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# Isolation and characterization of *Micromonospora* bacteria from various soil samples obtained around Lake Van

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**The objective of the study was to isolate and characterize the *Micromonospora* type bacteria from 24 soil samples collected from different localities around the Lake Van basin and Lake Erçek surroundings. Culturing was carried out on selective media following the completion of various physical-chemical analyses of the soil samples. Purified 65 *Micromonospora* isolate color grouping and numerical taxonomic analyses were carried out. In addition, complete cell protein profiles of 10 *Micromonospora* test isolates selected as representative of the color groups for characterization studies were determined via sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and the obtained results were presented as dendograms. Whereas the pHs of the soil samples were generally at neutral values, their organic material content varied from 4.181 to 15.248%. Colony counts in the isolation study varied between $0.9\times10^4$ and $14.3\times10^4$ cfu/g soil.**

**Key words:** *Micromonospora,* numeric analysis, simple matching coefficient, SDS PAGE.

## INTRODUCTION

There are very few studies carried out on *Micromonospora* bacteria worldwide however nowadays, studies have started to increase (Kawamoto, 1989; Hernandez et al., 2000). *Micromonospora* genus is characterized as real actinomycetes with its Gram-positivity, chemo-organotropic, aerobic and high % G+C ratio (Vobis, 1992). Even though *Micromonospora* displays a significant physiological variety, it is a genus that is well defined chemotaxonomically, phylogenically and morphologically (Kawamoto, 1989). *Micromonospora* is common both in water and soil and mostly lives in aquatic environments (Hernandez et al., 2000; Gutierrez-Lugo et al., 2005). *Micromonospora* species play an important role in the mineralization of organic material found in soil and water. In addition, they produce some important antibiotics (Wagman and Weinstein, 1980; Houge-Frydrych et al., 2000). Studies started regarding whether *Micromonospora* species are sources of new antibiotics and over 300 antibiotics have been defined (Bèrdy, 1984). *Micromonospora* genus produces many antibiotics which include aminoglycosides, macrolids, maquarimisids and anticancer antibiotics (Antal et al., 2005). *Micromonospora* species are well known especially since they synthesize aminoglycosides, enedien (natural toxin produced by bacteria and used as an antitumor) and oligosaccharide antibiotics. Hence, their significance in the drug industry is quite high (Bèrdy, 2005; Igarashi et al., 2007).

In this study, *Micromonospora* species were isolated in

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soil and mud samples obtained from different localities and were subjected to color grouping, biochemical, and developmental tests along with characterization via sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis. These analyses were presented as dendograms and differences between species were determined.

## MATERIALS AND METHODS

Soil samples were collected from 24 different localities around the Van lake basin in order to isolate the *Micromonospora* genus bacteria. Three soil and mud samples were collected from each region.

### Biochemical and phenotypic tests

Strains of *Micromonospora* genus in the soils were isolated in Medium 65 culture medium and were purified in Bennet's agar. Following the color group determination of pure colonies, diaminopimelic acid determination, morphological character determination along with antimicrobial activity studies, degradation activities, physiologic and chemical inhibitor tests, nitrogen source tests and carbon source test were carried out after which computer based numerical analysis study was made. Analysis was carried out in Multi-Variate Statistical Package (MVSP 3.2) software in accordance with $S_{SM}$ coefficients with unweighted pair group method with arithmetic average cluster (UPGMA) software and the results were presented as dendograms (Kovach Computing Service, Anglesey, UK).

### SDS electrophoresis method and analysis of cell proteins

Ten (10) *Micromonospora* isolates were selected to represent the sets that formed as a result of the color grouping carried out for cell protein analysis. Protein extraction was carried out from these isolates via QB Buffer (which contains 100 mM potassium phosphate buffer pH= 7.8, 1 mM EDTA, 1% Triton-X-100, and 10% glycerol along with several protease inhibitors). Spot test was carried out after which electrophoretic processes were done in accordance with Laemmli (1970). Protein profiles of all cell proteins were determined by SDS electrophoresis method and the results were presented as dendograms in MVSP 3.2 software in accordance with $S_{SM}$ with UPGMA program.

## RESULTS AND DISCUSSION

Sixty-five (65) purified *Micromonospora* isolates were divided into 14 color groups as a result of culturing in Modified Bennet's agar and pepton yeast extract iron agar culture medium. Ten (10) *Micromonospora* test isolates were selected in representation of color groups. pH, moisture and organic matter content of the collected soil samples were measured prior to isolation. The pH values of the samples varied between 6.56 and 8.86 and it was found that they constituted soil type with different pH values whereas moisture ratios varied between 0.74 and 63.94% and the samples mostly had high moisture ratio. Organic matter content varied between 4.181 and 15.248%.

In this study, isolation of *Micromonospora* bacteria was carried out in 24 soil samples. *Micromonospora* colonies cultured in cycloheximid (50 µg/ml) and nystatin (50 µg/ml) antibiotics with M3 agar medium were selected according to their characteristic micelle and pigment status and were isolated (Figure 1). Our results are in agreement with those from previous studies (Hernandez et al., 2000; Gutierrez-Lugo et al., 2005).

*Micromonospora* test isolates were moved on a layer of cellulose acetate using *meso*-diamino pimelic acid standard thin paper chromatography and it showed that they were clearly separated. According to the results, *meso*-diamino pimelic acid was determined in the cell wall structures of *Micromonospora* isolates. For the purified 65 *Micromonospora* strains, six pathogenic and nonpathogenic microorganisms tested showed antimicrobial activity of 53.85 to 33.8%. Of the *Micromonospora* strains, 76.92% displayed the ability to degrade xanthine, 75.2% tween 80, 69.23 % elastine, 80% casein and 72.30% guanin. Of the *Micromonospora* test isolates, B002, B003, B009, B013, B015, B016, B024, B028, B039, B040 and B063 strains were able to degrade all selected materials whereas test isolates B002, B037, B051 and B063 samples displayed development at 4°C; B003, B002, B009, B017, B037, B047, B051 and B063 samples displayed development at 10°C and no development was observed in other isolates at these temperatures. Even though all of the *Micromonospora* isolates displayed development at pH 7.3, no development was observed at pH 4.3.

No development was observed in 4, 7 and 10% NaCl mediums during the development tests carried out by adding different concentrations of various chemical materials to the culture medium. No development was observed in B004, B005 and B023 numbered isolates placed in sodium azide containing (0.01%) medium. However, development was observed in no isolate in sodium azide (0.02%) containing medium.

Development was observed in 53.84% of the *Micromonospora* isolates in phenol (0.1%) containing medium. 68% of the *Micromonospora* isolates used sisteine as source for nitrogen, 84.60% valine, 80% treonine, 78.46% phenylaniline, 70.77% arginine, 76.92% serine, 64.7% hydroxyproline and 86.15% histidine for development. It was observed that for the *micromonospora* isolates, 50.76% used sucrose, 73.46% maltose, 66.15% arabinose, 80% inositole, 69.23% mannitole, 81.53% fructose, 76.92% lactose, 70.7% trehalose, 63.07% melibiose, 78.46% dextrane, 75.38% galactose and 69.23% used xylose as carbon source and reproduced in the medium. Our results agree with those of previous studies (Hernandez et al., 2000; Gutierrez-Lugo et al., 2005).

UPGMA analyses were carried out for the results of the 67 different tests carried out for the 65 selected *Micromonospora* test strains using MVSP 3.2 software in accordance with $S_{SM}$ coefficients. Figure 2 shows the microorganisms selected according to $S_{SM}$-UPGMA

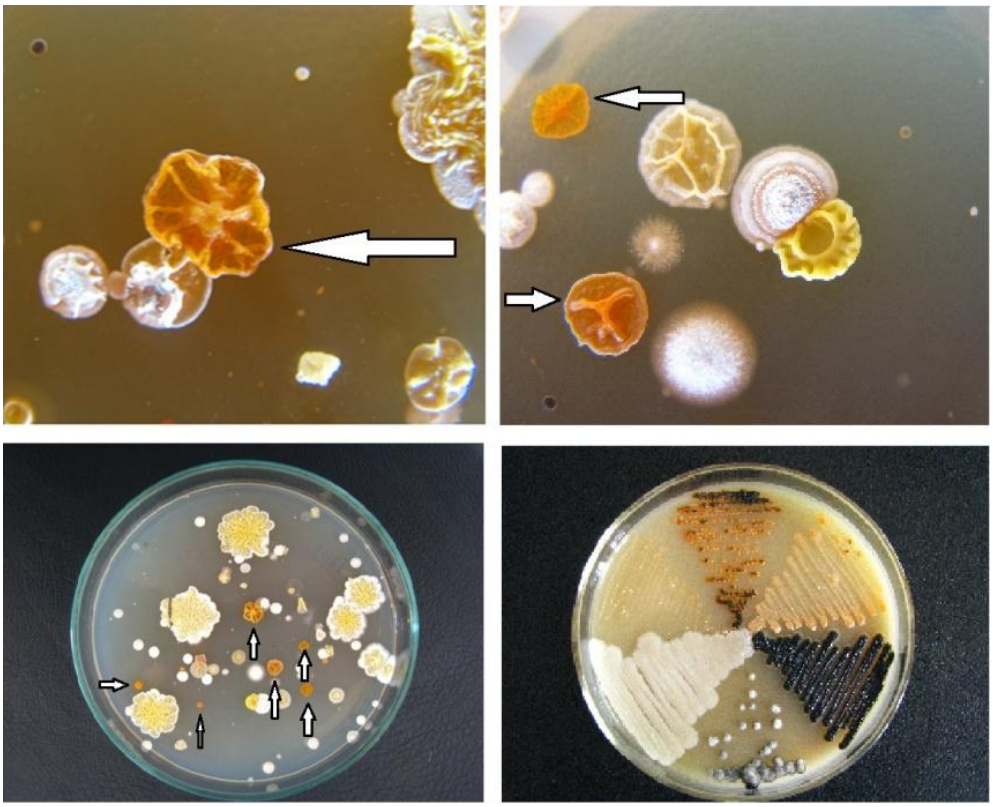

**Figure 1.** The view of *Micromonospora* strains (Arrows show *Micromonospor*colonies).

coefficient analyses. A total of seven sets were formed and all of these clusters contained more than twice isolate except for the 1st. Five major clusters, two minor clusters and two single item clusters were formed according to dendogram; 75% similarity ratio. There were more than 18 test isolates in two major clusters and less number of test isolates were found in three of the clusters. Test strains showed similarity commonly in 72 and 85% ratio. Analyses of the protein profiles of 10 isolates selected according to color grouping were carried out using MVSP 3.2 $S_{SM}$-UPGMA coefficient (Figures 3 and 4).

The *Micromonospora* test isolates formed four clusters in the dendogram. Among these, six test isolates showed over 85% similarity in the 3rd cluster whereas the 1st and the 4th groups contained two test isolates. Single member of the 2nd group took place in the dendogram. Whereas some protein bands of the test isolates were found in all of them according to the analysis results, some bands were observed to be strain specific. As can be seen in Figure 3, whereas the bands shown with arrows numbered 1, 2, 6, 7, 8 and 9 are specific to test organisms B006, B030, B057, B010, B052 and B057, respectively, the bands shown by arrows numbered 3, 4, 5 are those that are common to *Micromonospora* test isolates.This band was found in all isolates and was probably a structural protein.

In this study, a test error of 2.306% was calculated as a result of statistical analyses. This test error was quite low when compared with the 10% test error accepted as normal by Sneath and Johnson (1972) and the test errors in some of the previous numerical studies.

Similarity coefficient is used in numerical taxonomy studies in order to determine the suitability of the data in hierarchical clustering. Similarity values between 60 and 95% (Sackin and Jones, 1993) show that the taxonomic structure has resulted well in numerical classification. As a result of the analyses carried out, the similarity ratio between SSM-UPGMA strains was determined to be 75%. 65 test strains were classified into five large (four to 26 strains), two small (two to three strains) and two single item sets in terms of unit character. The highest value in the large and small inner set similarities defined according to SSM-UPGMA analyses was 98% in the 5th set whereas the smallest value was 74% for the 3rd set. Different bacteria sampled from different soils were generally in different sets.

In our study, 10 *Micromonospora* isolates were selected to represent color groups for the analysis of all cell protein profiles. It was found that there were 28 protein bands in *Micromonospora* strains and their molecular weight varied between 25 to 100 kDa. Four sets were formed according to the dendogram analysis of the protein band profiles in SSM-UPGMA. The 3rd cluster contained five isolates, the 1st and 4th clusters contained

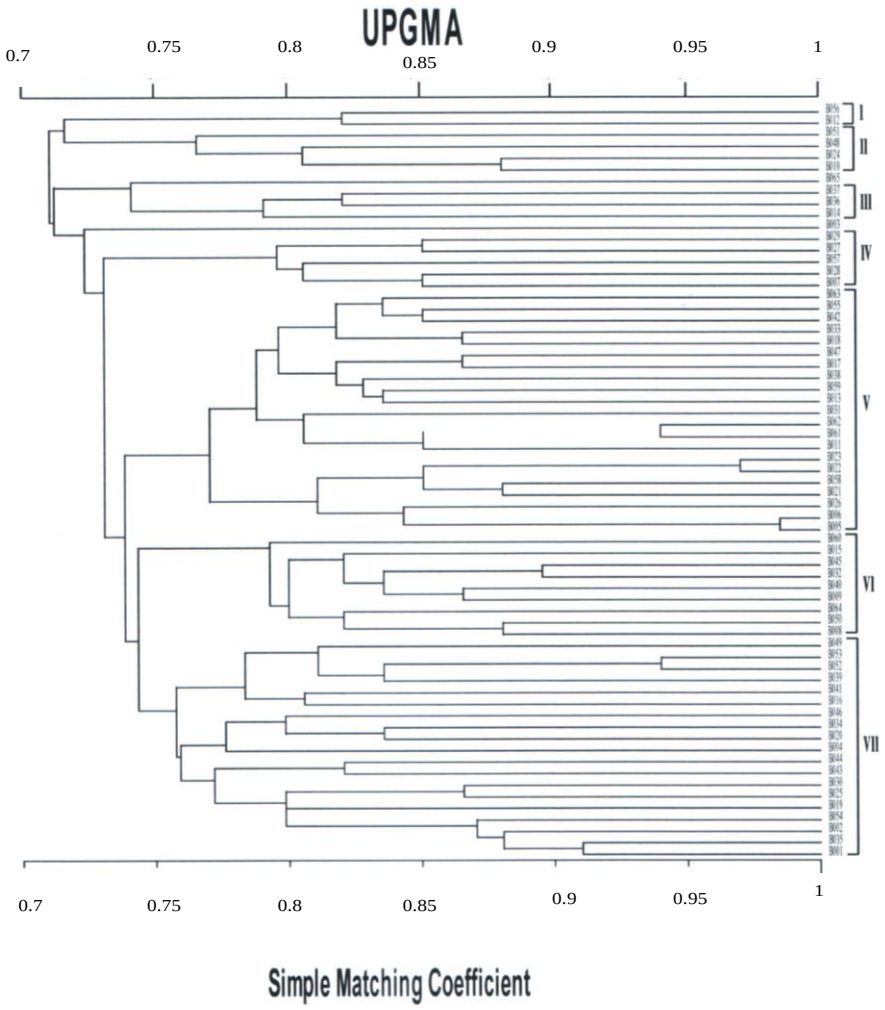


**Figure 2.** UPGMA dendogram derived from the combination of tests of *Micromonospora* sp.

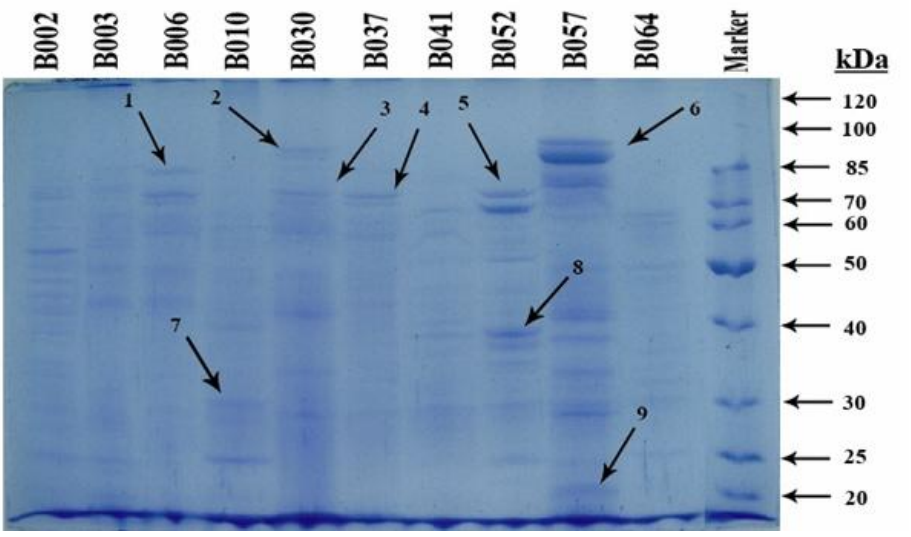


**Figure 3.** Total protein profiles of *Micromonospora* strains. Molecular weights are kDa.

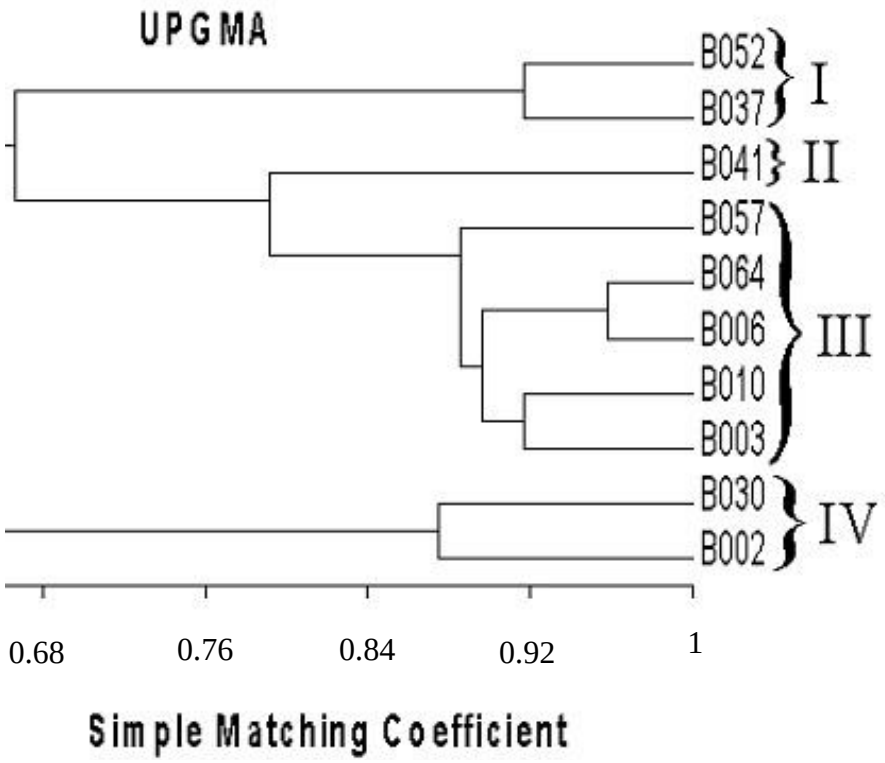


**Figure 4.** UPGMA dendogram derived from the combination of SDS-PAGE profiles of *Micromonospora* sp.

2 isolates and the 2nd cluster contained 1 isolate.

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