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Prevalence of *Thermoactinomyces thalpophilus* and *T. sacchari* strains with biotechnological potential at hot springs and soils from West Anatolia in Turkey

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Abstract: Hot spring sediment and soil samples from West Anatolia in Turkey were investigated for the occurrence of thermophilic Actinomycetes. Isolation was done using Actinomycetes isolation agar (AIA), starch casein agar and glycerol yeast extract agar at 55 °C. Extracellular protease activity of the isolates were screened by using AIA plus 1% casein according to the hydrolysis zones surrounding the colonies. Antimicrobial activities of the isolates were also screened by using streaked plate method against a panel of test bacteria. Identification of the isolates was made by cultural, physiological characteristics and 16S rDNA sequence similarity. Sixty-seven thermophilic Actinomycetes isolates were classified in *Thermoactinomyces thalpophilus* and *T. sacchari* species. Among these, 62 isolates (92.5%) were found to be extracellular protease producers and 38 isolates (56.7%) were found active against methicillin resistant *Staphylococcus aureus* and *Enterococcus faecalis*. Based on these results, we suggest that the thermophilic actinomycetes, which are a part of the biodiversity of the hot springs and soils from West Anatolia in Turkey, are promising sources for novel enzymes and antimicrobial compounds.

Key words: *Thermoactinomyces*, *T. thalpophilus T. sacchari,* thermophilic Actinomycetes, protease, 16S rDNA sequence, phylogenetic analysis, hot springs

Türkiye Batı Anadolu sıcak su kaynakları ve topraklarındaki biyoteknolojik potansiyele sahip *Thermoactinomyces thalpophilus* ve *T. sacchari* suşlarının prevalansı

Özet: Türkiye, Batı Anadolu'dan alınan sıcak su kaynağı sedimenti ve toprak örnekleri termofilik aktinomiset mevcudiyeti bakımından araştırıldı. İzolasyon, 55 °C'de Aktinomiset izolasyon agar (AIA), Nişasta kazein agar ve Gliserol maya özütü agar kullanılarak yapıldı. İzolatların hücre dışı proteaz aktiviteleri %1 kazein içeren AIA kullanılarak kolonileri çevreleyen hidroliz zonlarına göre tarandı. İzolatların antimikrobiyal aktiviteleri de çizgi plaka yöntemi ile bir seri test bakterisine karşı tarandı. İzolatların tanılanmaları kültürel fizyolojik karakteristiklere ve 16S rDNA dizi benzerliklerine göre yapıldı. Altmış yedi termofilik aktinomiset izolatı *Thermoactinomyces thalpophilus* ve *T. sacchari* türleri içinde sınıflandırıldı. Altmış iki izolatın (%92,5) hücre dışı proteaz üreticisi olduğu ve 38 izolatın da (%56,7) metisillin dirençli *Staphylococcus aureus* ve *Enterococcus faecalis*'e karşı aktif oldukları belirlendi. Bu sonuçlardan hareketle, Türkiye, Batı Anadolu'daki sıcak su kaynakları ve topraklarındaki biyoçeşitliliğin bir parçası olan termofilik aktinomisetlerin yeni enzimler ve antimikrobiyal maddeler için umut vaat edici kaynaklar olabilecekleri öngörülmektedir.

Anahtar sözcükler: *Thermoactinomyces*, *T. thalpophilus T. sacchari,* termofilik aktinomisetler, proteaz, 16S rDNA dizisi, filogenetik analiz, termal kaynaklar

Introduction

Actinomycetes are gram positive, mostly aerobic, largely mycelial and primarily soil organisms that have high GC contents. They have an important ecological role for biodegradation of organic materials; they are also a commercially important class of microorganisms. The major attention has been focused mainly on the more commonly isolated genera such as *Streptomyces* and *Micromonospora* (1,2).

In recent years, researchers have shown great interest in thermophilic Actinomycetes because of their economical potential, either in useful biological processes such as biodegradation (3), or in the production of antibiotics (4) and enzymes (5,6). Thermophilic Actinomycetes exist in the genera *Saccharomonospora*, *Saccharopolyspora*, *Streptomyces*, *Thermoactinomyces*, *Thermobifida*, and *Thermomonospora* (7). Among these thermophilic Actinomycetes, the genus *Thermoactinomyces* has industrial and clinical importance. Some *Thermoactinomyces* strains are known as potent protease producers and can contribute to hypersensitivity pneumonitis including farmer's lung diseases and bagassosis (1,8).

Isolation and identification of thermophilic Actinomycetes from different sources have been studied in many countries and their identification has been made by various methods such as numerical taxonomy (9), fatty acid composition analysis (10), ribosomal protein analysis (11), and 16S rDNA sequence analysis (7,12,13). Among these methods, 16S rDNA sequence analysis has been established as a powerful technique for phylogenetic investigations of microorganisms (14). The 16S rDNA sequence based phylogenetic analysis can be made from genomic DNA isolated from bacteria or directly from total DNA isolated from environment. There are many reports on 16S rDNA sequence based phylogenetic analysis of thermophilic Actinomycetes from different countries (1,7,9,12) but not from West Anatolia in Turkey. This study was done to investigate the prevalence of *Thermoactinomyces* spp. in some hot springs and soils from West Anatolia in Turkey.

Materials and methods

Samples: A total of 27 samples (14 hot spring sediments and 13 soils) were collected from West Anatolia in Turkey. The sample stations are shown in the Table. The sediment samples taken from the hot springs were collected in sterile glass bottles and soil samples were taken from 15-20 cm below the surface with a sterile spoon into clean polyethylene bags and deposited in an icebox until arrival at the microbiology laboratory within 24 h.

Isolation of *Thermoactinomyces* strains: A portion of the sediment and soil samples was mixed with 1% $CaCO_3$ and dried at 100 °C for 1 h to provide selectivity for the isolation of *Thermoactinomyces* spp. since most of the strains of this genus were spore forming and thermophilic/thermotolerant microorganisms. The following media were used for isolation: (i) Actinomycetes isolation agar (AIA) (Difco), (ii) starch casein agar (pH 7.2) supplemented with cycloheximide (50 mg mL^{-1}) and filter sterilised rifampicin (0.5 mg mL^{-1}) and (iii) glycerol yeast extract agar. Samples were serially diluted and spread on to isolation media. The plates were incubated at 55 °C in humidified atmosphere for 2-3 days. The isolates were subcultured on AIA until obtaining pure cultures with incubation at 55 °C. Stock cultures were prepared with 50% (v/v) glycerol and stored at -70 °C.

Identification

The isolates were identified according to *Bergey's Manual of Systematic Bacteriology* and 16S rDNA sequence analysis (15). Colony morphology, colour of aerial and substrate mycelium, sporophore morphology, endospore formation (endospore staining and survival after heat treatment at 90 °C for 30 min), soluble pigment production on CYC agar supplemented with L-tyrosine (0.5% w/v) and novobiocin tolerance (25 µg/mL) were tested. Culture odours, growth at different temperatures (30, 45, 50, 55, 60, and 65 °C) were investigated. Growth at pH 11 and 5% (w/v) salt concentration was also determined.

DNA extraction: High-molecular-weight genomic DNA was purified from isolates using the guanidium thiocyanate method described by Pitcher et al. (16). Briefly, Actinomycetes cells (25-50 µL pellet volume in a microcentrifuge tube) cultured on a broth medium (soluble starch; 20 g/L, Peptone 5 g/L) were treated with 100 µL of a solution of lysosyme 2 mg/mL in sucrose 25%, 50 mM Tris/HCl, pH 8.0. After the incubation for 10 min at 20 °C, cells were dissolved

Table. Sample locations, 16S rDNA sequence based identity, and bioactivity properties of the isolates.

Sample no.	Location	Sample type	Isolate no.	Protease activity	Antimicrobial activity (mm)			
					S. aureus	*E. coli*	*E. faecalis*	*K. pneumonia*
1	Salihli Sart	Sediment	9 – 2	+	0	0	0	0
1	Salihli Sart	Sediment	9 – 3	+	7	0	12	0
1	Salihli Sart	Sediment	9 – 4	+	4.5	0	4	0
1	Salihli Sart	Sediment	9 – 5	+	5	0	5	0
2	Salihli Sart	Sediment	10 – 2	+	4	0	4.5	0
3	Salihli Kurşunlu	Sediment	13 – 1	-	6	0	4	0
4	Salihli Kurşunlu	Sediment	15 – 2	+	3.5	0	3.5	0
4	Salihli Kurşunlu	Sediment	15 – 3	+	0	0	0	0
5	Salihli Kurşunlu	Soil	16 – 2	+	0.5	0	0	0
5	Salihli Kurşunlu	Soil	16 – 3	+	0	0	0	0
5	Salihli Kurşunlu	Soil	16 – 7	-	3	0	1.5	0
5	Salihli Kurşunlu	Soil	16 – 15	+	8	0	10.5	0
6	Balıkesir Sındırgı	Soil	27 – 1	+	0	0	0	0
7	Balıkesir Sındırgı	Soil	31 – 1	+	0	0	0	0
7	Balıkesir Sındırgı	Soil	31 – 3	+	0	0	0	0
8	Balıkesir Sındırgı	Sediment	32 – 1	+	4	0	3	0
8	Balıkesir Sındırgı	Sediment	32 – 2	+	3	0	2	0
9	Balıkesir Sındırgı	Soil	33 – 2	+	1	0	2	0
9	Balıkesir Sındırgı	Soil	33 – 3	+	8	0	5	0
10	Balıkesir Bigadiç	Soil	34 – 1	+	5	0	4.5	0
10	Balıkesir Bigadiç	Soil	34 – 1b	+	6	0	4.5	0
10	Balıkesir Bigadiç	Soil	34 – 2	+	6	0	4.5	0
10	Balıkesir Bigadiç	Soil	34 – 4	+	0	0	0	0
10	Balıkesir Bigadiç	Soil	34 – 7	+	0	0	0	0
10	Balıkesir Bigadiç	Soil	34 – 10	+	0	0	0	0
10	Balıkesir Bigadiç	Soil	34 – 11	+	2	0	3	0
10	Balıkesir Bigadiç	Soil	34 – 12	+	7.5	0	7	0
10	Balıkesir Bigadiç	Soil	34 – 13	+	10	0	7.5	0
10	Balıkesir Bigadiç	Soil	34 – 17	+	1.5	0	0	0
10	Balıkesir Bigadiç	Soil	34 – 18	+	2	0	1.5	0
10	Balıkesir Bigadiç	Soil	34 – 36	+	7	0	7	0
11	Balıkesir Bigadiç	Soil	35 – 2	+	0	0	4	0
12	Balıkesir Bigadiç	Sediment	37 – 2	+	0	0	2	0

Table. Contunied

Sample no.	Location	Sample type	Isolate no.	Protease activity	Antimicrobial activity (mm)			
					S. aureus	*E. coli*	*E. faecalis*	*K. pneumonia*
12	Balıkesir Bigadiç	Sediment	37 – 2b	+	0	0	0	0
12	Balıkesir Bigadiç	Sediment	37 – 3a	+	0	0	0	0
12	Balıkesir Bigadiç	Sediment	37 – 7	+	0	0	0	0
13	İzmir Bornova	Soil	40 – 1	+	10	0	11	0
13	İzmir Bornova	Soil	40 – 4	+	10	0	8	0
13	İzmir Bornova	Soil	40 – 5	+	3	0	1	0
14	İzmir Bostanlı	Soil	43 – 2	+	4	0	3	0
14	İzmir Bostanlı	Soil	43 – 5	+	6	0	6	0
15	İzmir Bostanlı	Soil	44 – 2	-	0	0	0	0
15	İzmir Bostanlı	Soil	44 – 4	+	0	0	0	0
16	İzmir Bostanlı	Soil	45 – 1	+	7	0	7	0
17	Balıkesir Ayvalık	Sediment	46 – 1	+	7	0	2	0
17	Balıkesir Ayvalık	Sediment	46 – 2	+	0	0	0	0
18	Çanakkale Ayvacık	Soil	48 – 2	+	0	0	0	0
18	Çanakkale Ayvacık	Soil	48 – 3	+	0	0	0	0
18	Çanakkale Ayvacık	Soil	48 – 6	+	0	0	0	0
18	Çanakkale Ayvacık	Soil	48 – 8	+	0	0	1	0
18	Çanakkale Ayvacık	Soil	48 – 9	+	0	0	7	0
19	Çanakkale Tuzla	Sediment	51 – 9	+	0	0	0	0
20	Çanakkale Kestanbol	Soil	58 – 1	+	2	0	0	0
20	Çanakkale Kestanbol	Soil	58 – 2a	+	0	0	0	0
20	Çanakkale Kestanbol	Soil	58 – 26	+	0	0	0	0
20	Çanakkale Kestanbol	Soil	58 – 3	+	3	0	3	0
21	Çanakkale Bayramiç	Sediment	60 – 1	+	2	0	2	0
23	Çanakkale Etili tepe	Soil	67 – 1	+	0	0	0	0
23	Çanakkale Etili tepe	Sediment	67 – 20	+	0	0	1	0
24	Çanakkale Biga Kırgeçit	Sediment	71 - 1	+	0	0	0	0
24	Çanakkale Biga Kırgeçit	Sediment	71 – 2	+	4	0	0	0
24	Çanakkale Biga Kırgeçit	Sediment	71 – 3	+	4	0	0	0
24	Çanakkale Biga Kırgeçit	Sediment	71 – 6	+	0	0	0	0
25	Çanakkale Yenice Hıdırlar	Sediment	73 – 1	+	3	0	3	0
25	Çanakkale Yenice Hıdırlar	Sediment	73 – 2	+	0	0	0	0
26	Dikili Nebiler	Sediment	85 – 1	-	0	0	0	0
27	Dikili Nebiler	Sediment	86 – 1	+	0	0	0	0

by addition of 200 µL of 5 M guanidine isothiocyanate, 0.1 M EDTA, pH 7.0, and 150 µL of ammonium acetate was added to the cleared solution. The lysate was emulsified with an equal volume of chloroform:isoamyl alcohol (24:1, v/v) and the phases were separated by centrifugation at 10,000 × *g* for 2 min. The aqueous phase was then transferred to an Eppendorf tube and the DNA was precipitated by addition of 0.54 volumes of propan-2-ol, collected by centrifugation, and washed twice in 70% (v/v) ethanol. The final pellet was dried and resuspended in TE buffer (10 mM Tris/HCl, pH 8.0, 1 mM EDTA). The genomic DNA was quantitated and assessed for integrity by agarose gel electrophoresis.

Sequencing of 16S rDNA and phylogenetic tree construction

All PCR reactions were performed with the Gene Amp®PCR system (Applied Biosystems). PCR reactions were performed in 50 µL mixtures containing 10 ´ PCR buffer, 1.5 mM $MgCl_2$, 0.4 mM dNTP, 0.2 mM primer, 1.25 U of *Taq* DNA polymerase (Amplitaq Gold, Applied Biosystems), and 50 to 500 ng of genomic DNA template. The primers used for the amplification of the 16S rDNA were FC27 (5'- AGAGTTTGATCCTGGCTCAG-3) and RC1492 (5'-TACGGCTACCTTGTTACGACTT - 3') (17). PCR conditions for 16S rDNA reactions were 1 cycle of denaturation for 5 min at 94 °C; 30 amplification cycles consisting of denaturation (94 °C for 30s), primer annealing (49 °C for 30 s), and primer extension (72 °C for 90s); and a final extension of 7 min at 72 °C.

16S rDNA amplicons were cleaned with Nucleospin Extract Kit (Takara Inc.) and bidirectionally sequenced on an ABI 3100 automated sequencer (Applied Biosystems, Foster City, California). Cycle sequencing conditions for all reactions involved 40 to 60 ng of template DNA, 3.2 pmol of primer, 4 mL of Big Dye (Applied Biosystems), and water to a final volume of 20 mL. 16S rDNA reactions were primed with primers FC27 and RC1492. The following internal primers were used to ensure overlapping sequences for analysis of 16S rDNA sequences: F514 (5' GTGCCAGCAGCC GCGGTAA-3') and F1114 (5'-GCAAC GAGCGC AACCC-3) and the reverse primers R530 (5'- CCG CGGCTGCTGGCACGTA-3') and R936 (5'- GTGC GGGCCCCCGTCAATT-3').

Sequence files in ABI format were transformed to FASTA format by using ChromasPro v1.22 (Technelysium Pty Ltd). Nucleotide sequences of reference species were downloaded from NCBI Gene Bank. BLAST analysis was performed for isolates by using 16S rDNA sequence data. Bio Edit sequence alignment editor v7.0.1 (Isis Pharmaceutical Inc.) was used for Multiple Sequence Alignments (18). Maximum sequence differences were set as 0.75 and neighbour joining tree method was used with Mega v2.1 for phylogenetic tree constructions (19).

Screening of Extracellular Protease Production and Antimicrobial Activity: *Thermoactinomyces* isolates were screened for their protease and antimicrobial activities. For the determination of the protease activity, isolates were streaked onto AIA containing 1% (g/L) casein. Clear hydrolysis zones surrounding the colonies were evaluated as protease producers.

For the determination of the antimicrobial activity, isolates were streaked across one-third of the Mueller Hinton plates (Oxoid) and incubated at 50 °C for 48 h. After the incubation period, the test bacteria were streaked perpendicular to the thermophilic Actinomycetes and the plates were further incubated at 37 °C for 24 h. Methicillin resistant *Staphylococcus aureus* (MRSA) RSSK 95047, enteropathogenic *Escherichia coli* O157:H7 RSSK 232, *Enterococcus faecalis* ATCC 29212, and *Klebsiella pneumoniae* CCM 2318 were used as test bacteria. The inhibition zone between the test bacterium and actinomycetes isolates was recorded.

Results and discussion

AIA, starch–casein agar, and glycerol yeast extract agar were used for *Thermoactinomyces* isolation. AIA yielded a great number of Actinomycetes colonies compared to other media and was found more useful for isolation. A total of 67 *Thermoactinomyces* strains were isolated from 14 hot spring sediments and 13 soil samples.

Isolates were identified according to the phenotypic characteristics (data not shown) and 16S rDNA gene sequence analysis (Figure). The genus *Thermoactinomyces* currently consists of 8 species; *T.*

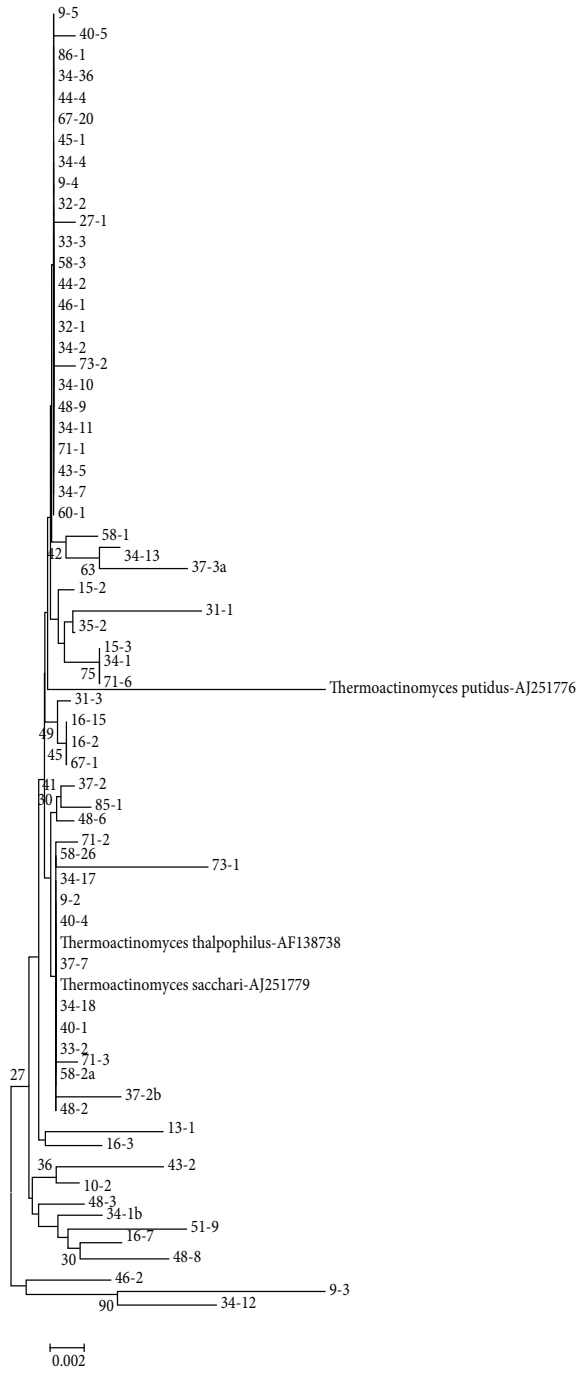

Figure. 16S rDNA sequence based phylogenetic analysis of thermophilic Actinomycetes isolated from hot springs and soil samples from West Anatolia in Turkey. The phylogenetic tree shows a close relatedness among the *T. thalpophilus* and *T. sacchari* strains. The scale bar presents 2 nucleotide substitutions per 1000 nucleotides. Bootstrap values (expressed as percentages of 1000 replications) greater than 20% are shown at the branch points.

candidus, *T. dicotomicus*, *T. intermedius*, *T. peptonophilus*, *T. putidus*, *T. sacchari*, *T. thalpophilus*, and *T. vulgaris* (8). These species are thermotolerant except the *T. peptonophilus* (11). From the phylogenetic tree, it can be concluded that all of the isolates could be classified in 2 species, *T. thalpophilus* and *T. sacchari*. While 26 (38.8%) of the strains were isolated from the sediment samples, 41 strains (61.2%) were isolated from the soil samples. Most of the strains were also able to grow at pH 11 except 3 strains (9-2, 32-2, and 37-3). In addition, the strain 58-2 was found resistant to 5% (w/v) NaCl concentration and considered as moderate halotolerant.

A selective enrichment was applied to the samples before isolation since the aim of the study was to investigate the prevalence of *Thermoactinomyces* spp. in some hot springs and soils from West Anatolia in Turkey. Therefore, other thermophilic Actinomycetes genera were not isolated in our study since the only endospore forming genus was *Thermoactinomyces*. Among the *Thermoactinomyces* isolates only *T. sacchari* and *T. talpophilus* strains were recovered from the samples, which were considered as synonyms among other *Thermoactinomyces* species. Our findings indicate that *T. sacchari* and *T. thalpophilus* strains have a high prevalence in soil and hot spring sediment samples from West Anatolia.

Yoon and Park (12) studied the 16S rDNA sequence based phylogenetic analysis of the genus *Thermoactinomyces* and showed that some *Thermoactinomyces* species are very closely related. They showed that the *T. thalpophilus* KCTC 9789 and *T. sacchari* KCTC 9790 reference strains showed almost 100% 16S rDNA sequences similarity. Yoon et al. (13) also suggest that *T. thalpophilus* should be considered as a synonym of *T. sacchari* according to DNA-DNA relatedness and 16S rDNA sequence similarities. In the present study *T. thalpophilus* and *T. sacchari* strains also showed a close relatedness (Figure). We found similar 16Sr DNA sequences among the *Thermoactinomyces* isolates. The GenBank accession numbers of the isolates were as follows: 9-2;GU441696, 9-3; GU441697, 9-4; GU441698, 9-5; GU441699, 10-2; GU441700, 13-1; GU441701, 15-2; GU441702, 15-3; GU441703, 16-2; GU441704, 16-3;

GU441705, 16-7; GU441706, 16-15; GU441707, 27-1; GU441708, 31-1; GU441709, 31-3; GU441710, 32-1; GU441711, 32-2; GU441712, 33-2; GU441713, 33-3; GU441714, 34-1; GU441715, 34-1b; GU441716, 34-2; GU441717, 34-4; GU441718, 34-7; GU441719, 34-10; GU441720, 34-11; GU441721, 34-12; GU441722, 34-13; GU441723, 34-17; GU441724, 34-18; GU441726, 34-36; GU441727, 35-2; GU441728, 37-2; GU441729, 37-2b; GU441730, 37-3a; GU441731, 37-7; GU441732, 40-1; GU441733, 40-4; GU441734, 40-5; GU441735, 43-2; GU441736, 43-5; GU441737, 44-2; GU441738, 44-4; GU441739, 45-1; GU441740, 46-1; GU441741, 46-2; GU441742, 48-2; GU441743, 48-3; GU441744, 48-6; GU441745, 48-8; GU441746, 48-9; GU441747, 51-9; GU441748, 58-1; GU441749, 58-2a; GU441750, 58-26; GU441751, 58-3; GU441752, 60-1; GU441753, 67-1; GU441754, 67-20; GU441755, 71-1; GU441756, 71-2; GU441757, 71-3; GU441758, 71-6; GU441759, 73-1; GU441760, 73-2; GU441761, 85-1; GU441762, 86-1; GU441763.

Actinomycetes are used by industry for mainly 2 purposes, namely in the production of antibiotics and enzymes. Hence, enzymatic screening for extracellular protease and antibacterial activity were performed for these isolates. Results showed that most of the strains are extracellular protease producers (92.3%). All of the isolates showed protease activity except for 5 strains, which showed no activity. The main protease producers are *Bacillus* members in industry (20). The genus *Thermoactinomyces* may be a promising source for industrial protease production. The genus *Thermoactinomyces* is more closely related to *Bacillus* species then the other Actinomycetes. *Thermoactinomyces* species produce endospores as shown in bacilli (12) and have lower G+C content than those of Actinomycetes (13). However, *Thermoactinomyces* species produce aerial and substrate mycelium as other Actinomycetes. This close relatedness with the genus *Bacillus* may explain the high level extracellular protease production and further investigations with *Thermoactinomyces* protease may reveal enzymes that have novel properties.

There is an undoubted need for new antibiotics effective against drug resistant microorganisms since antibiotic resistance is a disseminative phenomenon among dangerous pathogens (21). Although methods like site directed mutagenesis or chemical modification of existing antimicrobials are powerful, the search of natural products is also important for discovering novel bioactive compounds. As efforts for antibiotic discovery in industry are focused mainly on soil microorganisms, investigations result in the isolation of previously isolated chemical classes with known modes of action. In view of the fact that the microorganisms living in extreme conditions produce stress molecules to survive, antibiotic discovery research is headed toward extremophilic microorganisms in order to obtain novel molecules. Therefore, the antimicrobial activities of the thermophilic Actinomycetes isolates against a panel of bacteria were also screened since the Actinomycetes are the primary antibiotic producers.

The *Thermoactinomyces* isolates did not show any activity against *E. coli* and *K. pneumoniae*. However, 38 strains (56.7%) were found active against Methicillin resistant *S. aureus* and *E. faecalis*. Gram positive test bacteria were inhibited in different ranges but gram negative test bacteria did not show any inhibition zone. The further characterization of bioactive molecules responsible for the inhibition of gram positive bacteria may be useful.

The genus *Thermoactinomyces* has important properties and should be exploited for bioactive molecules. Recently, a number of tryptamine derivatives have been isolated from *T. sacchari* 66-2 strain (17). To the best of our knowledge this is the first study on the isolation and 16S rDNA sequence based identification of thermophilic actinomycetes from the hot spring and soil samples of Western Anatolia in Turkey. The results indicate that *T. thalpophilus* and *T. sacchari* are the prevalent species among the thermophilic Actinomycetes in hot spring and soil samples in Western Anatolia. Based on these results, we think that the hot springs of West Anatolia are good sources to find thermophilic Actinomycetes, especially *Thermoactinomyces* species, which might be very useful for discovery of novel enzymes and antimicrobial molecules in the future.

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