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sa međunarodnim učešćem

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with international participation

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MALDI-TOF-MS Characterization of Environmental Bacterial Isolates

MALDI-TOF-MS karakterizacija bakterija izolovanih iz životne sredine

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Introduction

Various traditional and modern microbiological methods are available for the analysis and characterization of pure bacterial cultures. However, for some of them sample preparation can be very time-consuming. Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF-MS) has the advantage of short measuring time, fast sample preparation and negligible sample consumption. With the utilization of MALDI-TOF MS biomolecules, such as proteins, peptides, oligosaccharides and oligonucleotides in the range of 400 and up to 350,000 Da, can be analysed within a few seconds [1]. Recently, determination of the bacterial samples as intact (whole) cells and also bacterial extracts has been developed [2]. Ten bacterial environmental isolates were characterized using 16SrRNA and subsequently MALDI-TOF-MS, with the use of the Maldi Biotyper database.

Material and Methods

Isolation and media. Hydrocarbon degrading bacteria were isolated as a pure culture using media with diesel as a sole source of carbon. Ferrous iron and sulphur oxidizing thionic bacteria were isolated as a pure culture after enrichment in 9K liquid medium and growth in microwell plates using the method of most probable number.

Analysis of 16S rRNA gene sequences. The genomic DNA of bacteria was extracted with the use of DNeasy Blood & Tissue Kit (Qiagen, Germany). The 16S rRNA genes were amplified by PCR using 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-CGGCTACCTTGTTACGACTT-3') primers and amplified fragments were sequenced using the commercial MACROGEN (Netherlands) service.

MALDI-TOF MS. Autoflex II Bruker Daltonics, and Microflex Bruker Daltonics MSTM, software flexControl, flexAnalysis and Maldi biotyper were used. Matrix α-Cyano-4-hydroxycinnamic acid; shots: 300; laser intensity: 35-40%; masses

in the range 2-20 kDa were determined in linear positive mode at an accelerated voltage of 19 kV. Bacterial samples were analysed as intact (whole) cells and also as ethanol extracts.

Results

According to 16S rRNA gene analysis, 10 strains were *Brachybacterium sp*. CH-KOV3, *Rhodococcus sp.* H33-7, *Rhodococcus sp*. H63-1, *Acidithiobacillus ferrooxidans* strains B1 & B2, *Planomicrobium sp.* RNP01, *Micrococcus sp.* RNP04, *Rhodococcus sp.* RNP05, *Planococcus sp.* RNP07, and *Pseudomonas sp.* NS22. Through the comparison of the identification made by 16S rRNA gene sequencing and MALDI Biotyper software, positive identification was made only for two samples. *Rhodococcus erythropolis* (RNP05) and *Micrococcus luteus* (RNP04) were identified positive as secure genus identification and probable species identification. However, software was not able to identify genera *Brachybacterium, Acidithiobacillus, Planomicrobium, Planococcus* and *Pseudomonas.* In this case, the spectral/protein profile is referenced to a compiled database with 3,900 strains from over 2,000 well-characterized microbial species. However, it was shown that this database was not reliable for environmental samples and that the database was optimized for clinical samples. In addition, differentiation between two different phyla (Proteobacteria and Actinobacteria), genus (*Planomicrobium* and *Planococcus)*, species (*Rhodococcus rhodochrous* and *Rhodococcus erythropolis*) and strains (*Acidithiobacillus ferrooxidans* strain B-1 and B-2) was studied. It was confirmed that the mass spectra of evolutionary close related environmental bacteria are more similar than between phylogenetically remote bacteria. The similarity of mass spectra was about 15% for evolutionarily distant phyla versus more than 90% similarity for close related strains. Dominant protein peaks are present in genus and species spectra, suggesting that the method is reliable for genus and species differentiation and identification.

Conclusion

The results have confirmed that MALDI-TOF-MS is a fast and reliable automated method for clinical isolates but that the current database is not appropriate for the identification of bacterial environmental isolates. It is necessary to expand the database and analyse a number of bacteria from different habitats. MALDI-TOF-MS is an excellent method for a quick fingerprinting of intact bacterial cells.

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