DEVELOPMENT OF A SURFACE ENHANCED RAMAN SCATTERING METHOD FOR THE QUANTIFICATION OF BACTERIA: APPLICATION TO THE CHARACTERIZATION OF PROBIOTICS ENCAPSULATED IN MICROSPHERES

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Probiotics are increasingly used to improve, maintain or restore homeostasis of the gut microbiota. For these purposes, they are administrated to human via various pharmaceutical formulations improving their stability and preservation, such as lyophilized powder in capsules or microparticle-based delivery systems. The development of such dosage forms requires a reliable quantification method of the bacteria to determine formulation characteristics such as encapsulation efficiency, release kinetics or microorganism viability. The conventional counting method using plating and culturing is the most straightforward and widely used method for these purposes, but it appears tedious and time consuming (requiring at least 24h).

Conventional Raman spectroscopy appears as an interesting alternative method by providing many advantages, in particular easy and rapid sample preparation, rapid analysis in aqueous matrices (biological fluids, tissues or food matrices) and also the acquisition of spectral data characteristics of the bacteria. However, this technique is limited by the weakness of the inelastic light scattering phenomena, thus requiring the use of highly concentrated samples. Therefore, Surface Enhanced Raman Scattering (SERS) technique, allowing to provide a high enhancement of Raman scattering from molecules adsorbed on a nanostructured noble metal surface (silver, gold), was investigated for the characterization of bacteria. The developed SERS method allowed, within 30 min, the quantification of probiotic *Lactobacillus rhamnosus GG* (LGG) suspended in water over a concentration range consistent with pharmaceutical applications (Figure. 1). LGG were then encapsulated in spray-dried microspheres. The encapsulation efficiency was determined by both the SERS and the conventional counting methods indicating that the developed analytical procedure should be very useful for the characterization of probiotics-based pharmaceutical formulations. The SERS method was also successfully used in combination with chemometric techniques i) to highlight LGG modifications induced by applying deleterious process conditions (solvent, shear stress…) and ii) to discriminate different bacterial strains (Figure. 1).

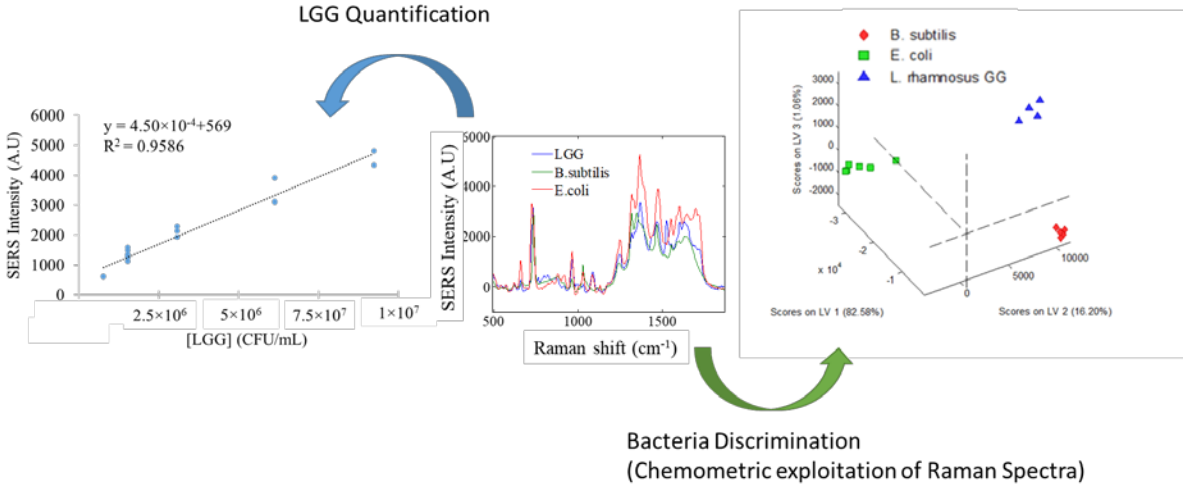

Figure. 1 Quantification and discrimination of bacteria from Raman SERS spectra

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