Bacterial barcoding - a SERS mapping technique for ultrasensitive detection of pathogens

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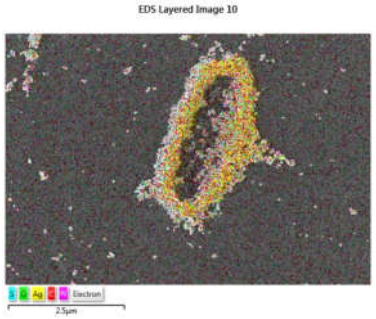
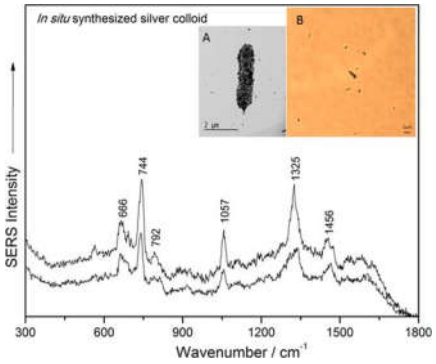
Conventional bacteria identification assays typically require several hours or even days to provide accurate results. Thus, sensitivity, fast response and discrimination at strain level of pathogens are keywords in establishing the appropriate treatment for an infection.

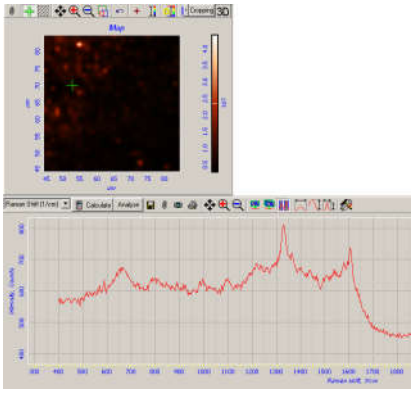
In this work, **surface-enhanced Raman spectroscopy (SERS) mapping** technique was employed with the final aim of identification and discrimination of pathogenic bacteria at single-cell level, based on their detected fingerprint features. Several genera of bacteria that are found in most of the isolated infections in bacteremia were successfully identified without the use of any antibody or other specific receptors. The key element of the SERS direct detection platform was low cost, accessible substrates (poly-slides), which facilitated single-cell events. Moreover, the SERS detection assay was successfully tested both on **Gram-negative and Gram-positive microorganisms.**



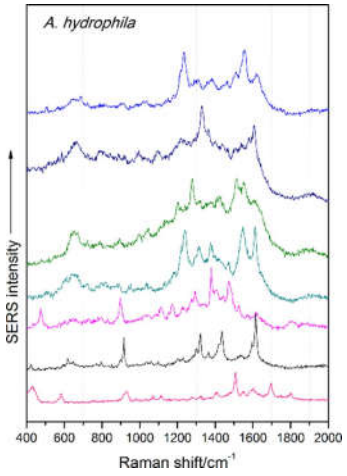
SEM/EDS image showing all elements present in and around a Gram-negative bacterium (M. morganii), a few minutes after generating AgNPs by in situ synthesis.

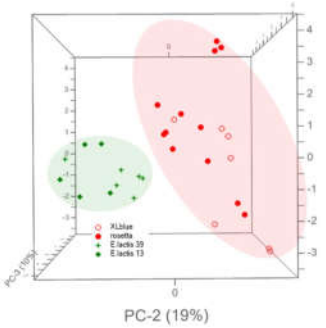


Raw single-cell SERS spectra of E. coli XL1-Blue irradiated with 633 nm laser line. Inset – TEM micrograph (A) and 100× microscopic image (B) of E. coli, showing the in situ synthesized silver colloid coverage of the cell membrane.

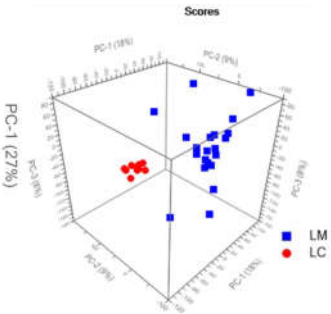


Reproducible single-cell SERS spectra of irradiated *A. hydrophila* cells with 532 nm laser line by using SERS-mapping technique (scanned surface - 50µm x 50 µm, by using 1 µm step).

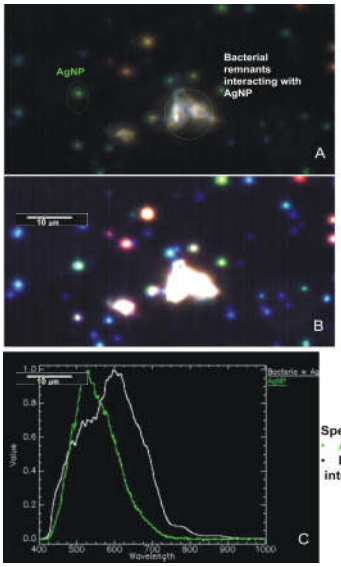




PCA scores 3D plot showing the grouping of two Gram-positive (*E. lactis* CE13 and CE39, respectively) and two Gram-negative species (*E. coli* ROSETTA (DE3)pLysS and *E. coli* XL1Blue).

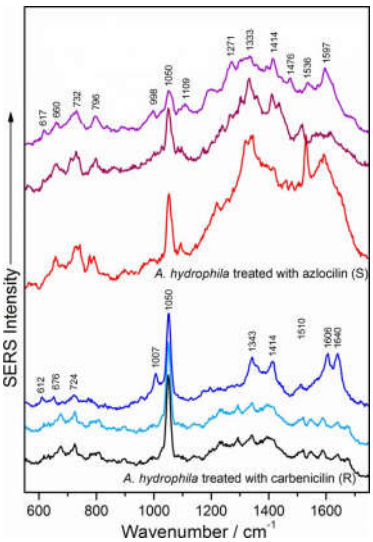


3D plot of PCA scores for the first three PCs showing a grouping tendency of spectral data (Raman) of two Gram-positive species (LM – *L. monocytogenes* and LC – *L. casei* , respectively).

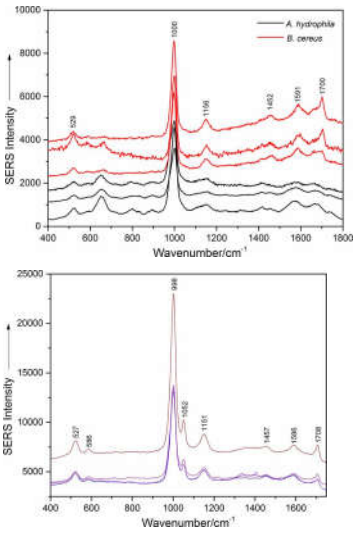


CytoViva Enhanced Darkfield Hyperspectral Microscope analysis (100× microscopic image plus 4× digital zoom on AgNPs and Bacteria@AgNPs **(A)**, higher illuminated image showing brighter the metallic reflexions of AgNPs and silver covered biomass **(B)** and the spectral response of both AgNPs and Bacteria@AgNPs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

Wavenumbers (cm^{-1})	Assignments
564-576	carbohydrates
642-683	δ(COO−) guanine
720-740	ν(Adenine), glycosidic ring)
792-831	ν(CN) tyrosine
863-872	ν(C–C) skeletal proteins
923-1005	"Breathing" in aromatic rings
1038	carbohydrates
1050-1059	ν(C–C)
1151-1166	(=C–C=) lipids
1223-1231	Amide III
1291	δ(CH) proteins
1324-1328	Adenine
1337-1346	δ(CH) and ν_s(COO−) proteins
1440-1488	δ(CH_2) saturated lipids
1565	δ(NH, CH), ν(CC)
1577-1620	ν(DNA)



SERS spectra recorded on *A. hydrophila* cells treated either with antibiotics for which this species has a **resistivity (R)**, either a **sensitivity (S)**, in saline buffer solution.



SERS spectra recorded in spiked artificial urine with *A. hydrophila* (Gram-negative) and *B. cereus* (Gram-positive) species - top; SERS spectra recorded for pure urea, in the same concentration as found in the artificial urine recipe - bottom.

Conclusions: The SERS-based detection of pathogens at single-cell level was successfully carried out both on Gram-negative and Gram-positive species, despite the fact that their cell wall structure is significantly different. The specific spectral profiles can be used for discrimination between various species, different strains of a pathogen, or drug-induced stress conditions by using chemometrics as an unbiased analysis tool of the single-cell SERS spectra.

References:

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