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Türk Hijyen ve Deneysel Biyoloji Dergisi

Determination of diversity of actinomycete in forest soil subject to different blood groups; Classical approaches before metagenomic

Farklı kan gruplarına bağlı orman topraklarında aktinomiset çeşitliliğinin belirlenmesi; Metagenomik öncesi klasik yaklaşımlar

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ABSTRACT

Objective: Forensic microbiology is the developing field of forensic science. Forensic microbiologist can use trace evidence to match people to crime scenes, to investigate bioterrorism incidents, and to determine cause and time of death. In recent years, important studies on the environmental microbiota have been carried out. These studies can be associated with many legally valuable data as well as being related to the microbiota of environmental events. Actinobacteria are gram-positive micelle bacteria with enormous diversity, common in soil, and have high economic value. So far 47 families and 711 species of Actinobacteria were isolated and identified in variety of habitats. Increasing forensic studies in recent years show that microbial profiles can be used as evidence.

Methods: In this study, actinobacteria were isolated from forest soil samples exposed to eight different blood groups for two weeks and control group. Genomic DNA isolation from colonies selected by considering

ÖZET

Amaç: Adli mikrobiyoloji, adli bilimin gelişen alanıdır. Adli mikrobiyolog, insanları suç mahalli ile eşleştirmek, biyoterörizm olaylarını araştırmak ve ölüm nedenini ve zamanını belirlemek için iz kanıtları kullanabilir. Son yıllarda çevresel mikrobiyota ile ilgili önemli çalışmalar yapılmaktadır. Bu çalışmalar, çevresel olayların mikrobiyotası ile ilgili olabileceği gibi, yasal olarak değerli birçok veri ile ilişkilendirilebilir. Aktinobakteriler, toprakta yaygın olarak bulunan ve ekonomik değeri yüksek, muazzam çeşitliliğe sahip gram pozitif miselli bakterilerdir. Şimdiye kadar çeşitli habitatlarda 47 familya ve 711 Actinobacteria türü izole edilmiş ve tanımlanmıştır. Son yıllarda artan adli tıp çalışmaları mikrobiyal profillerin kanıt olarak kullanılabileceğini göstermektedir.

Yöntem: Bu çalışmada, sekiz farklı kan grubuna iki hafta süreyle maruz bırakılan orman toprağı örneklerinden ve kontrol grubundan Aktinobakteriler izole edilmiştir. Karşılaştırmalı koloni morfolojisi dikkate

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comparative colony morphology, PCR amplification targetted 16S rRNA and sequence analysis were carried.

Results: A total of five Actinobacteria type bacteria (three *Micromonospora* sp., one is *Streptomyces* sp., and one *Actinomadura* sp.) were obtained from the soil samples mixed with the B Rh (+) blood group in the 7th day. No Actinobacteria growth was observed neither in the control group nor in the soils to which other blood groups were added. According to the *p-distance* values of all isolates, they were determined as subspecies rather than a new species.

Conclusion: This is a preliminary study to identify bacterial communities that may be present and differ in soil exposed to blood. The inability to isolate Actinobacteria from most of the soil samples may be due to the chemical or enzymatical properties of the bloods that can degrade the bacterial spores. In the further studies, different media and genomic techniques should be tried in different types of soils.

Key Words: Actinomycete, forensic microbiology, soil microbiome

alınarak seçilen kolonilerden genomik DNA izolasyonu, 16S rRNA hedefli PCR amplifikasyonu ve dizi analizi gerçekleştirilmiştir.

Bulgular: 7. günde B Rh (+) kan grubu ile karıştırılan toprak örneklerinden toplam beş Actinobacteria türü bakteri (üç *Micromonospora* sp., bir *Streptomyces* sp. ve bir *Actinomadura* sp.) elde edildi. Ne kontrol grubunda ne de diğer kan gruplarının eklendiği topraklarda Actinobacteria üremesi gözlenmedi. Tüm izolatların *p-distance* değerlerine göre yeni bir tür olmadığı ancak var olan türlere ait alt tür oldukları belirlenmiştir.

Sonuç: Bu çalışma, kana maruz kalan toprakta bulunabilecek ve farklılık gösterebilecek bakteri topluluklarını belirlemeye yönelik bir ön çalışmadır. Aktinobakterilerin toprak örneklerinin çoğundan izole edilememesi, bakteri sporlarını bozabilen kanların kimyasal veya enzimatik özelliklerinden kaynaklanabilir. Bundan sonraki çalışmalarda, farklı toprak türlerinde farklı ortam ve genomik teknikler denenmelidir.

Anahtar Kelimeler: Actinomycete, adli mikrobiyoloji, toprak mikrobiyomu

INTRODUCTION

Microorganisms are abundant in and on the human body. It is estimated that microbial cells outnumber the total number of human somatic cells in crime-related environments and on objects (1,2). On the other hand, using microbiology in forensic science is quite new (3). In the early 1990s, amplified viral DNA sequencing was used to support a case from a dentist in Florida, United States, who claimed that several patients were infected with Human Immunodeficiency (HIV) Virus (4).

Forensic microbiology could be used for several purposes such as geolocation and surface analysis, identification, biological sex determination, trace proof, type and cause of death and (thanatomicrobiome) postmortem (5). Increasingly widespread forensic studies show that relevant microbial profiles can be used as evidence or complement traditional research methods at least (6, 7). Although using of microbial profiles as evidence via advanced computational tools, bioinformatics, processing tools and traditional protocols, innovative approaches are needed in this emerging field (5).

In recent years, the analysis of soil microbial communities has been considered potentially useful in forensics because of its ability to discriminate at fine-scale resolution. Since the structure of the microbial community has been determined by various factors such as soil type, seasonal variation, site management, vegetation, and environmental conditions; bacterial and fungal communities in a particular region have a highly specific profile (8). Recent advances in Mass Parallel Sequencing (MPS)

can exhibit more comprehensive view of the microbial communities in a soil sample, via generating thousands of sequences reads per sample. It is possible to gain to 8.3 million unique sequences from one gram of soil depending on the type of the ground (9).

Actinomycetes are gram-positive bacteria commonly founding in many ecosystems, primarily soil. Although the diversity of Actinomycetes in the soil habitat varies according to the location, organic substances in the soil, soil cultivation and climatic factors, some researchers have stated that it is mostly found in dry alkaline soil (10).

Forensic microbiologists can use trace evidence to match people to crime scenes, to investigate incidents of bioterrorism, and to determine cause and time of death. There are different methods for studies on biodiversity determination from a soil ecosystem such as the use of metabarcoding technologies and *in vitro* bacterial biodiversity determination methods. It is thought that this phylogenetic study can be an important reference in interpreting the event in the crime scene investigation or biodiversity determination of a possible blood-exposed region in the future.

In this preliminary study for the phylogenetic evaluation of forensic sciences, bacterial isolation using selective media and phylogenetic analyses based on 16S rRNA gene region were performed and the effect of different blood groups on Actinomycete biodiversity in the soil ecosystem was examined.

MATERIAL and METHOD

Field Studies

Untreated soil far from the closest settlement was selected as study area (40°34'14.2"N, 34°59'05.0"E) and 8 units of different blood groups [A Rh (+), A Rh (-), B Rh (+), B Rh (-), AB Rh (+), AB Rh (-), 0 Rh (+), and 0 Rh (-)], obtained from the Hitit University Erol Olçok Training and Research Hospital Blood Center, were poured into the designated area separately (Figure 1) to determine a possible bacterial change depending on blood group. Soil without blood treatment was used as the control group (The graphic about the weather conditions in Supplementary File 1).

The first blood treatment to the soil was carried out in November-December 2020 when the temperature change is limited, and the weather is stable and cold in order to isolate the soil bacteria in the dormant form. Then, soil samples were collected on the 1st, 7th and 15th days and stored in polyethylene ziplock bags in the refrigerator at +4°C until the bacterial isolation process.

Figure 1. Under forest soil treated with blood, Çorum, 2021.

Establishment of Studying Groups

Soil samples treated with eight different blood groups and control groups, collecting in three different periods were grouped as in Table 1.

Bacteria Isolation from Soil

The soil samples of 1 g stored at +4°C was weighed and put into 15 ml tubes containing beads, and 9 ml of sterile Ringer's solution was added. The 10^{-1} soil solutions were vortexed for 30 minutes to separate the micelles and spores of the microorganisms attached to the soil colloids. Then, it was kept in a water bath at 55°C for 30 minutes to remove gram-negative bacteria and other microorganisms. It was homogenized by vortexing again. After incubation, 10-1 homogeneous solutions were diluted by serial dilution technique and 10^{-2} and 10^{-3} solutions were prepared (11).

Selection, Purification and Storage of Isolates

Actinomycete and rare Actinomycete isolates were selected by considering the comparative

Table 1. The name and the collecting date of the soil samples treated with eight different blood groups and control groups, Çorum, 2021

	Groups	Collecting Date	Name
I. Period	A Rh (+)	03.11.2020	AP1
	A Rh (-)	03.11.2020	AN1
	B Rh (+)	03.11.2020	BP1
	B Rh (-)	03.11.2020	BN1
	AB Rh (+)	03.11.2020	ABP1
	AB Rh (-)	03.11.2020	ABN1
	0 Rh (+)	03.11.2020	OP1
	0 Rh (-)	03.11.2020	ON1
	Control 1st day	03.11.2020	K1
II. Period	A Rh (+)	10.11.2020	AP2
	A Rh (-)	10.11.2020	AN2
	B Rh (+)	10.11.2020	BP2
	B Rh (-)	10.11.2020	BN2
	AB Rh (+)	10.11.2020	ABP2
	AB Rh (-)	10.11.2020	ABN2
	0 Rh (+)	10.11.2020	OP2
	0 Rh (-)	10.11.2020	ON2
	Control 7th day	10.11.2020	K2
III. Period	A Rh (+)	17.11.2020	AP3
	A Rh (-)	17.11.2020	AN3
	B Rh (+)	17.11.2020	BP3
	B Rh (-)	17.11.2020	BN3
	AB Rh (+)	17.11.2020	ABP3
	AB Rh (-)	17.11.2020	ABN3
	0 Rh (+)	17.11.2020	OP3
	0 Rh (-)	17.11.2020	ON3
	Control 15th day	17.11.2020	K3

colony morphology in petri dishes incubated on GYME agar at 28°C for 14 days. Selected colonies were inoculated with sterile loop and streaked on GYME agar supplemented with cycloheximide. Petri dishes were incubated at 28°C for 14 days. The spores and mycelium of the pure isolates were transferred to screw cap tubes containing 35% sterile glycerol under aseptic conditions and stored at -20°C.

Genomic DNA Isolation, PCR amplification and Sequence Analysis

Genomic DNA of the isolates was obtained using the CTAB protocol (12). For 16S rRNA PCR amplification and sequence analysis, PCR was performed on the Biorad 100X device using the universal primers 27F and 1525R of the 16S rRNA gene region. The reaction conditions are given in Table 2. Amplification products were run on a 1.5% agarose gel at 100 volts for 30 minutes. It was observed with a UV-transilluminator. The sequences of the relevant primers are given in Table 3. The obtained PCR products were stored at -20 °C.

The pcr sequences of the products were performed by PRZ Biotech (www.przbiotech.com) Company. After the 16S rRNA gene region sequence analysis of the isolates was completed, their genus and species were determined. Sequence data of closely related organisms were manually combined with the PHYDIT program and the correlation of isolates with most closely related organisms was performed using global alignment algorithms available on the EzTaxon Server (http://eztaxon-e.ezbiocloud.net/) (13). 16S rRNA nucleotide similarity was determined. MEGAX was used for phylogenetic analysis and CLUSTRAL_W option was used for alignment.

Table 2. Reaction Conditions

Denaturation	Amplification			Finish	
	Denaturation	Annealing	Extention	Extention	
95 °C	95 °C	60-66 °C	72 °C	72 °C	25 °C
5 min	30 s	30 s	90 s	10 min	1 min
1 cycle		30 cycle		1 cycle	

Table 3. The sequences of the relevant primers

Primers	Sequence (5'-3')	Base size	Reference
27F	AGAGTTTGATCCTGGCTCAG	20	(14)
1525R	AAGGAGGTGATCCAGCCGCA	20	(14)

RESULTS

Field Studies, DNA Isolation and PCR

Bacterial isolations were performed in three replicates from soil samples for the 1st, 7th and 15th days and isolates that could distinguish between blood groups were not obtained. All of the isolates were obtained from soil treated with B Rh (+) blood group on the 7th day. Five different isolates with different colony morphology were obtained in three repetitive isolation processes. DNA isolation of isolates was carried out according to the CTAB protocol. Nanodrop results are given in Table 4.

Table 4. Genomic DNA amount and purity of the isolates, Çorum, 2021

Isolates	DNA amount (ng/µl)	Purity A280/A260
Streptomyces sp. BPM1	324,57	1,81
Micromonospora sp. BPM2	652,48	1,80
Micromonospora sp. BPM3	400,36	1,82
Actinomadura sp. BPM4	362,45	1,80
Micromonospora sp. BPM5	374,20	1,81

Phylogenetic Analysis of 16S rRNA Gene Sequence Data

Sequence analyzes of the isolates obtained after bacterial isolation from soils exposed to blood were performed as service procurement. 16S rRNA gene region sequences were compared using EzTaxon (http://eztaxon-e.ezbiocloud.net/;) (13) and percent similarity was determined with the closest type species. MEGAX (15) program was used for phylogenetic analysis. Alignment of the sequences was performed using the ClustralW option via MEGAX. Phylogenetic trees of the isolates based on the 16S rRNA sequence were created with a bootstrap value of 1,000 based on the Neigbour joining method (16,17). The *p-distance* tables of the isolates were obtained by using the *p-distance* option of the same program. The cut off values of the group in which each isolate is included were calculated over the program with the same algorithms. (All sequence results are in Supplementary File 3).

As a result of the 16S rRNA sequence analysis of the isolate with the BPM1 code, *Streptomyces* sp. genus was determined. The white mycelium colony morphology of the isolate is also supporting this finding (Figure 2). The % nucleotide similarities with the closest type species and the different nucleotide numbers are given in Table 5.

Thus, the sequence similarity of the BPM1 isolate with the closest type species is 96.69%. There are 39 nucleotide differences in the 1180 nucleotide-long gene region between the two strains. This difference ensures that the BPM1 isolate shows different branching from the type species in the phylogenetic dendogram (Figure 3) drawn with the closest type species. There are differences of 43, 43 and 44 nucleotides with the other closest type species, *Streptomyces halstedii*, *Streptomyces fulvorobeus* and *Streptomyces flavovirens*, respectively (16, 17). Due to these differences, the isolate clustered differently from the type species.

Figure 2. Colony morphology of *Streptomyces* sp. of BPM1 isolate, Çorum, 2022

Table 5. Nucleotide similarities and different nucleotide numbers of *Streptomyces* sp. *BPM1* isolate with the closest type species, Çorum, 2021

	No	Bacteria	Accession Number	Nucleotide Similarity (%)	Different Nucleotide / Total Nucleotide
Streptomyces sp. BPM1	1	*Streptomyces pratensis*	JQ806215	96,69	39/1180
	2	*Streptomyces halstedii*	AB184142	96,44	43/1210
	3	*Streptomyces fulvorobeus*	AB184711	96,44	43/1209
	4	*Streptomyces flavovirens*	AB184834	96,36	44/1210
	5	*Streptomyces microflavus*	AB184284	96,36	44/1210
	6	*Streptomyces anulatus*	DQ026637	96,36	44/1210
	7	*Streptomyces cyaneofuscatus*	JOEM01000050	96,36	44/1210
	8	*Kitasatospora papulosa*	JNYQ01000038	96,36	44/1210
	9	*Streptomyces globisporus*	AB184203	96,36	44/1209
	10	*Streptomyces pluricolorescens*	AB184162	96,28	45/1210

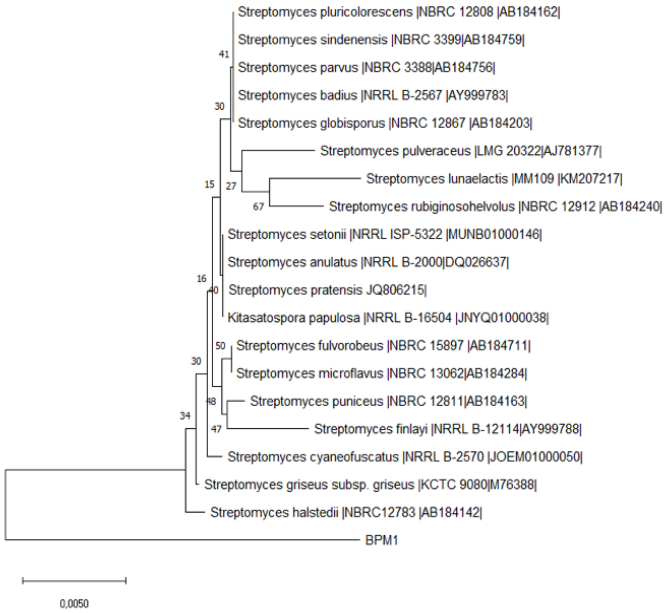


Figure 3. Neighbor joining phylogenetic family tree based on 16S rRNA sequence of *Streptomyces* sp. *BPM1* and type species

The *p-distance* analyzes performed with the sequence data genetic distance values of 15 subspecies are smaller than 0.0055, *Streptomyces* sp. BPM1 isolate is thought to be a subspecies (Supplementary Files 2).

Other bacteria isolated from the soil treated with B Rh (+) blood group was *Micromonospora* genus, generally forms orange colonies (Figure 4). The % nucleotide similarity of the *BPM2* isolate with the closest type species and the differences in nucleotide numbers are given in Table 6.

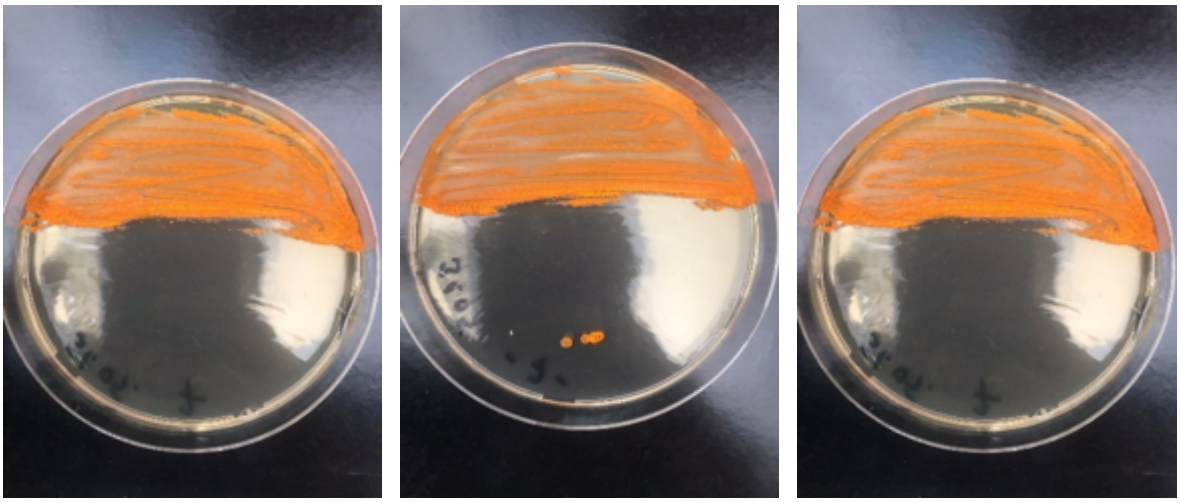


Figure 4. Colony morphologies of isolates of *Micromonospora* genus, *BPM2*, *BPM3* and *BPM5*, respectively, Çorum, 2021

Table 6. Nucleotide similarities and different nucleotide numbers of *Micromonospora* sp. *BPM2* isolate with the closest type species, Çorum, 2021

	No	Bacteria	Accession Number	Nucleotide Similarity (%)	Different Nucleotide / Total Nucleotide
Mikromonospora sp. BPM2	1	*Micromonospora arida*	MG725912	98,08	23/1204
	2	*Micromonospora saelicesensis*	AJ783993	97,92	25/1204
	3	*Micromonospora ureilytica*	FN658641	97,84	26/1204
	4	*Micromonospora profundi*	KF494813	97,75	27/1204
	5	*Micromonospora noduli*	FN658649	97,67	28/1204
	6	*Micromonospora taraxaci*	VIWZ01000001	97,50	30/1204
	7	*Micromonospora chokoriensis*	LT607409	97,42	31/1204
	8	*Micromonospora inaquosa*	MG725913	97,42	31/1204
	9	*Micromonospora vinacea*	FN658651	97,42	31/1204
	10	*Micromonospora violae*	KC161209	97,33	32/1203

According to these results, *Micromonospora arida* is the closest type of *Micromonospora* sp. BPM2 isolate with 98.08% similarity. There are 23 nucleotide difference between the type of strain and the isolate. There is 97.92%, 97.84%, 97.75% and 97.67% nucleotide similarity with *Micromonospora saelicesensis*, *Micromonospora ureilytica*, *Micromonospora profundi*, and *Micromonospora nodule*, respectively (16,17). These five species are also grouped in the same cluster in the phylogenetic tree (Figure 5).

The cut off value of *Micromonospora* sp. BPM2 isolate according to the p-distance table was 0.0097. Organisms with a genetic distance value greater than 0.009 can be considered as different species. Since it is likely to be a subspecies. (Supplementary File 2).

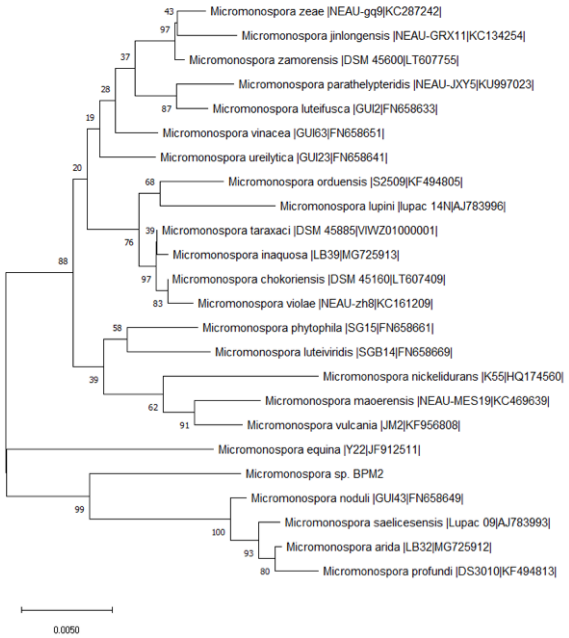


Figure 5. Neighbor joining phylogenetic family tree based on 16S rRNA sequence of *Micromonospora* sp. *BPM2* and type species

The closest type of *Micromonospora* sp. isolate BPM3 is identified as *Micromonospora phytophila*. There is 30 nucleotide difference and 97.33% nucleotide similarity between isolate and type. The other closest type species with a difference of 31 nucleotides are *Micromonospora saelicesensis*, *Micromonospora noduli* and *Micromonospora luteifusca*. There is approximately 97% of 16S rRNA gene region sequence similarity with these types (Table 7).

Table 7. Nucleotide similarities and different nucleotide numbers of *Micromonospora* sp. *BPM3* isolate with the closest type species, Çorum, 2021

	No	Bacteria	Accession Number	Nucleotide Similarity (%)	Different Nucleotide / Total Nucleotide
Micromonospora sp. BPM3	1	*Micromonospora phytophila*	FN658661	97,33	30/1127
	2	*Micromonospora saelicesensis*	AJ783993	97,29	31/1146
	3	*Micromonospora noduli*	FN658649	97,28	31/1140
	4	*Micromonospora luteifusca*	FN658633	97,27	31/1138
	5	*Micromonospora arida*	MG725912	97,20	32/1146
	6	*Micromonospora vinacea*	FN658651	97,16	32/1129
	7	*Micromonospora parathelypteridis*	KU997023	97,12	33/1146
	8	*Micromonospora vulcania*	KF956808	97,11	33/1145
	9	*Micromonospora luteiviridis*	FN658669	97,10	33/1138
	10	*Micromonospora purpureochromogenes*	LT607410	97,03	34/1146

According to the phylogenetic dendogram drawn with *Micromonospora* sp. BPM3 and the closest type species, it is seen that the isolate clustered in close branches with the closest type species (16,17) (Figure 6). According to the genetic distance data obtained through the MEGAX program, the cut-off value of the group including the BPM3 isolate; it is set at 0.0749. Organisms with a genetic distance value above this value are considered as different species. Since the genetic distance values of the BPM3 isolate are less than 0.07, it is considered to be a subspecies (Supplementary File 2).

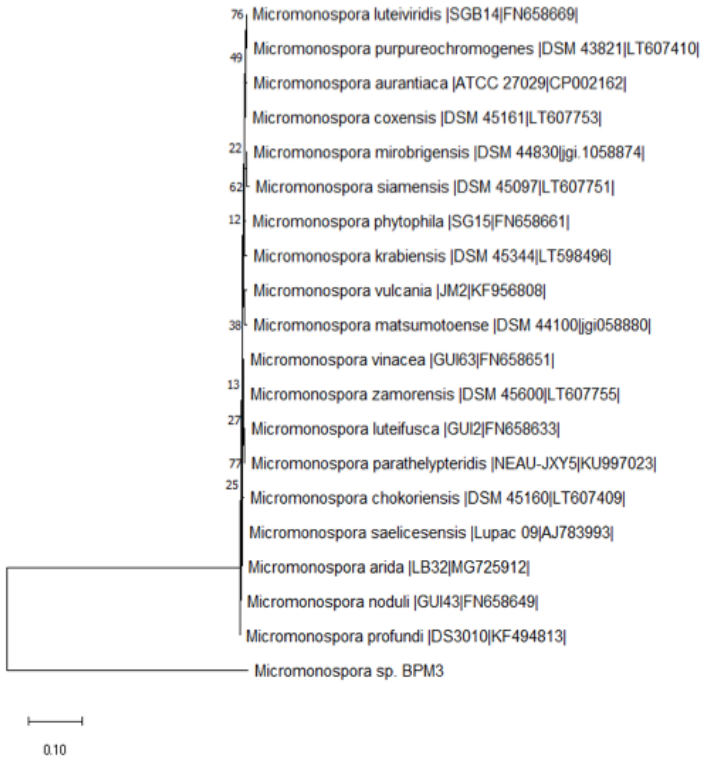


Figure 6. Neighbor joining phylogenetic family tree based on 16S rRNA sequence of *Micromonospora* sp. *BPM3* and type species

Micromonospora sp. with 97.90% sequence similarity of the BPM5 isolate, the most common type is *Micromonospora arida* (16,17). There are 25 nucleotide difference between the isolate and the types. Organisms with a difference of 27, 28, 29 and 30 nucleotides, respectively, are *Micromonospora saelicesensis*, *Micromonospora ureilytica*, *Micromonospora profundi*, *Micromonospora noduli* and *Micromonospora vinacea* (Table 8). The cut-off value of the isolate, which shows close clustering in the phylogenetic dendogram with all these types (Figure 7), was determined as 0.0157. This isolate is considered to be a subspecies since it has a genetic distance value of less than 0.01 with the closest type species (Supplementary File 2).

Table 8. Nucleotide similarities and different nucleotide numbers of *Micromonospora* sp. *BPM5* isolate with the closest type species, Çorum, 2021.

	No	Bacteria	Accession Number	Nucleotide Similarity (%)	Different Nucleotide / Total Nucleotide
Mikromonospora sp. *BPM5*	1	*Micromonospora arida*	MG725912	97,90	25/1196
	2	*Micromonospora saelicesensis*	AJ783993	97,74	27/1196
	3	*Micromonospora ureilytica*	FN658641	97,65	28/1196
	4	*Micromonospora profundi*	KF494813	97,57	29/1196
	5	*Micromonospora noduli*	FN658649	97,49	30/1196
	6	*Micromonospora vinacea*	FN658651	97,49	30/1196
	7	*Micromonospora taraxaci*	VIWZ01000001	97,32	32/1196
	8	*Micromonospora chokoriensis*	LT607409	97,24	33/1196
	9	*Micromonospora inaquosa*	MG725913	97,24	33/1196
	10	*Micromonospora zamorensis*	LT607755	97,15	34/1196

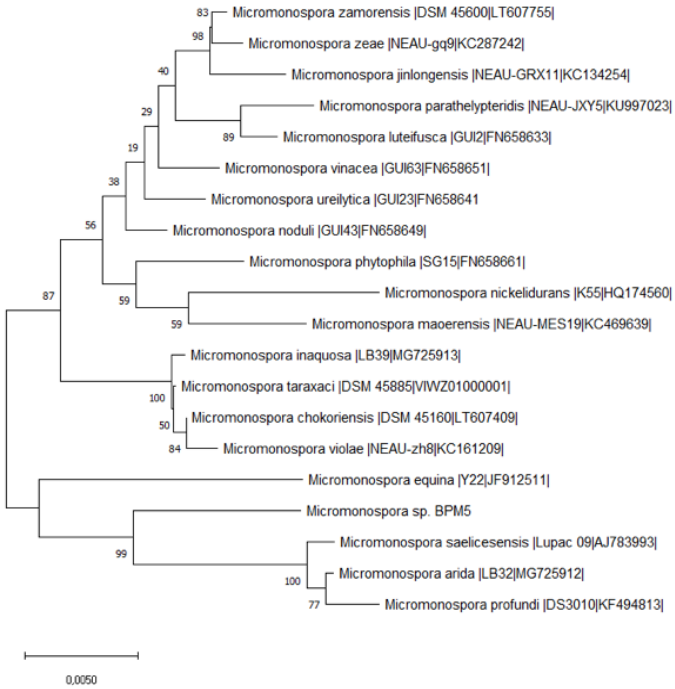


Figure 7. Neighbor joining phylogenetic family tree based on 16S rRNA sequence of *Micromonospora* sp. *BPM5* and type species

The isolate with the BPM4 code is an organism with white dots, mycelium and spore colonies (Figure 8). According to the 16S rRNA sequence data, the closest type of the organism, which was determined to belong to the genus *Actinomadura*, was determined as *Actinomadura cremea* with 98.40% nucleotide similarity. Sequence similarity and nucleotide differences with other type species are given in Table 9.

Although it shows close clustering in the phylogenetic tree with the closest type species (Figure 9), the genetic distance of the BPM4 isolate, which still has a different distance, is 0.0879. According to the genetic distance table; Due to the p-distance values of approximately 0.01 with the type species, *Actinomadura* sp. It is thought that the BPM4 isolate may also be a subspecies.

Figure 8. Colony morphologies of isolates of *Actinomadura* sp. *BPM4*, Çorum, 2021

Table 9. Nucleotide similarities and different nucleotide numbers of *Actinomadura* sp. *BPM4* isolate with the closest type species, Çorum, 2021.

	No	Bacteria	Accession Number	Nucleotide Similarity (%)	Different Nucleotide / Total Nucleotide
Actinomadura sp. BPM4	1	*Actinomadura cremea*	AF134067	98,40	19/1189
	2	*Actinomadura sediminis*	JF272484	97,73	27/1191
	3	*Actinomadura lepetitiana*	MH061375	97,64	28/1190
	4	*Actinomadura algeriensis*	KT259320	97,64	28/1189
	5	*Actinomadura mexicana*	AF277195	97,64	28/1188
	6	*Actinomadura maheshkhaliensis*	AB331731	97,60	28/1169
	7	*Actinomadura deserti*	MF972517	97,56	29/1190
	8	*Actinomadura apis*	AB557596	97,48	30/1191
	9	*Actinomadura formosensis*	AF002263	97,39	31/1191
	10	*Actinomadura madurae*	jgi.1068094	97,39	31/1190

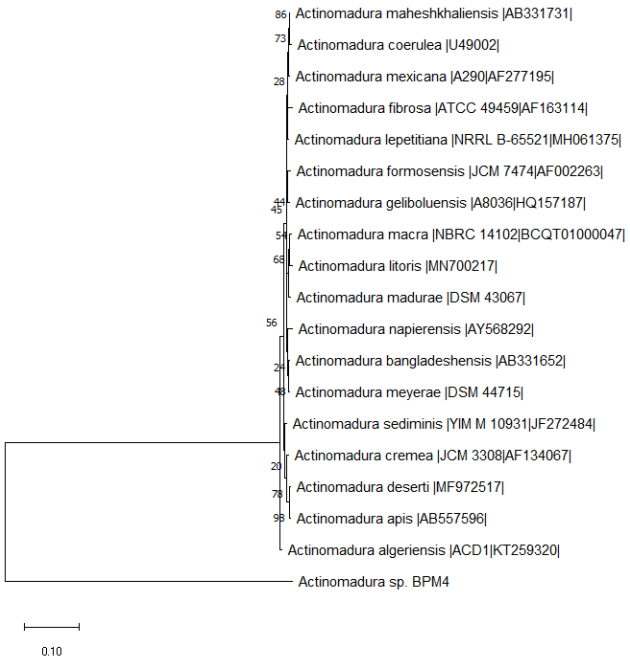


Figure 9. Neighbor joining phylogenetic family tree based on 16S rRNA sequence of *Actinomadura* sp. *BPM4* and type species

DISCUSSION

For nearly 100 years, microbiology has played a relatively minor role in forensic science (3). The emergence of PCR-mediated bacterial genotyping was recognized as a valuable tool in the future of forensic science (18). Fungal, pollen and spore analyses developed in the mid-1990s have enabled forensic scientists to relate to many fields (19,20). However, until the rise of bioterrorism in the early 2000s, microbial forensics or forensic microbiology was not used in forensic analysis (21,23).

The primary goal of forensic microbiology is to reference data from evidence samples comparison with examples. Microbial forensic research focuses on the detection and characterization of the biological agent in addition to non-biological evidence. Biological agents consist of bacteria, viruses, protists, fungi and toxins, additives, growth medium, habitat, growing conditions, intelligence, etc. Non-biological evidence such as microbial forensic science can be useful and potentially provide clues to researchers (22).

Microbial forensic evidence can be found in a wide variety of sample matrices, including food, water, air filters, swabs and swabs, soil, animal tissue, and clinical samples (eg, tissue, sputum, blood, feces, urine). In this context, fingerprints, human or animal DNA; traditional forensic evidence such as heat, fibers and hair can also be analyzed. However, determining the effects of humans and their secretions on microbial systems is one of the new approaches of forensic microbiology. Both the determination of the biodiversity of a region and the interaction of various bodily fluids with the environment can provide evidence that affects forensic processes (21,23). In our study, it was aimed to determine the change in Actinobacteria species, which are indispensable members of the soil, by pouring blood samples

from eight different blood groups into forest soil.

Forensic microbial methods; culture, microscopy, immunoassays, mass spectrometry, real-time PCR, microarray, genetic typing, whole genome sequencing, and more. Culture is still considered the gold standard for pathogen detection (24). However, culture many times cannot provide a solution beyond the genus or species level and may not be effective for response, especially when the safety of individuals is a pressing concern, as there can be significant latency due to the growth requirements of the microorganism. In addition, approximately 99% of microorganisms cannot be cultured by current methods; therefore, culture is not a reliable method for susceptible and possibly new/uncharacterized microorganisms. In addition, the microbes may have been exposed to environmental attack and may no longer live. Therefore, microorganism culture techniques should be performed with great care. While culture and immunoassays are adequate methods for initial testing and sample screening, nucleic acid typing is generally more analytical. An unbiased, more comprehensive method of genome screening is needed to extract the most analytical information possible (25). As it is mentioned by some of the authors, it was faced with the difficulty of culturing in our study as well. Although all isolation studies were performed in triplicate, no Actinobacteria growth was observed neither in the control group nor in the soils to which other blood groups were added except B Rh (+) one. We could obtain only five Actinobacteria type bacteria from the samples taken from eight different blood groups and control groups. Bacterial isolation should be attempted at different pH, temperature and salt concentrations, because of the limitation of the budget, we only used GYME (ISP2) agar, which is a selective media for Actinobacteria. However, different media should be tried for getting more reliable results in addition to this one.

Interestingly, all bacteria isolated were obtained from soil belonging to the B Rh (+) blood group belonging to the 7th day. The inability to isolate Actinobacteria from the majority of soils exposed to blood may be due to the chemical properties of the blood or the enzymes in it that can degrade bacterial spores. Although it has been studied in the pH range (~7.3-7.4) equivalent to blood in the preparation of the medium, some enzymes in the blood (lactate dehydrogenase, aspartate transaminase, etc.) may show weak acidic properties (26). This can cause bacterial spores to disappear or lose their viability.

Recent studies show that bacterial communities obtained from surface soil and buried soil exhibit different behaviors during the decomposition process of the corpse. While microbial communities from the surface soil show a decreasing trend in taxon richness, diversity and smoothness; microbial communities in close contact with buried cadavers exhibit contrasting characteristics (increased taxon richness, consistent diversity and decreased regularity, etc.). Also, Proteobacteria is the most abundant phylum in burial soil samples, while the relative abundance of Acidobacteria decreases and Firmicutes species increase in surface cadaver-soil assemblages. Microbial diversity remains fairly constant in buried soil communities (27). Delgado-Baquerizo et al. (28) argues that creating comprehensive maps of the geographical distribution of microbial communities will be useful in forensic microbiology. In our study, we aimed to determine the change of soil bacteria by pouring blood samples from eight different blood groups into forest soil.

Actinobacteria are mycelial, gram-positive bacteria with enormous diversity, they have high economic value and they are rich in Guanine-Cytosine (GC). They are found in a wide variety of soil habitats. They survive as endophytes in plant tissues and contribute to their growth by nutrient assimilation (29). Actinobacteria protect their hosts and food sources from pathogen attacks via colonizing the outside or inside of insects (30). Moreover, they can survive even in extreme environments such as the deep ocean and maintain nutrient cycles under harsh conditions (31,32). The adaptation and interaction

of Actinobacteria to diverse environments have led to the evolution of their distinct biosynthetic potential. Therefore, they can produce a wide range of secondary metabolites (33) and they have quite high economic values. That is why we have selected Actinobacteria as an indicator bacterium for our study.

So far 47 families and 711 species of Actinobacteria were isolated and identified in variety of habitats. Actinobacterial families such as Streptomycetaceae, Pseudonocardiaceae,Nocardia ceae, Micromonosporaceae, Streptosporangiaceae and Thermomonosporaceae have the widest diversity and highest economic value. Streptomycetaceae has 26.5 % of the new actinobacteria reported in the last five years. The enormous increase in the number of recently identified species indicates that the Pseudonocardiaceae, Nocardioidaceae, Micromonosporaceae, Nocardiaceae and Streptosporangiaceae are also no longer rare (34). In our study, *Micromonospora* sp., *Streptomyces* sp., and *Actinomadura* sp. were identified as strains belonging to this genus.

Metagenomic methods provide a culture-independent method to study microorganisms and DNA samples collected directly from environmental samples and they enable to microbial diversity determination studies (35). The most widely used method in bacterial metagenomic studies is metabarcoding. Diversity determination based on 16S rRNA metabarcoding gives more reliable results than culture. Many forensic applications are limited to individual taxon analysis. The most challenging concept for forensic microbiology is the lack of cost-effective sequencing technologies (36,37). Advances in genomic sequencing technology and new methods for processing complex datasets have led to the emergence of a new microbiomic field (38).

Bacterial taxonomy is important to provide adequate information about the identity and uniqueness of a species in ecological, clinical and industrial niche settings. Bacterial taxonomy consists of classification, naming and identification steps. There are two basic approaches to classification. The first is the % GC content based on the similarity between microorganisms and the morphological and physiological characters including DNA-DNA homology. The other is based on chemotaxonomic markers such as protein, fatty acid profiles, sugar contents (39).

In phylogenetic systematics, it uses nucleotide sequences of conserved genes called molecular chronometers. A combination of both phenotypic and phylogenetic is called polyphasic taxonomy which is usefull for identifying new species and genera Numerical analysis of small subunit ribosomal RNA (rRNA) genes has formed the basis of microbial phylogenetics, resulting in branching trees representing the distance from a common ancestor although some limitations, especially in distinguishing closely related taxa (40).

DNA-DNA hybridization (DDH) is a technique based on comparative analysis between the total DNA of two bacterial species (41). DDH is considered the gold standard for bacterial species classification. The DNA molecule can be denatured and renatured by changing the temperature. The main parameters that play an important role in this technique are (1) % G+C content, (2) The ionic strength of the solution, (3) Its melting temperature (Tm) (Raina et al., 2019). More than 5,000 bacterial species have been effectively identified using this technique (42).

Another hybridization method is the DNA microarray technique involving the use of DNA fragments. A set of fragmented DNAs can be hybridized on a single microarray plate and can generate a large amount of data. This method is used for virulence/pathogenicity detection by identifying strain among pathogenic bacterial strains (43).

The 16S rRNA gene is a highly conserved gene consisting of nine hypervariable domains separated by more conserved fragments that can be used to design universal primers. Thanks to the 16S rRNA gene, many pre-existing taxa have been reclassified and more species have been identified. Two strains were considered to belong to different species if they shared less than 97% 16S rRNA gene sequence similarity

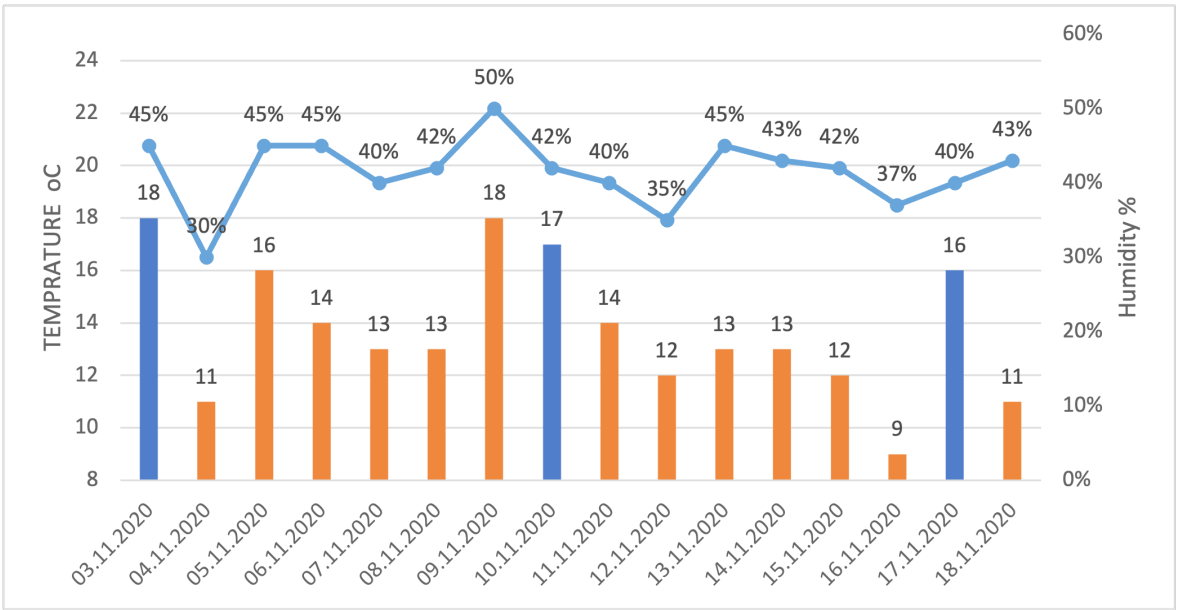
(44), and if this value was less than 95%, they were considered to distinguish between two genera (45).

This study aimed to show the effect of blood groups on soil biodiversity. However, very limited data were obtained due to the small number of bacteria isolated, It could be related with the use of stable growing conditions during the isolation process and the preference for a single medium. In future studies, different isolation conditions (different pH, temperature, salt concentration and different medium) are planning to be tried for culture.

Of the five isolates, three of them are *Micromonospora* sp., one is *Streptomyces* sp., and the other one is *Actinomadura* sp. According to the analyzes made with the data obtained from 16S rRNA sequencing, % nucleotide similarities with the closest relatives of all isolates and different nucleotide numbers were determined, and phylogenetic trees and genetic distance graphs were drawn. According to the *p-distance* values of all isolates, they were considered as subspecies rather than new species.

This study is only a preliminary study to identify bacterial communities that may be present in soil exposed to blood. In order to evaluate the further results accurately, other genomic techniques to determine the exact location of the test isolates in a phylogenetic system should be tried in addition to alternative cultures. Such as carbon and nitrogen sources, degradation, pH, temperature and salt tolerance, phenotypic characteristics like spore morphology, chemotaxonomic analyzes like DAP (diaminopimelic acid test), determination of fatty acid profiles, cell wall analysis, and defining sugar could be used additionally for complete discrimination with 16S rRNA metabarcoding.

Supplementary File 1



Supplementary File 2

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. Streptomyces sp. BPM1		0,0046	0,0046	0,0047	0,0048	0,0047	0,0047	0,0047	0,0047	0,0047	0,0047	0,0048	0,0049	0,0045	0,0047
2. Streptomyces pratensis JQ806215|	0,0263		0,0014	0,0011	0,0011	0,0000	0,0010	0,0000	0,0007	0,0007	0,0000	0,0014	0,0023	0,0011	0,0007
3. Streptomyces halstedii |NBRC12783 |AB184142|	0,0265	0,0029		0,0014	0,0014	0,0014	0,0014	0,0014	0,0015	0,0015	0,0014	0,0017	0,0023	0,0012	0,0015
4. Streptomyces fulvorobeus |NBRC 15897 |AB184711|	0,0265	0,0015	0,0028		0,0000	0,0010	0,0010	0,0010	0,0012	0,0012	0,0010	0,0010	0,0024	0,0010	0,0012
5. Streptomyces microflavus |NBRC 13062|AB184284|	0,0273	0,0015	0,0028	0,0000		0,0010	0,0010	0,0010	0,0012	0,0012	0,0010	0,0010	0,0024	0,0010	0,0012
6. Streptomyces anulatus |NRRL B-2000|DQ026637|	0,0273	0,0000	0,0028	0,0014	0,0014		0,0010	0,0000	0,0007	0,0007	0,0000	0,0014	0,0022	0,0010	0,0007
7. Streptomyces cyaneofuscatus |NRRL B-2570 |JOEM01000050|	0,0273	0,0015	0,0028	0,0014	0,0014	0,0014		0,0010	0,0012	0,0012	0,0010	0,0014	0,0022	0,0010	0,0012
8. Kitasatospora papulosa |NRRL B-16504 |JNYQ01000038|	0,0273	0,0000	0,0028	0,0014	0,0014	0,0000	0,0014		0,0007	0,0007	0,0000	0,0014	0,0022	0,0010	0,0007
9. Streptomyces globisporus |NBRC 12867 |AB184203|	0,0273	0,0007	0,0035	0,0021	0,0021	0,0007	0,0021	0,0007		0,0000	0,0007	0,0012	0,0021	0,0012	0,0000
10. Streptomyces pluricolorescens |NBRC 12808 |AB184162|	0,0281	0,0007	0,0035	0,0021	0,0021	0,0007	0,0021	0,0007	0,0000		0,0007	0,0012	0,0021	0,0012	0,0000
11. Streptomyces setonii |NRRL ISP-5322 |MUNB01000146|	0,0273	0,0000	0,0028	0,0014	0,0014	0,0000	0,0014	0,0000	0,0007	0,0007		0,0014	0,0022	0,0010	0,0007
12. Streptomyces puniceus |NBRC 12811|AB184163|	0,0289	0,0029	0,0041	0,0014	0,0014	0,0028	0,0028	0,0028	0,0021	0,0021	0,0028		0,0025	0,0013	0,0012
13. Streptomyces lunaelactis |MM109 |KM207217|	0,0318	0,0073	0,0085	0,0085	0,0085	0,0070	0,0070	0,0070	0,0063	0,0063	0,0070	0,0085		0,0024	0,0021
14. Streptomyces griseus subsp. griseus |KCTC 9080|M76388|	0,0257	0,0015	0,0021	0,0014	0,0014	0,0014	0,0014	0,0014	0,0021	0,0021	0,0014	0,0028	0,0085		0,0012
15. Streptomyces badius |NRRL B-2567 |AY999783|	0,0281	0,0007	0,0035	0,0021	0,0021	0,0007	0,0021	0,0007	0,0000	0,0000	0,0007	0,0021	0,0063	0,0021	

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. Micromonospora sp. BPM2		0,0036	0,0038	0,0038	0,0039	0,0040	0,0040	0,0042	0,0043	0,0043	0,0043	0,0043	0,0044	0,0045	0,0046
2. Micromonospora arida |LB32|MG725912|	0,0151		0,0012	0,0013	0,0014	0,0016	0,0019	0,0018	0,0019	0,0020	0,0021	0,0021	0,0022	0,0024	0,0025
3. Micromonospora saelicesensis |Lupac 09|AJ783993|	0,0167	0,0021		0,0017	0,0019	0,0018	0,0020	0,0018	0,0019	0,0020	0,0021	0,0021	0,0023	0,0026	0,0026
4. Micromonospora ureilytica |GUI23|FN658641|	0,0176	0,0028	0,0049		0,0020	0,0021	0,0023	0,0022	0,0023	0,0024	0,0019	0,0025	0,0020	0,0028	0,0025
5. Micromonospora profundi |DS3010|KF494813|	0,0184	0,0028	0,0049	0,0056		0,0019	0,0022	0,0020	0,0019	0,0022	0,0026	0,0022	0,0027	0,0029	0,0030
6. Micromonospora noduli |GUI43|FN658649|	0,0192	0,0035	0,0042	0,0063	0,0049		0,0020	0,0019	0,0020	0,0021	0,0023	0,0023	0,0024	0,0022	0,0024
7. Micromonospora vinacea |GUI63|FN658651|	0,0192	0,0049	0,0056	0,0078	0,0063	0,0056		0,0023	0,0024	0,0024	0,0020	0,0025	0,0022	0,0025	0,0024
8. Micromonospora taraxaci |DSM 45885|VIWZ01000001|	0,0209	0,0049	0,0049	0,0077	0,0063	0,0056	0,0071		0,0007	0,0007	0,0026	0,0012	0,0027	0,0028	0,0029
9. Micromonospora chokoriensis |DSM 45160|LT607409|	0,0217	0,0056	0,0056	0,0084	0,0056	0,0063	0,0078	0,0007		0,0010	0,0027	0,0010	0,0028	0,0029	0,0030
10. Micromonospora inaquosa |LB39|MG725913|	0,0217	0,0056	0,0056	0,0084	0,0070	0,0063	0,0078	0,0007	0,0014		0,0027	0,0014	0,0028	0,0029	0,0030
11. Micromonospora zamorensis |DSM 45600|LT607755|	0,0226	0,0070	0,0063	0,0056	0,0097	0,0077	0,0063	0,0097	0,0104	0,0104		0,0028	0,0012	0,0024	0,0021
12. Micromonospora violae |NEAU-zh8|KC161209|	0,0226	0,0070	0,0070	0,0098	0,0070	0,0077	0,0092	0,0021	0,0014	0,0028	0,0118		0,0030	0,0031	0,0031
13. Micromonospora zeae |NEAU-gq9|KC287242|97.07|	0,0234	0,0077	0,0084	0,0063	0,0104	0,0084	0,0071	0,0111	0,0118	0,0118	0,0021	0,0132		0,0025	0,0022
14. Micromonospora parathelypteridis |NEAU-JXY5|KU997023|	0,0251	0,0090	0,0097	0,0119	0,0118	0,0070	0,0099	0,0125	0,0132	0,0132	0,0090	0,0146	0,0097		0,0018
15. Micromonospora luteifusca |GUI2|FN658633|	0,0251	0,0084	0,0091	0,0091	0,0112	0,0077	0,0078	0,0119	0,0126	0,0126	0,0063	0,0140	0,0070	0,0049	

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. Micromonospora sp. BPM2		0,0036	0,0038	0,0038	0,0039	0,0040	0,0040	0,0042	0,0043	0,0043	0,0043	0,0043	0,0044	0,0045	0,0046
2. Micromonospora arida |LB32|MG725912|	0,0151		0,0012	0,0013	0,0014	0,0016	0,0019	0,0018	0,0019	0,0020	0,0021	0,0021	0,0022	0,0024	0,0025
3. Micromonospora saelicesensis |Lupac 09|AJ783993|	0,0167	0,0021		0,0017	0,0019	0,0018	0,0020	0,0018	0,0019	0,0020	0,0021	0,0021	0,0023	0,0026	0,0026
4. Micromonospora ureilytica |GUI23|FN658641|	0,0176	0,0028	0,0049		0,0020	0,0021	0,0023	0,0022	0,0023	0,0024	0,0019	0,0025	0,0020	0,0028	0,0025
5. Micromonospora profundi |DS3010|KF494813|	0,0184	0,0028	0,0049	0,0056		0,0019	0,0022	0,0020	0,0019	0,0022	0,0026	0,0022	0,0027	0,0029	0,0030
6. Micromonospora noduli |GUI43|FN658649|	0,0192	0,0035	0,0042	0,0063	0,0049		0,0020	0,0019	0,0020	0,0021	0,0023	0,0023	0,0024	0,0022	0,0024
7. Micromonospora vinacea |GUI63|FN658651|	0,0192	0,0049	0,0056	0,0078	0,0063	0,0056		0,0023	0,0024	0,0024	0,0020	0,0025	0,0022	0,0025	0,0024
8. Micromonospora taraxaci |DSM 45885|VIWZ01000001|	0,0209	0,0049	0,0049	0,0077	0,0063	0,0056	0,0071		0,0007	0,0007	0,0026	0,0012	0,0027	0,0028	0,0029
9. Micromonospora chokoriensis |DSM 45160|LT607409|	0,0217	0,0056	0,0056	0,0084	0,0056	0,0063	0,0078	0,0007		0,0010	0,0027	0,0010	0,0028	0,0029	0,0030
10. Micromonospora inaquosa |LB39|MG725913|	0,0217	0,0056	0,0056	0,0084	0,0070	0,0063	0,0078	0,0007	0,0014		0,0027	0,0014	0,0028	0,0029	0,0030
11. Micromonospora zamorensis |DSM 45600|LT607755|	0,0226	0,0070	0,0063	0,0056	0,0097	0,0077	0,0063	0,0097	0,0104	0,0104		0,0028	0,0012	0,0024	0,0021
12. Micromonospora violae |NEAU-zh8|KC161209|	0,0226	0,0070	0,0070	0,0098	0,0070	0,0077	0,0092	0,0021	0,0014	0,0028	0,0118		0,0030	0,0031	0,0031
13. Micromonospora zeae |NEAU-gq9|KC287242|97.07|	0,0234	0,0077	0,0084	0,0063	0,0104	0,0084	0,0071	0,0111	0,0118	0,0118	0,0021	0,0132		0,0025	0,0022
14. Micromonospora parathelypteridis |NEAU-JXY5|KU997023|	0,0251	0,0090	0,0097	0,0119	0,0118	0,0070	0,0099	0,0125	0,0132	0,0132	0,0090	0,0146	0,0097		0,0018
15. Micromonospora luteifusca |GUI2|FN658633|	0,0251	0,0084	0,0091	0,0091	0,0112	0,0077	0,0078	0,0119	0,0126	0,0126	0,0063	0,0140	0,0070	0,0049	

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. Actinomadura sp. BPM4		0.0142	0.0142	0.0142	0.0142	0.0142	0.0141	0.0142	0.0142	0.0141	0.0143	0.0141	0.0141	0.0143	0.0142
2. Actinomadura cremea |JCM 3308|AF134067|	0.5629		0.0031	0.0035	0.0033	0.0038	0.0036	0.0032	0.0030	0.0038	0.0037	0.0040	0.0040	0.0039	0.0038
3. Actinomadura sediminis |YIM M 10931|JF272484|	0.5621	0.0140		0.0031	0.0029	0.0038	0.0034	0.0033	0.0036	0.0035	0.0036	0.0039	0.0041	0.0037	0.0033
4. Actinomadura lepetitiana |NRRL B-65521|MH061375|	0.5626	0.0196	0.0146		0.0037	0.0027	0.0023	0.0041	0.0039	0.0030	0.0032	0.0028	0.0037	0.0033	0.0021
5. Actinomadura algeriensis |ACD1|KT259320|	0.5588	0.0154	0.0125	0.0216		0.0042	0.0040	0.0040	0.0038	0.0042	0.0040	0.0045	0.0038	0.0041	0.0041
6. Actinomadura mexicana |A290|AF277195|	0.5618	0.0226	0.0218	0.0106	0.0268		0.0021	0.0042	0.0041	0.0033	0.0031	0.0028	0.0040	0.0033	0.0033
7. Actinomadura maheshkhaliensis |AB331731|	0.5618	0.0192	0.0176	0.0077	0.0247	0.0064		0.0043	0.0040	0.0031	0.0035	0.0021	0.0040	0.0034	0.0030
8. Actinomadura deserti |MF972517|	0.5654	0.0140	0.0160	0.0250	0.0216	0.0261	0.0261		0.0023	0.0044	0.0040	0.0047	0.0043	0.0042	0.0043
9. Actinomadura apis |AB557596|	0.5645	0.0126	0.0188	0.0236	0.0195	0.0261	0.0233	0.0076		0.0043	0.0041	0.0045	0.0041	0.0041	0.0042
10. Actinomadura formosensis |JCM 7474|AF002263|	0.5621	0.0224	0.0194	0.0125	0.0264	0.0155	0.0141	0.0285	0.0271		0.0038	0.0035	0.0040	0.0036	0.0033
11. Actinomadura madurae |DSM 43067|	0.5634	0.0232	0.0202	0.0146	0.0265	0.0141	0.0176	0.0244	0.0257	0.0209		0.0040	0.0037	0.0030	0.0031
12. Actinomadura coerulea |U49002|	0.5628	0.0242	0.0227	0.0114	0.0299	0.0114	0.0065	0.0313	0.0299	0.0177	0.0214		0.0044	0.0039	0.0035
13. Actinomadura napierensis |AY568292|	0.5655	0.0238	0.0246	0.0179	0.0216	0.0216	0.0216	0.0276	0.0246	0.0208	0.0186	0.0254		0.0040	0.0034
14. Actinomadura macra |NBRC 14102|BCQT01000047|	0.5597	0.0260	0.0223	0.0160	0.0265	0.0169	0.0177	0.0279	0.0272	0.0195	0.0132	0.0214	0.0216		0.0034
15. Actinomadura bangladeshensis |AB331652|	0.5626	0.0231	0.0173	0.0069	0.0257	0.0169	0.0141	0.0271	0.0271	0.0173	0.0132	0.0177	0.0164	0.0167	

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. Micromonospora sp. BPM5		0,0035	0,0036	0,0037	0,0037	0,0038	0,0038	0,0040	0,0041	0,0041	0,0041	0,0042	0,0042	0,0043	0,0043
2. Micromonospora arida |LB32|MG725912|	0,0151		0,0012	0,0038	0,0013	0,0038	0,0033	0,0040	0,0040	0,0040	0,0044	0,0041	0,0044	0,0045	0,0042
3. Micromonospora saelicesensis |Lupac 09|AJ783993|	0,0167	0,0021		0,0040	0,0017	0,0038	0,0033	0,0039	0,0039	0,0040	0,0042	0,0041	0,0044	0,0045	0,0042
4. Micromonospora ureilytica |GUI23|FN658641	0,0176	0,0210	0,0224		0,0041	0,0018	0,0021	0,0026	0,0027	0,0027	0,0019	0,0029	0,0021	0,0028	0,0025
5. Micromonospora profundi |DS3010|KF494813|	0,0184	0,0028	0,0049	0,0238		0,0039	0,0034	0,0041	0,0040	0,0041	0,0046	0,0042	0,0046	0,0047	0,0044
6. Micromonospora noduli |GUI43|FN658649|	0,0192	0,0217	0,0217	0,0056	0,0231		0,0020	0,0024	0,0025	0,0025	0,0021	0,0027	0,0022	0,0023	0,0023
7. Micromonospora vinacea |GUI63|FN658651|	0,0192	0,0155	0,0155	0,0071	0,0169	0,0056		0,0025	0,0026	0,0026	0,0020	0,0028	0,0020	0,0026	0,0023
8. Micromonospora taraxaci |DSM 45885|VIWZ01000001|	0,0209	0,0230	0,0223	0,0105	0,0244	0,0084	0,0092		0,0007	0,0007	0,0028	0,0011	0,0029	0,0032	0,0032
9. Micromonospora chokoriensis |DSM 45160|LT607409|	0,0217	0,0237	0,0230	0,0112	0,0237	0,0091	0,0099	0,0007		0,0010	0,0029	0,0009	0,0030	0,0033	0,0032
10. Micromonospora inaquosa |LB39|MG725913|	0,0217	0,0237	0,0230	0,0112	0,0251	0,0091	0,0099	0,0007	0,0014		0,0029	0,0013	0,0030	0,0033	0,0032
11. Micromonospora zamorensis |DSM 45600|LT607755|	0,0226	0,0285	0,0271	0,0056	0,0313	0,0070	0,0056	0,0125	0,0132	0,0132		0,0031	0,0012	0,0024	0,0021
12. Micromonospora violae |NEAU-zh8|KC161209|	0,0226	0,0251	0,0244	0,0126	0,0251	0,0105	0,0113	0,0021	0,0014	0,0028	0,0146		0,0032	0,0034	0,0034
13. Micromonospora zeae |NEAU-gq9|KC287242|	0,0234	0,0292	0,0292	0,0063	0,0320	0,0077	0,0063	0,0139	0,0146	0,0146	0,0021	0,0160		0,0025	0,0021
14. Micromonospora parathelypteridis |NEAU-JXY5|KU997023|	0,0251	0,0307	0,0307	0,0119	0,0334	0,0070	0,0099	0,0153	0,0160	0,0160	0,0090	0,0174	0,0097		0,0018
15. Micromonospora luteifusca |GUI2|FN658633|	0,0251	0,0259	0,0259	0,0091	0,0287	0,0077	0,0078	0,0154	0,0161	0,0161	0,0063	0,0175	0,0070	0,0049	

Supplementary File 3

BPM1 16S rRNA Forward

GCCGGGGGGTGCTTACACATGCAAGTCGAACGATGAAGCCTTTCGGGGTGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTG
CCCTTCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATAACACTCTGTCCCGCATGGGACGGGGTTAAAAGCTCCGGCGGTG
AAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCC
ACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCG
CGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAAC
TACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCG
GATGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCATTCGATACGGGCTAGCTAGAGTGTGGTAGGGGAGATCGGAATTCCTGGTGTAGCG
GTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGGGGAGC
GAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGTTGGGAACTAGGTGTTGGCGACATTCCACGTCGTCGGTGCCGCAGCTAACG
CATTAAGTTCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCAGCGGAGCATGTGGCT
TAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATATACCGGAAAGCATCAGAGATGGTGCCCCCTTGTGGTCGGTATACAGTGG
TGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCACGAGCGCAACCATTGTTCTGTGTTGCAGCATGCCTTTCGG
GTGATGGGACTCACAGGAACTGCGGGTCACTCGAGAGTGGGGACGACGTCAGTCATCATGGCGTTATGTTCTTGGGCGTGCCACTGCTAC
ATTGGACTGATACATGACTTGCATGGCCAGGGCTGGACCGAATCA

BPM1 16S rRNA Reverse

CGGGGGGCTCGTTGTTACGACTTCGTCCAATCGCCAGTCCCACCTTCGACAGCTCCCTCCCACAAGGGGTTGGGCCACCGGCTTCGGGTG
TTACCGACTTTCGTGACGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCAGCAATGCTGATCTGCGATTACTAGCAACTCC
GACTTCATGGGGTCGAGTTGCAGACCCCAATCCGAACTGAGACCGGCTTTTTGAGATTCGCTCCGCCTCGCGGCATCGCAGCTCATTGTAC
CGGCCATTGTAGCACGTGTGCAGCCCAAGACATAAGGGGCATGATGACTTGACGTCGTCCCCACCTTCCTCCGAGTTGACCCCGGCAGTCT
CCTGTGAGTCCCCATCACCCCGAAGGGCATGCTGGCAACACAGAACAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACAC
GAGCTGACGACAGCCATGCACCACCTGTATACCGACCACAAGGGGGGCACCATCTCTGATGCTTTCCGGTATATGTCAAGCCTTGGTAAGG
TTCTTCGCGTTGCGTCGAATTAAGCCACATGCTCCGCTGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTTAGCCTTGCGGCCGTACTC
CCCAGGCGGGGAACTTAATGCGTTAGCTGCGGCACCGACGACGTGGAATGTCGCCAACACCTAGTTCCCAACGTTTACGGCGTGGACTAC
CAGGGTATCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTAATGGCCCAGAGATCCGCCTTCGCCACCGGTGTTCCTCC
TGATATCTGCGCATTTCACCGCTACACCAGAAATTCCCGATCTCCCCCTACCACACTCTAGCTAGCCCGTATCGAATGCAGACCCGGGTTAA
AGCCCCGGGGCTTTCACATCCGACGTGACAGCCGCCGTACGAGCTCTTTACGCCCAATAATTCCTGACACGCTGCGCCCTACGTATACCGC
GCTGCTGCCCGTAGTAGCGGCGCTTCTTTCTGCAGTACGTCATTTTCGCTCCTCCCTTGCCTGAAGAGTACAATCCGAGCCGTTCATGCTT
CAGCGGCGTCCTGCATAAGCCTTTCGCCATTGGCCAATTT

BPM2 16S rRNA Forward

GCAGGGCGCGTGCTACACATGCAGTCGAGCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGAGTAACACGTGAGCAACCTGC
CCCAAGCTTTGGGATAACCCTCGGAAACGGGGGCTAATACCGAATATTACTTCTGGCCGCATGGCTGGTGGTGGAAAGTTTTTCGGCTTGG
GATGGGCTCGCGGCCTATCAGCTTGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACA
CTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGGAAGCCTGATGCAGCGACGCCGCG
TGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGACGAAGCGAGAGTGACGGTACCTGCAGAAGAAGCACCGGCCAACTA
CGCCAGCAGCCGCGGTAAGACGTAGGGTGCGAGCGTTGTCCGGATTTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACCG
TGAAAACTTGGGGCTCAACTCCAAGCCTGCGGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGA
AATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGGTCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAAC
AGGATTAGATACCCTGGTAGTCCACGCTGTAAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCCTGTGCCGCAGCTAACGCATTA
AGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTC
GATGCAACGCGAAGAACCTTACCTGGGTTTGACATGGCCGCAAAACTCGCAGAGATGTGAGGTCCTTTCGGGGCGGTCACAGGTGGTGCA
TGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGATAGTCCCGCAACGAGCGCAACCCTCGCTCGATGTTGCAGCGCGTTATGGCGGGGAC
TCATCGAGACTGCGGGTCACCTCGGAGAGGTGGGGATGACGTCAGTCATCATGCCCTTATGTTCAGGCTTCACGCATGGCTACATGGATGC
ATACAATGGGCTTGCATAACCGTGAAGGGTGACCAAATC

BPM2 16S rRNA Reverse

TAGGCTCTTGTTACGACTTCGTCCAATCGCCAGCCCCACCTTCGACGGCTCCCTCCACAAGGGTTGGGCCACCGGCTTCGGGTGTTGCCG
ACTTTCGTGACGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCAGCGTTGCTGATCTGCGATTACTAGCGACTCCGACTT
CACGGGGTCGAGTTGCAGACCCCGATCCGAACTGAGACCGGCTTTTTGGGATTCGCTCCACCTCACGGTATCGCAGCCCATTGTACCGGCC
ATTGTAGCATGCGTGAAGCCCTGGACATAAGGGGCATGATGACTTGACGTCATCCCCACCTTCCTCCGAGTTGACCCCGGCAGTCTTCGAT
GAGTCCCCGCCATAACGCGCTGGCAACATCGAACGAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACA
GCCATGCACCACCTGTGACCGCCCCCGAAGGACCTCACATCTCTGCGAGTTTTGCGGCCATGTCAAACCCAGGTAAGGTTCTTCGCGTTGC
ATCGAATTAATCCGCATGCTCCGCCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTTAGCCTTGCGGCCGTACTCCCCAGGCGGGGCG
CTTAATGCGTTAGCTGCGGCACAGGGAACCGGAGAGGCCCCCCACACCTAGCGCCCAACGTTTACAGCGTGGACTACCAGGGTATCTAATC
CTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTATCGGCCCAGAGACCCGCCTTCGCCACCGGTGTTCCTCCTGATATCTGCGCATT
TCACCGCTACACCAGGAATTCCAGTCTCCCCTACCGAACTCTAGCCTGCCCGTATCGACCGCAGGCTTGGAGTTGAGCCCCAAGTTTTCAC
GGTCGACGCGACAAGCCGCCTACGAGCTCTTTTACGCCCAATAAATCCCGGACACGCTCGCACCCTACGTCTTACCGCGGCTGCTGGCACG
TAGTTGGCCGGTGCTTCTTTCTGCAGGTACCGTCACTCTCGCTTCGTCCCTGCTGGAAAGAGTTACACCCGAAGCGTCATCCCTCAGCGG
CGTCGCTGCATCAAGCTCCGCCAATTTGCCAAAGATTCCACTGCTGCCTTCCGTAGAGTCTTGGCCGGGG

BPM3 16S rRNA Forward

GAGGGAGCTTACCATGCAGTCGAGCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGAGTAACACGAGAGCAACCTGCCCCA
AGCTTTGGGATAACCCTCGGAAACGGGGGCTAATACCGAATATTACTTCTGGCCGCATGGCTGGTGGTGGAAAGTTTTTCGGCTTGGGA
TGGGCTCGCGGCCTATCAGCTTGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACAC
TGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGGAAGCCTGATGCAGCGACGCCGCG
TGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGACGAAGCGAGAGTGACGGTACCTGCAGAAGAAGCACCGGCCAACT
ACGTGCCAGCAGCCGCGGTAAGACGTAGGGTGCGAGCGTTGTCCGGATTTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCG
ACCGTGAAAACTTGGGGCTCAACCCCAAGCCTGCGGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGGAATTCCTGGTGTAG
CGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGGTCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGG
AGCGAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCCTGTGCCGCAGC
TAACGCATTAAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCAT
GCGGATTAATTCGATGCAACGCGAAGAACCTTACCTGGGTTTGACATGGCCGCAAAACTCACAGAGATGTGAGTCCTTTCGGGGCGGTC
ACAGGTGTGCATGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGTAGTCCCGCACGAGCGCATCCCTCGATCGATGGTGCAGCGCGTATG
GCGGGACTCATCGAGACTGGCTGGTTCACTCGAGAAGTGGGATGACGTCCAGTCATCATGCCCTTTATGTCCAGCTCAGGCATGGCCTAC
ATGCGGTCAATCGACTGCAATCCGCGAGGTGAACCGATTCCAAAAAGCGT

BPM3 16S rRNA Reverse

GTTGGGCTCTTGATTACGACTTCGTCCATCGCCAGCCCCACCTTCGACGGCTCCCTCCACAAGGGTTGGGCCACCGGCTTCGGGTGTTGC
CGACTTTCGTGACGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCAGCGTTGCTGATCTGCGATTACTAGCGACTCCGAC
TTCACGGGGTCGAGTTGCAGACCCCGATCCGAACTGAGACCGGCTTTTTGGGATTCGCTCCACCTCGCGGTATCGCAGCCCATTGTACCG
GCCATTGTAGCATGCGTGAAGCCCTGGACATAAGGGGCATGATGACTTGACGTCATCCCCACCTTCCTCCGAGTTGACCCCGGCAGTCTTC
GATGAGTCCCCGCCATAACGCGCTGGCAACATCGAACGAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGAC
GACAGCCATGCACCACCTGTGACCGCCCCCGAAGGACCTCACATCTCTGTGAGTTTTGCGGCCATGTCAAACCCAGGTAAGGTTCTTCGC
GTTGCATCGAATTAATCCGCATGCTCCGCCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTTAGCCTTGCGGCCGTACTCCCCAGGCG
GGGCGCTTAATGCGTTAGCTGCGGCACAGGGAACCGGAGAGGCCCCCCACACCTAGCGCCCAACGTTTACAGCGTGGACTACCAGGGTAT
CTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTATCGGCCCAGAGACCCGCCTTCGCCACCGGTGTTCCTCCTGATATCTG
CGCATTTCACCGCTACACCAGGAATTCCAGTCTCCCCTACCGAACTCTAGCCTGCCCGTATCGACCGCAGGCTTGGGGTTGAGCCCCAAGT
TTTCACGGTCGACGCGACAAGCCGCCTACGAGCTCTTTTACGCCCCAATAAATCCGGACAACGCTCGCACCCTACGTCTTACCCGCGGCTG
CTGGCACGTAGTTGGCCGGTGCTTCTCTGCAGGTACCGTCACCTCTCGCTCGTCCCTGCTGAAAGAGGTTACACCGAGCCGTCATCCCCT
CAGCGCCGTCGCTGCATCAGCTCCGGTCAATGTGCCATATTCCCCACTGCTGCTTCCGGTAGGATCTGGCCTGTCTCTCTCCAAGTTCCCA

BPM4 16S rRNA Forward

GCATGGGCGGGTGCTTACCATGCAGTCGAGCGGAAGGCCCCTTCGGGGGTACTCGAGCGGCGAACGGGTGAGTAACACGTGAGCAACC
TGCCCCCGACTCTGGGATAAGCCCGGGAAACCAGGTCTAATACCGGATACGACCATCTCTCTCCTGAGACGATGGTGGAAAGATTCATCGG
TTGGGGATGGGCTCGCGGCCTATCAGCTTGTTGGTGGGGTAACGGCCTACCAAGGCGACGACGGGTAACCGGCCTGAGAGGGCGACCG
GTCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCGCAATGGGCGGAAGCCTGACGCAGCGAC
GCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGACGAAGCGCAAGTGACGGTACCTGCAGAAGAAGCGCCGGC
TAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCG
TCTGTCGTGAAAGCCCACGGCTTAACCGTGGGTCTGCGGTGGATACGGGCAGACTAGAGGCAGGTAGGGGAGAATGGAATTCCCGGTGT
AGCGGTGAAATGCGCAGATATCGGGAGGAACACCGGTGGCGAAGGCGGTTCTCTGGGCCTGTACTGACGCTGAGGAGCGAAAGCGTGG
GGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGTTGGGCGCTAGGTGTGGGGTCCTTCCACGGATTCCGCGCCGTAGCT
AACGCATTAAGCGCCCCGCCTGGGGAGTACAGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGT
TGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATCGCCGGAATCCATCAGAGATGGTGGGTCCTTTTTGGGCCGGTGAC
AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTCCATGTGCCAGCACG
TAATTGGTGGGGACTCATGGGAGAACGCCGGGGTTCACTCGAGATGGTGGGATGACGTCAAGTCATCATGCCCGTTATGTCTGGACTGCA
ACATGCTAATTGTCGGGTTTACAGAAAGGCCTTGCCCAATTACTCGTTGAA

BPM4 16S rRNA Reverse

TCTGAGGGCAAATTGTTTCGACTTCGTCCAATCGCCGGCCCCACCTTCGACCGCTCCCCCCACAAGGGTTGGGCCACGGGCTTCGGGTG
TTGCCGACTTTCGTGACGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCAGCGTTGCTGATCTGCGATTACTAGCGACT
CCGACTTCACGAAGTCGAGTTGCAGACTTCGATCCGAACTGAGACCGGCTTTAAGGGATTCGCTCCACCTCACGGTATCGCAGCCCTCTG
TACCGGCCATTGTAGCATGTTTGCAGCCCAAGACATAAGGGGCATGATGACTTGACGTCATCCCCACCTTCCTCCGAGTTGACCCCGGCGG
TCTCCCATGAGTCCCCACCATTACGTGCTGGCAACATGGAACGAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGC
TGACGACAGCCATGCACCACCTGTCACCGGCCCAAAAAGGACCCACCATCTCTGATGGATTTCCGGCGATGTCAAGCCTTGGTAAGGTTCT
TCGCGTTGCGTCGAATTAAGCAACATGCTCCGCCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTTAGCCTTGCGGCCGTACTCCCCA
GGCGGGGCGCTTAATGCGTTAGCTACGGCGCGGAATCCGTGGAAGGACCCCACACCTAGCGCCCAACGTTTACGGCGTGGACTACCAGGG
TATCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTACAGGCCCAGAGAACCGCCTTCGCCACCGGTGTTCCTCCCGATATC
TGCGCATTTCACCGCTACACCGGGAATTCCATTCTCCCCTACCTGCCTCTAGTCTGCCCGTATCCACCGCAGACCCACGGTTAAGCCGTGG
GCTTTCACGACAGACGCGACAAACCGCCTACGAGCTCTTTACGCCCAATAATTCCGGACACGCTTGCGCCCTACGTATTACCGCGGGCTGC
TGGCACGTAGTTAGCCCGGCGCTTCTTCTGCAGGTACCGTCACTTGCGCTTCGTCCCTGCTGAAAGAGGTTACAACCGAAGGCGTCATCC
TCACGCGGCGTCGCTGCGTCAGCTTCCGCCATGCGCAATATTCCCCCACTGCTGCCTCCGTAGGAGTCTTGGATCGTTTCTCAGTTCAGT
GTGACGGATCGCCATCTTCAAGGGCCCGGTTACCCGATCGGTTCTCGACCTG

BPM5 16S rRNA Forward

TTATAGGGTGCTTACCATGCAGTCGAGCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCC
AAGCTTTGGGATAACCCTCGGAAACGGGGGCTAATACCGAATATTACTTCTGGCCGCATGGCTGGTGGTGGAAAGTTTTTCGGCTTGGGA
TGGGCTCGCGGCCTATCAGCTTGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACT
GGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGGAAGCCTGATGCAGCGACGCCGCGTG
AGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGACGAAGCGAGAGTGACGGTACCTGCAGAAGAAGCACCGGCCAACTACGT
GCCAGCAGCCGCGGTAAGACGTAGGGTGCGAGCGTTGTCCGGATTTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACCGT
GAAAACTTGGGGCTCAACTCCAAGCCTGCGGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGA
AATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGGGTCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAAC
AGGATTAGATACCCTGGTAGTCCACGCTGTAAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCCTGTGCCGCAGCTAACGCATTA
AGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTC
GATGCAACGCGAAGAACCTTACCTGGGTTTGACATGGCCCGCAAAACTCGCAGAGATGTGAGGTCCTTTCGGGGGCGGTCACAGGTGGT
GCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGGTAAGTCCCGCAACGAGCGCACCCTCGTTCGATGTGCAGCGCGTTATGGCGG
GGACTCATCGAGACTGCGGGTCACTCGGAGATGGTGGGGATGACGTCAGTCATCATGCCCTTATGTCAAGGCTCCAGCATGCTACATGGCG
GTACAGGTCTGCATACCGTCAGTGAGCGAATCCA

BPM5 16S rRNA Reverse

CCCTGGAGCTCTTGTTACGACTTCGTCCAATCGCCAGCCCCACCTTCGACGGCTCCCTCCACAAGGGTTGGGCCACCGGCTTCGGGTGTT
GCCGACTTTCGTGACGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCAGCGTTGCTGATCTGCGATTACTAGCGACTCCG
ACTTCACGGGGTCGAGTTGCAGACCCCGATCCGAACTGAGACCGGCTTTTTGGGATTCGCTCCACCTCACGGTATCGCAGCCCATTGTAC
CGGCCATTGTAGCATGCGTGAAGCCCTGGACATAAGGGGCATGATGACTTGACGTCATCCCCACCTTCCTCCGAGTTGACCCCGGCAGTCT
TCGATGAGTCCCCGCCATAACGCGCTGGCAACATCGAACGAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTG
ACGACAGCCATGCACCACCTGTGACCGCCCCCGAAGGACCTCACATCTCTGCGAGTTTTGCGGCCATGTCAAACCCAGGTAAGGTTCTTC
GCGTTGCATCGAATTAATCCGCATGCTCCGCCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTTAGCCTTGCGGCCGTACTCCCCAGG
CGGGGCGCTTAATGCGTTAGCTGCGGCACAGGGAACCGGAGAGGCCCCCCACACCTAGCGCCCAACGTTTACAGCGTGGACTACCAGGG
TATCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTATCGGCCCAGAGACCCGCCTTCGCCACCGGTGTTCCTCCTGATA
TCTGCGCATTTCACCGCTACACCAGGAATTCCAGTCTCCCCTACCGAACTCTAGCCTGCCCGTATCGACCGCAGGCTTGGAGTTGAGCCC
CAAGTTTTCACGGTCGACGCGACAAGCCGCCTACGAGCTCTTTACGCCCAATAAATCCCGGACAACGCTCGCACCCTACGTCTTACCGCGG
CTGCTGGGCACGGTAGTTGGGCGGTGCTTCTCTGCAGTACGTCACTCTCGCTTCGTCCTGCTGAAAGAGGTTTACCACCGACGCGGTCAT
CCCTCAGCGGCGTCGCTGCATCAGCTCGGCCAATGTGCATATTCCACTGCTGCTTCTGTAGAATCTGGTCGTGTCTCTCAAGTTCCA

AUTHOR CONTRIBUTIONS

FSG: Investigation, Methodology, Formal analysis, Writing review & editing. MD: Investigation, Formal analysis. GAA: Investigation, Methodology, Formal analysis, Writing review & editing. SA: Formal analysis. DK: Methodology, Investigation, Writing review & editing. ATÖ: Methodology, Formal analysis, Writing original draft & editing.

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ETHICS COMMITTEE APPROVAL

* This study does not require Ethics Committee Approval.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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