SYNTHETIC INTERMEDIATE RESEARCH REPORT 2016

PROJECT CODE: PN-II-RU-TE-2014-4-0862 CONTRACT NO: 381/10-11-2015

Report on project achievement in the period January 1st, 2015 – December 30th, 2016

O1). A) The label-free character of the developed detection protocol was assured by the unspecific receptor immobilization procedure of the tested microorganisms. In this regard, the sample supports used were microscope adhesion slides, which are chemically covered with a layer of poly-L-lysine (**polyslides**). This surface chemistry enhanced the efficiency of the bacterial immobilization process, without the use of antibodies or other specific receptors. Moreover, the **reduced cost** (0.31 EUR/slide) and the **reduced sample volume** necessary (3 µl) for analysis at single-cell level justified the usage of this sample support.

B) By using the label-free SERS detection optimized protocol (Bacteria@AgNPs approach), a potential pathogen yeast, *R. mucilaginosa*, was investigated. In this case, the results obtained were confronted with the resonant Raman spectra obtained by exciting with the 532nm laser line. In addition to the vibrational analysis of the Raman and SERS spectra obtained, **the influence of the growth conditions** was monitored by changing the pH of the growth medium used for cultivation. The excitation with an alternative laser line (532nm) was applied combined with the Bacteria@AgNPs approach, in order to monitor the influence of the selected excitation laser line in the reproducibility of the SERS spectra.

C) The SERS spectral database was built up and now is available on the project's webpage. It contains the specific SERS fingerprint of **more than 20 pathogenic bacterial strains**, both Gram-positive and Gram-negative, including new species, as mycobacteria. The relevant spectral domain from the whole SERS profile, which contains the major SERS marker bands, was determined in order to be analyzed by multidimensional analysis (Principle Component Analysis - PCA) for unbiased classification and discrimination of the investigated pathogens.

O2). A **PCA algorithm for accurate discrimination** (<80 % specificity and sensitivity) of SERS single-cell bacterial fingerprints was optimized. The developed PCA model was applied on both Gram-positive and Gram-negative species and inter-species as well as intra-species discrimination was successfully performed. The future improvement could be found in more precise validation models included in our developed PCA model, such as "leave-one-out" principle.

O3). As a preliminary attempt to detect bacteria at single-cell level in **real-life samples**, the centrifugation of a synthetic matrix which mimics the human plasma (contains its main characteristic components) was required. A centrifugal concentrator was used and the remaining cellular content was spiked with bacteria and then scanned by using a label-free real-time SERS monitoring approach.

Continuing to pursue of the project's objectives, in our next investigations we will collect more relevant pathogenic spectral SERS fingerprints and will optimize the detection protocol in real-life conditions. The effect of old and new antibiotic formulas will be monitored by using the Bacteria@AgNPs approach and ultimately the detection method will be validated by obtaining the fast and reliable antibiogram.

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