Journal of Animal and Veterinary Advances 13 (16): 974-977, 2014
ISSN: 1680-5593
© Medwell Journals, 2014

Use of Random Amplified Polymorphic DNA PCR to Distinguish *Micromonospora* Species Isolated from Soil

[1]Metin Ertas, [1]Kerem Ozdemir and [2]Ekrem Atalan
[1]Department of Biology, Faculty of Science, Yuzuncu Yil University, 65080 Van, Turkey
[2]Department of Biology, Faculty of Arts and Sciences, Inonu University, 44100 Malatya, Turkey

Abstract: Recently with the advancement of molecular methods, studies that determine intraspecific genetic polymorphisms have yielded substantial results. The aim of this study was to use Random Amplified Polymorphic DNA PCR (RAPD-PCR) analysis to determine the differences among *Micromonospora* species isolated from the soil. M3 medium with antibiotics was used to isolate the species from the soil and 65 *Micromonospora* species were identified. Total DNA analysis was performed for the different color groups which were obtained as a result analysis of the species. Sm5R, Sm6F, DAF4, M13, 27F and 1492R primers were used for RAPD-PCR analysis and the band profiles of the species were obtained. The dendrogram generated according to the simple matching coefficient method clearly showed the differences detected among the species. A total of seven groups were identified along with two main groups. Five of these groups had a single member, one had 3 and one had 2 members.

Key words: Micromonospora, RAPD-PCR, simple matching coefficient, soil, genetic polymorphism

INTRODUCTION

Among molecular methods, Random Amplified Polymorphic DNA PCR (RAPD-PCR) analysis yields significant results in identification of intraspecific genetic polymorphisms. This method has been used for comparing several bacterial isolates (Alves *et al*., 2002). Repetitive sequence-based PCR (RepPCR) Restriction Fragment Length Polymorphism (RFLP) and RAPD-PCR are commonly used in order to identify micro-variations among the species of a genus. One advanced stage of these methods is DNA sequence analysis. However, RAPD-PCR is more commonly used among the other methods due to its applicability and low cost.

Micromonospora sp. have the ability to degrade polymers such as cellulose and chitin in organic matter. These bacteria can be found in many different habitats but they mostly prefer aquatic environments. The spores of these bacteria have been found in the soil and muddy bottoms of lakes and seas and germinate under suitable conditions to form vegetative hyphae (Antal *et al*., 2005; Atalan *et al*., 2000). Primary and secondary metabolites produced by the Actinomycetes members (also containing the *Micromonospora* genus) have occupied a prominent place in the field of medicine in recent years. Micromonospora is the genus that produces the strongest antibiotics after the *Streptomyces* genus (Ausubel *et al*., 1994). The fact that the metabolites produced by them are used in anticancer treatments make them all the more important (Coenye *et al*., 2005; Cross, 1981; Erikson, 1941).

MATERIALS AND METHODS

Isolation of bacterial strains: Soil samples from various locations in the Lake Van basin (Eastern Turkey) were collected in order to isolate the bacteria belonging to the *Micromonospora* species. The areas where the samples were collected are indicated on the map. The M3 medium containing the antibiotics cycloheximide (50 μg mL^{-1}) and novobiocin (50 μg mL^{-1}) was used for the selection of *Micromonospora* species (Fani *et al*., 1993; Goodfellow and Haynes, 1984).

Genomic DNA isolation: Color groups of the purified species were determined and genomic DNA was isolated from selected strains in these groups using a modified version of the method by Ausubel *et al*. (1994) and Holt *et al*. (2000). The genomic DNA samples were stained with ethidium bromide (1%) and electrophoresed, after which they were visualized using a UV transilluminator gel imaging system (Fotodyne Inc., USA). The DNA quantity was determined by measuring the absorbance at 260 nm on a spectrophotometer (Novaspec II Pharmacia-Biotech, Biochrom Limited, Cambridge, UK). All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Corresponding Author: Kerem Ozdemir, Department of Biology, Faculty of Science, Yuzuncu Yil University, 65080 Van, Turkey

RAPD-PCR analysis: The RAPD-PCR Method was applied to the isolated genomic DNA samples. During this analysis, PCR products were produced using Sm5R, Sm6F, DAF4, M13, 27F and 1492R primers (OPERON Technologies, USA) in an automated thermal cycler (DNA Thermal Cycler 480; Perkin Elmer, Norwalk, CT, USA). The primer numbers were sequentially increased and the most suitable band profile was obtained using all six primers together. PCR products were subjected to electrophoresis in a 1.5% agarose gel at 100 V for ~45 min.

Similarity coefficient: Analysis of the band profiles from RAPD-PCR was carried out using MVSP 3.2 (Multivariate Statistical Software Package) and an S_{sm} (Simple matching coefficient)-Unweighted Pair Group Method with an Arithmetic average (UPGMA) dendrogram was constructed (Kovach Computing Service, Anglesey, UK).

RESULTS

Isolation of Micromonospora strains: The Micromonospora colonies in the collected soil samples that were grown in the M3 medium containing the antibiotics cycloheximide (50 μg mL^{-1}) and nystatin (50 μg mL^{-1}) were selected according to their micelle and pigment status in comparison with the other colonies. The selected colonies were purified in medium 65 and were stored at -20°C in a 20% glycerol solution.

DNA isolation and RAPD-PCR analysis: Analysis of the band profiles from RAPD-PCR was carried out using the Multi Variate Statistical Package Software (Version 3.2) and Simple Matching Coefficient-UPGMA and a dendrogram was constructed. The dendrogram consisted of two main clusters. In the first cluster there were five small groups and in the second cluster there were two small groups.

According to the 85% similarity ratio, seven groups were formed during the dendrogram analysis of the RAPD-PCR results from the Micromonospora test isolates. RAPD-PCR results are displayed in Fig. 1 and the relevant dendrogram is shown in Fig. 2. Isolates B006, B010 and B003 exhibited similarity of 90%. Isolates B010 and B003 displayed similarity of over 95%. Isolates B002 and B010 were collected from the same location and were grouped into different clusters. They were in different groups in the dendrogram of RAPD-PCR results whereas B010 was in the first main group, the B002 isolate was in the second main group. The 1st, 2nd, 4th, 6th and 7th sets

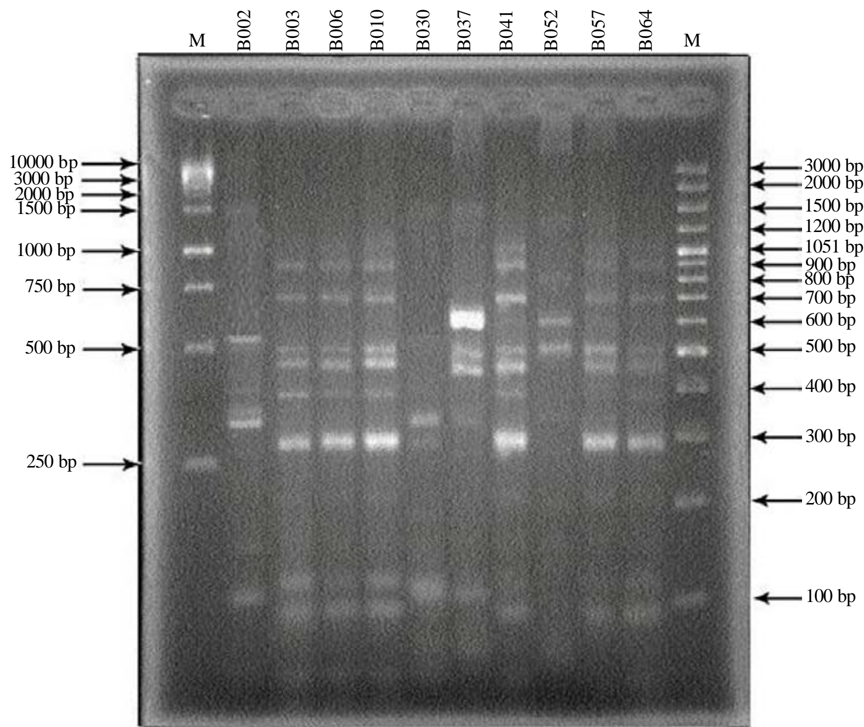

Fig. 1: Random amplified polymorphic DNA PCR (RAPD-PCR) fingerprinting profile of Micromonospora isolates. Agarose gel electrophoresis of the isolates amplified using specific primers (M: Marker)

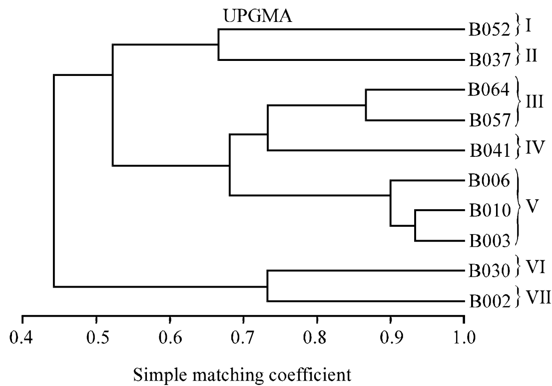

Fig. 2: The Unweighted Pair Group Method with an Arithmetic average (UPGMA) dendrogram. The latter was derived from the combination of primer sets. Isolate numbers correspond to the different *Micromonospora* sp.

formed groups with a single member whereas the 3rd set contained two test isolates and the 5th set contained three test isolates.

DISCUSSION

RAPD-PCR is one of the most commonly used methods to identify phylogenetic relationships among bacterial species. Moreover, it is a DNA-typing Method that facilitates identification of the inter and intraspecies similarities among bacteria (Jensen *et al*., 2005; Martin *et al*., 2000; Ozdemir, 2008; Roberts and Crawford, 2000). In this study, the similarity among 10 *Micromonospora* strains (selected to represent the color groups) has been examined by means of RAPD-PCR using the Sm5R, Sm6F, DAF4, M13, 27F and 1492R universal primers. A total of 10 isolates (18 different bands) were observed and the size of these amplicons was between 100 and 1200 bp. Two main groups formed according to the S_{sm}-UPGMA dendrogram analysis. Those in the 6th and 7th set formed the 1st cluster while the rest formed the 2nd cluster. This second cluster was further divided into two groups with a similarity ratio of 52%. The group containing the 1st and 2nd set showed similarity of 67%. The 3rd set showed similarity of 86% whereas the 5th set containing the B006, B003 and B010 isolates displayed similarity of over 90%. Isolates B010 and B003 displayed similarity of 94%. Isolates B002 and B030 showed similarity of 76% in RAPD-PCR analyses.

CONCLUSION

The aim of this study was to use RAPD-PCR to determine the differences among the *Micromonospora* species isolated from the soil. These analyses are presented as a dendrogram and the differences among the species were successfully found.

REFERENCES

Alves, A., O. Santos, I. Henriques and A. Correia, 2002. Evaluation of methods for molecular typing and identification of members of the genus *Brevibacterium* and other related species. FEMS Microbiol. Lett., 213: 205-211.

Antal, N., H.P. Fiedler, E. Stackebrandt, W. Beil, K. Stroch and A. Zeeck, 2005. Retymicin, galtamycin B, saquayamycin Z and ribofuranosyllumichrome, novel secondary metabolites from *Micromonospora* sp. Tu 6368. I. Taxonomy, fermentation, isolation and biological properties. J. Antibiot., 58: 95-102.

Atalan, E., G.P. Manfio, A.C. Ward, R.M. Kroppenstedt and M. Goodfellow, 2000. Biosystematic studies on novel *Streptomycetes* from soil. Ann. Rev. Microbiol., 77: 337-353.

Ausubel, F.M., R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith and K. Struhl, 1994. Current Protocols in Molecular Biology. John Wiley and Sons, New York.

Coenye, T., D. Gevers, Y. van de Peer, P. Vandamme and J. Swings, 2005. Towards a prokaryotic genomic taxonomy. FEMS Microbiol. Rev., 29: 147-167.

Cross, T., 1981. The Monosporic Actinomycetes. In: The Prokaryotes: A Handbook on Habitats, Isolation and Identification of Bacteria, Star, M.P., H. Stolp, H.G. Truper, A. Balows and H.G. Schlegel (Eds.). Springer-Verlag, Berlin, pp: 2091-2102.

Erikson, D., 1941. Studies on same lake-mud strains of Micromonospora. J. Bacteriol., 41: 277-300.

Fani, R., G. Damiani, C. Di Serio, E. Gallori, A. Grifoni and M. Bazzicalupo, 1993. Use of random amplified polymorphic DNA (RAPD) for generating specific DNA probes for microorganisms. Mol. Ecol., 2: 243-250.

Goodfellow, M. and J.A. Haynes, 1984. Actinomycetes in Marine Sediments. In: Biological, Biochemical and Biomedical Aspects of Actinomycetes, Oritz-Oritz, L., C.F. Bojali and V. Yakoleff (Eds.). Academic Press, New York, London, pp: 453.

Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley and S.T. Williams, 2000. Bergey's Manual of Determinative Bacteriology. 9th Edn., Lippincott Williams and Wilkins, Philedelphia, pp: 653-655.

Jensen, P.R., T.J. Mincer, P.G. Williams and W. Fenical, 2005. Marine actinomycete diversity and natural product discovery. Antonie van Leeuwenhoek, 87: 43-48.

Martin, P., A. Dary, A. Andre and B. Decaris, 2000. Identification and typing of Streptomyces strains: Evaluation of interspecific, intraspecific and intraclonal differences by RAPD fingerprinting. Res. Microbiol., 151: 853-864.

Ozdemir, K., 2008. Isolation, characterisation and identification of *Streptomyces* sp. isolated from rhizosfere of *Lens orientalis* (Boiss.) Hand and Mazz and Cicer anatolicum Alef. Ph.D. Thesis, Yuzuncu Yil University, Institute of Science, Turkey.

Roberts, M.A. and D.L. Crawford, 2000. Use of random amplified polymorphic DNA (RAPD) as a means to develop genus and strain specific Streptomyces DNA probes. Applied Environ. Microbiol., 66: 2555-2564.