Rapid single-cell detection and identification of pathogens by using surface-enhanced Raman spectroscopy

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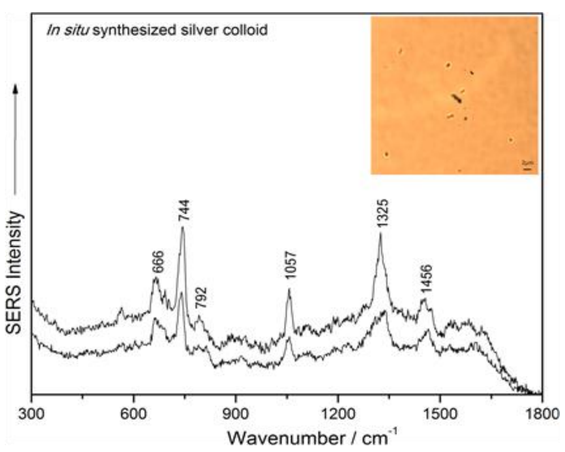
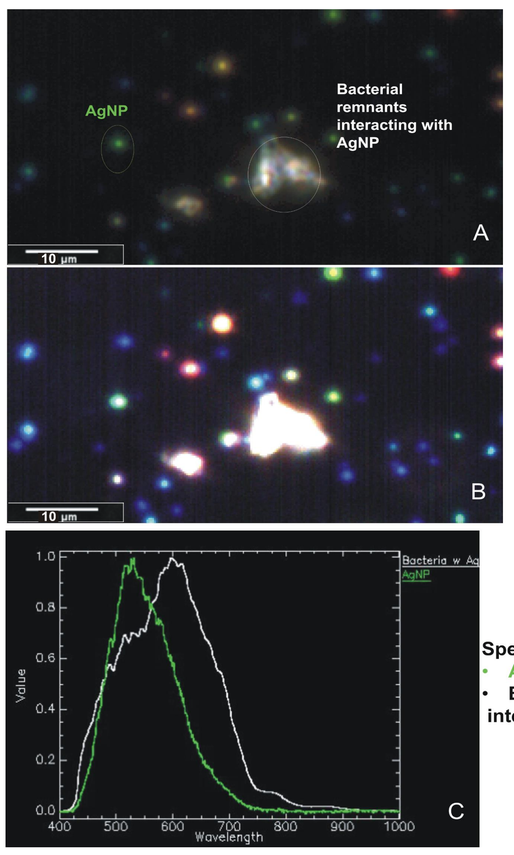
Conventional bacteria identification assays typically require several hours or even days to provide accurate results. Thus, sensitivity, fast response and discrimination at strain level of pathogens are keywords in establishing the appropriate treatment for an infection.

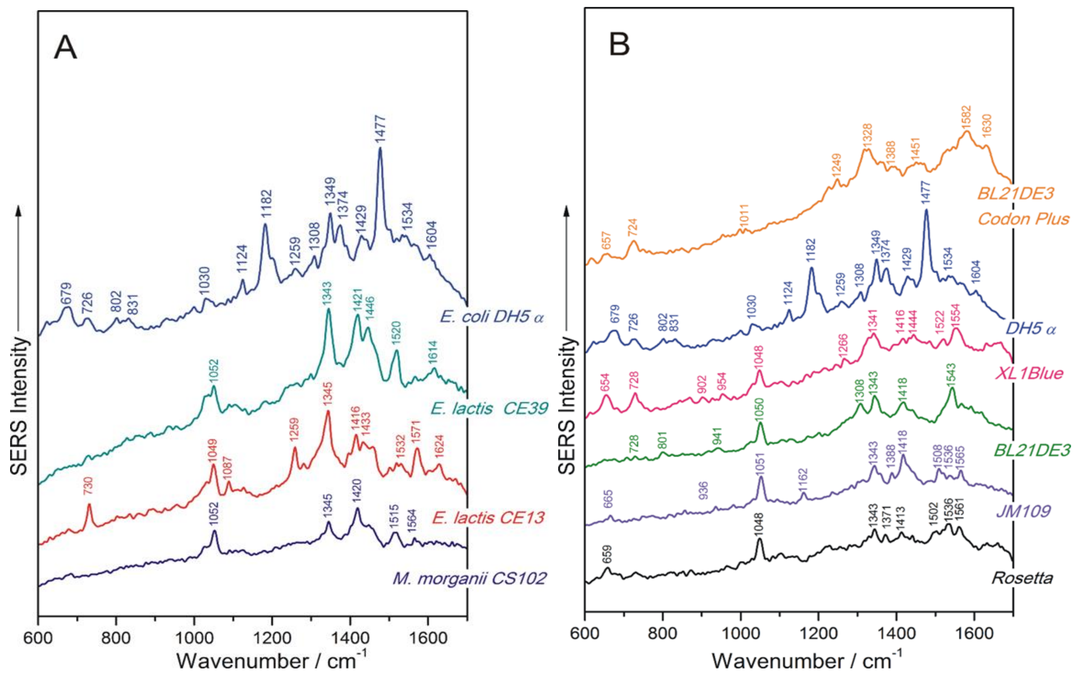
In this work, **surface-enhanced Raman spectroscopy (SERS)** technique was employed with the final aim of identification and discrimination of pathogenic bacteria at single-cell level, based on their detected fingerprint features. Several genera of bacteria that are found in most of the isolated infections 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.**

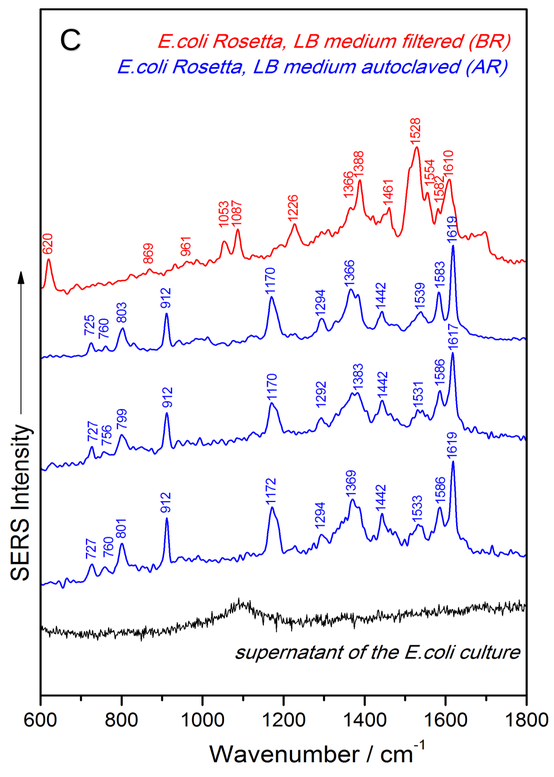


Reproducible SERS spectra of single cell of *E. coli* irradiated with 633 nm laser line. Inset – 100× microscopic image of *E. coli*, showing the *in situ* synthesized AgNPs coverage of the cell membrane.



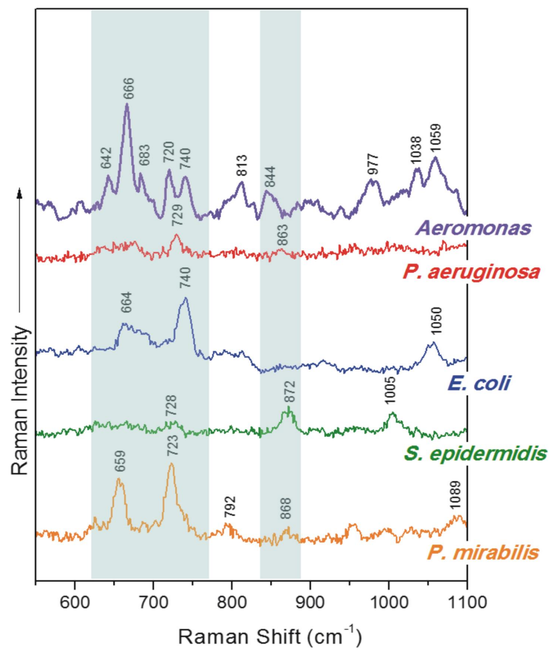
CytoViva Enhanced Darkfield Hyperspectral Microscope analysis (100× microscopic image plus 4× digital zoom on AgNPs and Bacteria@AgNPs **(A)**, higher illuminated image showing brighter the metallic reflexions of AgNPs and silver covered biomass **(B)** and the spectral response of both AgNPs and Bacteria@AgNPs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

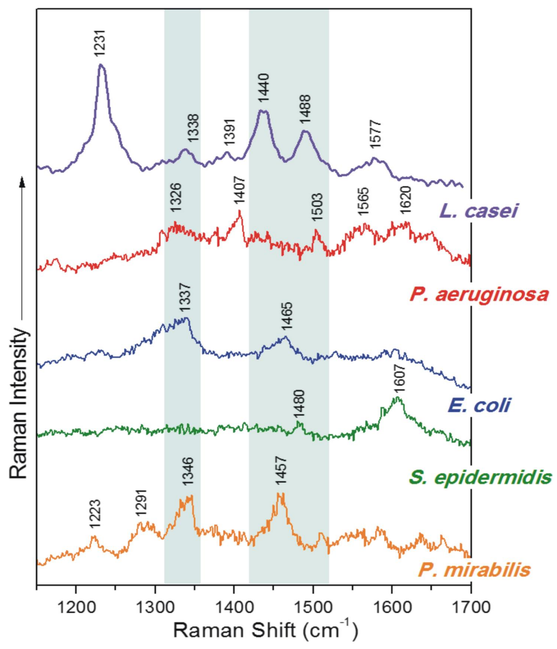




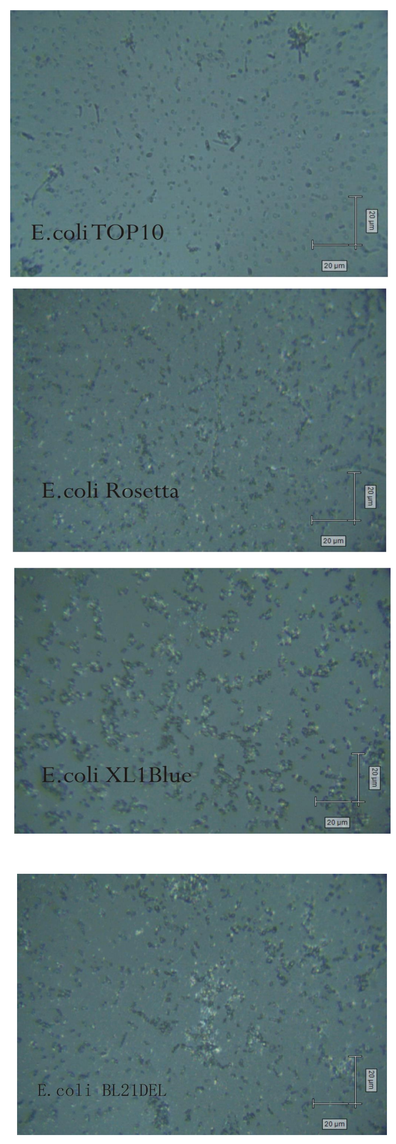
Wavenumbers (cm^{-1})	Assignments
642-683	δ(COO−) guanine
720-740	ν(Adenine, glycosidic ring)
792-831	ν(CN) tyrosine
863-872	ν(C–C) skeletal proteins
923-1005	“Breathing” in aromatic rings
1038	carbohydrates
1050-1059	ν(C–C)
1151-1166	(=C–C=) lipids
1223-1231	Amide III
1291	δ(CH) proteins
1324-1328	Amide II
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 of different microorganisms at single-cell level irradiated with 633 nm laser line (**right** – wavenumbers below 1100 cm^{-1}; **left** – wavenumbers above 1100 cm^{-1} of the SERS spectra of five pathogens are displayed, by highlighting the SERS marker bands).





Single-cell SERS spectra of several pathogens compared to a *E.coli* strain **(A),** of different strains of *E.coli* **(B)**, and of *Rosetta* strain of *E.coli* cultivated in different growth media **(C)**, respectively, recorded by using the Polysine™ Microscope Adhesion Slides (poly-slides) as substrate for Bacteria@AgNPs, irradiated with the 532 nm laserline.



Microscopic images (50× objective) of some selected *E.coli* strains immobilized on the polyslides and covered with the *in situ* synthesized AgNPs.

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 the two types of pathogens and even between different strains of a pathogen, by using chemometrics as an unbiased analysis tool of the single-cell SERS spectra.

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