18	-	-	+	-
Y136	1S	*Bacillus pumilus* / **JQ773350.1/99**	+	+	8	+	-	-	-
Y138	1S	*Bacillus* sp. / **MG470694.1/99**	+	+	19	+	-	+	-
Y140	1Y	*Bacillus pumilus* / **CP058911.1/99**	+	+	9	+	-	-	-
Y141	1Y	*Bacillus safensis* / **MT642941.1/100**	+	-	18	+	-	+	-

Isolate code	Sample area	Bacterial division/Accession (Nearest relative)/ % Similarity	$MgCO_3$ dissolution	$CaCO_3$ dissolution	Production of Crystal (Day)	Crystal observed away from colonies	PKS	NRPS	
								MT	A3/ A7
Y143	1Y	*Bacillus safensis* / **KR054086.1/99**	+	-	15	+	-	+	-
Y146	1Y	*Bacillus safensis* / **MT501806.1/99**	+	+	1	-	-	-	-
Y147	1Y	*Micrococcus aloeverae* / **MN401101.1/99**	-	-	10	+	-	+	-
Y149	1Y	*Bacillus pumilus* / **KC182057.1/99**	+	-	19	+	-	-	-
Y150	1Y	*Serratia myotis* / **KJ739884.1/99**	+	+	18	-	-	-	-
Y152	1Y	*Microbacterium* sp. / **MG228460.1/99**	+	+	18	+	-	+	-
Y153	1Y	*Bacillus pumilus* / **LT906438.1/99**	+	+	8	+	-	-	-
Y154	1Y	*Micrococcus luteus* / **MH142592.1/98**	-	+	8	+	ND	ND	ND
Y157	1Y	*Bacillus pumilus* / **KC182057.1/99**	+	+	18	+	-	+	-
Y162	2Y	*Bacillus zhangzhouensis* / **MN826587.1/99**	-	+	18	+	-	+	-
Y165	2Y	*Bacillus pumilus* / **MT102721.1/100**	-	+	7	-	-	-	-
Y168	2Y	ND	+	+	1	+	+	-	-
Y169	2Y	*Bacillus pumilus* / **CP054310.1/99**	+	+	15	+	+	-	-
Y171	2Y	ND	-	+	5	+	-	-	+
Y172	2Y	*Brevundimonas vesicularis* / **MH283789.1/99**	+	+	15	+	-	-	-
Y173	2Y	*Brevundimonas vesicularis*/ **KJ540983.1/99**	+	-	18	+	-	+	-
Y175	2Y	*Bacillus zhangzhouensis* / **MT538321.1/100**	-	-	21	+	-	+	-
Y177	2Y	*Bacillus* sp. / **MK491013.1/100**	-	+	3	+	-	-	-
Y178	2Y	*Sphingomonas mucosissima* / **MK415046.1/99**	-	-	2	+	-	-	-
Y179	1S	*Bacillus* sp. / **MF373581.1/100**	-	-	18	-	-	+	-
Y181	1Y	*Bacillus pumilus* / **CP016784.1/99**	+	+	2	-	-	+	-
Y184	2Y	*Pseudomonas* sp. / **MT626781.1/99**	-	-	8	+	+	-	+
Y186	2Y	*Brevundimonas vesicularis*/ **MN932333.1/100**	+	+	18	+	-	-	+
Y187	2Y	*Brevundimonas vesicularis* / **KJ540983.1/99**	+	+	18	+	-	-	-
Y188	2Y	*Bacillus pumilus* / **LT906438.1/99**	+	+	18	-	-	-	-

Isolate code	Sample area	Bacterial division/Accession (Nearest relative)/ % Similarity	$MgCO_3$ dissolution	$CaCO_3$ dissolution	Production of Crystal (Day)	Crystal observed away from colonies	PKS	NRPS	
								MT	A3/ A7
Y189	2Y	*Bacillus zhangzhouensis* / **MN826587.1/100**	-	+	18	-	-	+	-
Y193	2Y	*Bacillus* sp. / **MG470705.1/99**	-	-	1	+	-	-	-
Y196	2S	*Bacillus pumilus* / **KC182057.1/99**	+	+	18	+	-	+	-
Y199	2S	ND	-	+	9	+	-	+	-
Y200	2S	*Bacillus pumilus* / **CP054310.1/99**	+	-	18	+	-	+	-
Y201	2S	*Bacillus safensis* / **MT642941.1/100**	-	+	15	+	-	+	-
Y202	2S	*Bacillus* sp. / **MT808965.1/98**	+	-	17	+	-	-	-
Y205	2S	*Paenibacillus apiarius* / **FR877661.1/100**	-	+	21	+	+	-	-
Y208	2S	*Staphylococcus epidermidis*/ **MT445363.1/99**	-	+	1	+	-	-	-
Y211	2S	*Bacillus pumilus* / **MN181331.1/100**	-	+	18	+	-	-	-
Y212	2S	*Bacillus zhangzhouensis* / **MN826587.1/99**	-	-	1	+	-	-	-
Y213	2S	*Bacillus safensis* / **MT733991.1/99**	-	-	1	+	-	+	-
Y218	1Y	*Bacillus safensis* / **MT501806.1/100**	-	-	14	+	-	-	-
Y222	2S	*Bacillus safensis* / **MT501806.1/100**	-	-	8	+	-	+	+
Y227	1Y	ND	-	+	3	+	-	-	-
Y228	1Y	*Bacillus pumilus* / **LT906438.1/100**	+	+	18	-	-	+	-
Y229	1Y	*Bacillus safensis* / **MT642941.1/100**	+	+	21	-	-	-	-
Y232	1Y	*Bacillus pumilus* / **LT906438.1/99**	+	-	18	+	ND	ND	ND
Y271	2S	*Bacillus safensis* / **MH321592.1/99**	+	+	3	+	-	+	-
Y276	2S	*Bacillus* sp. / **LC488930.1/99**	-	+	18	+	-	+	-
Y281	2S	*Bacillus pumilus* / **MN581193.1/99**	-	+	17	+	-	-	-

S: soil; Y: surface; 1: -80 m, 2:-300 m, ND : Non determined