DISCRIMINATION BETWEEN HALOARCHAEA GENERA USING RAMAN SPECTROSCOPY AND PCA

Anda Leș[1], Nicoleta E. Dina[1], Andreea Baricz[2], Horia Banciu[2] and Nicolae Leopold[1]

[1]*Faculty of Physics, Babeş-Bolyai University, Cluj-Napoca, Romania*

[2]*Faculty of Biology and Geology, Babeș-Bolyai University, Cluj-Napoca, Romania*

INTRODUCTION

- Halophilic archaea of Halobacteriaceae family have been investigated as models for understanding life at extreme conditions, such as high salinity.
- Haloarchaeal strains tested in the present study, *Haloferax 1R9*, *Halobacterium 103.5-1* and *Halorubrum 5F8-2*, were isolated from perennially stratified Transylvanian salt lake.
- The Raman spectra, all showing similar features, attest the presence of bacterioruberin biomarkers (Fig. 1).

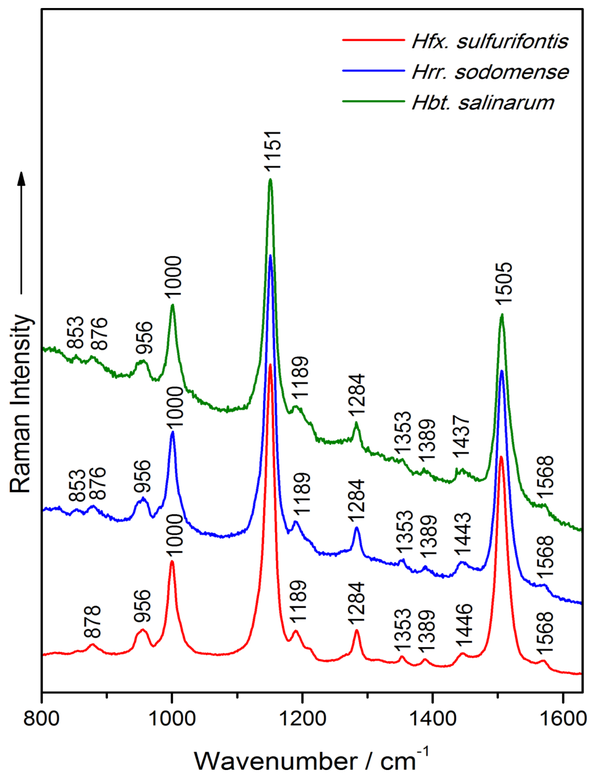

Fig. 1 Raman spectra of the haloarchaea samples, genera: *Haloferax*, *Halorubrum* and *Halobacterium*, respectively, recorded with the 785 nm laser line.

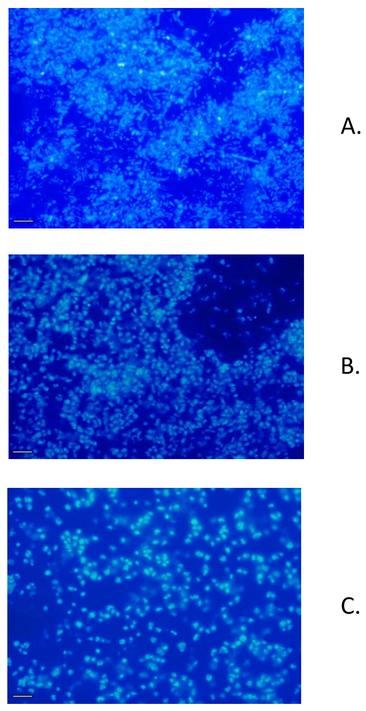

Fig. 2 Epifluorescence micrographs of DAPI stained cultures of *Halobacterium* (A), *Haloferx* (B), *Halorubrum* (C). The scale bar indicates 10 μm.

- This work exhibits the Principal Components Analysis (PCA) of the Raman data as a potential tool to quantify the differences in the spectral profile of all strains in order to achieve the best discrimination between them.
- Moreover, a Raman mapping was done on a membrane filter on which different microorganisms were randomly distributed. Particularly, this analysis enabled visualizing the specific spots from the material at which the known haloarchaea strains were present and to make a signal-based image of the whole area of interest.

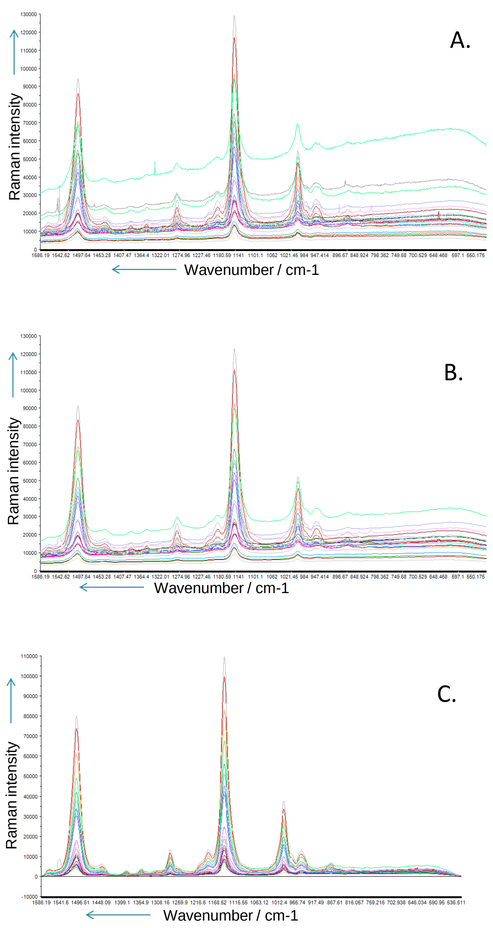

Fig. 4 Effects of the preprocessing techniques on the Raman spectra: raw spectra (A), after smoothing (B) and baseline correction (C).

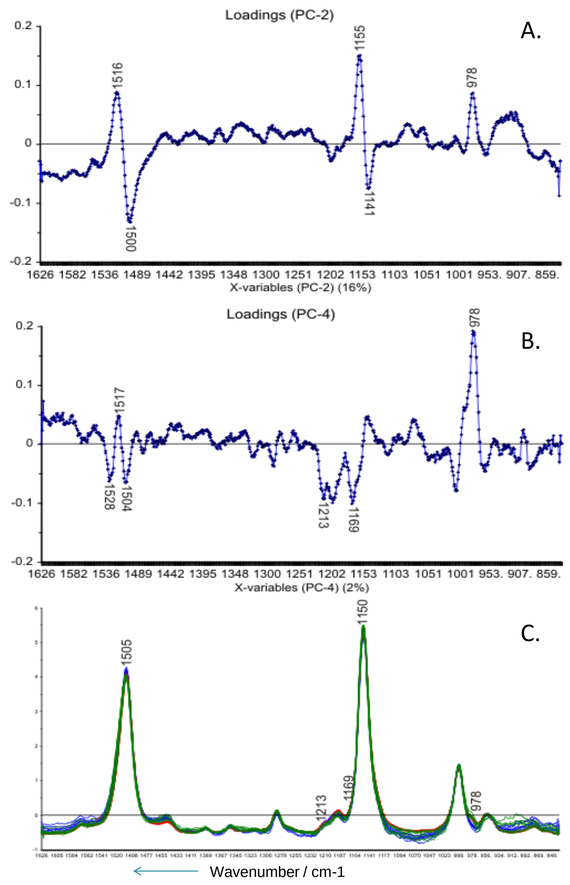

Fig. 5 Loadings plot for the PC2 (A) and PC4 (B). Also the final corrected spectra, after smoothing, baseline correction and SNV are represented (C)

EXPERIMENTAL AND THEORETICAL METHODS

- The Raman spectra were recorded with a Renishaw inVia Raman Microscope, using the 785 nm laser line by co-adding two acquisitions, each of 40 s exposure time at a power of 1 mW.
- The samples were stained with DAPI, 5 μg ml^{-1} and examined by epifluorescence microscopy using an Olympus XC50 microscope equipped with a UV filter with excitation light set at 330-385 nm and a digital camera.
- The PCA was performed in a dedicated software, UnscramblerX, version 10.1 (Camo), using cross-validation method. All the spectral data were preprocessed, several transformation algorithms being trialed.
- Also, the data collected from the Raman mapping of a membrane filter embedded with a solution of hypersaline environment were used to recreate the original scanned area.
- For this part, the paper was left to dry and then was Raman mapped (1h scanning time) with the resonant 532 nm laser line at a power of 20mW.

RESULTS

- The resonant Raman spectra of the haloarchaea samples exhibit similar features, the main peaks being present at the mean wavenumbers: 1000 δ(C=CH), 1151 υ(C-C), 1284 δ(CH2), 1446 δ(CH3) and 1506 cm^{-1} in-phase υ(C=C) bacterioruberin with specific band shifts for each strain.
- The PCA was employed only after most relevant preprocessing techniques were applied: smoothing procedure (using the Savitsky-Golay algorithm with seven points of smoothing) followed by baseline correction (linear baseline correction algorithm) and finally applying the standard normal variate (SNV) transformation (Fig. 4).

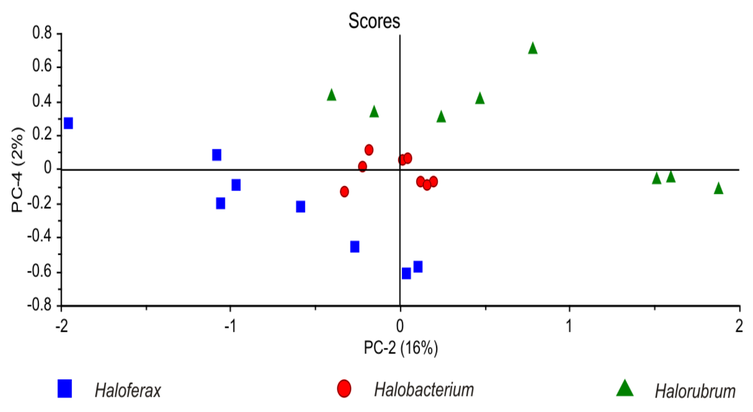

Fig. 6 PCA scores plot showing the grouping of the three studied halophilic genera *Haloferax, Halobacterium* and *Halorubrum.*

- The best strain discrimination could be done based on the variance explained by the PC4 plotted against PC2 (Fig. 3).
- The analysis shows a good grouping tendency for the three strains investigated.

- The loadings plot of these two principal components underscore the wavenumbers of the Raman shifts on the horizontal axis and their new coordinates upon the direction of PC2 (Fig. 5A) and PC4 (Fig.5B).

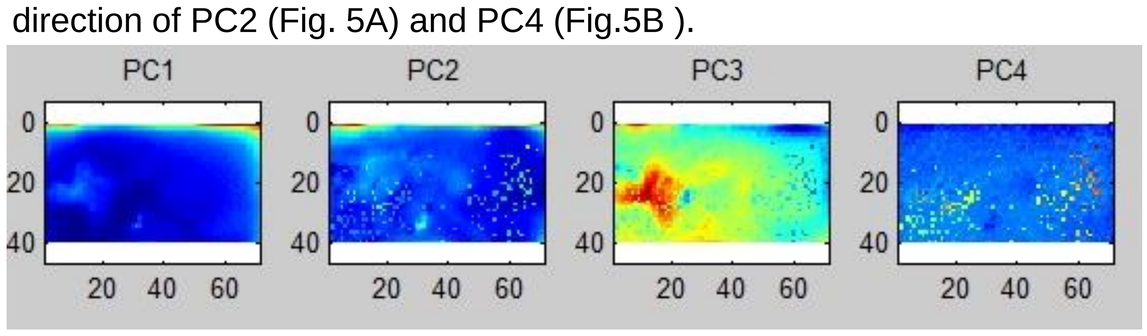

Fig. 7 Reconstructed images of the membrane filter, on which the haloarchaea microorganisms are represented with dark blue color in PC3 picture.

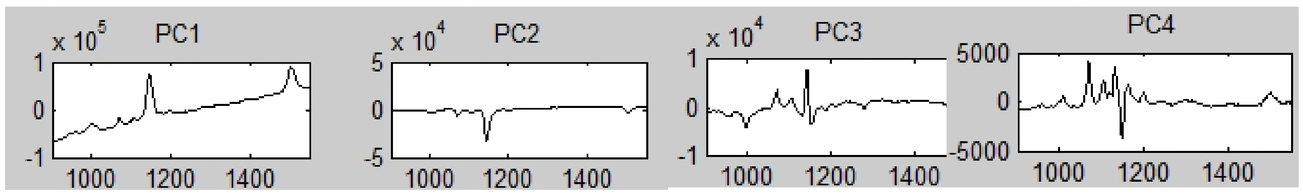

Fig. 6 Loadings plot for the first four principal components, corresponding to the processed images.

- For reproducing the image of the membrane filter, PC3 was used to distinguish the zones with strong signal from the bacterioruberin (blue spotted), which have negative values on the loadings plot at 1000 and 1506 cm^{-1} wavenumbers (Fig. 6).

CONCLUSIONS

- This study shows the utility of the combined method - Raman spectroscopy and PCA for haloarchaea strain discrimination.
- The accuracy of the technique depends on the preprocessing techniques applied.
- Reconstructed images essay the correlation of the chemical fingerprints of the sample with its morphological shape.
- Being given the results, this multivariate analysis of the spectral data has good prospects for life science applications.

ACKNOWLEDGMENTS

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