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Isolation and Molecular Identification of Bacteria from Deteriorated Sandstone Monuments

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A B S T R A C T

Cultural heritage monuments can be compromised as well as obscured by the turn of occasions and activity of living organic entities. Bio-structures framework microbial biofilm on surface areas of sandstone, producing injury to design as well as greatness. Living beings involved with this are bacteria, algae, fungi, as well as lichen. Among all the opportunities of bacteria in the development of all-natural spots and shades, the most essential task of weakening as well as fragmentation of different kinds of sandstone in cultural heritage. In the existing exam eight examples were collected from different destinations which are included sandstone. The 3 biocommunities were divided which have control over sandstone layouts of the monuments. The exam demonstrated that the strain exposed substantial level of 16s comparability with Bacillus cereus (MT994833), Bacillus paralicheniformis (MT994830), Bacillus subtilis subsp. Stercoris (MT994828).

***Key words*:** Cultural heritage, Deterioration, Sandstone, Bacillus sp. bacteria, Algae, Fungi

A number of varieties of macro as well as biocommunities can observed an ideal domain for their growth on sandstone, monuments and also historical remain. The necessary tasks of these living organisms create undesirable alterations in the resources of building stones-this is called as biodeterioration and organisms responsible for this procedure as biodeteriogens. In ecological succession pattern on stone biodeteriogens include Microbes, cyanobacteria, algae, fungi, actinomycetes, lower plants, greater plants and animals. Cyanobacteria are normally there in association with diatoms, eco-friendly algae and red algae. Algae with cyanobacteria can create on monument surface area at really little light. Cyanobacteria and also algae can create biofilm on surface area of the rock and also crust that are dark black shade when they completely dry and also light green color below problems of damp. Other than the clear classy injury on a shallow level, there is a lot of evidence of crucial biophysical and biochemical decay of the material by discharge of sugar inferred carbonic acids and also chelating natural acids that start the penetrating action. Bacteria and also Actinomycetes result in biochemical degeneration by developing not natural acids like H_2So_4 and also HNO_3. The acids trigger dissolution of stone and also causes regards to crusts of insoluble and also soluble salts and also powdering. Consequently, the scientist recently explained that there is not a close relationship in between high variety of biocommunities and also biodeterioration capacity, as a result of commonly biocommunities' adaptation to live on the rock has an extreme sluggish development pattern as well as not easily to detectable with typical laboratory procedure.

Microorganisms have lot of domain of prokaryotic biocommunities. Microbes are a lot of in shapes, reaching from balls to spirals as well as poles, they are typically a few μm in size. Microbes were the 1st life kind showed up on Earth as well as they are located primarily in its habitats. Microorganisms can reside in all sphere of life like water, dirt, waste located from radioactive product. Microbes are responsible for the decomposition of raw materials specially the normal anaerobic Microbes in the nutrient cycle. In the biocommunities enclosed cool seeps which is an area of ocean flooring where H_2S (Hydrogen sulphide), CH_4 (methane) some other hydrocarbon which liquid discharge happens in Salt Lake and hydrothermal vents which is a splits on the sea flooring which geothermally water problem, extremophile mycoflora provide the nutrients required to sustain life. Microorganisms are able to identify on the basis of O_2 need for their expanding. High or reduced O_2 web content needed for expanding of anaerobic microorganisms as well as are more versatile microbes. Conversely, anaerobic

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microorganisms can develop specifically in atmosphere where negligible or no O_2 existing.

Bacteroides are the examples of anaerobes and exist in large intestinal tract. Pseudomonas aeruginosa, a microorganism, is an example of a stringent aerobe. Microaerophilic bacteria create in scenarios of minimized O_2 and also in some cases also require large quantity of carbon dioxide level. *Neisseria sp.* are e.g., of micraerophilic microorganisms. For that reason, the researcher just recently explained that there is not a close relationship between high variety of biocommunities and also biodeterioration potential, because of commonly biocommunities adaptation to survive the rock has a severe slow-moving development pattern and also not conveniently to obvious with normal research laboratory treatment.

The Wear and tear of all the sandstones normally consists of the separation of different layers leading, at times, to splitting of stone pitting and also powdering of the surface In sandstone with calcite and dolomite as cementing material though has great sealing buildings, is conveniently struck by carbon -di-oxide and also other acidic gases existing airborne or dissolved in the water The dissolution of a fairly small amount of cementing product loosens up a fairly a great deal of quartz grains and therefore they are weathered quickly. In a similar way the sandstone that have clay as binding medium are the poorest when it comes to resistance to weathering. Because clay has no good cementing properties as well as when the rock is wetted by rainfall or wetness [5] Sandstone surfaces are over and over presented to natural, artificial and actual debasement. Various specialists consisting of material, physical as well as organic specialists operate in co-affiliation and going from collaborating to aggressive, triggering the oddball. The growth of transmittable on object of social legacy most of the time causes a significant aesthetical wrecking due to colonization and pigmentation on parasitical. Besides organisms' damages sandstones and also along these lines impact object significantly. The chemical corruption of all-natural reason loss of paint layers and even reduce. Microorganisms move and enter under layers of paint appropriately separation [9] The biocommunities in the museum is particularly impacted by atmospheres carrying mineral carbonate, and also others [1]. The objective of this research is to impact of the biocommunities on sandstone monuments by using microscopic and also molecular method.

MATERIALS AND METHODS

The current paper takes care of various methods made use of in examination of biodeterioration of sandstone monolith. The techniques consist of the bacterial recognition.

Sampling

Trial of sandstone are accumulated from eleven domains: Red Fort, Akbar Tomb, Fatehpur Sikri, Mariam Tomb, Etma Ud Daula, St. Johns, Kailash Temple, 64 Khamba, Ochha Temple, Khas Mahal, Fatehpur Sikri and some unidentified tourist spots. Under the discernment evident corruption and change were arranged and after that the models are accumulated. Sandstone test from different objections use for mycological concentrate by cleaning surfaces with sterile q-tips. The sandstone tests are taken care of at 4°C.

Isolation of microflora

The examples were assembled with the help of purified contraptions (brushes, careful instruments, cellophane tape and swab) and directed at 4◦C until the hour of examination in the lab. In the current assessment bound was performed clearly from the milestones and from accumulated debilitating sandstone tests.

Historical monument

(i) *Scrapping method*: The area showed plain and passionate shaded game spread on the sandstone surface area. These designs were drawn from the rock surface using a clean cautious instrument and also lancet and also the surface area product was scraped to a relevance of 1-- 3 mm, and a short time later transported to the exploration area in clean vials.

(ii) *Cellophane tape method*: Tests of bacterial development straightforwardly from the influenced sandstone divider utilizing tacky tape. The tape in a split second eliminates powdered stone with microflora fruiting bodies. Along these lines, direct distinguishing proof of the bacterial turns out to be simple. These examples were refined in the research facility for additional assessment with the assistance of a magnifying lens.

(iii) Swabbing and serial dilution method: In this system the outside of disintegrated sandstone test was cleaned by purified saturated cotton and shaken in 10 ml of sterilized refined water. Sequential weakenings 10-2, 10-3… … 10-7 were made by pipetting assessed volumes (1ml) into additional debilitating spaces (having 9ml sterile water). Finally, 1 ml aliquots of various debilitating were added 20 ml of the perfect, cool fluid (45°C) media (Nutrient agar for microorganisms). The weakening 10-4 to 10-7 for microscopic organisms. Upon hardening, the plate were hatched 35±1°C (for microscopic organisms) and 24 - 72 hours individually. The convenient methods utilized for microbes and parasites were pertinent to tiny green growth as well. Just with the qualification bring forth conditions, 30-35◦C temperature, light of 60W tungustun, 15-20 days and filled in Beneck's stock priegsheim and changed Knop's stock.

Molecular and morphological identification of bacteria: DNA confinement, PCR using comprehensive presentations for amplicons, cycle sequencing reactions, cleansing and run them on a mechanized restricted based Sanger DNA Sequencing system. At every movement, there is in-house quality check to ensure accomplishment of the sequencing reactions. Post sequencing, areas are truly checked and simply extraordinary quality game plans are used to shape contigs, which are then organized in well-curated informational indexes for designating closest neighbor as the temporary distinguishing proof of life form.

RESULTS AND DISCUSSION

Microbial assortment of stones is relies upon the ecological just as climatic elements, for example, accessibility of water, pH, climate, petrologic boundaries, and on wellspring of supplements, for example, porousness, porosity and mineral convergence of the material [3]. Be that as it may, these cycles cause irreversible harm to old

stone monuments of social and chronicled importance. The microflora at external surfaces of stone means a mind-boggling biological system with not just green growth, lichens, parasites and microbes just as protozoa. Despite the fact that gathered from locales with comparative indications of microbial colonization and biodeterioration, the microbial states in this investigation shifted immensely in piece on account of contrasting discovery measures and natural conditions. By and large, culture-autonomous strategies are viewed as extra educational just as advantageous than culture-subordinate techniques, which just give us the discovery of 1–5% of the whole microbial local community [6]. Some analyst expressed that the plating brings about an overestimated number of microscopic organisms which are spore-framing in contrast with detached vegetative express these are less and handily refined, yet they are essentially recognize by culture-free techniques [2]. These restrictions of microorganism rely upon natural states of the zone, which changes from geological territory to region [8]. During screening for search of biocommuities, all out 3 types of Microorganisms were segregated from various sandstone monuments. In these monuments Bacillus shows maximum frequency Other researcher reported bacterial communities consisted of *Acaryochloris*, *Chroococcidiopsis*, *Coccomyxa*, *Flavisolibacter*, *Hymenobacter*, *Leptolyngbya*, *Lutibacterium*, *Lysobacter*, *Methylobacterium*, *Modestobacter*, *Planctomyces*, *Pseudonocardia*, *Pseudozobellia*, *Rhodocytophaga*, *Roseomonas*, *Rubrobacter*, *Rudanella*, *Scytonema*, *Sphingomonas*, *Spirosoma*, *Truepera*, Unclassified, and Others (<0.5%)[7]. Some examination shows that the microbial settlements distinguished in the biofilm on sandstone surface in jungle Asia included parasites, green growth, protozoa, cyanobacteria and archaea [6].

Molecular identification of the isolated bacteria

Eight bacteria isolates were distinguish based on their sub-atomic qualities. The 16s rRNA quality has been chosen as an objective for PCR enhancement as an extensive sub-atomic investigation of succession information to remake the transformative history of biocommunities is utilized to. In the phylogenetic tree, 16srRNA succession investigation uncovered that the isolates are close relative of *Bacillus cereus* ATCC (gene bank accession no. MT994826) with 99.4% comparability, next bactearial strain indicated undeniable degree of similitude (100%) with close neighbor of *Bacillus paralicheniformis* KJ-16 (T) (gene bank accession no. MT994827).

Table 1 Identification of bacteria

PRN	Strain No.	Closest Neighbor	Accession No.	Percent similarity
A_FEB_20_215	Un. I	*Bacillus cereus*	MT994826	99.74
A_FEB_20_216	M.T. II	*Bacillus paralicheniformis*	MT994827	100.00
A_FEB_20_217	64K. III	*Bacillus subtilissubsp. Stercoris*	MT994828	100.00
A_FEB_20_218	F.S. IV	*Baclus cereus*	MT994829	100.00
A_FEB_20_219	Sikandra V	*Bacillus paralicheniformis*	MT994830	100.00
A_FEB_20_220	G.D. VI	*Bacillus cereus*	MT994831	100.00
A_FEB_20_221	E. VII	*Bacillus cereus*	MT994832	100.00
A_FEB_20_222	A.T. VIII	*Bacillus cereus*	MT994833	100.00

Another strain similar (100%) with *Bacillus subtilis sub sp*. *Stercoris* D7XPN1 (T) isolates (gene bank accession MT994828). Next strain is 100% similarity with *Bacillus cereus* ATCC (gene bank accession no. MT994829). Other bacterial strain indicated undeniable degree of closeness (100%) with close neighbor of *Bacillus paralicheniformis* KJ-16(T) (gene bank accession no. MT994830). Next three strain close comparative with *Bacillus cereus* ATCC with 100% closeness (Table 1). The (Table 2) shows the aftereffects of the entire genome ID of isolates of bacterial separates got from the sandstone landmarks.

Table 2 Nucleotide sequence of 16S rRNA gene of bacteria

Bacteria name	Sequence
Bacillus cereus ATCC14579 (T)	TCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAATGGATT AAGAGCTTGCTCTTATGAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCC ATAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATAACATTTTGAACCGCA TGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAG CTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGA TCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAAT CTTCCGCAATGGACGAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGG TCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACG GTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGTGG CAAGCGTTATCCGGATTATTGGGCGTAAGCGCGCGCAGGTGGTTCTTAGTCTGATGTGAA AGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGG AAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGC GAAGGCGACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAG GATTAGATACCCTG
Bacillus paralicheniformis KJ-16 (T)	GCTGCAGCACTAAAGGGCGGAAACCCTCTAACACTTAGCACTCATCGTTTACGGCGTGGA CTACCAGGGTATCTAATCCTGTTCGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTTACAGA CCAGAGAGTCGCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTACACG TGGAATTCCACTCTCCTCTTCTGCACTCAAGTTCCCCAGTTTCCAATGACCCTCCCCGGTT GAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCTGCGCGCGCTTTACGCCCAATAA

	TTCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGG CTTTCTGGTTAGGTACCGTCAAGGTACCGCCCTATTCGAACGGTACTTGTTCTTCCCTAAC AACAGAGTTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTT CGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTC CCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTAC CTCACCAACTAGCTAATGCGCCGCGGGTCCATCTGTAAGTGGTAGCTAAAAGCCACCTTT TATAATTGAACCATGCGGTTCAATCAAGCATCCGGTATTAGCCCCGGTTTCCCGGAGTTA TCCCAGTCTTACAGGCAGGTTACCCACGTGTTACTCACCCGTCCGCCGCTAACATCAGGG AGCAAGCTCCCAT
Bacillus subtilis subsp. Stercoris D7XPN1 (T)	CCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGGACAGATG GGAGCTTGCTCCCTGATGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGT AAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATGCTTGTTTGAACCGCATG GTTCAAACATAAAAGGTGGCTTCGGCTACCACTTACAGATGGACCCGCGGCGCATTAGCT AGTTGGTGAGGTAATGGCTCACCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTGATC GGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCT TCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGAT CGTAAAGCTCTGTTGTTAGGGAAGAACAAGTACCGTTCGAATAGGGCGGTACCTTGACGG TACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGG CAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATG TGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAA GAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAG TGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGA ACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTG
Bacillus cereus ATCC 14579 (T)	TGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGTGTCAGTTA CAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTACGCATTTCACCGCTA CACATGGAATTCCACTTTCCTCTTCTGCACTCAAGTCTCCCAGTTTCCAATGACCCTCCAC GGTTGAGCCGTGGGCTTTCACATCAGACTTAAGAAACCACCTGCGCGCGCTTTACGCCCA ATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCC GTGGCTTTCTGGTTAGGTACCGTCAAGGTGCCAGCTTATTCAACTAGCACTTGTTCTTCCC TAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGA CTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTC AGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCG TTACCTCACCAACTAGCTAATGCGACGCGGGTCCATCCATAAGTGACAGCCGAAGCCGCC TTTCAATTTCGAACCATGCGGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGT TATCCCAGTCTTATGGGCAGGTTACCCACGTGTTACTCACCCGTCCGCCGCTAACTTCATA AGAGCAAGCTCTTAATCCATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCA TCCTGAGCCAGG
Bacillus paralicheniformis KJ-16 (T)	CGTGGACTACCAGGGTATCTAATCCTGTTCGCTCCCCACGCTTTCGCGCCTCAGCGTCAGT TACAGACCAGAGAGTCGCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTCACCGC TACACGTGGAATTCCACTCTCCTCTTCTGCACTCAAGTTCCCCAGTTTCCAATGACCCTCC CCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCTGCGCGCGCTTTACGCC CAATAATTCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAG CCGTGGCTTTCTGGTTAGGTACCGTCAAGGTACCGCCCTATTCGAACGGTACTTGTTCTTC CCTAACAACAGAGTTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCA GACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTC TCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGC CGTTACCTCACCAACTAGCTAATGCGCCGCGGGTCCATCTGTAAGTGGTAGCTAAAAGCC ACCTTTTATAATTGAACCATGCGGTTCAATCAAGCATCCGGTATTAGCCCCGGTTTCCCGG AGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTACTCACCCGTCCGCCGCTAACATC AGGGAGCAAGCTCCCATCTGTCCGCTCGACTTGCATGTATTAGCACGCCGCCAGCGTTCG TCCTGAGCCAGA
Bacillus cereus ATCC 14579 (T)	TATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGT CGCCTTCGCCACTGGTGTTCCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCC ACTTTCCTCTTCTGCACTCAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGG GCTTTCACATCAGACTTAAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATA ACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTT AGGTACCGTCAAGGTGCCAGCTTATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTT TACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCG GAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGC CGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTA GCTAATGCGACGCGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACC

	ATGCGGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATG GGCAGGTTACCCACGTGTTACTCACCCGTCCGCCGCTAACTTCATAAGAGCAAGCTCTTA ATCCATTCGCTCGACTTGCATGTATTA
Bacillus cereus ATCC 14579 (T)	ATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTC GCCTTCGCCACTGGTGTTCCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCA CTTTCCTCTTCTGCACTCAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGG CTTTCACATCAGACTTAAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAA CGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTA GGTACCGTCAAGGTGCCAGCTTATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTT ACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCG GAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGC CGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTA GCTAATGCGACGCGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACC ATGCGGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATG GGCAGGTTACCCACGTGTTACTCACCCGTCCGCCGCTAACTTCATAAGAGCAAGCTCTTA ATCCATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATCCTGA
Bacillus cereus ATCC 14579 (T)	TCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAATGGATT AAGAGCTTGCTCTTATGAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCC ATAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATAACATTTTGAACCGCA TGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAG CTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGA TCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAAT CTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGG GTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGAC GGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGT GGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGA TGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAG AAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACC AGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTG

CONCLUSION

The taxonomy and diversity of *Bacillus* have been primarily studied using traditional morphological methods and 16rRNA sequences. These studies identified that the type of bacteria that deteriorate sandstone monuments a highly reliable indicator about the evolutionary relationships among these species. Currently, available genomic sequences of *Bacillus* have made it possible for the evolutionary study at genomic level. Identified connections provide proof to the complicated relationships amid the organisms that develop explained biocenoses, however, the functional evaluation of the procedures occurring on the surface of the historical sandstone in various seasons needs further study. In the following phase we want to validate, which metabolic pathways control in an offered season. Bacterial species were segregated from different sandstone landmarks. The segregated species initially recognized morphologically and a short time later attempted *Bacillus paralicheniformis* KJ-16(T), *Bacillus cereus* ATCC 14579(T), *Bacillus subtilis subsp. Stercoris* D7XPN1 (T) were perceived genetically by sequencing ~700 base pair.

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