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Molecular identification of different actinomycetes isolated from East Black Sea region plateau soil by 16S rDNA gene sequencing

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In order to obtain different actinomycetes isolates from the East Black Sea Region plateau soil, an isolation study was carried out by using sucrose gradient method and different growth media. A molecular taxonomic method, 16S rDNA gene sequencing, was applied to obtain different actinomycetes isolates from East Black Sea region plateau soil. Phylogenetic dendrograms based on 16S rDNA nucleotide sequences were constructed by using neighbour-joining algorithm. In the light of phylogenetic analyses, it was determined that out of 15 organisms, two belong to *Actinomadura*, three *Kribbella*, three *Nocardia,* six *Micromonospora* and an organism of *Microbacterium*. Soil isolates obtained from this study contribute to taxonomy of actinomycetes and also many species will emerge.

Key words: *Actinomadura, Kribbella, Microbacterium, Micromonospora, Nocardia,* 16S rDNA gene sequencing.

INTRODUCTION

Most common groups of actinomycete have the capability to produce secondary metabolites with biological activities such as anticancer, antibiotic, antiviral, antifungal, enzyme, immunosuppressant and other industrially beneficial compounds (Baltz, 2007; Kekuda et al., 2010; Naine et al., 2011). Antibiotics have been isolated from almost all the suborders of actinomycetes. Considering increase in antibiotic resistance against widely used medicines, there is still a source of continuous novel antimicrobial agents from actinomycetes isolated from aquatic and terrestrial habitats (Baltz, 2006; Yang et al., 2011).

Actinomycete taxonomy was formerly thought to be associated with morphology, which is inadequate in differentiating between different species of many genera. The use of phylogenetic and molecular evolutionary approaches has been of great importance to the classification methods (Babalola et al., 2009; Hozzein and Goodfellow, 2011). Some organisms that are in advertently placed in an ineligible group are now classified appropriate owing to the advent of molecular techniques (Zhi et al., 2009). Recently, the identification of the species and phylogenies are commonly derived from 16S rDNA and the use of polymerase chain reaction (PCR) for sequence analyses (Wood et al., 2007; Zhi et al., 2009).

Majority of actinomycetes are commonly found in both aquatic and terrestrial habitats (Macagnan et al., 2006). The best known actinomycetes in soil is the genus *Streptomyces* although others, like *Norcardia, Microbispora*, *Micromonospora*, *Actinomyces*, *Actinoplanes* and *Streptosporangium,* have also been isolated from soil. The number and variety of actinomycetes present in any soil sample would be

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Table 1. Sources and strain histories of the test organisms.

Source of soil	Location	pH
Plateau soil	Gençbay, T., Plateau soil, Meşeli, Şavşat, Artvin	6.3
Plateau soil	Gençbay, T., Plateau soil, Aydıntepe, Bayburt	6.4
Plateau soil	Gençbay, T., Plateau soil, Kop dağı, Bayburt	6.5
Plateau soil	Gençbay, T., Plateau soil, Durundas, Çamoluk, Giresun	6.4
Plateau soil	Gençbay, T., Plateau soil, Çevrepınarı, Şiran, Gümüşhane	6.3
Plateau soil	Gençbay, T., Plateau soil, Şemsahat, Çayeli, Rize	6.5
Plateau soil	Gençbay, T., Plateau soil, Kaskar, Maçka, Trabzon	6.2

significantly influenced by geographical location, soil temperature; type and pH, organic matter content, agricultural activities, aeration, nutrient availability, moisture content and soil vegetation (Arifuzzaman et al., 2010).

Presently, different antimicrobial compounds likely to be obtained are decreasing day by day from actinomycete groups. Nowadays, obtaining new antibiotics from rare actinomycetes is industrially important (Hayakawa, 2008).

Actinomadura produce a stable, extensively branched substrate mycelium and aerial hyphae, which when formed, differentiate into chains of spores (Lechevalier and Lechevalier, 1970). Members of genus *Actinomadura* have a mesophilic structure and growth occurs at 25-40°C. Some types of thermophilic *Actinomadura* require 50-65°C optimum temperature. *Actinomadura* was established by Lechevalier and Lechevalier (1970) and was placed in the family *Thermomonosporaceae* containing four other genera, *Actinocorallia*, *Spirillospora*, *Thermomonospora* and *Actinoallomurus* (Zhang et al., 1998; Trujillo and Goodfellow, 2003).

The genus *Nocardia* is a member of the family *Nocardiaceae* and the order *Actinomycetales* (Stackebrandt et al., 1997). *Nocardia* form part of the soil microflora and play a role in the turnover of organic matter (Orchard, 1981); there is also evidence that some strains produce secondary metabolites of potential industrial value (Kinoshita et al., 2001). *Nocardia* grow slowly on non-selective culture media, and are strict aerobes with the ability to grow in a wide temperature range. Some species are non-pathogenic while others are responsible for nocardiosis (Conville and Witebsky, 2010).

Kribbella was established by Park et al. (1999) to accommodate nocardia form of actinomycetes that contain LL-diaminopimelic acid in the cell wall. *Kribbella* is aerobic, Gram-positive, motionless and vegetative mycelium appears cream in colour with highly branched hyphae, which fragment in both liquid and agar cultures (Urzi et al., 2008).

The genus *Micromonospora* included in *Micromonosporaceae* family are distributed widely in different environments, such as soils, water, sandstone and root nodules. *Micromonaspora* is presumably the second most prolific producer of antibiotics. This genus enables the production of gentamycin micromonosporin, megalomycin, maduramycin, halomycin, quinolidomycin, arisostatin A and B, and anthraquinone antibiotics (Qiu et al., 2008).

Microbacterium includes a diverse collection of Gram-positive, non-spore-forming rods that were isolated from various environmental habitats including water, soil, steep liquor, milk products and also from humans. Members of the genus *Microbacterium* were charac-terized largely by their marked heat resistance, presence in dairies, and production of small amounts of L(+) lactic acid from glucose (Brennan et al., 2001; Young et al., 2010)

Our literature search showed that there was no isolation study of rare actinomycetes of East Black Sea region in Turkey until now. Therefore, the present preliminary study was aimed to identify rare actinomycetes strains isolated from different localities of plateau soil and to place them in phylogeny by 16S rDNA gene sequencing.

MATERIALS AND METHODS

Selection of soil samples and isolation of microorganisms

For this study, in the six provinces of the Eastern Black Sea region, soil samples obtained from seven different localities were put in sterile container with sterile plastic bags. Collected samples were taken to the laboratory, numbered and stored at 4°C. Each soil sample weighed 20-25 g and was added to 100 ml beaker. Sufficient amount of distilled water was added and after 24 h, pH was measured with pH meter for each soil samples and values are also shown in Table 1.

Sucrose gradient method was applied as a selective isolation method (Yamamura et al., 2003). This application achieved 20% sucrose gradients to help in selection of different soil bacteria. 20% solution of sucrose was prepared in a screw cap centrifuge tube (105 mm).

This solution was added to 1 ml of purified spore suspensions of the test actinomycete strains prepared by using conventional technique and the tube was centrifuged (room temperature, 30 min, 240 g) in a swinging bucket rotor. After centrifugation, each sucrose layer (1 ml) was transferred sequentially from the top of the gradient using a different sterile pipette, and then diluted in a 10-fold series in sterile Ringer's solution (Merck, Darmstadt, Germany) (Hayakawa and Nonomura, 1987; Yamamura et al., 2003).

Table 2. Oligonucleotide primers used in the PCR amplification and sequencing of 16S rDNA.

Primary name	Sequence (5' to3')[a]	Binding Site[b]		Usage		Source
		5'	3'	PCR	Seq	
27f	AGAGTTTGATCTGGCTCAG	8	27	√		Lane, 1991
MG3f	CAGCAGCCGCGGTAATAC	520	536		√	Kagayema et al., 2004
MG5f	AAACTCAAAGGAATTGACGG	907	926		√	Chun, 1995
800r	TACCAGGGTATCTAATCC	800	782		√	Chun, 1995
1115r	AGGGTTGCGCTCGTTG	1115	1131		√	Gyobu and Miyadoh, 2001
1492r	TACGG**Y**TACCTTGTTACGACT	1492	1474		√	Gyobu and Miyadoh, 2001
1525r	AAGGAGGTG**W**TCCA**R**CC	1544	1525	√		Lane, 1991

[a]Degeneracies according to lane (1991): Y = C:T; R = A:G; W = A:T. [b]Binding site on the 16S rDNA molecule: Numbering according to *Escherichia coli* numbering system (Brosius et al., 1978).

Aliquots (200 µl) of this diluted suspensions were plated in triplicate on the surface of five solid media, that is, humic acid-vitamin (HV; Hayakawa and Nonomura, 1987), tryptone-yeast glucose extract (TYG; Blackall et al., 1989), glucose-yeast extract agar (GYEA; Gordon and Mihm, 1962) and oatmeal agar (Küster, 1959) plates supplemented with filter sterilised cycloheximide (50 µg ml^{-1}), nalidixic acid (10 µg ml^{-1}) and rifampicin (0,5 µg ml^{-1}), and then plates were incubated at 28°C for 14-21 days. Selective mediums were used to collect desired rare actinomycetes.

Isolates putatively assigned to the different actinomycetes on the basis of colony morphology, notably aerial spore mass colour, substrate mycelial pigmentation and the colour of diffusible pigments, were sub-cultured on glucose yeast extract agar, glucose yeast extract-malt extract agar (ISP 2), oatmeal agar (ISP 3) and tryptone-yeast extract agar and incubated at 28°C for 10 days. Observed morphological characteristics were compared with those of type strains by light microscopy under described conditions (Brennan et al., 2001).

Spore suspensions and mycelial fragments of the isolates were preserved in 20% glycerol (v/v) at -20°C until required.

Culture conditions and DNA extraction

Test strains were maintained on glucose-yeast extract agar (GYEA; Gordon and Mihm, 1962) slopes and as glycerol suspensions (20%, v/v) at -20°C. Before DNA extraction, biomass were grown in shake flasks containing modified tryptone-yeast glucose extract (TYG; Blackall et al., 1989) broth; these cultures were incubated for 6 to 8 days at 28°C. Chromosomal DNA was isolated by using a method by modifying "guanidine thiocyanate DNA isolation method" of Pitcher et al. (1989).

16S rDNA sequence analysis

The 16S rRNA genes (rDNA) were amplified by using universal primers 27f (5'-AGA GTT TGA TCM TGG CTC AG-3'; positions 8 to 27; (Lane, 1991) and 1525r (5'-AAG GAG GTG WTC CAR CC-3'; (Lane, 1991). Each PCR mixture (50 µl) contained primers (each at a concentration of 20 µM, Invitrogen, USA), a mixture of deoxynucleoside triphosphates (Promega) (each at a concentration of 25 µM), and Taq polymerase buffer (HotStarTaq®, Qiagen, Hilden, Germany). Chromosomal DNA (50-300 ng) and Taq polymerase (2.5 U, HotStarTaq®, Qiagen, Hilden, Germany) were added to the solution. The DNA thermal cycler (PCR Express, ThermoHybaid, Middlesex, UK) used for thermal amplification was programmed as follows: The conditions consisted of an initial denaturation at 95°C for 5 min; 35 cycles at 95°C for 1 min, 55°C for 2 min, and 72°C for 3 min; and a final extension step consisting of 72°C for 10 min. The PCR products were electrophoresed on 1% agarose gel (Merck, Germany) in 1×TBE buffer stained with ethidium bromide and were visualized with the Gene Genius Bio imaging system.

In order to assess the identities of the strains isolated, the nearly full-length sequences of the 16S rDNA gene were analyzed. The PCR products of the selected isolates were purified (QIAquick purification kits, Qiagen, Hilden, Germany) and sequencing was performed by ABI PRISM BigDye Terminator Cycle Sequencing kits (Macrogen, Netherland) with previously described oligonucleotide primers (Table 2).

The resulting 16S rDNA gene sequences (1.325-1.435 nucleotides) were used to search the GenBank/EMBL/DDBJ database with the BLAST program (http://www.ncbi.nlm.nih.gov/) to determine relative phylogenetic positions. Phylogenetic analysis was conducted using MEGA 5.0 (Tamura et al., 2011) by first generating a complete alignment of 16S rDNA gene sequences of the isolates and type strains of all valid species. A phylogenetic tree was inferred using neighbour-joining tree algorithms (Jukes and Cantor, 1969). 16S rDNA datasets were cooperatively analysed using MEGA 5.0 (Tamura et al., 2011) which was used to calculate evolutionary distances and similarity values. Topography of the constructed tree was evaluated by bootstrap analysis with 1000 replicates (Saitou and Nei, 1987). Only nodes with bootstrap values over 50% were considered to be significant.

Phylogenetic analysis

The current version of the EzTaxon-e (http://eztaxon-e.ezbiocloud.net/; Kim et al., 2012) server provides multiple sequence alignment by CLUSTAL W 1.8 (Thompson et al., 1994). The resultant sequence alignment can then be used for the neighbour-joining (Saitou and Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods using the MEGA 5.0 (Tamura et al., 2011) package; all were implemented within the server. The alignment can be exported for use by external programs including MEGA 5.0 (Tamura et al., 2011) and BioEdit (version 7.2.0, Hall, 1999). An evolutionary distance matrix was generated for the neighbour-joining as described by

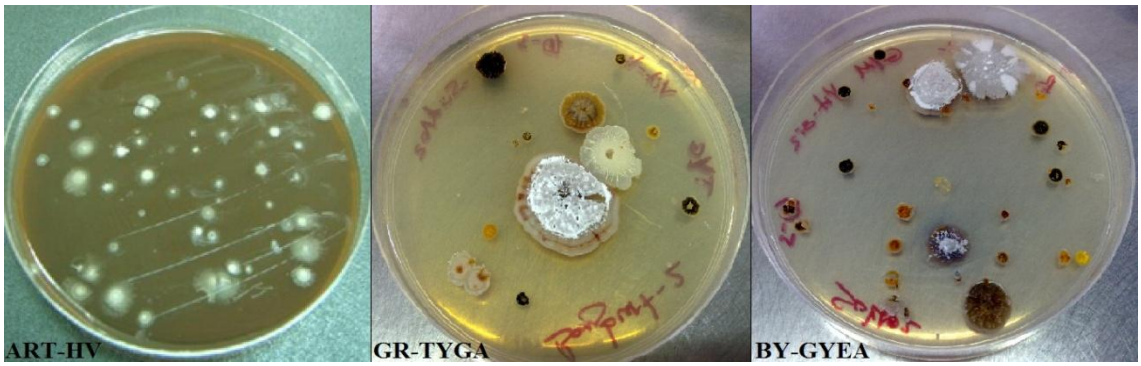

Figure 1. Isolation of soil samples taken from 3 different locations of plateau soil in the East Black Sea region; GR: Giresun, BY: Bayburt, ART: Artvin; HV: humic acid vitamin agar, TYGA: triptone yeast glucose extract agar, GYEA: glucose yeast extract agar.

Jukes and Cantor (1969). The resultant tree topology was evaluated by a bootstrap analysis (Felsenstein, 1985) with 1000 resamplings from the neighbour-joining dataset using Seqboot and Consense from the Phylip package (Felsenstein, 1993).

RESULTS

Many studies have been performed using several selective isolation techniques in order to assess the diversity of actinomycetes in natural habitats (Goodfellow and Fiedler, 2010). In this study, various pretreatment techniques and selective media have also been used for actinomycete isolation from plateau soil samples. Totally one hundred and one strain were isolated on humic acid-vitamin (HV), tryptone-yeast glucose extract (TYG) and glucose-yeast extract (GYEA) agars supplemented with nalidixic acid, rifampicin and cycloheximide (Yamamura et al., 2003) and incubated at 28°C for about two to three weeks (Figure 1).

The organisms obtained by using humic acid-vitamin agar (HV; Hayakawa and Nonomura, 1987) were more easily observed than by using tryptone-yeast glucose extract agar (Blackall et al., 1989). The organisms selected based on colony morphology were inoculated on oatmeal agar medium. Sporulating colonies with grey pigmentation on this medium were eliminated due to having high probability of being members of the genus *Streptomyces.*

Isolates putatively assigned to the rare actinomycetes on the basis of colony morphology-notably spore mass colour, substrate mycelial pigmentation and the colour of diffusible pigment were subcultured on oatmeal agar (Küster, 1959) and tryptone-yeast glucose extract agar (Blackall et al., 1989) and incubated 28°C for 10 days (Table 3). Indication of different pigmentation of fifteen rare actinomycetes colonies were selected to study further molecular techniques. All of the strains were maintained on glucose-yeast extract agar (GYEA; Gordon and Mihm, 1962) at 28°C and as glycerol suspensions (20%, v/v) stored at -20°C for future work.

Genomic DNA from bacteria likely to be involved in different actinomycetes groups is obtained by modifying “guanidine thiocyanate DNA Isolation Method” (Pitcher et al., 1989). The 16S ribosomal RNA gene was amplified by using the PCR method with Taq DNA polymerase and primers 27f and 1525r. The molecular-based methods using polymerase chain reaction (PCR) is suitable for taxonomic studies. Samples of PCR product patterns of some representatives of test strains are given in Figure 2. During the analysis based on 16S rDNA gene sequencing 800R, MG3f, MG5f, 1115r and 1492r primers were also used to get a nearly complete 16S rDNA sequence data of test starins.

16S rDNA gene sequence data was determined for the fifteen test strains according to Blast analysis and a 1309 nucleotide base pair was used for phylogenetic analysis and compared with 16S rDNA gene sequences of tree representatives of closely related type strains of Actinomycetes (Figure 3). Obtained results of nucleotide similarities (%) and differences based on 16S rDNA gene sequences data are given in Table 4.

Plateau soil samples *Actinomadura* sp. ART34 shares same 98.2 and 97.5% of 16S rDNA gene sequence similarity with the type strains of *A. glucoflavus* and *A. mexicana,* value corresponding to 18 and 25 nt difference. *Actinomadura* sp. ART64 shares 97.8% and 97.1% 16S rDNA gene sequence similarity with the type strains of *Actinomadura glucoflavus* and *Actinomadura mexicana*, value corresponding to 22 and 29 nt difference. *Actinomadura* sp. ART34 also shares 99.6% 16S rDNA gene sequence similarity with *Actinomadura* sp. ART64, value corresponding to 4 nt difference, respectively (Figure 3 and Table 4).

Kribbella sp. BY909 shares 98.7 and 97.0% of 16S rDNA gene sequence similarity with the type strain of *Kribbella sandramycini* and *Kribbella swartbergensis*, value corresponding to 12 and 30 nt difference, respectively. *Kribbella* sp. GR10 shares 97.4 and 97.2% of 16S rDNA gene sequence similarity with the type strain of *K. sandramycini* and *K. swartbergensis*, value

Table 3. Growth and cultural characteristics of the test organisms in different culture.

Isolate no:	Growth and cultural characteristics	TYGA	Oatmeal agar
Actinomadura sp. ART34	Growth:	+++	+
	Spore colour:	None	None
	Substrate mycelium colour:	Brown	Dark brown
	Colony morphology :	Spherical-spiral	Spherical- spiral
Actinomadura sp. ART64	Growth:	++	+
	Spore colour:	None	White
	Substrate mycelium colour:	Brown	Brown
	Colony morphology :	Spherical- spiral	Spherical- spiral
Kribbella sp. GR10	Growth:	+++	++
	Spore colour:	None	None
	Substrate mycelium colour:	Light brown	Light brown
	Colony morphology :	Curved-soft	Curved - soft
Kribbella sp. GR148	Growth:	+++	++
	Spore colour:	None	None
	Substrate mycelium colour:	Light brown	Light brown
	Colony morphology :	Curved - Soft	Curved - soft
Kribbella sp. BY909	Growth:	++	++
	Spore colour:	None	None
	Substrate mycelium colour:	Light brown	Light brown
	Colony morphology :	Curved - soft	Curved - soft
Microbacterium sp. BY803	Growth:	++	No growth
	Spore colour:	None	
	Substrate mycelium colour:	White	
	Colony morphology :	Soft -smooth	
Micromonospora sp. BY707	Growth:	++	+
	Spore colour:	Black	Dark brown
	Substrate mycelium colour:	Orange	Orange
	Colony morphology :	Spherical- puffy	Spherical- puffy
Micromonospora sp. BY300	Growth:	++	+
	Spore colour:	Black	None
	Substrate mycelium colour:	Orange	Orange
	Colony morphology :	Spherical- puffy	Spherical- puffy
Micromonospora sp. BY700	Growth:	++	+
	Spore colour:	Black	Dark brown
	Substrate mycelium colour:	Orange	Orange
	Colony morphology	Spherical -puffy	Spherical- puffy
Micromonospora sp. BY351	Growth:	++	+
	Spore colour:	Black	None
	Substrate mycelium colour:	Orange	Orange
	Colony morphology :	Spherical - puffy	Spherical - puffy
Micromonospora sp. BY368	Growth:	++	+
	Spore colour:	Black	None
	Substrate mycelium colour:	Orange	Orange
	Colony morphology :	Spherical - puffy	Spherical - puffy

Table 3. Contd.

Micromonospora sp. BY298	Growth:	++	+
	Spore colour:	Black	Dark brown
	Substrate mycelium colour:	Orange	Orange
	Colony morphology :	Spherical - puffy	Spherical - puffy
Nocardia sp. BY352	Growth:	+++	+
	Spore colour:	None	None
	Substrate mycelium colour:	White	Light brown
	Colony morphology :	Soft - smooth	Soft - smooth
Nocardia sp. GR130	Growth:	+++	+
	Spore colour:	None	None
	Substrate mycelium colour:	Light brown	Light brown
	Colony morphology :	Soft - smooth	Soft - smooth
Nocardia sp. GR14	Growth:	+++	+
	Spore colour:	None	None
	Substrate mycelium colour:	Light brown	Light brown
	Colony morphology :	Soft - smooth	Soft - smooth

Media: +++, Abundant; ++, moderate; +, poor.

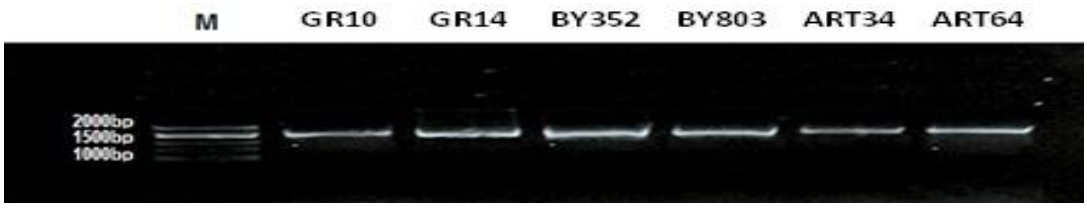

Figure 2. PCR amplification image of the 16S rDNA genes of actinomycetes isolated from different localities. M, DNA Marker (Fermentas™; 100kb Gene Ruler), GR: Giresun, BY: Bayburt, ART: Artvin.

corresponding to 26-28 nt difference. *Kribbella* sp. GR148 shares 98.6 and 97.7% of 16S rDNA gene sequence similarity with *Kribbella* sp. GR10 and *K. sandramycini*, value corresponding to 14-23 nt difference (Figure 3 and Table 4).

Micromonospora sp. BY707, BY351, BY368, BY300 and BY700 isolates showed that close 16S rDNA gene sequence similarity with the type strain of *M. chokoriensis* which are 97.7, 98.9, 99.0, 99.3 and 99.5%, values corresponding to 30, 11, 10, 7 and 5 nt differences, respectively. *Micromonospora* sp. BY298 shares 96.9% 16S rDNA gene sequence similarity with the type strain of *M. lupini,* value corresponding to 31 nt differences (Figure 3 and Table 4).

An isolate of *Microbacterium* sp. BY803 shares 99.5% 16S rDNA gene sequence similarity with the type strain of *M. paraoxydans*, value corresponding to 5 nt difference (Figure 3 and Table 4).

Soil sample *Nocardia* sp. BY352 shares 99.1% of 16S rDNA gene sequence similarity with the type strain of *Nocardia rhamnosiphila,* value corresponding to 8 nt difference. *Nocardia* sp. GR130 shares 99.6 and 98.0% of 16S rDNA gene sequence similarity with the type strain of *N. cyriacigeorgica* and *N. carnea,* value corresponding to 3 and 19 nt difference. *Nocardia* sp. GR14 shares 99.7 and 98.1% of 16S rDNA gene sequence similarity with the type strain of *Nocardia cyriacigeorgica* and *Nocardia carnea*, value corresponding to 2 and 19 nt difference, respectively (Figure 3 and Table 4).

DISCUSSION

Conventional culture methods are not satisfactory for analysis of actinomycetes. Therefore, 16S rDNA gene studies became the most popular, routine and convenient method for many research laboratories. 16S rDNA part of the ribosomes are required by all prokaryotic organisms to synthesize proteins. The gene sequence encoding 16S

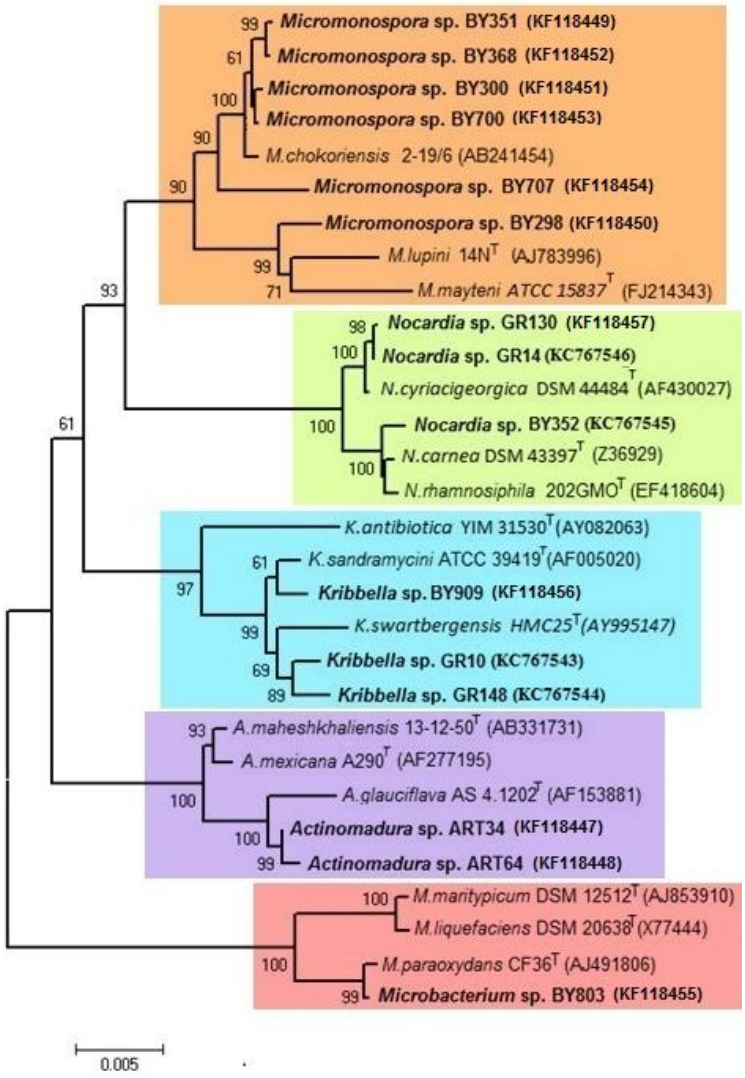

Figure 3. Neighbour-joining tree (Lane, 1991) based on nearly complete 16S rDNA gene sequences (1309 bp length) showing relationships between test isolate and closely related type strains of the genus *Actinomadura, Kribbella, Micromonospora, Microbacterium* and *Nocardia*. Numbers on branch nodes are bootstrap values (1000 resamplings; only values over 50% are given). Bar: 0.005 substitutions per nucleotide position. GenBank accession numbers are also given in parenthesis.

rDNA is a very suitable molecular marker to clarify phylogenetic relationships. 16S rDNA gene sequences have played a vital role in microbiology and can be utilized in many ways in various disciplines, notably taxonomy and ecology. It has led to a wealth of information concerning prokaryotic diversity (Zhi et al., 2009; Tindall et al., 2010).

The order *Actinomycetales* is considered the most promising source of bioactive natural products. The recent researchers screened intensively the marine, plant and medicinal plant, sediment and soil environments of actinomycetes to elucidate the structures of bioactive molecules produced by them (Qin et al., 2009; Kumar et al., 2010; Baskaran et al., 2011; Khanna et al., 2011; George et al., 2012; Kaewkla and Franco, 2013). Using 16S rDNA sequencing, in this preliminary study, we aimed at isolating rare actinomycetes, which could have high possibility of gene clusters for bioactive secondary metabolites. The strains from the genera *Actinomadura*, *Kribbella*, *Microbacterium, Micromonospora* and *Nocardia* obtained in our work might have great capacity for bioactive secondary metabolites such as antibiotics, antitumor agents, etc. In order to reveal biotechnological capabilities of these strains, further molecular and chemotaxonomic studies will be carried out.

In conclusion, preliminary isolation studies shows that

Table 4. Nucleotide similarities (%) and differences based on 16S rDNA gene sequences of the test and closely related valid strains of *Actinomadura, Kribbella, Micromonospora, Microbacterium* and *Nocardia.*

Taxon	*A. glucoflavus*	*A. maheshkhaliensis*	*A. mexicana*	*K. antibiotica*	*K. sandramycini*	*K. swartbergensis*	*M. chokoriensis*	*M. lupini*	*M mayteni*	*N. cyriacigeorgica*	*N.carnea*	*N. rhamnosiphila*	*M. maritypicum*	*M. liquefaciens*	*M. paraoxydan*	*Actinomadura* sp. ART34	*Actinomadura* sp. ART64	*Kribbella* sp.BY909	*Kribbella* sp.GR10	*Kribbella* sp.GR148	*Micromonospora* sp. BY351	*Micromonospora* sp. BY298	*Micromonospora* sp. BY368	*Micromonospora* sp. BY300	*Micromonospora* sp. BY700	*Micromonospora* sp. BY707	*Microbacterium* sp. BY803	*Nocardia* sp. GR130	*Nocardia* sp. GR14	*Nocardia* sp. BY352
A. glucoflavus	-	33	40	125	137	146	114	116	112	147	150	149	154	154	146	18	22	137	139	139	120	111	119	120	118	115	144	150	149	153
A. maheshkhaliensis.	96.7	-	8	133	101	113	81	123	120	110	113	112	162	163	145	26	30	102	106	105	87	118	86	87	85	82	144	113	112	115
A. mexicana	96.1	99.2	-	134	106	114	82	121	120	111	114	115	163	164	146	25	29	103	107	107	87	117	87	87	86	87	144	114	113	119
K. antibiotica	88.4	87.7	87.7	-	55	75	114	101	96	139	144	145	133	131	156	113	117	50	60	62	111	85	111	110	108	128	154	142	141	149
K. sandramycini	87.4	90.5	90.1	94.6	-	29	80	125	123	104	110	111	167	168	157	126	131	12	26	23	81	115	81	84	83	97	156	107	107	114
K. swartbergensis	86.7	89.5	89.3	92.8	97.1	-	86	135	133	113	120	124	182	183	166	136	141	30	28	26	93	128	94	93	91	109	165	116	115	125
M. chokoriensis	89.3	92.2	92.2	89.4	92.4	91.9	-	55	73	84	93	93	164	165	154	101	106	82	85	86	11	47	10	7	5	30	151	87	87	96
M. lupini	89.2	88.6	88.8	90.5	88.4	87.6	94.7	-	50	131	138	139	150	148	160	101	106	128	130	132	61	31	60	59	58	75	158	133	133	137
M mayteni	89.6	88.9	88.9	90.9	88.6	87.7	93.0	95.1	-	129	135	137	143	141	151	98	102	127	131	132	75	45	75	74	75	64	148	132	131	137
N. cyriacigeorgica	86.6	89.7	89.6	87.3	90.2	89.5	92.0	88.0	88.1	-	16	18	177	178	168	134	139	109	112	111	87	121	87	86	87	94	167	3	2	23
N.carnea	86.4	89.5	89.3	86.8	89.7	88.9	91.2	87.3	87.6	98.3	-	6	186	187	177	137	142	115	118	123	93	127	94	95	95	100	175	19	19	10
N. rhamnosiphila	86.4	89.6	89.3	86.7	89.6	88.5	91.2	87.3	87.4	98.2	99.3	-	185	186	176	136	141	116	121	123	96	130	95	95	96	99	174	21	20	8
M. maritypicum	86.0	85.4	85.3	87.8	85.0	83.8	85.2	86.4	87.0	84.2	83.4	83.5	-	6	46	144	149	171	175	175	165	140	164	166	165	171	41	180	179	190
M. liquefaciens	86.0	85.3	85.2	88.0	84.9	83.7	85.1	86.5	87.1	84.1	83.4	83.4	99.3	-	50	143	147	172	176	176	166	138	165	167	166	172	45	181	180	191
M. paraoxydan	86.7	86.7	86.7	85.9	85.8	85.1	86.0	85.5	86.3	84.9	84.2	84.3	95.5	95.1	-	137	142	160	162	162	157	152	156	156	155	160	5	171	170	181
***Actinomadura* sp. ART34**	98.2	97.4	97.5	89.5	88.3	87.5	90.5	90.5	90.8	87.7	87.4	87.5	86.8	87.0	87.4	-	4	125	127	128	107	97	106	107	105	102	134	137	136	140
***Actinomadura*sp. ART64**	97.8	97.0	97.1	89.1	88.0	87.1	90.1	90.1	90.4	87.3	87.0	87.1	86.4	86.6	87.0	99.6	-	130	130	133	111	101	110	111	109	107	139	142	141	144
***Kribbella* sp.BY909**	87.4	90.4	90.3	95.1	98.7	97.0	92.2	88.2	88.3	89.8	89.3	89.2	84.7	84.6	85.5	88.4	88.0	-	19	18	85	120	87	87	86	98	159	107	107	114
***Kribbella* sp.GR10**	87.3	90.1	90.0	94.2	97.4	97.2	91.9	88.0	88.0	89.6	89.0	88.8	84.4	84.3	85.4	88.3	88.0	98.1	-	14	87	118	87	86	83	103	161	110	109	118
***Kribbella* sp.GR148**	87.3	90.2	90.0	94.0	97.7	97.4	91.9	87.9	87.9	89.6	88.6	88.6	84.4	84.3	85.4	88.2	87.8	98.2	98.6	-	89	123	89	88	86	106	161	109	108	121
***Micromonospora* sp. BY351**	88.9	91.8	91.7	89.6	92.2	91.2	98.9	94.1	92.8	91.7	91.2	90.9	85.1	85.1	85.8	90.0	89.6	91.9	91.8	91.5	-	38	2	7	6	34	154	91	90	97
***Micromonospora* sp. BY298**	89.6	89.0	89.1	91.9	89.3	88.2	95.4	96.9	95.6	88.8	88.3	88.0	87.2	87.3	86.2	90.9	90.5	88.9	89.0	88.6	96.3	-	38	43	42	69	149	123	123	130
***Micromonospora* sp. BY368**	88.9	91.9	91.8	89.6	92.2	91.1	99.0	94.1	92.8	91.7	91.1	91.0	85.2	85.1	85.9	90.1	89.7	91.8	91.8	91.5	99.7	96.2	-	6	5	33	153	91	90	98
***Micromonospora* sp. BY300**	88.9	91.8	91.7	89.7	92.0	91.2	99.3	94.3	92.9	91.9	91.0	91.0	85.1	85.0	85.9	90.0	89.6	91.7	91.9	91.6	99.3	95.7	99.3	-	2	31	153	89	88	98
***Micromonospora* sp. BY700**	89.0	91.9	91.9	89.9	92.1	91.4	99.5	94.4	92.8	91.8	91.0	90.9	85.1	85.1	86.0	90.2	89.8	91.9	92.1	91.9	99.3	95.8	99.4	99.7	-	32	152	90	89	99
***Micromonospora* sp. BY707**	89.3	92.2	91.8	88.2	90.9	89.8	97.0	92.8	93.8	91.1	90.6	90.7	84.7	84.6	85.5	90.4	90.0	90.8	90.3	90.1	96.7	93.4	96.7	96.9	96.8	-	158	92	92	98
***Microbacterium* sp. BY803**	86.9	86.9	86.8	86.0	85.9	85.1	86.3	85.7	86.5	85.0	84.4	84.0	96.0	95.6	99.5	87.7	87.3	85.6	85.4	85.4	86.0	86.4	86.1	86.1	86.2	85.7	-	170	169	179
***Nocardia* sp. GR130**	86.4	89.5	89.4	87.0	89.9	89.2	91.7	87.7	87.9	99.6	98.0	97.9	84.0	83.9	84.7	87.4	87.0	89.9	89.7	89.8	91.4	88.6	91.4	91.5	91.5	91.3	84.7	-	1	22
***Nocardia* sp. GR14**	86.4	89.6	89.5	87.1	90.0	89.3	91.8	87.8	88.0	99.7	98.1	98.0	84.1	84.0	84.7	87.5	87.1	90.0	89.8	89.9	91.5	88.6	91.5	91.6	91.5	91.3	84.8	99.9	-	21
***Nocardia* sp. BY352**	86.1	89.3	88.9	86.4	89.4	88.4	90.9	87.4	87.4	97.7	99.0	99.1	83.1	83.1	83.9	87.2	86.8	89.4	89.0	88.8	90.9	88.0	90.8	90.8	90.7	90.8	84.1	97.8	97.9	-

the obtained results are in harmony with literature of Yamamura et al. (2003) and also indicate that isolation of many novel actinomycetes from plateau soil of East Black Sea region have a great capacity to be added as a merit species to the literature from Turkey.

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