Optimized label-free detection of most common pathogens based on SERS mapping

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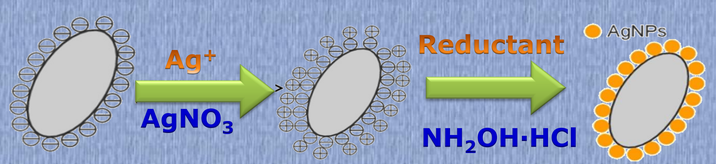
Recently, the possibility of creating surface-enhanced Raman scattering (SERS)-based biosensors for rapid detection of bacteria is widely explored. With this purpose, we used SERS spectroscopy along with chemometric techniques to detect and identify the most common pathogens' spectral profiles in different cultivation conditions by using *in situ* synthesized silver colloid (Bacteria@AgNPs) and incubation in silver colloid [1,2].

Enhanced darkfield hyperspectral microscopy analysis was employed for characterizing the interaction between the bacteria and silver nanoparticles (Bacteria@AgNPs system). The reduced required sample volume, the rapid spectral acquisition, and the use of chemometric techniques for analyzing the SERS maps, provided the optimum platform for developing SERS-based biosensors for food safety, water research, or health care real-life applications.

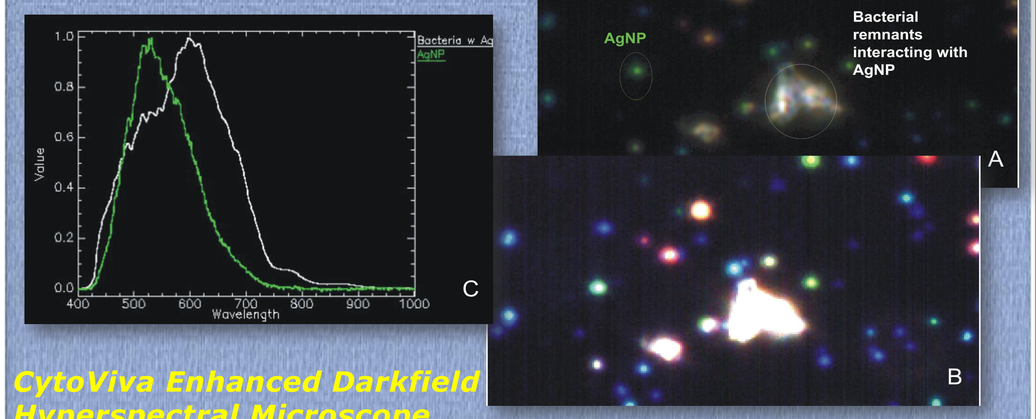
Experimental details:

I. Bacteria cultivation: 2 ml of Gram-positive *(E. lactis, L. monocytogenes)* and Gram-negative (*Aeromonas, M. morganii, E. lactis* and *E. coli – Rosetta, JM109, BL21DE3, XL1Blue, DH5α strains)* bacteria were centrifuged at 6000 rpm for 10 minutes and washed 3 times in saline buffer; the supernatant was discarded and the final pellet was resuspended in 200 µl saline buffer.

II. Sample preparation: 3 µl of washed and saline resuspended bacteria were used for the ***in situ* AgNPs synthesis (Bacteria@AgNPs),** as described in [1]. In this case, 5 µl of **Bacteria@AgNPs** were deposited on glass, Polysine™ Microscope Adhesion Slides and silanized glass slides prior to SERS analysis.



III. Characterization methods: SERS effect: Raman – AFM Ntegra platform, NT-MDT, lasers: 532 nm.



CytoViva Enhanced Darkfield Hyperspectral Microscope analysis (100× microscopic image plus 4× digital zoom on AgNPs and Bacteria@AgNPs (A), higher illuminated image showing brighter the metallic reflexions of AgNPs and silver covered biomass (B) and the spectral response of both AgNPs and Bacteria@AgNPs (C)).

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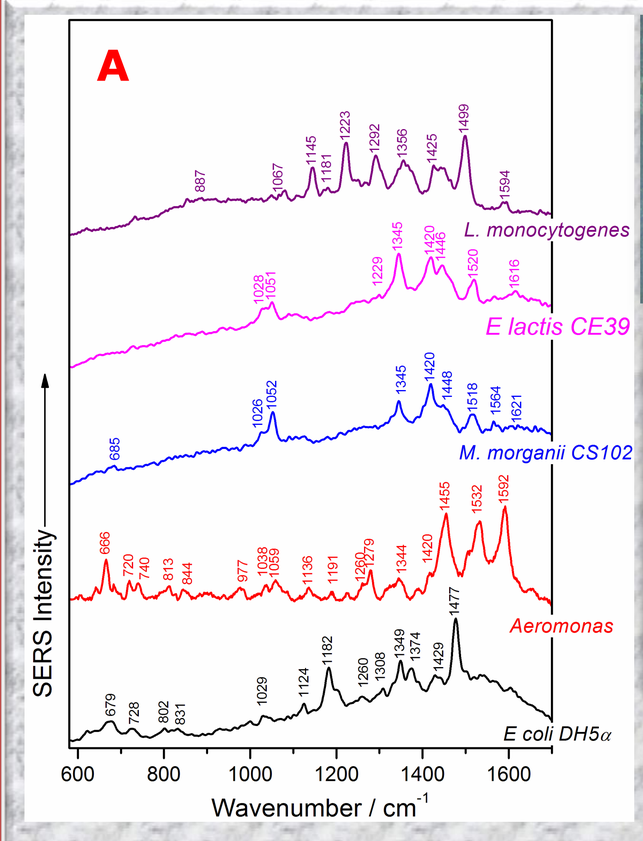
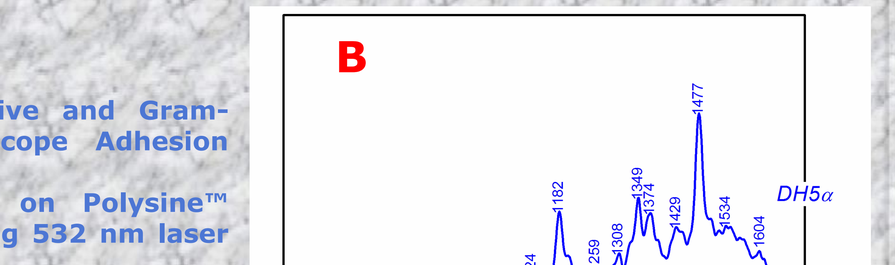
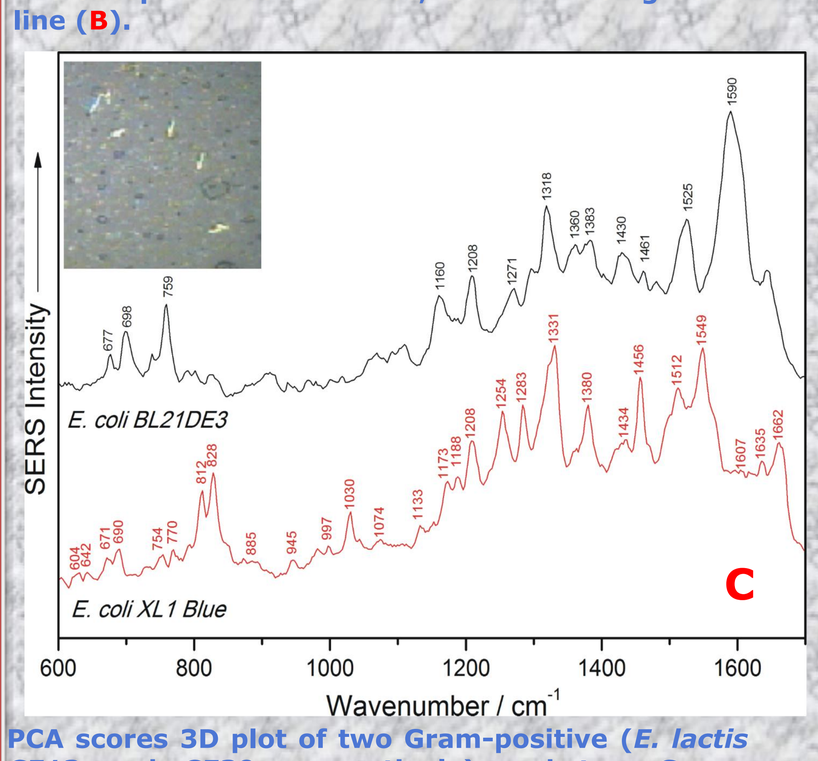


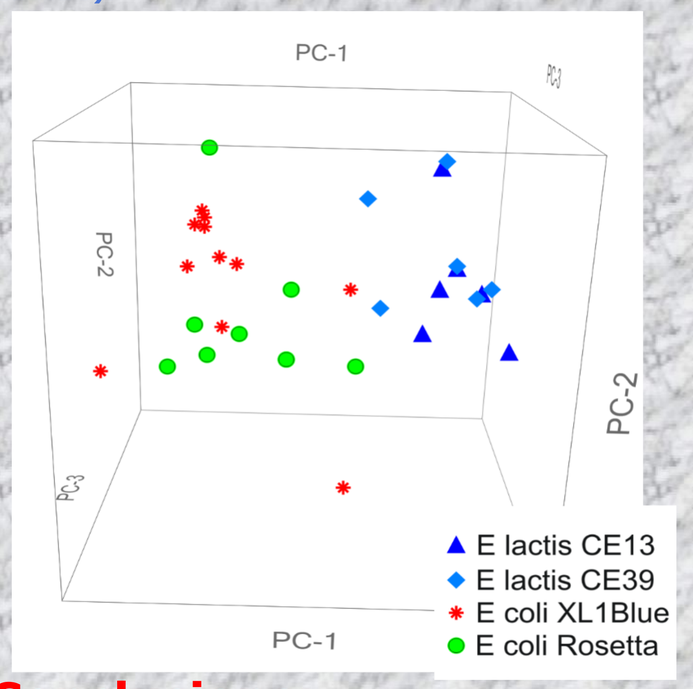
SERS spectra of the tested Gram-positive and Gram-negative bacteria on Polysine™ Microscope Adhesion Slides using 532 nm laser line (A).
SERS spectra of the *E. coli* strains on Polysine™ Microscope Adhesion Slides, obtained using 532 nm laser line (B).



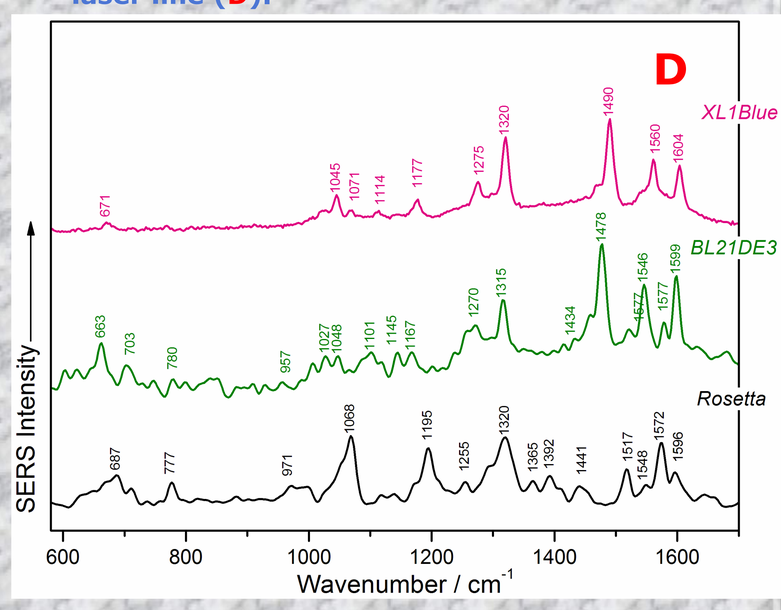


SERS spectra of *BL21DE3* and *XL1Blue E coli* strains recorded by using incubation in silver colloid (C).

PCA scores 3D plot of two Gram-positive (*E. lactis CE13* and *CE39*, respectively) and two Gram-negative species (*E. coli Rosetta* and *E.coli XL1Blue*).



SERS spectra of *E. coli* strains immobilized on silanized glass slides, obtained using 532 nm laser line (D).



Conclusions:

A label-free SERS-based protocol was optimized and the influence of taxonomic affiliation and time-dependent effects of incubation in silver colloid were monitored. The detection and identification of several common pathogens (*E. coli, Aeromonas, M. morganii, E. lactis and L. monocytogenes*) were successfully assessed by using this *in situ* approach.

[1] H. Zhou et al., Anal. Chem. (2014) 86(3), 1525-1533.
[2] D. Yang et al., Talanta (2016) 146, 457-463.

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