IDENTIFICATION OF BACTERIA BY COMBINATION OF PREPARATIVE ISOELECTRIC FOCUSING AND MALDI-TOF MS

J. Šalplachta[1], A. Kubesová[1], F. Růžička[2] and K. Šlais[1]

[1]Department of Fluid Phase Separations, Institute of Analytical Chemistry of the CAS, Veveří 97, 602 00 Brno, Czech Republic
E-mail: salplachta@iach.cz

[2]Department of Microbiology, Faculty of Medicine, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic

Introduction

Identification and monitoring of various microorganisms from a complicated matrix is required for diagnostic purposes in medicine, environmental studies, food industry, and many other areas. This study describes a preparative method based on isoelectric focusing of colored microorganisms, *Rhodotorula mucilaginosa*, *Micrococcus luteus*, and *Dietzia* sp., in a cellulose-based separation medium from a high conductivity matrix. These microorganisms are opportunistic human pathogens found especially in immunocompromised patients. The cells of *R. mucilaginosa* are coral pink, *M. luteus* produces bright yellow colonies and *Dietzia* sp. colonies are initially orange in color, becoming coral red with age. The isoelectric points found for the examined cells were 1.8 for *M. luteus*, 3.5 for *Dietzia* sp., and 4.7 for *R. mucilaginosa* using capillary isoelectric focusing. The final positions of the zones of colored microbial cells in the cellulose-bed are indicated by colored p*I* markers. Segments of the separation medium with cells were harvested with a spatula, simply processed and further analyzed by MALDI-TOF mass spectrometry.

Results

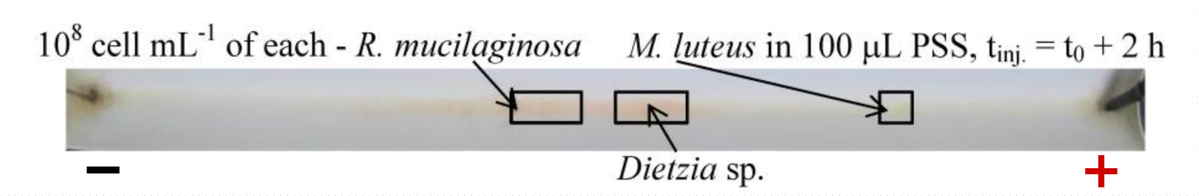

Fig. 2: Preparative IEF of the mixture of colored microbes (*R. mucilaginosa*, *Dietzia* sp., and *M. luteus*) in PSS, each of them 10^8 cell mL^{-1}.

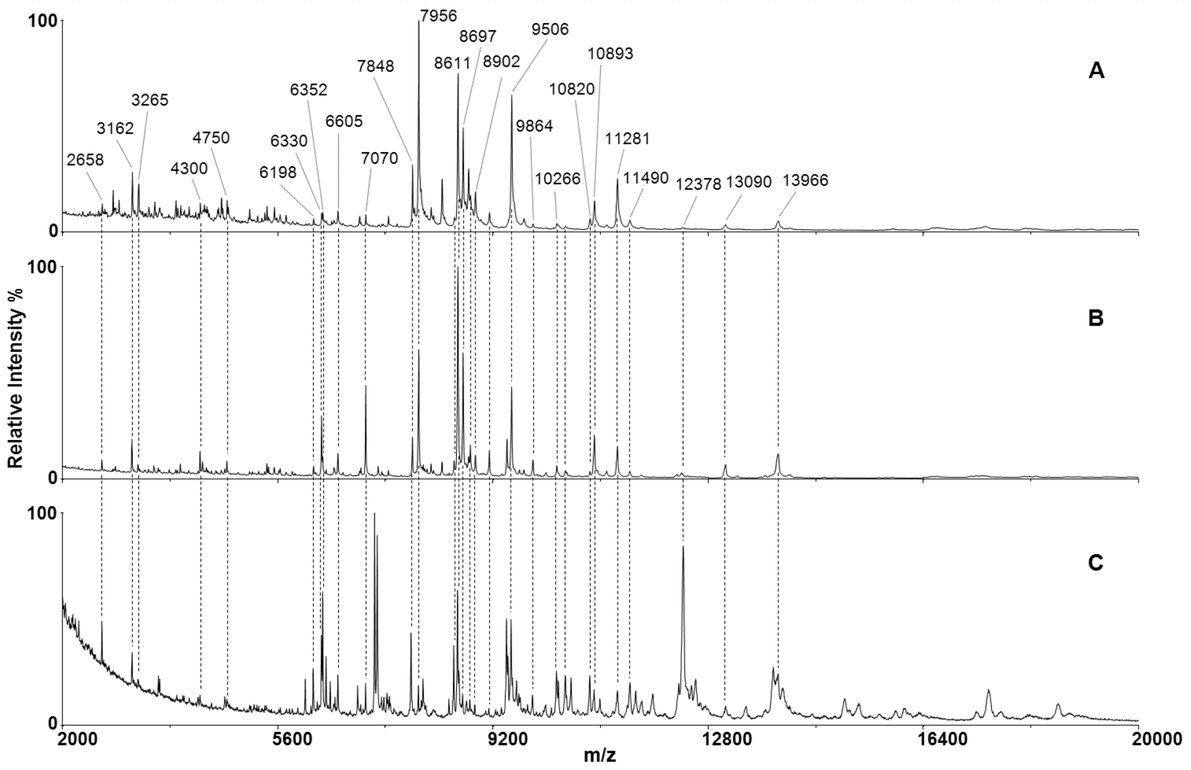

Fig. 3: MALDI-TOF mass spectra of *R. mucilaginosa*. (A) cultivated bacterial cells; (B) fraction collected after preparative IEF of single strain; (C) fraction collected after preparative IEF of the mixture of three microbial strains.

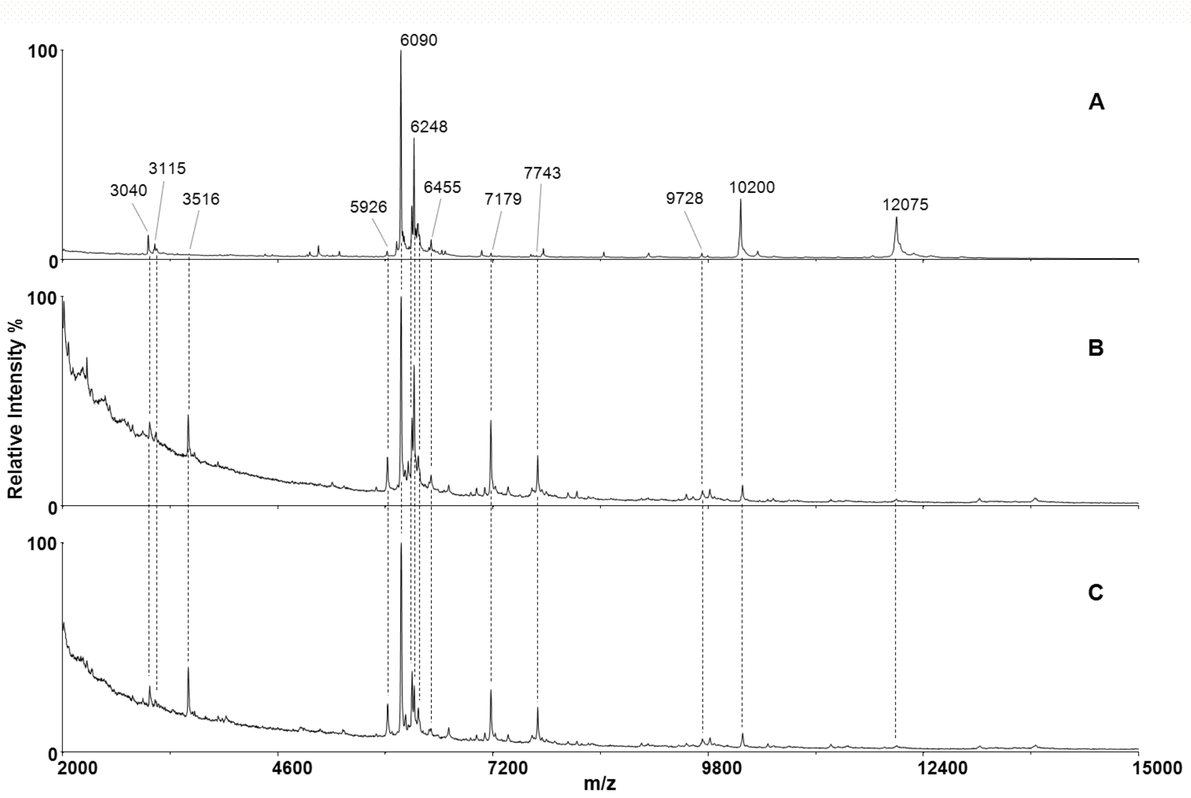

Fig. 4: MALDI-TOF mass spectra of *M. luteus*. (A) cultivated bacterial cells; (B) fraction collected after preparative IEF of single strain; (C) fraction collected after preparative IEF of the mixture of three microbial strains.

Experimental

Microorganisms

The strains of *M. luteus* CCM 169T, and *Dietzia* sp. CCM 2585 were obtained from the Czech Collection of Microorganisms (Brno, Czech Republic). The colored strain *R. mucilaginosa* M019 was isolated from clinical material and stored in a Collection of Microbiology, Masaryk University and St. Anne's University Hospital (Brno, Czech Republic).

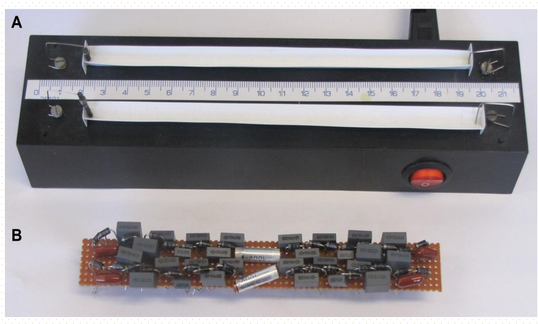

Fig. 1: Preparative IEF device with a pair of troughs (A) and power supply for IEF (B).

Preparative IEF

Preparative IEF device is shown in Fig. 1. The separation medium together with p*I* marker solution were introduced in an empty V-shaped plastic trough positioned on a power source. The power supply was turned on and the trough was covered to retard the evaporation of water from the separation medium. After two hours, the plastic lid was removed and 100 µL of the microbial sample was introduced along the central third of the trough. The sample was either individual microorganism or a mixture of the microorganisms resuspended in a physiological saline solution (PSS). The IEF device was left running for additional 18 h and then the fractions, defined by the positions of colored p*I* markers, were collected and analyzed by MALDI-TOF MS.

MALDI-TOF MS

All MALDI-TOF MS experiments were performed on AB Sciex TOF/TOF 5800 System operating in linear positive ion mode, sinapinic acid (SA) was used as a matrix.

Fifty microliters of the bacterial suspension (1×10^8 cells mL^{-1}) was centrifuged at 6000×g for 20 min, the supernatant was discarded and the pellet was resuspended in 50 µL of SA solution (20 mg mL-1 in ACN/0.1% TFA, 3:2, (v/v)).

IEF fractions - each fraction was resuspended in 100 µL of deionized water, the suspension was centrifuged at 6000 g for 20 min. The supernatant was discarded and the cells were resuspended in 10 µL of SA solution.

All bacterial samples were centrifuged again at 3000 g for 3 min and each supernatant was spotted onto a sample plate previously overlaid with the SA solution.

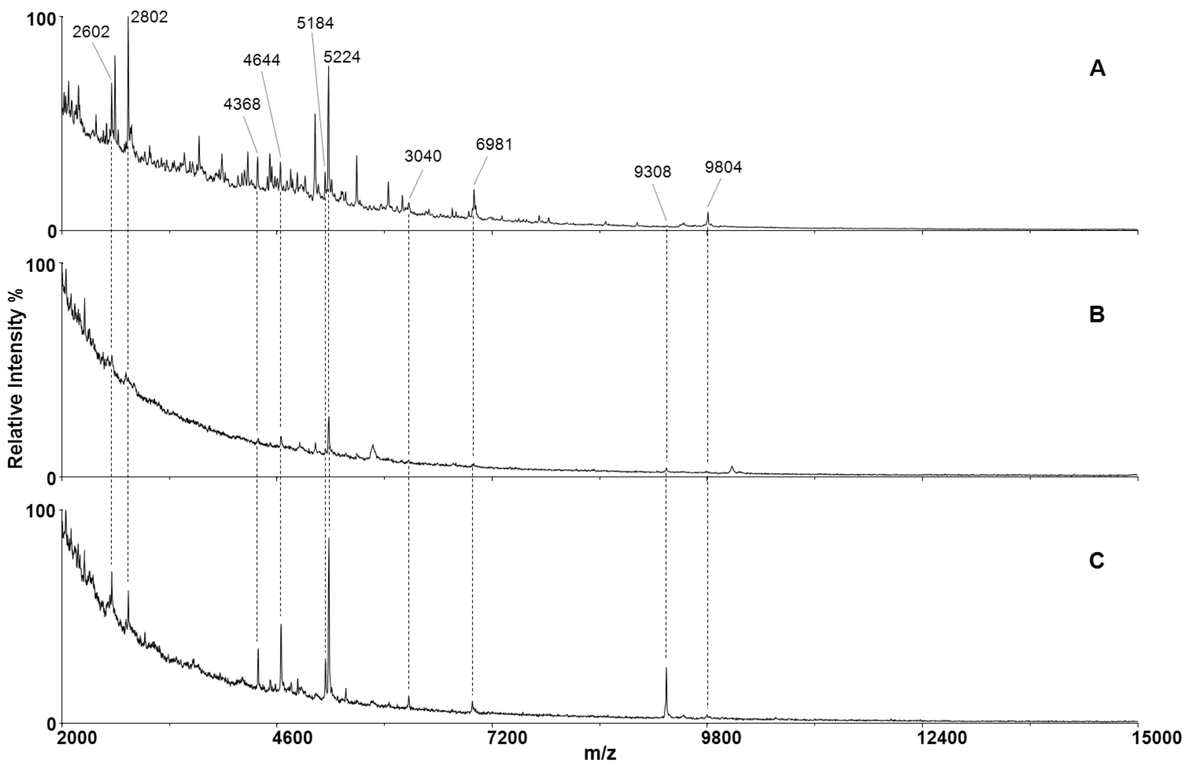

Fig. 5: MALDI-TOF mass spectra of *Dietzia* sp. (A) cultivated bacterial cells; (B) fraction collected after preparative IEF of single strain; (C) fraction collected after preparative IEF of the mixture of three microbial strains.

Conclusions

The colored microorganisms, *R. mucilaginosa*, *M. luteus*, *Dietzia* sp., both individually and in the mixture, were successfully separated by preparative IEF in cellulose-based separation medium according to their p*I* values. This is made possible by the pores of the separation medium which are large enough for the analytes of µm's dimensions to move in independently of their size. The focused microbial zones in the cellulose bed were collected according to the color of the zones or their isoelectric points. The cells from the zones were further analyzed by MALDI-TOF MS in order to verify the focusing ability of the preparative IEF.

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