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

"live microorganisms which, when administered in adequate numbers, confer a health benefit on the host"

Strains used : Lactic acid bacteria (*Lactobacillus, Bifidobacterium, Streptococus),* Yeast (*Saccharomyces Boulardii) ...*
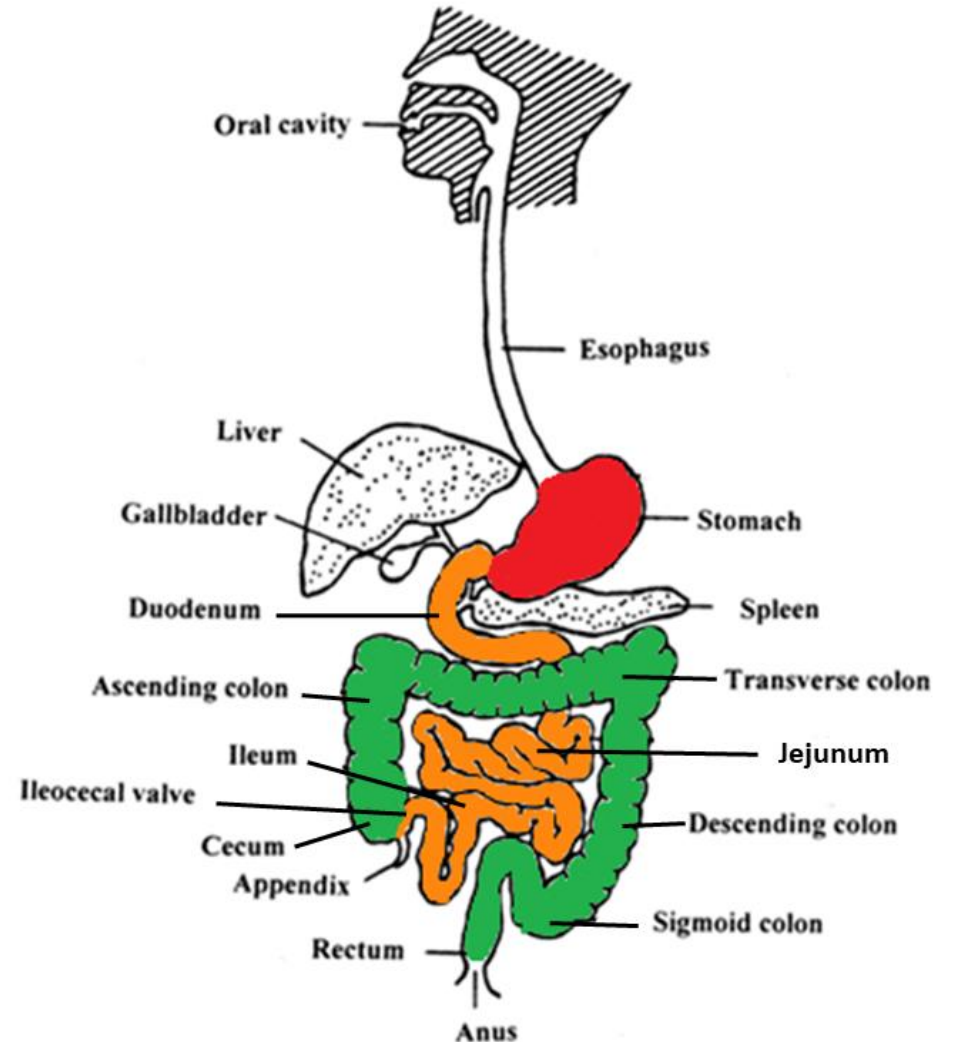
⇨ Restore intestinal microbiota
⇨ Homeostasis

Target site of action ➜ Large intestine

⇨ Deleterious conditions
- Acidic environment in the stomach
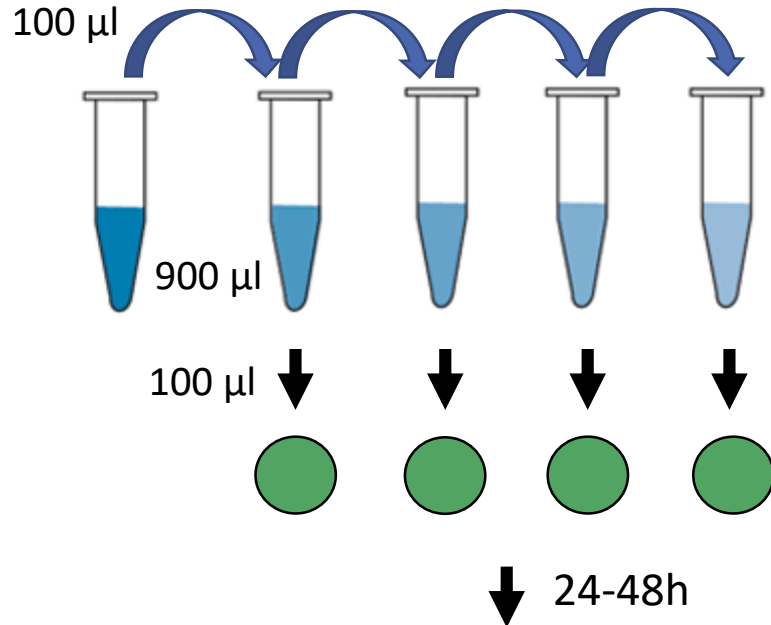- Enzymatic activities and bile acids in the small intestine
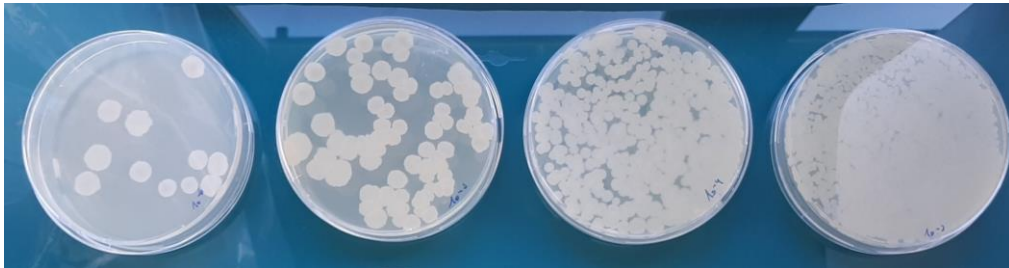
Microencapsulation systems

Characterization of formulation

⇨ **Encapsulation efficiency**, bacteria release kinetics, viability studies

100 μl

900 μl

100 μl

24-48h

B. subtilis

The **conventional counting method** : plating and culturing
⇨ most widely used **method**
⇨ tedious and time consuming

Conventional Raman Spectroscopy
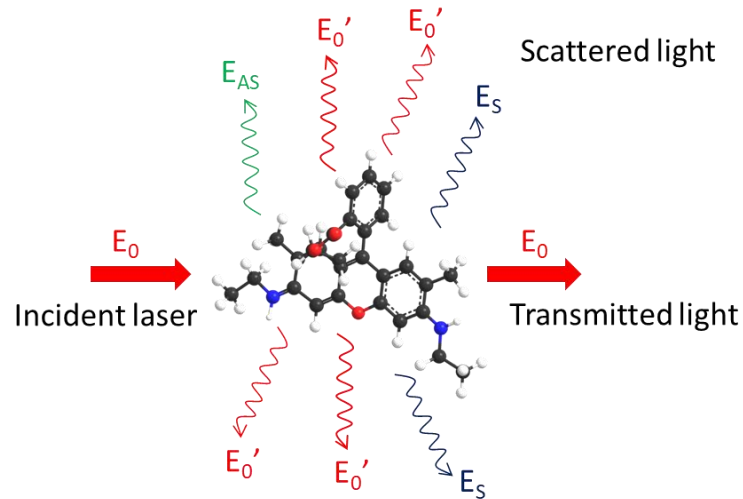
⇨ Rapid analysis (less than 1 hour)

⇨ Qualitative and quantitative analysis

⇨ Analyse in aqueous media or dry state

⇨ Easy and rapid preparation of samples

⇨ Spectral data characteristics of the analyte/microorganism

Surface Enhanced Raman Spectroscopy

Incident laser · Transmitted light · Scattered light

High enhancement of Raman scattering from molecules adsorbed on a **nanostructured noble metal surface** (Ag, Au, Cu)

- R6G 5 nM with AuNPs
- R6G 1 mM

Raman Intensity (U.A) vs Raman Shift (cm-1)

Raman Spectroscopy is based on the study of weak **inelastic light scattering phenomena**

⇨ concentrated samples

Surface Enhanced Raman Spectroscopy
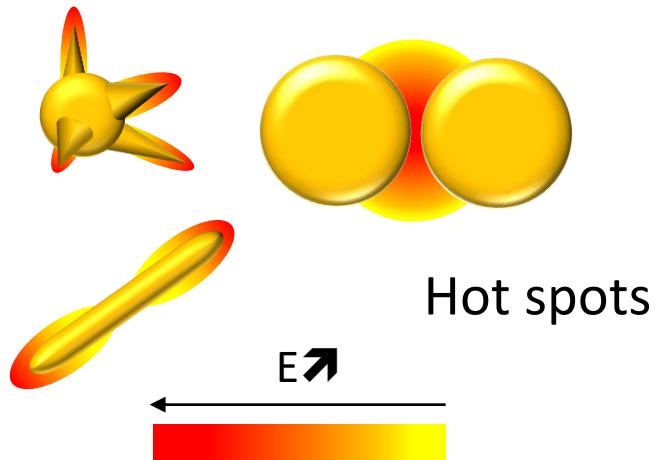
⇨ **SERS Effect**
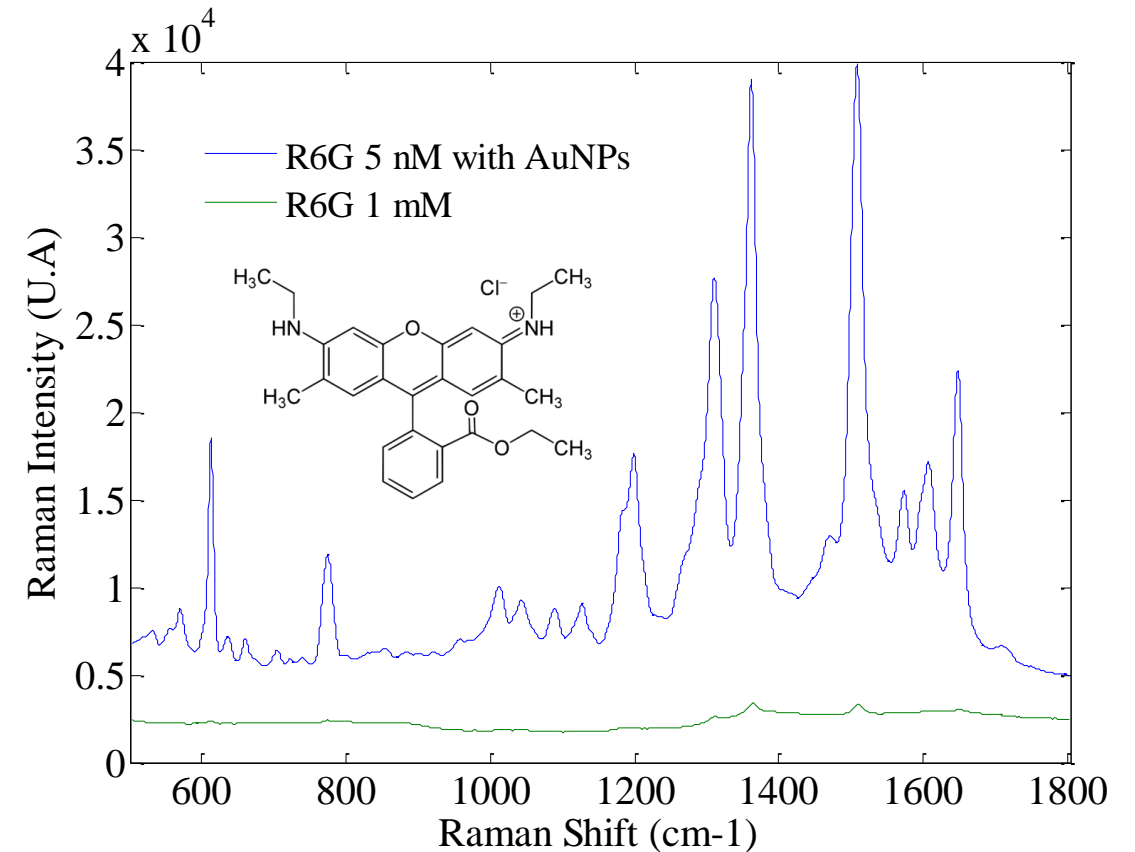
SERS Effect and Parameters influencing the exaltation phenomena

Electromagnetic effect (EM)

Localized surface Plasmon resonance
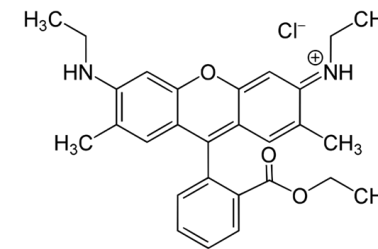
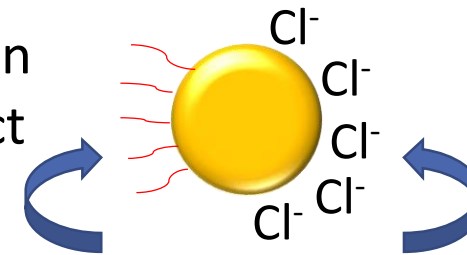↗ of electromagnetic field at NPs surface
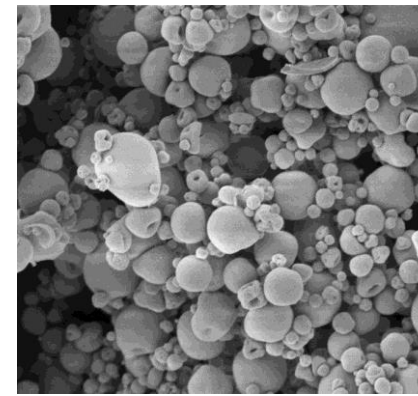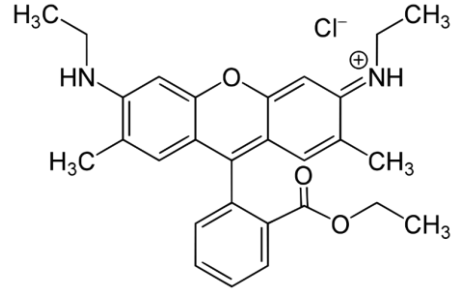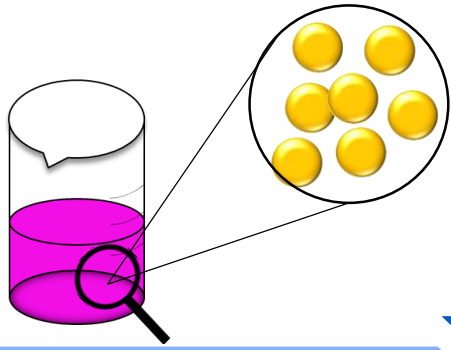
Hot spots

E ↗

Chemical effect (CM)

Charge-transfer between the chemisorbed analyte and the nanostructure

↗ molecule polarizability

Stabilization agent effect

Cl^-
Cl^-
Cl^-
Cl^- Cl^-
Cl^-

Co-adsorption with anion
➔ Charge Transfer

R6G Molecule

Strategy

Development of SERS substrates → SERS efficiency assays using Rhodamine 6G (R6G) model analyte → *Lactobacillus Rhamnosus GG* (LGG) Characterization → Development of an encapsulation system and characterization of the formulation
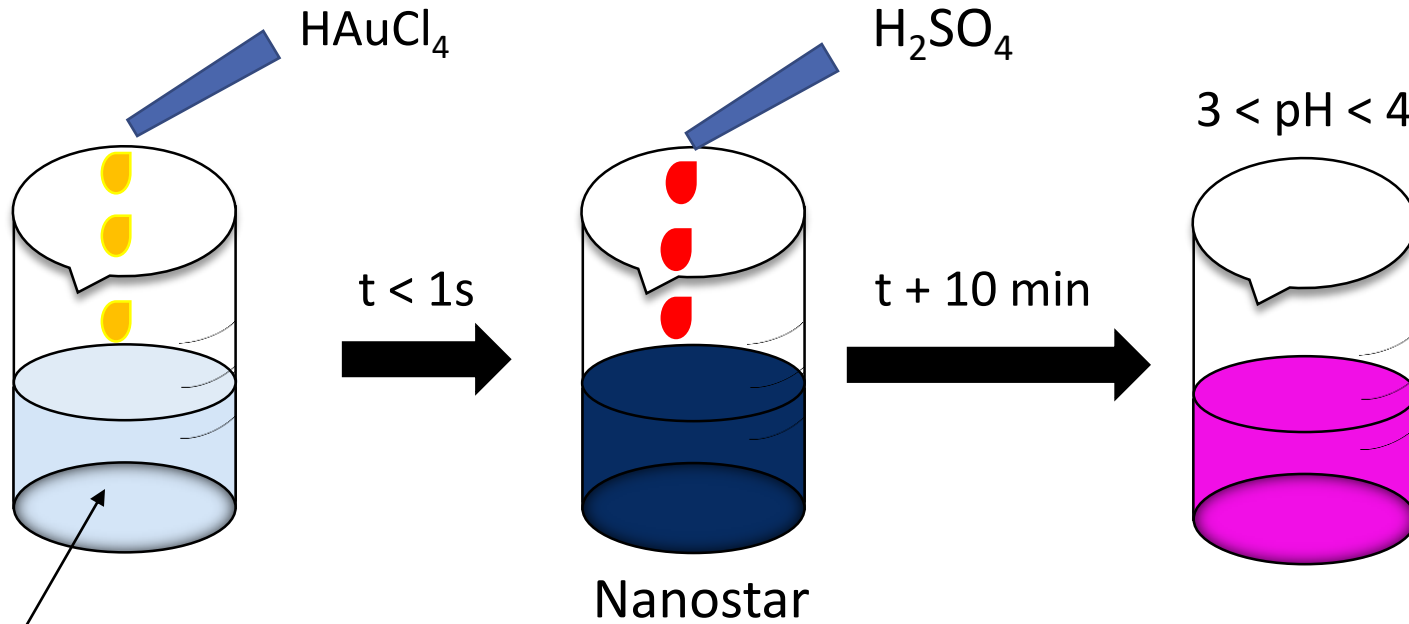
Gram-positive bacteria, indicated for the treatment or the prevention of antibiotic-associated diarrhea
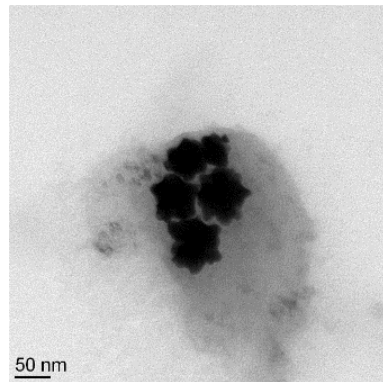

0.2 µm
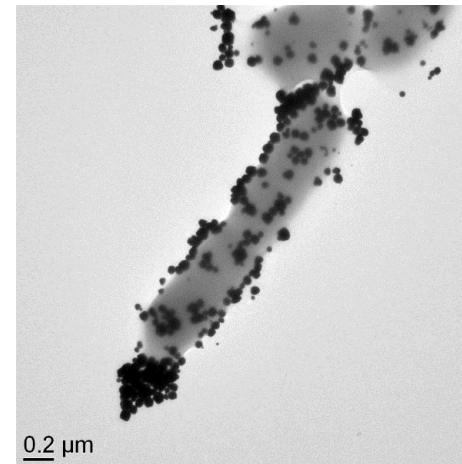
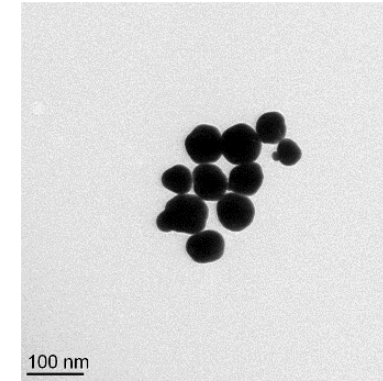*Encapsulation efficiency*

SERS method
vs
Conventionnal counting

SERS active uncoated spherical gold nanoparticles synthesis

HAuCl$_4$

H$_2$SO$_4$

$3 < $ pH $ < 4$

$t < 1s$

$t + 10$ min

Nanostar

Hydroxylamine aqueous solution (pH=12)

D$_H$ =70 (± 5) nm
(PDI ≈ 0.2)
ζ = - 42 mV

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(PDI ≈ 0.2)
ζ = - 42 mV

Au$_{nanostar}$ Au$_{spherical}$

D$_H$ = Hydrodynamic diameter ; ζ = Zeta potential

Acquisition of the bacteria SERS signal

0.2 μm
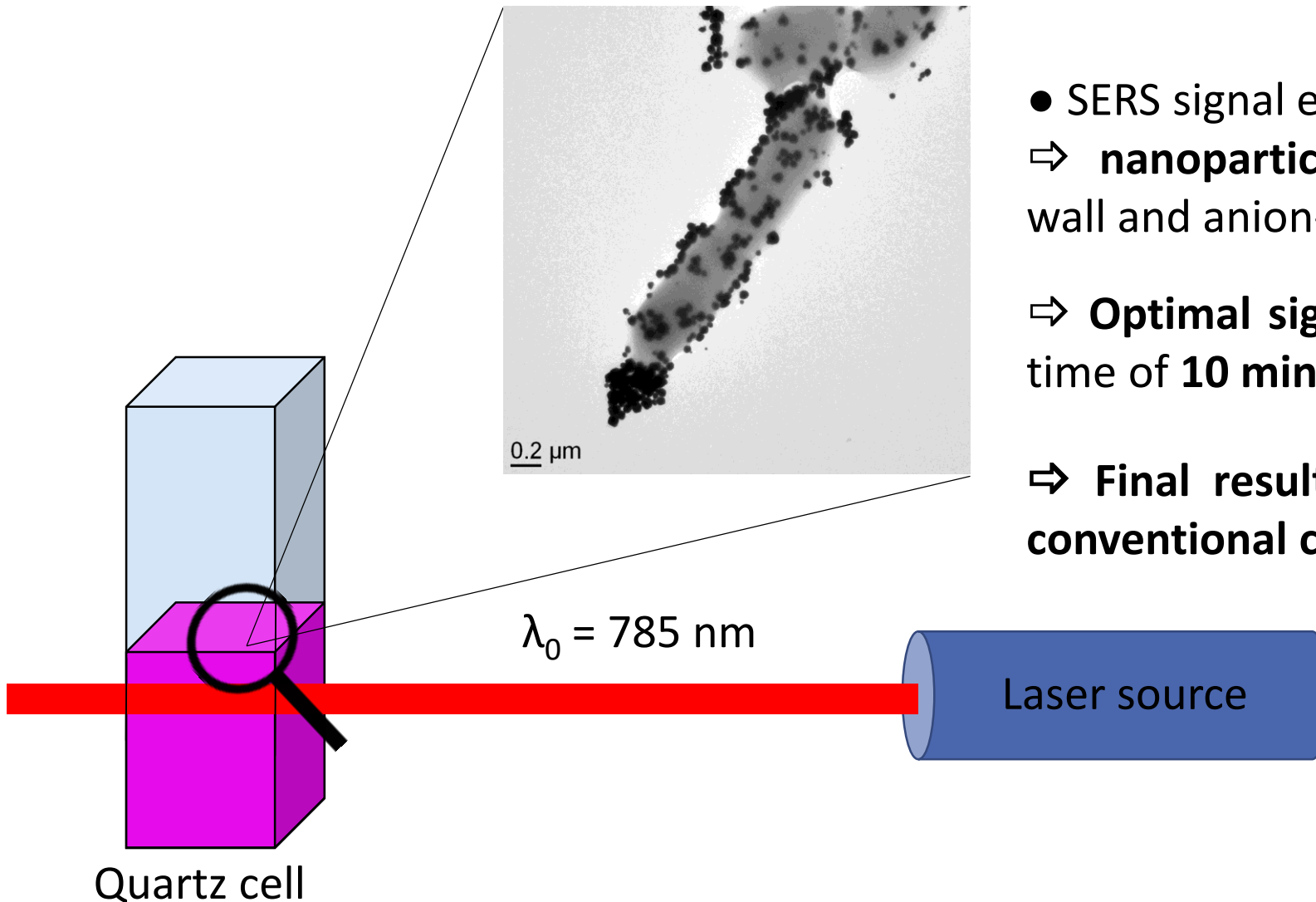
● SERS signal enhancement

⇨ **nanoparticles aggregation** on bacteria cell wall and anion-induced chemical enhancement

⇨ **Optimal signals** recorded after an incubation time of **10 min**

⇨ **Final result** under **30 min** Vs **1-2 days for conventional counting method**

λ_0 = 785 nm

Laser source

Quartz cell

Detection and quantification of LGG bacteria
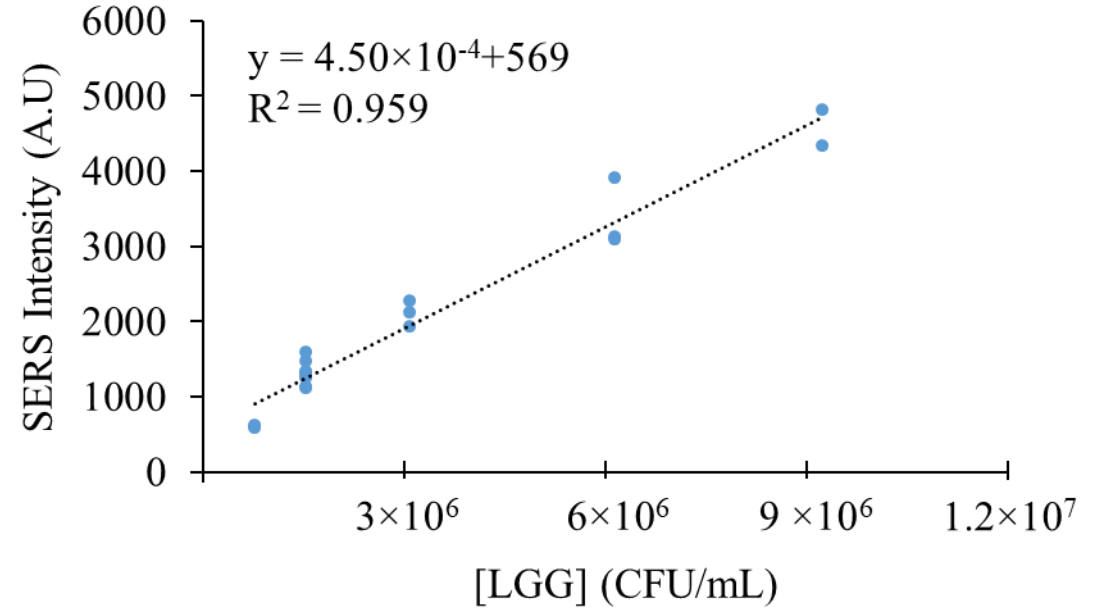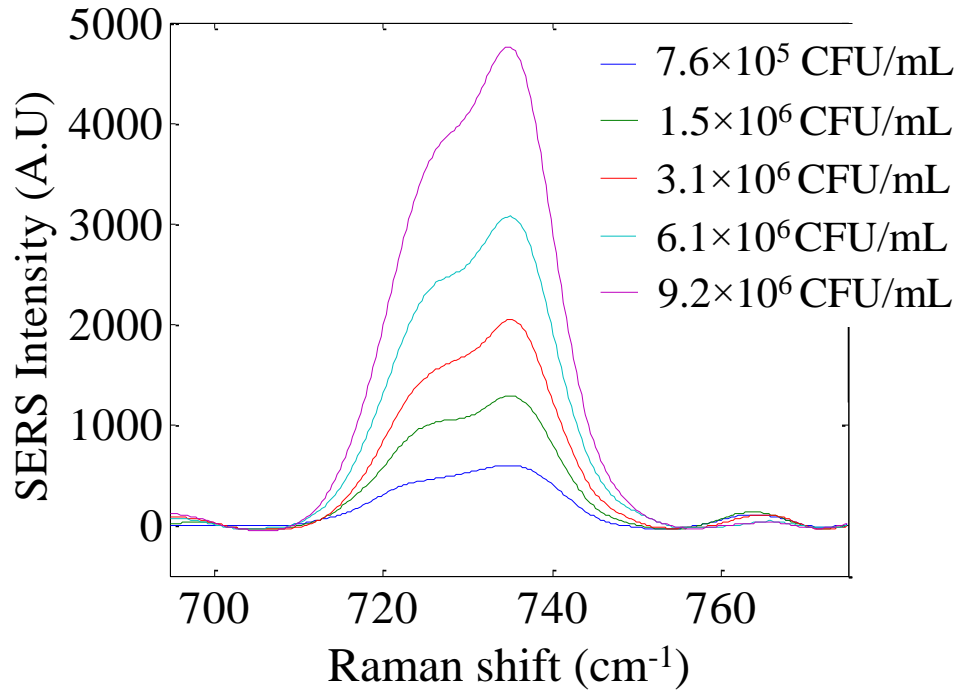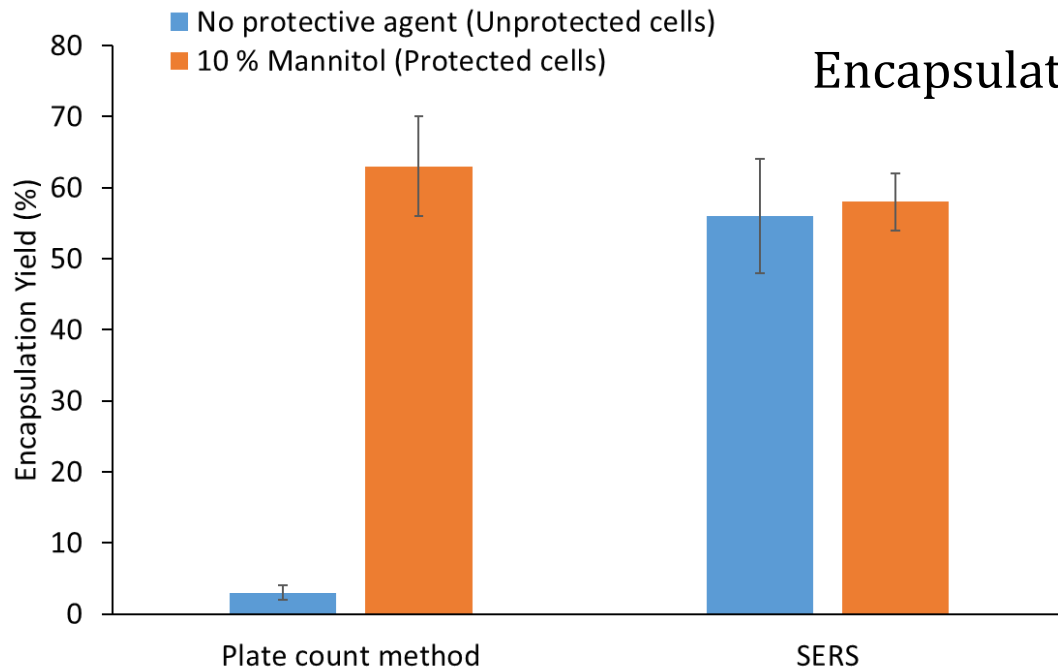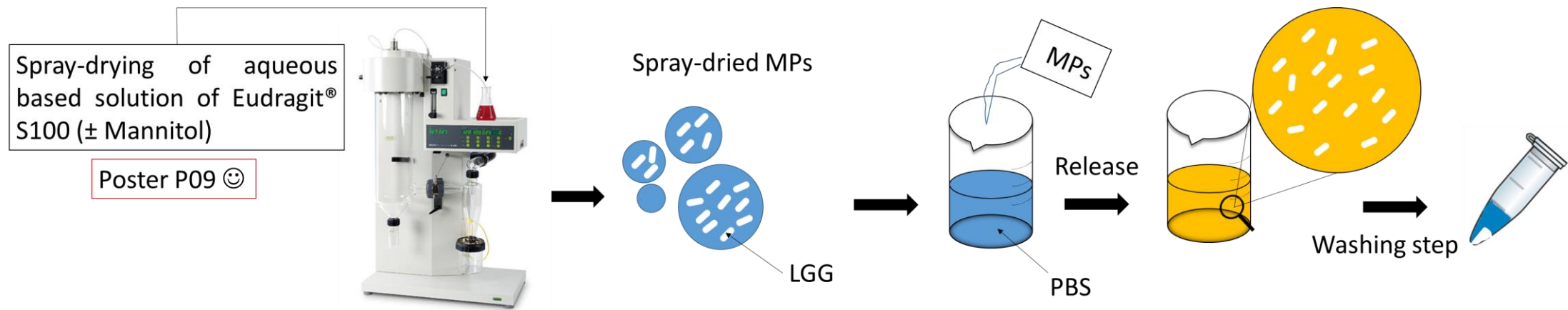

Most important feature in SERS spectra of LGG
➡ **Peak at ≈ 735 cm^{-1}** : peptidoglycan, purines molecules and/or adenine-containing molecules

➡**Satisfactory signal variability** : CV < 20%
➡**Linear relationship** between LGG concentration and Raman SERS signal

➡ Characterization of an encapsulation system

Application to the characterization of an encapsulation system

Spray-drying of aqueous based solution of Eudragit® S100 (± Mannitol)

Poster P09 ☺

Spray-dried MPs

MPs

Release

PBS

Washing step

LGG

No protective agent (Unprotected cells)
10 % Mannitol (Protected cells)

Encapsulation Yield (%)

Plate count method   SERS

$$\text{Encapsulation Yield (\%)} = \frac{\text{CFU/g of LGG after spray drying (N)}}{\text{CFU/g of LGG in the feed solution}(N_0)} \times 100$$

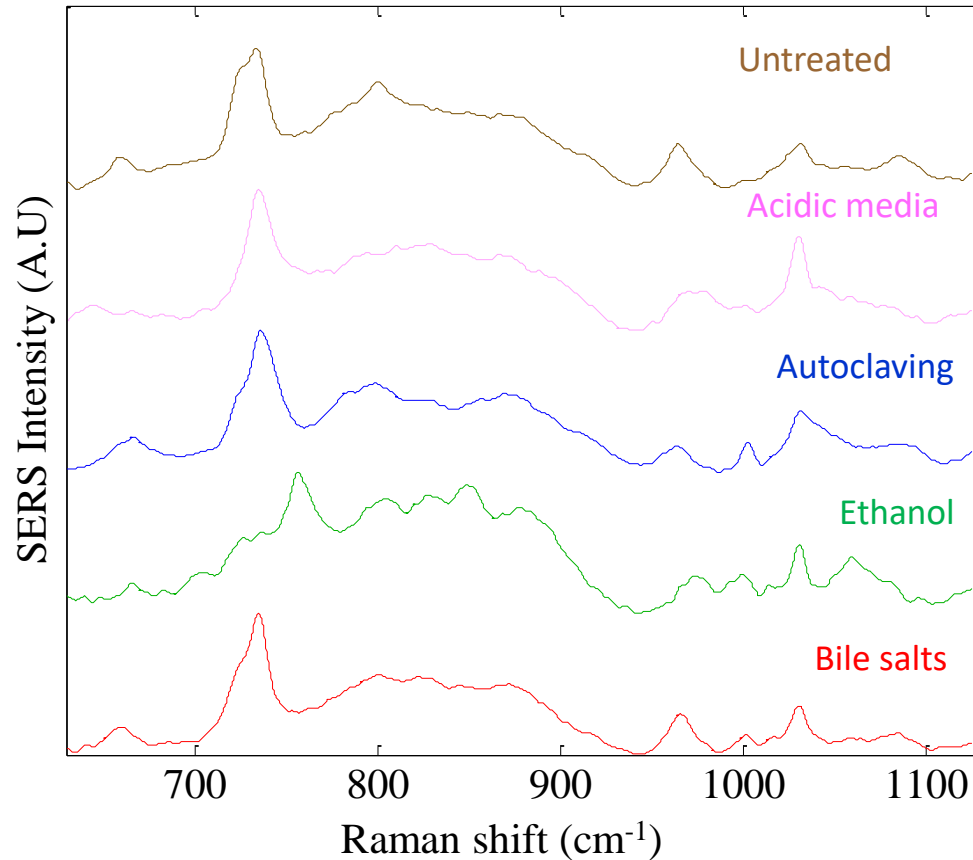SERS ➔ N = CFU/g of live + dead bacteria

Count plate method ➔ N = CFU/g of living cells
➔ Survival Ratio

SERS method : a real alternative ?
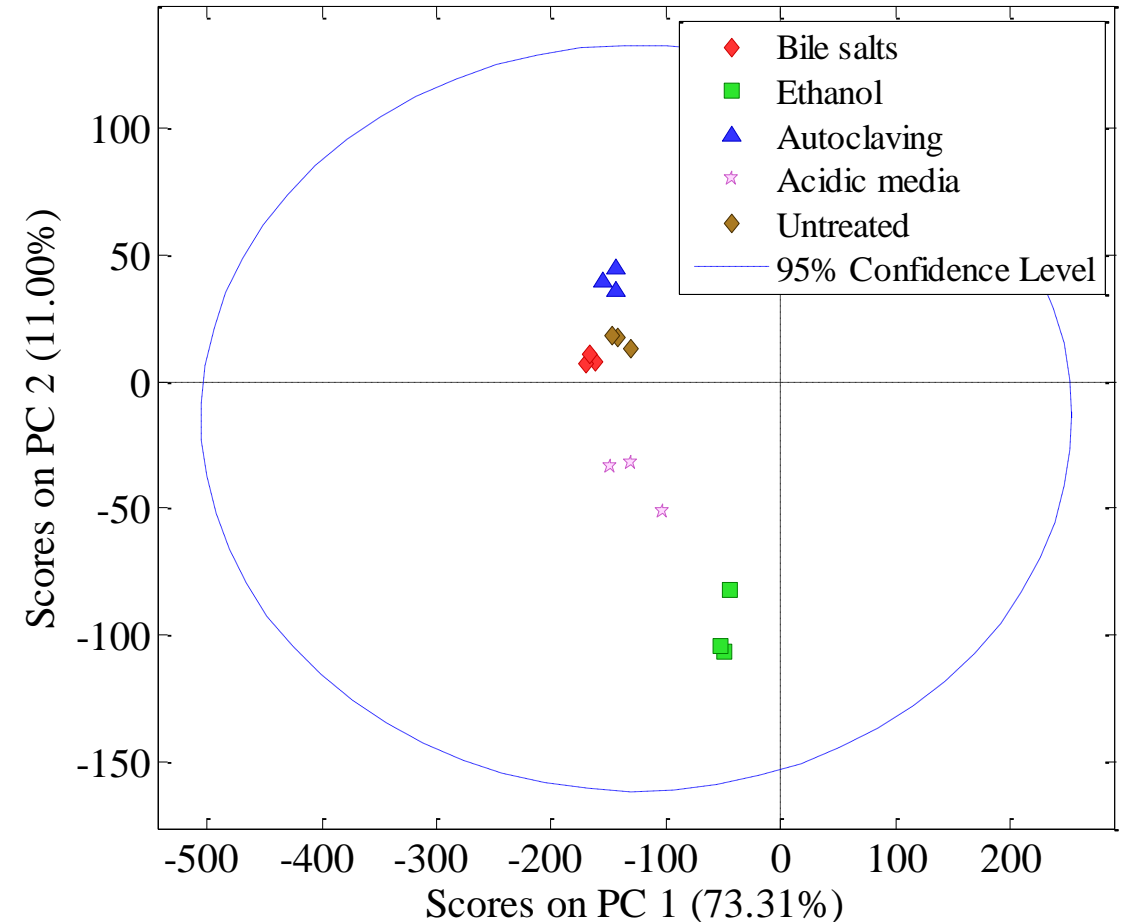
SERS method : A real alternative ?

Deleterious treatments : Autoclaving, ethanol, acidic media, bile salts...

➥ Changes in spectral features

Principal Component Analysis (PCA) scores plot showing the differentiation of the bacteria as a function of treatment

➔ Quantification ?

Conclusion

● Convenient synthesis NPs method and SERS analysis

⇨ 2 steps, under 20 min

⇨ ambient temperature

⇨ No stabilization agent added

⇨ Result obtained under 30 min

● Quantification of live/dead bacteria

● Discrimination based on spectral features : deleterious conditions and bacterial strains

Perspectives

● Specific quantification of live and dead bacteria

● Bacteria release kinetics, viability study…

● Colonic delivery : proof of concept

Thank you for your attention