Bacterial cell membrane barcoding, a SERS mapping methodology for iden fica on and detec on of poten al pathogenic bacteria

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Objec ves

- Determina on of SERS marker bands for iden fica on of poten al pathogenic microorganisms, isolated from the environment.
- Preliminary tests for iden fica on of bacteria from liquid media that mimic the complex biological sample.

Methods

- Bacterial strains were isolated from environmental samples and were iden fied based on 16S rRNA molecular markers, using 27FB-1492R primer pair.
- The SERS spectra was recorded with a confocal Renishaw inVia Reflex Raman Spectrometer using either the 532nm (Cobolt, Diode Pumped Solid State – DPSS – 200 mW) or 633 nm (He-Ne laser – 17 mW) excita on line, by using the 100× objec ve (Leica, NA 0.9, WD 3.4 mm).
- The SERS-ac ve silver clusters were generated by using the in situ synthesis, in the presence of the bacterial biomass.
- The SERS fingerprin ng is based on enhanced Raman signal arising from the "hot-spots" generated in the close proximity of the bacterial cell wall.

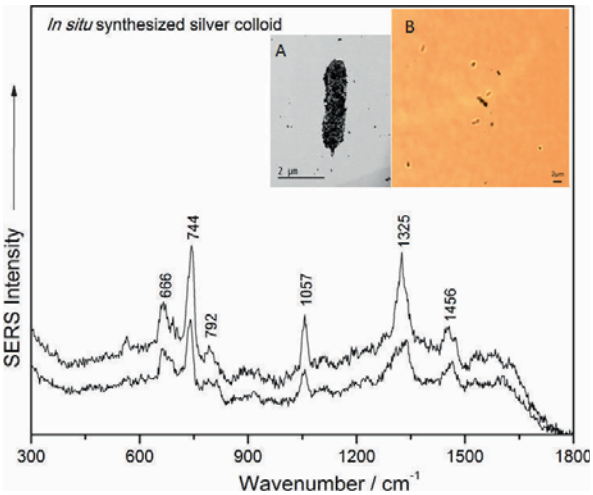

Fig. 1. Raw single-cell SERS spectra of *E. coli* XL1-Blue irradiated with 633 nm laser line. Inset – TEM micrograph (A) and 100× microscopic image (B) of *E. coli*, showing the in situ synthesized silver colloid coverage of the cell membrane (Dina et al., 2017)

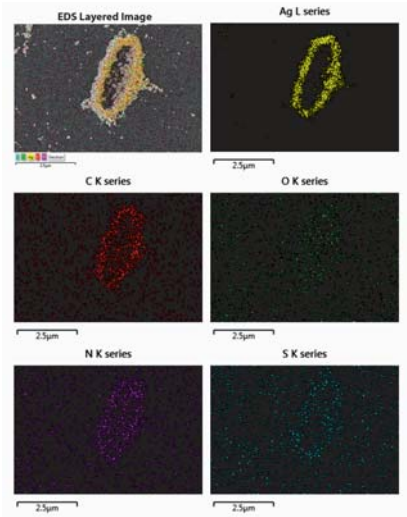

Fig. 2. SEM/EDS image showing all elements present in and around a Gram-nega ve bacterium (*M. morganii*) a few minutes a er genera ng AgNPs by in situ synthesis (Dina et al., 2017)

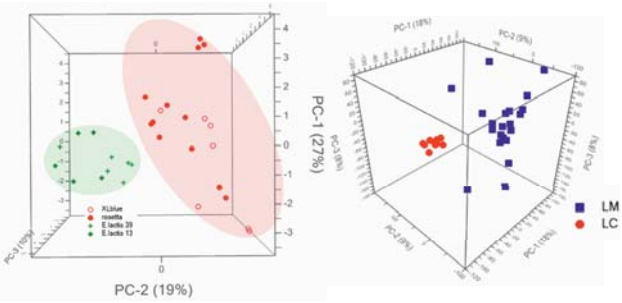

Fig. 3. PCA scores 3D plot showing the grouping of two Gram-posi ve (*E. lactis* CE13 and CE39, respec vely) and two Gram-nega ve species (*E. coli* ROSETTA (DE3)pLysS and *E. coli* XL1Blue).

Fig. 4. 3D plot of PCA scores for the first three PCs showing a grouping tendency of spectral data (Raman) of two Gram-pozi ve species (LM - *L. monocytogenes* and LC – *L. casei*, respec vely).

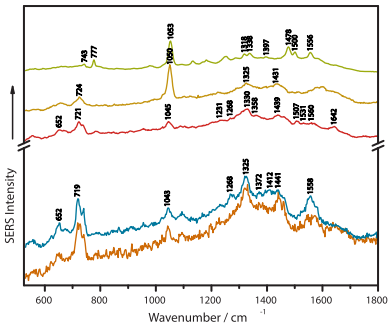

Fig. 5. SERS spectra single-cell level showing the reproducibility in five repeated experiments for the control sample (*A. hydrophila*).

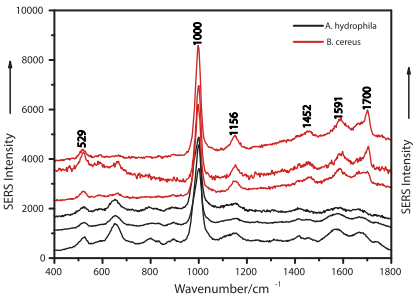

Fig. 6. SERS spectra recorded in spiked artificial urine with *A. hydrophila* (Gram-negative) and B. cereus (Gram-pozitive) species.

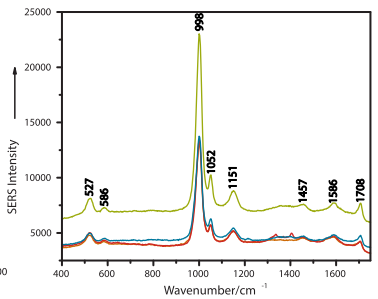

Fig. 7. SERS spectra recorded for pure urea, in the same concentration as found in the artificial urine recipe.

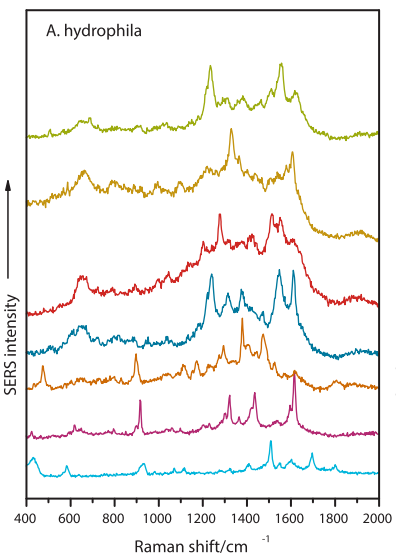

Fig. 8. Raw SERS spectra recorded on *A. hydrophila* cells by SERS-mapping technique (scanned surface - 50µm x 50 µm, by using 1 µm step).

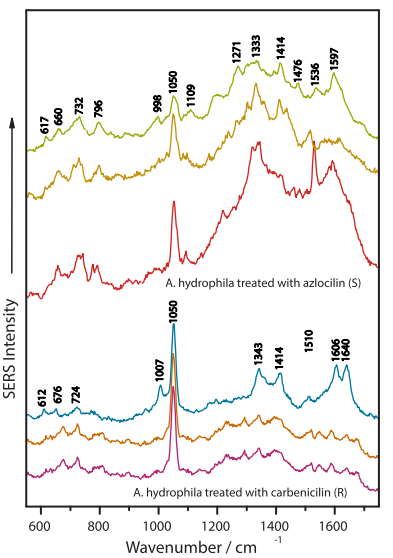

Fig. 9. SERS spectra single-cell level showing the reproducibility in five repeated experiments for the control sample (*A. hydrophila*).

Conclusions

- Specific SERS marker bands were iden fied for both Gram-posi ve and Gram-nega ve strains, specific for bacterial cell membrane components. By using robust chemometric data analysis tools, the discrimina on at strain level of bacteria was assessed; even predic ng their virulence or resistance to drug treatment is possible.

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References:

- N.E. Dina, H. Zhou, A. Colniță, N. Leopold, T. Szöke-Nagy, C. Coman, C. Haisch, *Analyst*, 2017,142, 1782-1789.
- H. Zhou, et al., *Microchim. Acta.*, 2015;182(13-14), 2259-66.
- N.E. Mircescu, et al., *Anal. Bioanal. Chem.*, 2014;406(13):3051-8.