**INTRODUCTION AND AIMS OF STUDY**

In this study we described a method for classifying strains of the genus *Pseudomonas*. Characterization of intact cells by Matrix-Assisted Laser Desorption Ionization - Time-of-Flight Mass Spectrometry (MALDI TOF MS) was proved to be effective for the detection of protein/peptide ions and biomarkers of bacterial cytoplasmic membrane and cytoplasm in less than five minutes with a high sensitivity.

**EXPERIMENTAL SECTION**

**Bacterial strains and optimal growth conditions**

*Pseudomonas* strains were obtained from the Czech Collection of Microorganisms (33 type strains) and from *Die Deutsche Sammlung von Mikroorganismen und Zellkulturen* (7 type strains). In total, there were analyzed 103 strains involved types and reference cultures. For a significant output, the strains were cultivated in two subcultures (two times for 24 hours) on Tryptone soya agar at 30°C, alternatively at 25°C and 37°C.

**Optimal sample preparation**

Cells were taken with sterile sampling loop (1ml) from the second subculture on Petri dish. Cell suspension was prepared in the solvent demi-Q water: ACN (1:1 v/v).

**MALDI-TOF MS analysis**

- Analysis of the MS spectra of intact cells using a Bruker instrument equipped with a nitrogen laser (337 nm).
- MALDI matrices: dithran (98% 2,5-dihydroxybenzoic acid) and 1% 2,5-dihydroxy-6-nitrobenzoic acid.
- MALDI-TOF MS targets were prepared by direct spotting method, each strain was spotted in 3 spots.
- Mass spectra recordings were measured in the linear positive mode under voltage 25kV.

**RESULTS**

MALDI-TOF MS profiling was used for taxonomic differentiation of strains of the genus *Pseudomonas*. The most reproducible spectra with high signal-to-noise ratios and appropriate distribution of peaks were obtained with cell density 2 µl of cells in 500 µl water: acetonitrile (1:1) in sample. For 50% ACN bacteria extracts, optimal MALDI matrix was dithran (1% TFA). Data analysis algorithms have been optimized. Cluster analysis of MALDI-TOF MS profiles showed taxonomic relationship and reference strains at the sub-species level. Positions of type strains in dendrogram correlated with 16S rRNA phyllogram published previously.

**CONCLUSIONS**

- Optimization process showed no influence of culture age, medium, prolonged cultivation and freeze sample storage on *Pseudomonas* type strains MALDI-TOF MS profiles.
- Cluster analysis of *Pseudomonas* strains showed the capability of MALDI-TOF MS to differentiate and to classify samples at the subspecies level. *Pseudomonas* species represented with two and more strains form one branch point as shown on Fig. 10, Fig. 11 and Fig. 12. Species forming one branch are pointed out in colour ellipses.

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