CHARACTERIZATION OF GRAM POSITIVE BACTERIA USING RAMAN SPECTROSCOPY

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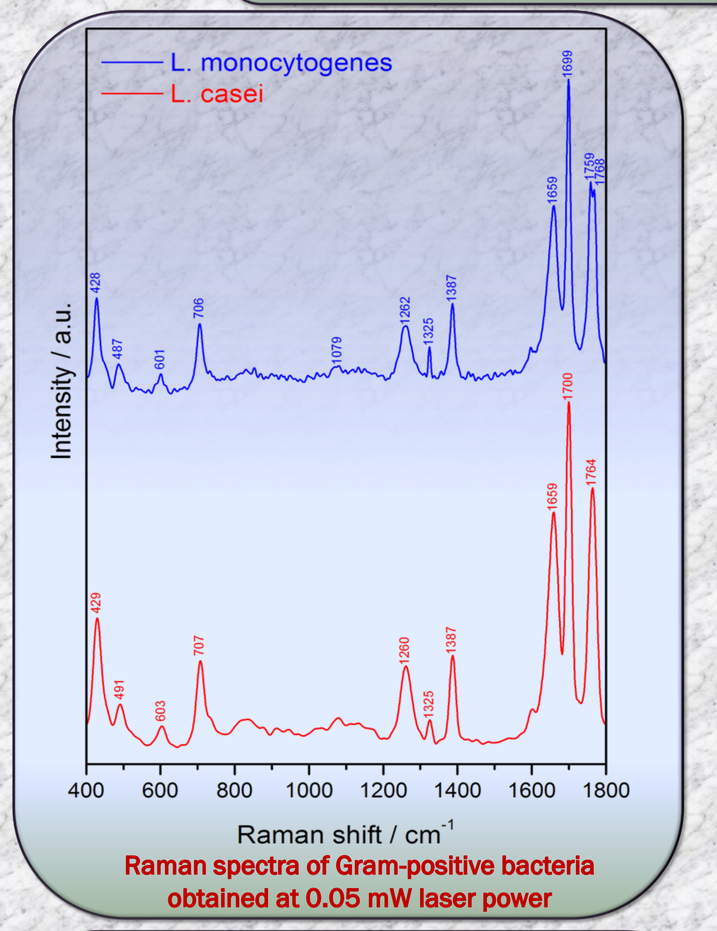
Abstract

In the last years, Raman spectroscopy has become a well-known physicochemical technique for the rapid identification of microorganisms. This powerful analytical method generates a spectroscopic fingerprint from the bacterial sample, which provides quantitative and qualitative information used to characterize, discriminate and identify different types of microorganisms. This work reports the use of Raman and surface-enhanced Raman scattering (SERS) spectroscopic methods for identification, characterization and discrimination of Gram-positive bacterial species. *Lactobacillus casei* and *Listeria monocytogenes* are rod shaped, nonsporing, anaerobic bacteria. While *L. casei* is a well-known probiotic, since it has numerous beneficial effects, *L. monocytogenes* is the agent of listeriosis, a serious infection caused by eating food contaminated with this type of bacteria. In order to obtain the Raman spectra, the bacteria were harvested and suspended in a small volume of water. The measurements were made on MgF_2 slides using 50x objective. As SERS active substrate we used silver colloids prepared by different reduction methods and the obtained SERS spectra were compared with the Raman spectra. The assignment of the main bands was made by taking into account the position and the relative intensities. We were able to optimize the bacteria sample preparation procedures in order to obtain reproducible SERS spectra that enable us to characterize and discriminate the two Gram-positive species.

Growth conditions - *L. casei ATCC 393*
• routinely cultured overnight at 37 °C in MRS broth (Oxoid, UK).
• sample concentration:
Sample preparation for Raman / SERS measurements
2 mL bacteria culture were centrifuged (6000 rpm, 10 min) and washed 3 times with deionized water; the harvested pellet was resuspended in 1 mL H_2O for all the measurements.

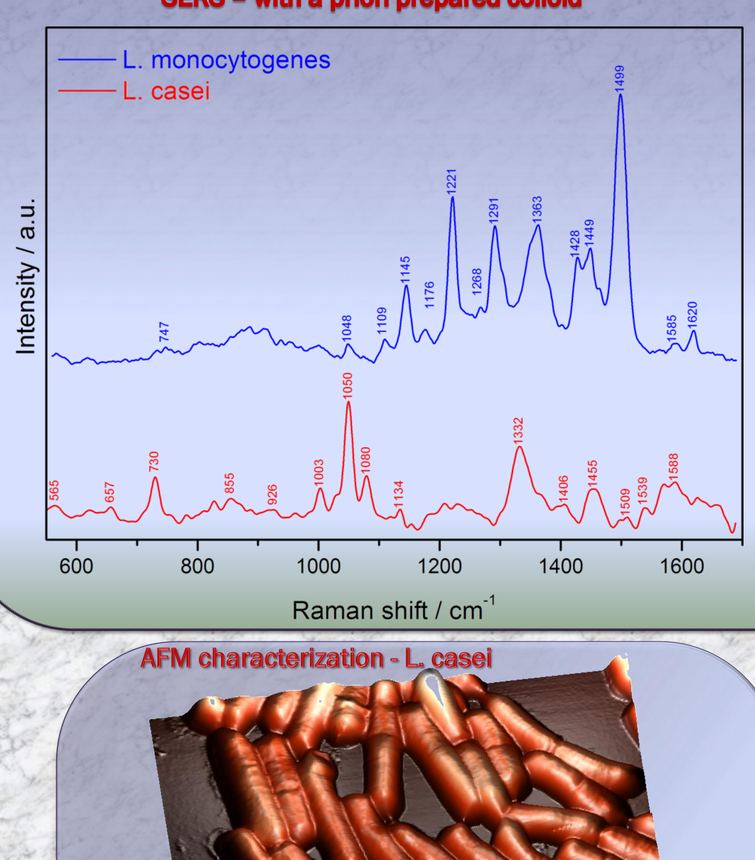
Growth conditions - *L. monocytogenes ATCC 19115*
• maintained on Oxford agar (Sifin, Germany) plates at 4 °C; a single colony of *L. monocytogenes* was inoculated into a tube of tryptic soy broth plus 0.7% yeast extract (TSBYE) (Difco Laboratories) and incubated at 35 °C for 24 h.
Sample concentration: 10^{11} CFU/ml. (O.D. 600nm=0.7)
Sample preparation for Raman / SERS measurements
2 mL bacteria culture were centrifuged (6000 rpm, 10 min) and washed 3 times with deionized water; the harvested pellet was resuspended in 250 µL H_2O for all the measurements.

Experimental methods
Raman / SERS spectra (Department of Biomolecular Physics, Faculty of Physics, Babeş-Bolyai University, Cluj-Napoca)
• Renishaw inVia Raman Microscope with He-Ne laser (632.8 nm); objective: 50x; total laser power: 50mW; resolution: 4 cm^{-1}
• SERS spectra – AgNPs were prepared by reduction of $AgNO_3$ with hydroxilamine hidrochloride (hya) [1].
- for in situ detection: to 100 µl $AgNO_3$ (10^{-3} M) we added bacteria suspension (5-30 µl) and 900 µl hya solution (~10^{-3} M) with basic pH [2].

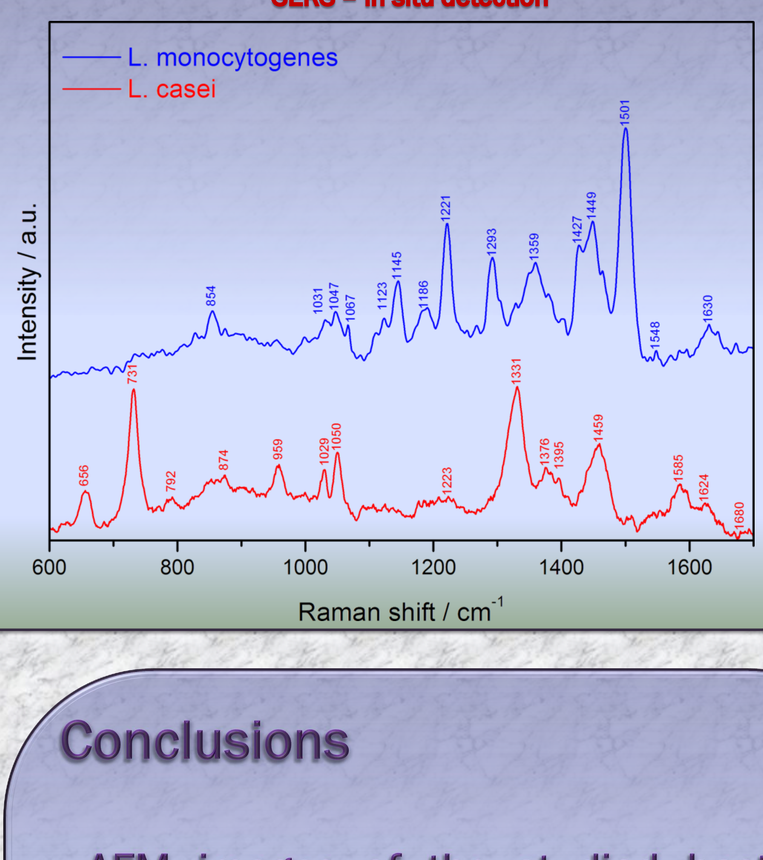


Raman spectra of Gram-positive bacteria obtained at 0.05 mW laser power

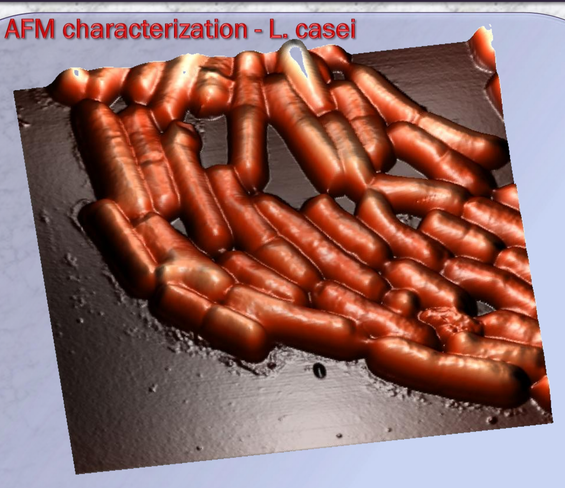
SERS – with a priori prepared colloid



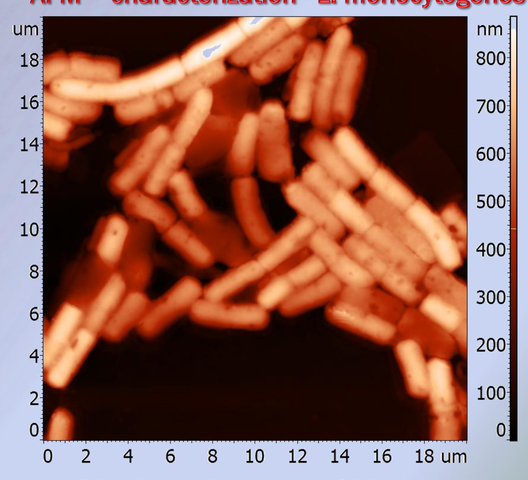
SERS – in situ detection



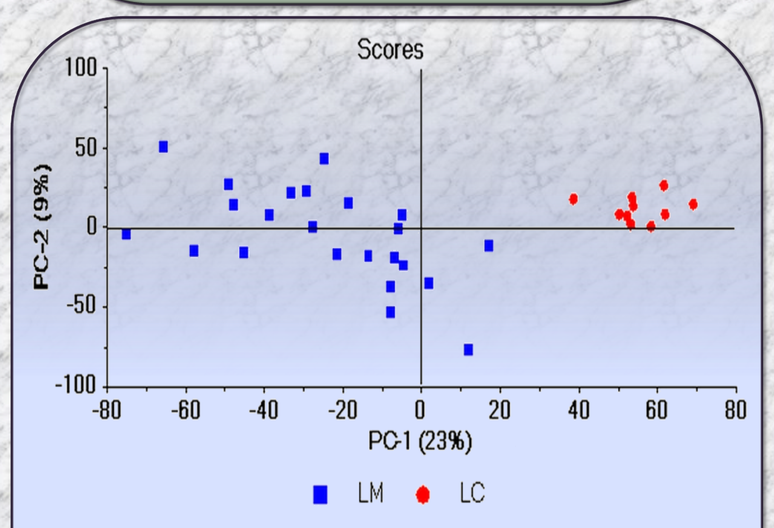
AFM characterization - L. casei



AFM – characterization - L. monocytogenes



* Courtesy of Dr. Diana Bogdan, INCDTIM Cluj-Napoca



PCA discrimination of Lactobacillus casei and Listeria monocytogenes based on first derivative Raman spectra
The results were obtained using the Unscrambler® software

Conclusions

- AFM images of the studied bacteria indicate their viability for the obtained Raman and SERS results.
- The Raman spectra of two Gram-positive bacterial species are very similar, an indicator of the same cell wall components.
- Based on the PCA analysis, the discrimination between the two Gram-positive bacteria is possible.
- The SERS bands located in the spectra of *L. casei* and *L. monocytogenes* arise from the peptidoglycan layer inside the cellular wall.

References:
[1] N. Leopold, B. Lendl, *J. Phys. Chem. B* 107 (2003) 5723.
[2] M. Kahraman, K. Keseroglu, M. Culha, *Appl. Spectrosc.* 65 (2011) 500.

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