Milk pathogens trapping with nanoparticles and detection by QCM

Chloé Richet[1,2], Delphine Larose[2], Philippe Trossat[2], Thérèse Leblois[1] , Vincent Humblot[1]

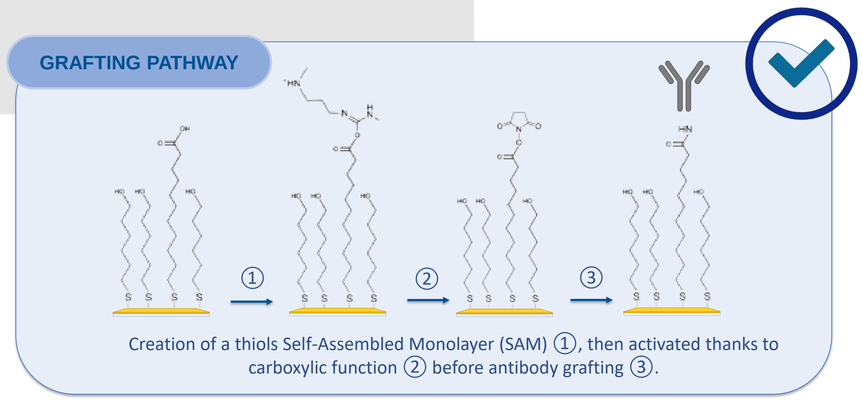
[1] Université Marie et Louis Pasteur, CNRS, Institut FEMTO-ST, F-25000 Besançon, France
[2] Actalia CECALAIT, F-39802 Poligny, France

CONTEXT & OBJECTIVES

The dairy sector can be faced with contamination of raw materials and transformed products by pathogenic bacteria [1]. Existing methods take a long time to detect the absence of contamination by these bacteria, ranging from one to several days depending on the method. In addition, these methods must have very low detection thresholds, regarding the microbiological criterion for these micro-organisms of **"absence of pathogen in 25 g of product"**. It is essential to set-up a method that can quickly confirm the absence of *Listeria monocytogenes*, a pathogenic germ chosen for this study, in these products.

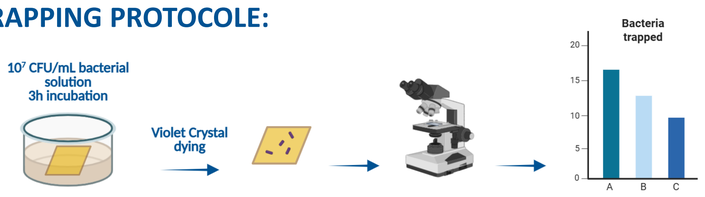
In this context, the overall objective of the **DEPLASP-BAAG** project (**DE**veloppement d'une **PL**ateforme de détection r**A**pide et **SP**écifique de **BA**ctéries sur matrices complexes **AG**roalimentaires) is to **significantly reduce the time required to obtain the first negative result in one day,** by improving the two main stages of the analytical process: the enrichment phase and the detection stage using a quartz crystal microbalance (QCM).

As the following functionalization pathway has been validated, biointerface can be used for the different bacteria trapping methods : on flat surfaces (test of ligands), on nanoparticles (magnetic nanoparticles – MNPs and gold nanoparticles GNPs) and for detection with QCM.

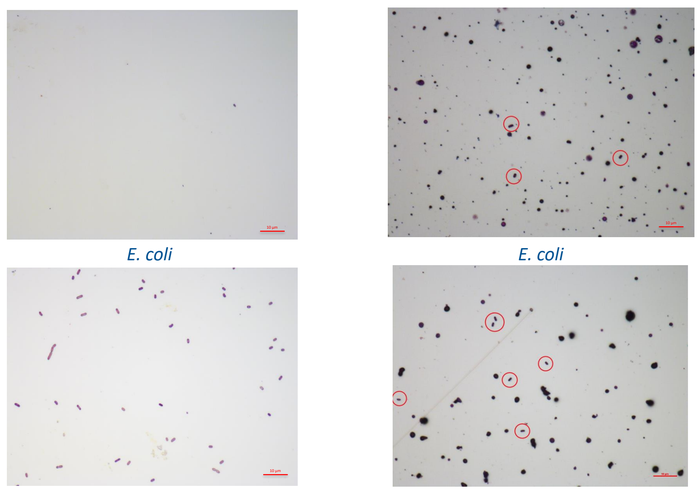


FLAT SURFACES

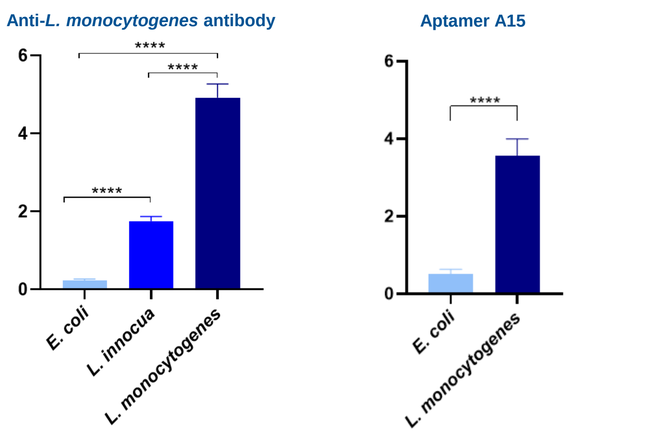
TRAPPING PROTOCOLE:



Anti-*L. monocytogenes* antibody | **Aptamer A15**



Number of bacteria trapped by unit of surface (a.u.)



Specie specificity(*L.mono/E.coli*) **= 94%**
Strain specificity(*L.mono/L. innocua*) **= 77%**

Specie specificity(*L.mono/E.coli*) **= 88%**

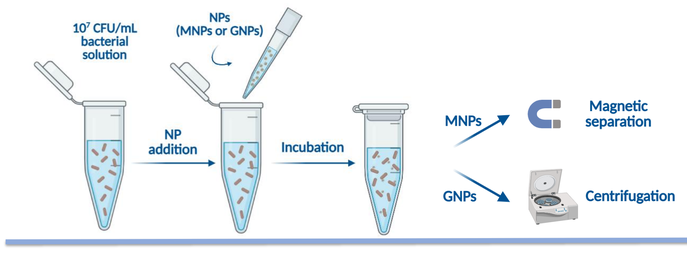
Antibody is not specific to the target only → could capture other bacteria in the sample at the same time

PROPOSITIONS

- Investigation of **more specific ligands** : monoclonal antibodies or other polyclonal antibodies, peptides or other aptamers directed against *L. monocytogenes*
- ELISA and contact capture tests for the different new ligands → **test the specificity with *Listeria monocytogenes***
- SPR measurement → characterization of the **interactions between ligand and target** : binding affinity, specificity and stability

NANOPARTICLES

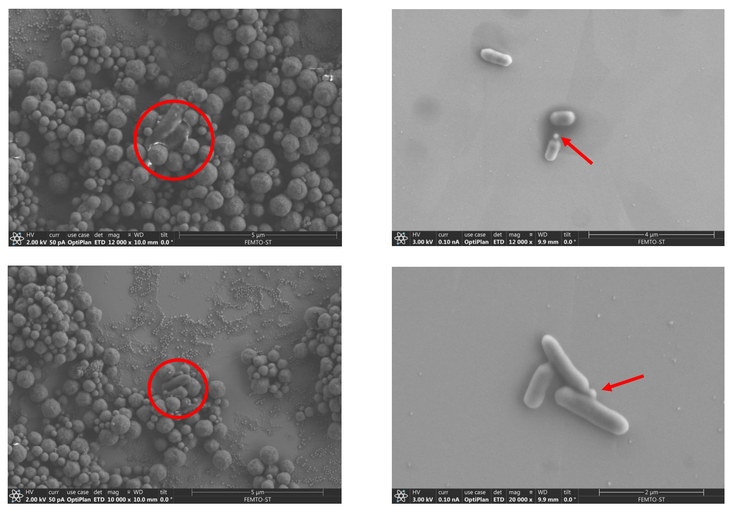
STATIC TRAPPING PROTOCOLE:



CHARACTERIZATION:

MEB observations after simple incubation with MNPs and GNPs, functionnalized with anti-*L. monocytogenes* antibodies, 3h incubation.

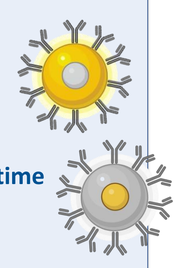
MNPs | **GNPs**



Non-optimal bacterium/particle ratios → bacteria wrapped with many MNPs, not enough GNPs
Size dispersion for MNPs → different capture depending on the size

PROPOSITIONS

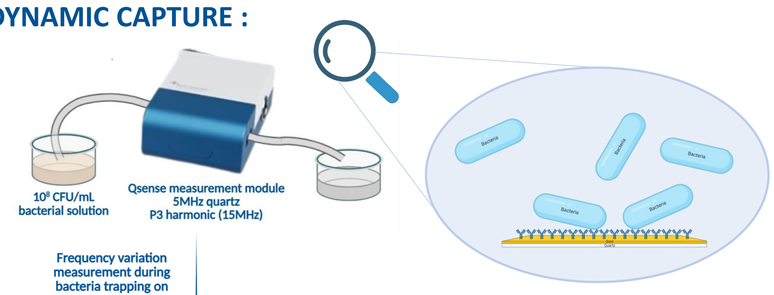
- Separation of MNPs using Nanopore or magnetic sorting → **calibrated particles batchs**
- Use of particles with gold core and magnetic shell (or the opposite) to reduce time, doing only one step → **magnetic separation & weighing at same time**



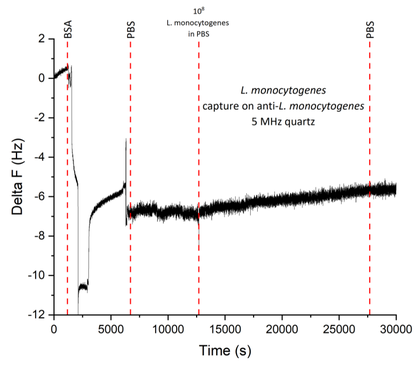
- Capture tests with different calibrated particles concentrations
  → **evaluation of the space requirement around bacteria & optimal ratio to use**
  → **evaluation of the optimal size for bacteria capture**

QUARTZ QCM SURFACES

DYNAMIC CAPTURE :



Frequency variation measurement during bacteria trapping on functionalized quartz

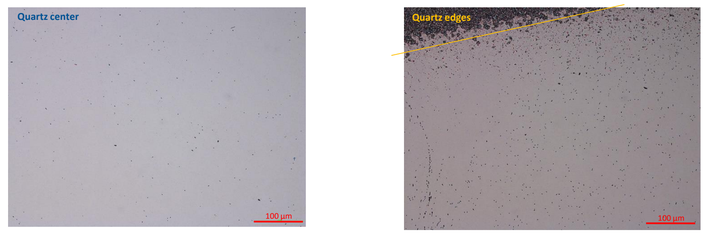


$\Delta f_{measured}$ = + 1.46 Hz

Positive frequency shift measurement → negative expected (mass gain)

CHARACTERIZATION:

After bacteria capture, violet cristal dying and optical microscopy observation, x20 magnification. ImageJ data treatment and counting.



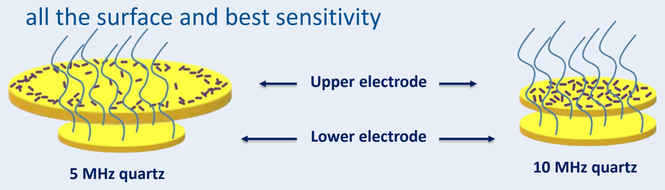
Presence of bacteria on quartz: **in the center and on the outer edges of quartz.**
Average bacterial density: 1.61 × 10^{-3} bacteria/µm²
According to Sauerbrey: $\Delta f_{theoretical}$ = - 8.2 Hz

Dispersion of bacteria across the surface and higher concentration at the edges → uneven coverage by bacteria & non homogeneous measurement

Different theoretical and measured signals → Sauerbrey approximation valid for a homogeneous surface

PROPOSITIONS

- Use of 10 MHz quartz or higher frequency → measurement between electrodes of same size (not the case for 5MHz), *i.e.* all the surface and best sensitivity



- Use of a theoretical equation that considers others parameters : microviscosity, ionic strength, cell surface hydrophobicity, …

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Contacts
Chloé RICHET
E-mail : chloe.richet@femto-st.fr – c.richet@actalia.eu
Institut FEMTO-ST, UMR CNRS 6174, UMLP
15B Avenue des Montboucons, Besançon, F-25000
www.femto-st.fr