SETSCI conference proceedings
SETSCI Conference Proceedings
4 (6), 420-422, 2019

4th International Symposium on Innovative Approaches in Engineering and Natural Sciences
November 22-24, 2019, Samsun, Turkey

https://doi.org/10.36287/setsci.4.6.108
2687-5527/ © 2019 The Authors. Published by SETSCI

ISAS WINTER - 2019

Phylogenetic Analysis of *Streptomyces* sp. H2AK isolated from soil in Kuşadası

Demet Tatar[1+*] and Aysel Veyisoğlu[2]

[1]*Department of Medical Services and Techniques, Osmancık Ömer Derindere Vocational School, Hitit University, Çorum, Turkey*
[2]*Department of Medical Services and Techniques, Vocational School of Health Services, Sinop University, Sinop, Turkey*
[*]*Corresponding author: demettatar@hitit.edu.tr*
[+]*Speaker: demettatar@hitit.edu.tr*
Presentation/Paper Type: Oral / Full Paper

Abstract – *Streptomyces* is a genus of Gram-positive bacteria that has a filamentous form similar to fungi. *Streptomyces* produces bioactive secondary metabolites, such as antifungals, antivirals, antitumorals and various antibiotics. The aim of this study is to carry out phylogenetic analysis of *Streptomyces* sp. H2AK isolated from soil sample taken from the Kuşadası located near Aydın province. *Streptomyces* sp. H2AK, was picked after 14 days of incubation at 28˚C on Humic acid-vitamin agar containing Cycloheximide (50 µg/ml) ve Nalidixic acid (10 µg/ml). Genomic DNA isolation was performed according to DNA isolation method [1]. The 16S rRNA gene was amplified by PCR using universal primers 27f and 1525r. Phylogenetic analyses were performed by using three different algorithms with MEGA 7 software. H2AK showed the highest 16S rRNA gene sequence similarity with *Streptomyces marokkonensis* Ap1T (98.90 %). When the polyphasic taxonomic analyses were completed, *Streptomyces* sp. H2AK isolate may be introduced into the literature as a new species of the genus *Streptomyces*. This study was supported by Hitit University (ODMYO19003.18.001).

Keywords *– Taxonomy, Phylogenetic Analysis, 16S rRNA Gene, Streptomyces*

I. Introduction

The borders of the city, which is 71 km away from Aydın, cover the coastal plain to the east and southeast of Kuşadası Bay and the low plateau behind it. The east and south are surrounded by mountains. Pigeon island area in front of the name of the county, tourism is one of the most important and advanced centers in terms of Turkey [2].

The genus *Streptomyces* are Gram-positive aerobic members of the *Actinomycetales* order of the *Actinobacteria* class [3]. *Streptomyces* produces bioactive secondary metabolites, such as antifungals, antivirals, antitumorals and various antibiotics [4]. The majority of antibiotics used in medicine, veterinary practice and agriculture are derived from *Streptomyces* bacteria [5]. The genus *Streptomyces* includes more than 800 species with validly published names [6].

The study aims to carry out the isolation and phylogenetic analysis of *Streptomyces* sp. H2AK isolate obtained from the Kuşadası located near Aydın province.

II. Materials and Method

Isolation and maintenance of the organism

Streptomyces sp. H2AK, was isolated from a soil sample from Kuşadası located near Aydın province using a dilution plate on on Humic acid-vitamin agar [7] containing Cycloheximide (50 µg/ml) ve Nalidixic acid (10 µg/ml). The organism was maintained on related agar slopes and stocked in glycerol (20 %, v/v) at -20°C. 1 gram soil sample was added to 9 ml Ringer's solution and then mixed for homogenization at room temperature. Then this 10^{-1} dilution was kept for 15 minutes at 55 ° C in a preheated water bath. Serial dilutions (0.1 ml of 10^{-1} to 10^{-2}) were spread over the surface of dried related agar plates and the plates were incubated at 28°C for 14 days.

Cultivation and DNA extraction

Streptomyces sp. H2AK isolate was subcultured onto related medium and incubated for up to 2 weeks at 28°C. Suspensions of spores and mycelia were maintained in 20 % glycerol (w/v) at -20°C. Genomic DNA isolation was performed according to the Guanidine thiocyanate DNA isolation method [1].

16S rRNA gene sequence

PCR mixture (50 µl) included primers (20 µM), Taq polymerase buffer (HotStarTaq®) and deoxynucleoside triphosphates mixture (Promega) (25 µM). Taq polymerase (2.5 U, HotStarTaq®) and chromosomal DNA (50-300 ng) were added to the solution. The 16S rRNA gene was amplified with specific primers 27F and 1525R. The DNA thermal cycler was utilized for PCR amplification. The PCR conditions were initial denaturation at 95°C (5 min), 35 cycles at 95°C (1 min), 55°C (2 min), and 72°C (3 min), and a final extension at 72°C (10 min). Then the PCR product was resolved using electrophoresis in 1 % agarose gel (Merck) and was imaged with the Gene Genius Bioimaging system.

Phylogenetic analysis

The PCR product of the test isolate was purified with QIAquick purification kit (Qiagen). 16S rRNA gene sequence of the test isolate was revealed using an ABI PRISM 3730 XL automatic sequencer with previously mentioned oligonucleotide primers (Table 1). Chromatogram files in ABI format are converted to FASTA format using Chromas 1.7.5. The determination of phylogenetic neighbors and computation of pairwise 16S rRNA gene sequence similarity were obtained using the Ezbiocloud server (https://www.ezbiocloud.net) [8]. Multiple alignments with sequences from closely related species were applied with the program CLUSTAL W in MEGA 7.0 [9]. Phylogenetic trees were formed with the neighbor-joining [10], maximum likelihood [11] and maximum parsimony [12] algorithms in MEGA 7.0 [9]. Evolutionary distances were computed with the model of Jukes and Cantor [13]. Topologies of the resultant trees were determined with bootstrap analyses [14] based on 1000 resamplings.

Table 1. Oligonucleotide primers employed for 16S rRNA PCR amplification and sequencing

Primer Code	Sequences (5'-3')	Length	References
27F	AGAGTTTGATC**M**TGGCTCAG	20	[15]
518F	CCAGCAGCCGCGGTAAT	17	[16]
800R	TACCAGGGTATCTAATCC	18	[17]
MG5F	AAACTCAAAGGAATTGACGG	20	[17]
MG6F	GACGTCAAGTCATCATGCC	19	[17]
1525R	AAGGAGGTG**W**TCCA**R**CC	17	[15]

M = A:S; R = A:G; W = A:T.

III. RESULTS

Streptomyces sp. H2AK, was isolated on Humic acid-vitamin agar [7] with Cycloheximide (50 µg/ml) and Nalidixic acid (10 µg/ml) and then incubated at 28°C for about 14 days (Figure 1).

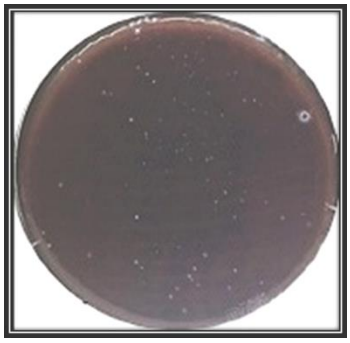

Figure 1. Isolation of a soil sample obtained from Kuşadası located near Aydın province on Humic acid-vitamin agar [7].

Streptomyces sp. H2AK isolate was obtained from a soil sample from Kuşadası located near Aydın province. 16S rRNA gene sequence of strain was detected with oligonucleotide primers (Figure 2).

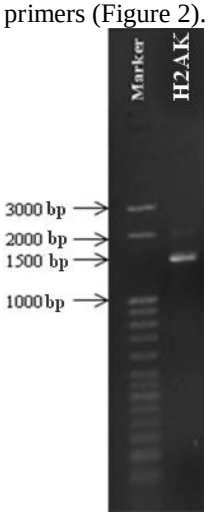

Figure 2. Amplification product of the 16S rRNA gene region of H2AK isolate M, DNA Marker (Sigma, 50 bp DNA Ladder).

The phylogenetic trees, according to the neighbor-joining, maximum parsimony and maximum likelihood algorithms indicated that the strain was member of the genus *Streptomyces* (Figures 3, 4 and 5).

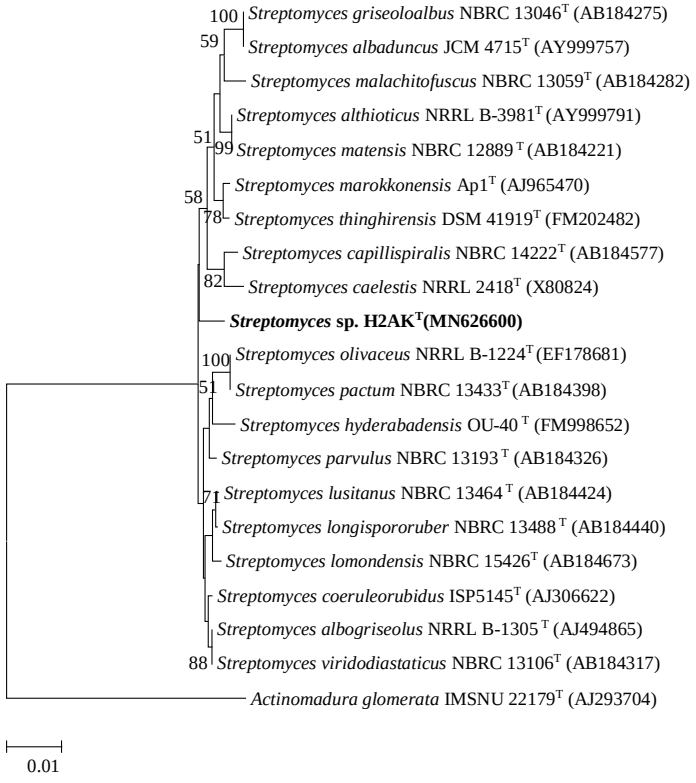

Figure 3. Neighbor-joining tree [10] based on 16S rRNA gene sequences showing the position of isolated H2AK strain among its phylogenetic neighbors. Numbers at the nodes demonstrate the levels of bootstrap support (%); only values ≥ 50% are shown. GenBank accession numbers are placed in parentheses. Bar, 0.01 substitutions per site.

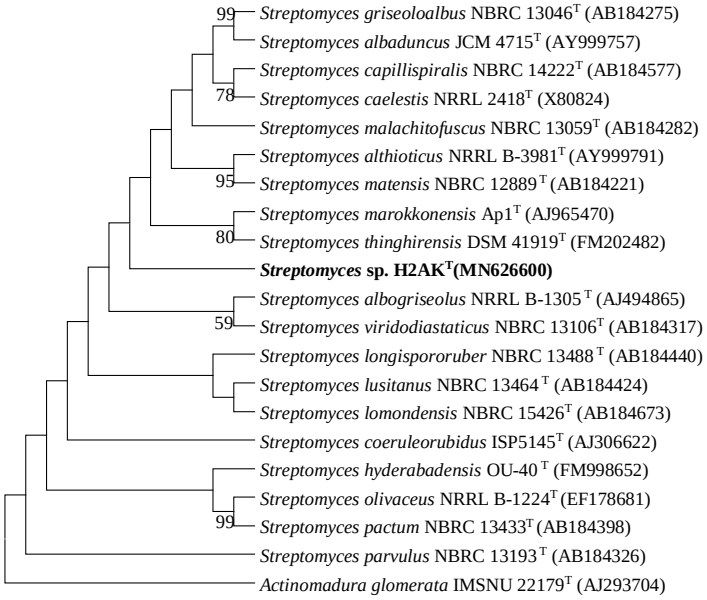

Figure 4. Maximum parsimony [12] based on 16S rRNA gene sequences showing the position of isolated H2AK strain among its phylogenetic neighbors.

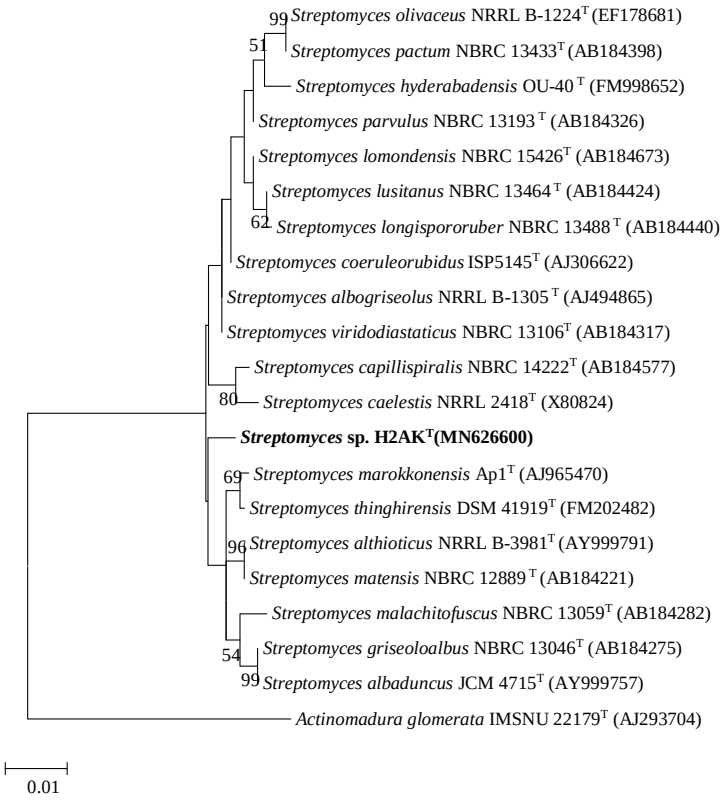

Figure 5. Maximum likelihood [11] based on 16S rRNA gene sequences showing the position of isolated H2AK strain among its phylogenetic neighbors.

IV. DISCUSSION

This *Streptomyces* sp. H2AK isolate was provided from a soil sample from Kuşadası located near Aydın province. Based on 16S rRNA gene sequence analysis, 1 *Streptomyces* isolate was detected. H2AK showed the highest 16S rRNA gene sequence similarity with *Streptomyces marokkonensis* Ap1^T (98.90 %).

V. CONCLUSION

In conclusion, isolation and phylogenetic analysis of *Streptomyces* sp. H2AK obtained from Kuşadası were performed.

According to the polyphasic characterization approach, in addition to the phylogenetic analysis of the 16S rRNA gene region, DNA-DNA hybridization studies, whole-genome analysis, phenotypic and chemotaxonomic analyses should be performed.

In subsequent studies, It is possible to make *Streptomyces* sp. H2AK obtained in this study as a new species in the literature by making the relevant analyzes.

ACKNOWLEDGMENT

This study was supported by Hitit University (ODMYO19003.18.001). I want to extend our gratitude to Prof. Dr. Nevzat Şahin (Faculty of Art and Science, Department of Molecular Biology and Genetics, Ondokuz Mayıs University) for his contributions to the study.

REFERENCES

[1] Pitcher D. G., Saunders N. A., and Owen R. J., Rapid extraction of bacterial genomic DNA with guanidium thiocyanate, Lett. Appl. Microbiol, 8, 151-156, 1989.

[2] URL-1: https://aydin.ktb.gov.tr/TR-64364/kusadasi.html (Available: 30 Nov 2019).

[3] Stackebrandt, E., Rainey, F. A. & Ward-Rainey, N. L., Proposal for a new hierarchic classification system, Actinobacteria classis nov. Int J Syst Bacteriol 47, 479–491, 1997.

[4] de Lima Procópio, R. E., Da Silva, I. R., Martins, M. K., De Azevedo, J. L., and De Araújo, J. M., Antibiotics produced by *Streptomyces*. Braz. J. Infect. Dis. 16, 466–471. doi: 10.1016/j.bjid.2012.08.014, 2012.

[5] Chater, K.F, Recent advances in understanding *Streptomyces* [version 1; referees: 4 approved] F1000Research, 5(F1000 Faculty Rev):2795 (doi: 10.12688/f1000research.9534.1), 2016.

[6] URL-2:http://www.bacterio.net/streptomyces.html (Available: 30 Nov 2019).

[7] Hayakawa, M., Nonomura, H, Humic acid-vitamin agar, a new medium for the selective isolation of soil actinomycetes. J Ferment Technol, 65, pp.501-509, 1987.

[8] Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, Seo H, Chun J, Introducing EzBioCloud: A taxonomically united database of 16S rRNA and whole genome assemblies. Int J Syst Evol Microbiol 67:1613-1617, 2017.

[9] Kumar S, Stecher G, Tamura K,. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33:1870-1874, 2016.

[10]Saitou N, Nei M,. The neighbor-joining method. A new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425, 1987.

[11]Felsenstein J,. Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17: 368–376, 1981.

[12]Kluge AG, Farris FS,. Quantitative phyletics and the evolution of anurans, Syst Zool 18: 1–32, 1969.

[13]Jukes TH, Cantor CR,. Evolution of protein molecules. *In Mammalian Protein Metabolism* vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press, 1969.

[14]Felsenstein J,. Confidence limits on phylogeny: an approach using the bootstrap. Evolution 39: 783–791, 1985.

[15] Lane DJ,. 16S/23S rRNA sequencing. In: Nucleic acid techniques in bacterial systematics. Stackebrandt, E., and Goodfellow, M., eds., John Wiley and Sons, New York, NY, pp. 115-175, 1991.

[16]Buchholz-Cleven BEE, Rattunde B & Straub KL,. Screening for genetic diversity of isolates of anaerobic Fe(II)-oxidizing bacteria using DGGE and whole-cell hybridization. Syst. Appl. Microbiol. 20: 301-309, 1997.

[17]Chun J, Goodfellow M,. A phylogenetic analysis of the genus *Nocardia* with 16S rRNA gene sequences. Int J Syst Bacteriol 45: 240–245, 1995.