In the Name of God

Journal Club Presentation

New Detection Platform for Screening Bacteria in Liquid Samples

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Article

New Detection Platform for Screening Bacteria in Liquid Samples

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JCR category rank: Q1: Chemistry, Analytical | Q1: Instruments & Instrumentation | Q2: Nanoscience & Nanotechnology

- What is new about the paper? **(Introduction)**
- Where does it fit in the context of prior work? **(Background)**
- What methods were used? **(Methods)**
- What were the primary results? **(Results)**
- What do the authors think these results mean? **(Conclusions)**
- What is your assessment of the paper? **(Critique)**

Introduction

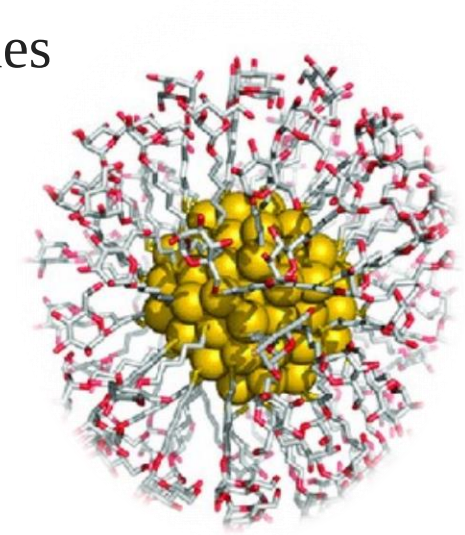
- Development of methods for the rapid detection and identification of bacteria
- Standardized methods (highly accurate and sensitive)

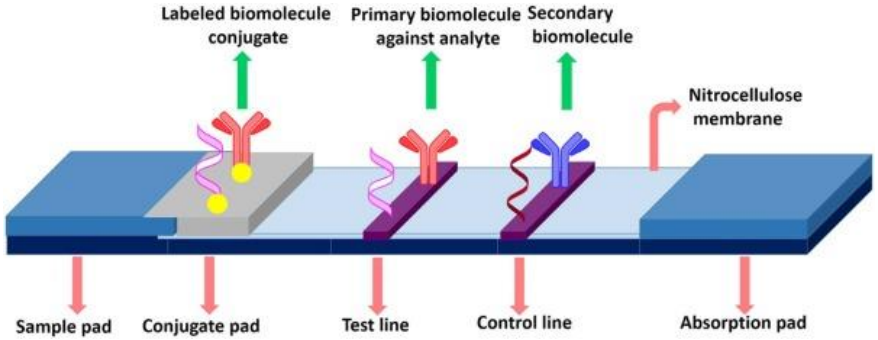
Genetic and immunological features of microorganisms

- Polymerase chain reaction (PCR)
- Quantitative PCR (qPCR)
- Enzyme-linked immunosorbent assay (ELISA)
- Lateral flow assay (LFA)

Introduction

- LFA is a rapid test format using antibodies as bioreceptors and colorimetry as detection method.
- Specific antibody-coated magnetic beads
- Gold nanoparticles bearing antibodies
- Flow cytometric methods





Introduction

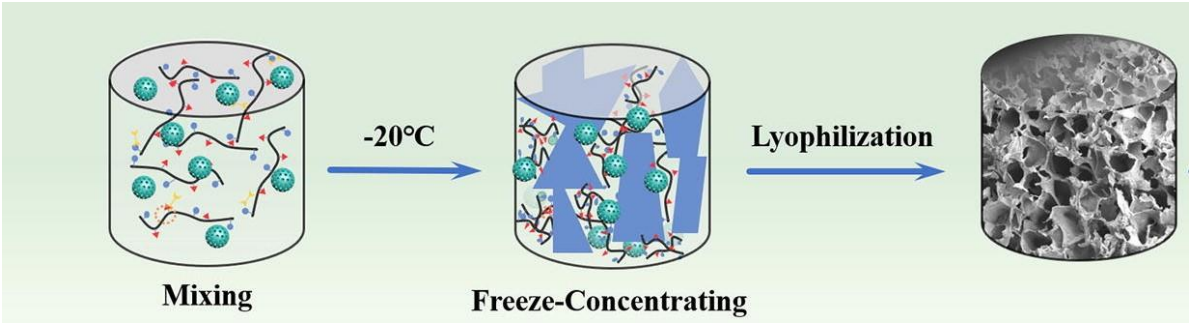
we present a new method combining:

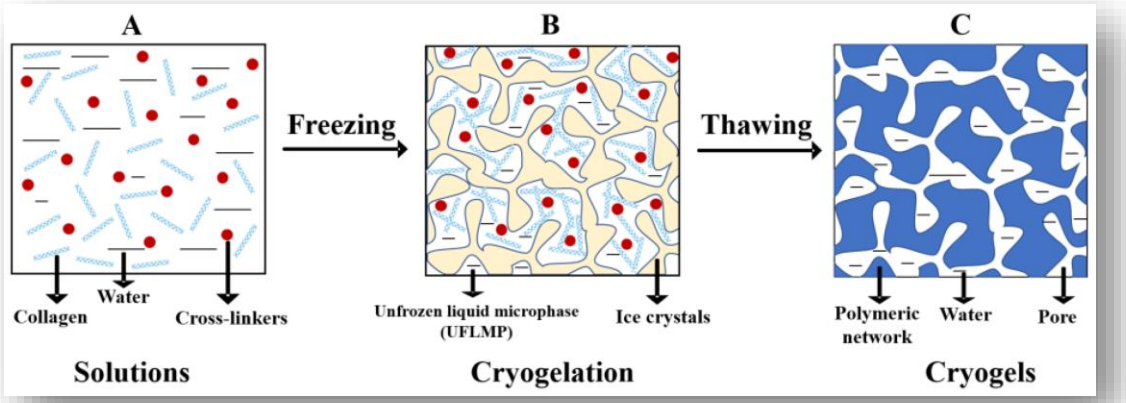
- pre-enrichment step using a microporous cryogel

  pHEMA-AEM 2-hydroxyethylmethacrylate (HEMA)
  2-Aminoethyl methacrylate hydrochloride (AEM)

- detection step using antimicrobial peptides (AMPs)

- labelled antibodies for identification





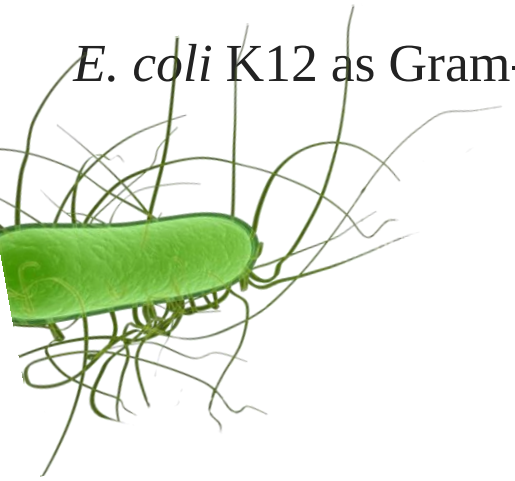
Introduction

The developed assay consists of three steps:

(i) entrapment of bacteria in the cryogel;

(ii) desorption of bacteria from the cryogel and measurement of their affinities toward immobilized AMPs

(iii) bacteria identification using specific labelled antibodies.



E. coli K12 as Gram-negative (Gram (−)) and *Bacillus sp.* 9727 Gram-positive (Gram (+)) bacteria using **cecropin B** and **cecropin P1** AMPs.

Materials and Methods

1. Chemicals

- 2-hydroxyethylmethacrylate **(HEMA)**
- 2-Aminoethyl methacrylate hydrochloride **(AEM)**
- *N*,*N*′-methylenebisacrylamide **(MBAA)**
- ammonium persulphate **(APS),**
- 1,2-bis(dimethylamino)ethane **(TEMED)**
- and **cecropin B** and **cecropin P1** were purchased from Merck KGaA (Darmstadt, Germany).
- Phosphate buffer saline **(PBS)** was purchased from Gibco Italia.
- **Noble Agar** were purchased from BD Diagnostics (Franklin Lakes, NJ, USA).
- **Antibodies against *E. coli*** (Polyclonal anti-*E. coli* ab13627) were purchased from Abcam (Cambridge, UK)
- **Quantum dots (QDs)** and **Qdot™ Incubation Buffer** were purchased from Invitrogen (code Q10101 MP and Q20001 MP, respectively).

Materials and Methods

2. Bacterial Cultures

- Bacterial strain ***E. coli* K12 (DSM No: 6897)**
- and ***Bacillus sp.* 9727 (DSM No: 9727)**
- kept at −20 °C for long storage
- washed in PBS before analysis

3. Synthesis and Physico-Chemical Characterization of the P(HEMA-AEM) Cryogels

Cryogel
- 2 mmol of AEM,
- 3.9 mmol of HEMA
- and 2 mmol of MBAA in 9 mL of water

- 30 min, 1% *w*/*w* APS/TEMED
- in 0.5 mL solution in a glass tube of 7 mm diameter
- washed with an of water and ethanol (0, 30, 50, 70, 90, 100%)
- dry at room temperature (RT)

Materials and Methods

4. Surface Characterization

- scanning electron microscopy (SEM)
- the cryogel was washed with an increasing percentage of ethanol
- treated using critical point drying (CPD)

5. Adsorption of Bacteria onto P(HEMA-AEM) Cryogels in PBS and Spiked Water Samples

- in buffer at RT with gentle shaking
- 45 mg of dried P(HEMA-AEM) cryogel were added to 4 mL of PBS containing bacteria
  - 10^4 to 10^8 cells *E. coli* (cells mL^{-1})
  - Bacillus sp. at 10^8 cells mL−1
- Experiments on adsorption kinetics
- the adsorption of bacteria in different matrices was evaluated by environmental water (Lago Maggiore, Italy) and bottled mineral water
- All experiments were performed in triplicates

Materials and Methods

6. *Quantification of Adsorbed Bacteria onto P(HEMA-AEM)*

$$A = \frac{C_e - C_0}{C_o} \times 100,$$

- C_0 is the initial concentration of viable *E. coli* bacteria (cells mL^{-1})
- C_e is the bacteria concentration remaining in supernatant solution at the equilibrium

7. *Quantification of Adsorbed Bacteria onto P(HEMA-AEM) in Flow Condition*

- in flow condition at RT, in PBS, at flow rates of 0.05, 0.1, or 0.3 mL min^{-1}
- 30 mL of bacteria was prepared at the initial concentration of 10^8 cells mL^{-1}
- turbidimetric analysis

Materials and Methods

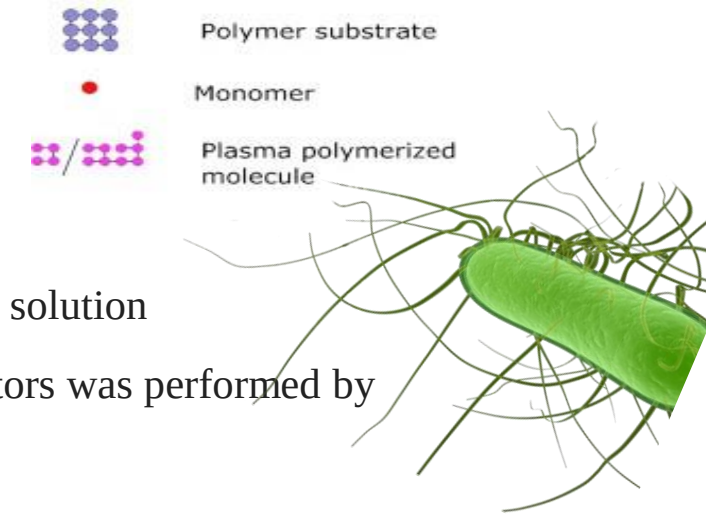
8. Elution of Adsorbed Bacteria from P(HEMA-AEM) Cryogel

- 45 mg of P(HEMA-AEM) cryogel was incubated for 6 h in 10 mL of *E. coli* and *Bacillus sp.*
- turbidimetric and CFU analysis.

then

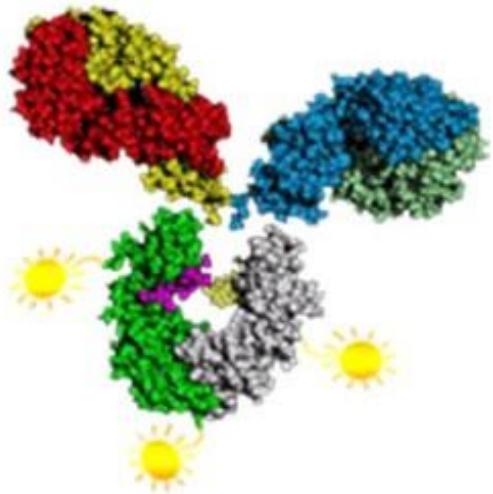
- 4 mL of 1 M NaCl
- and phosphate buffer at pH 12

9. Bacterial Motility Measurements



- A silicon wafer was coated with a 100 nm thick layer of PEO
- Plasma polymerization
- The PEO layer was functionalized by incubating the surface with 50 µg mL^{-1} AMPs solution
- Optimization and confirmation of the surface functionalization with AMPs bioreceptors was performed by surface plasmon resonance
- analyzed using ImageJ
- The motility of each strain interacting with each PEO-AMPs surface was compared

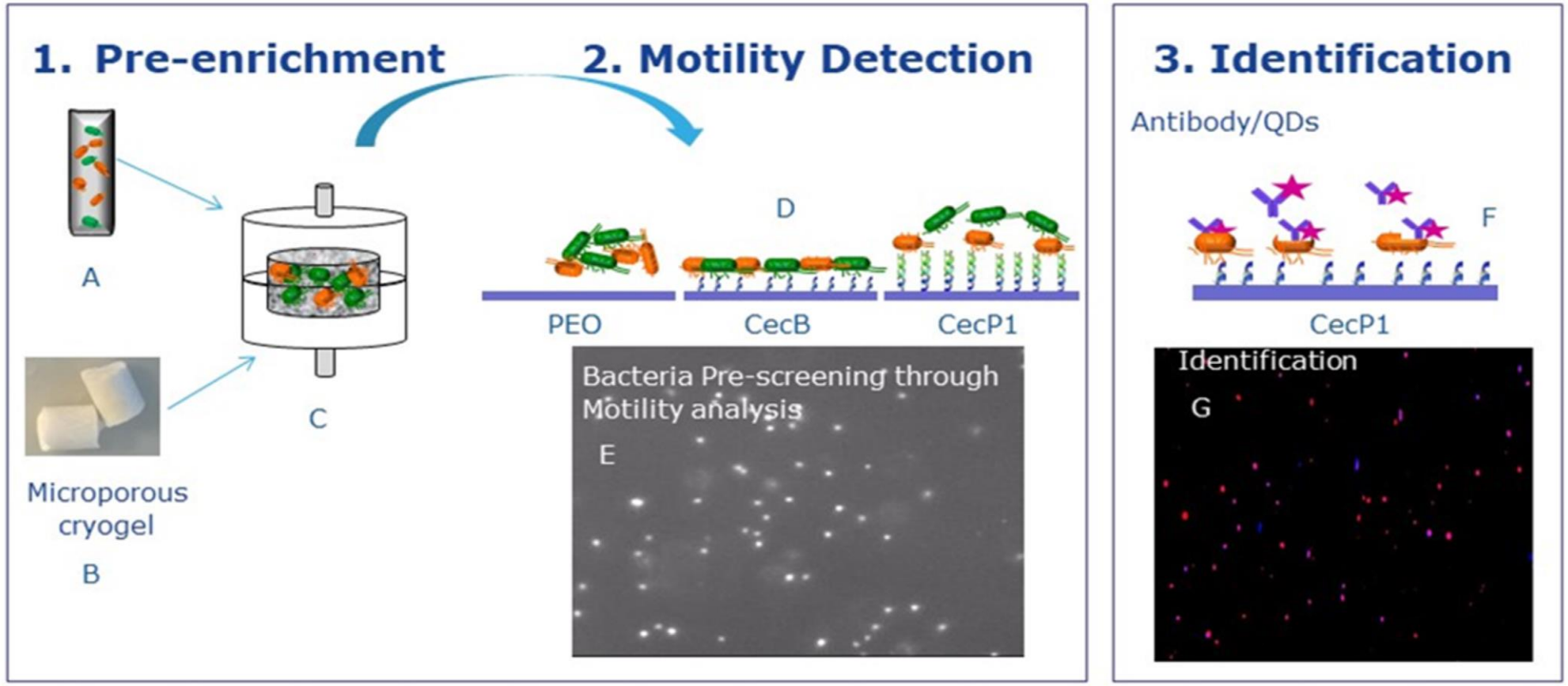
Materials and Methods



10. Bacteria Identification Test with Labeled Antibodies

- Anti-*E. coli*-QDs conjugates were prepared
- the test was only performed for the detection of *E. coli* .
- The comparison of the images obtained by DF
- The image acquisition was obtained in DF and FM

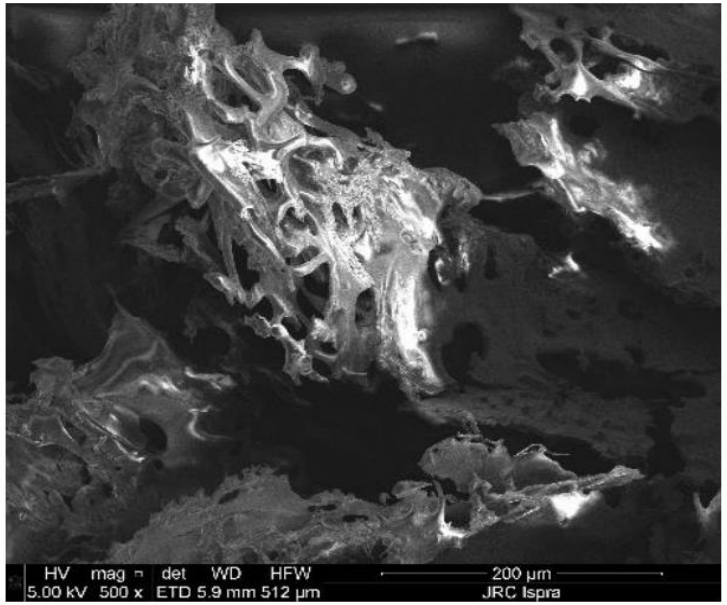
Results and Discussion



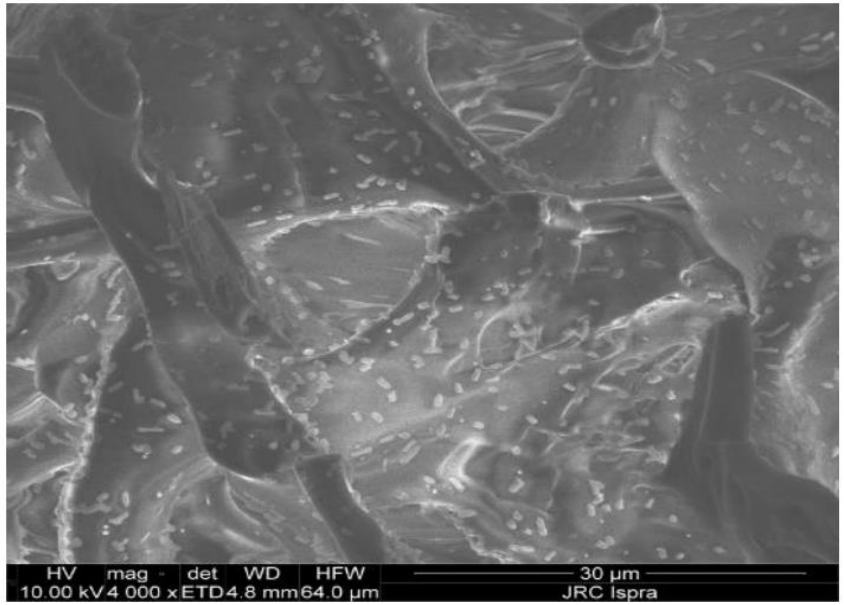
Results and Discussion

1. Physico-Chemical Characterization of P(HEMA-AEM) Cryogel

2. Adsorption of Bacteria in PBS and Spiked Water Samples



(a)

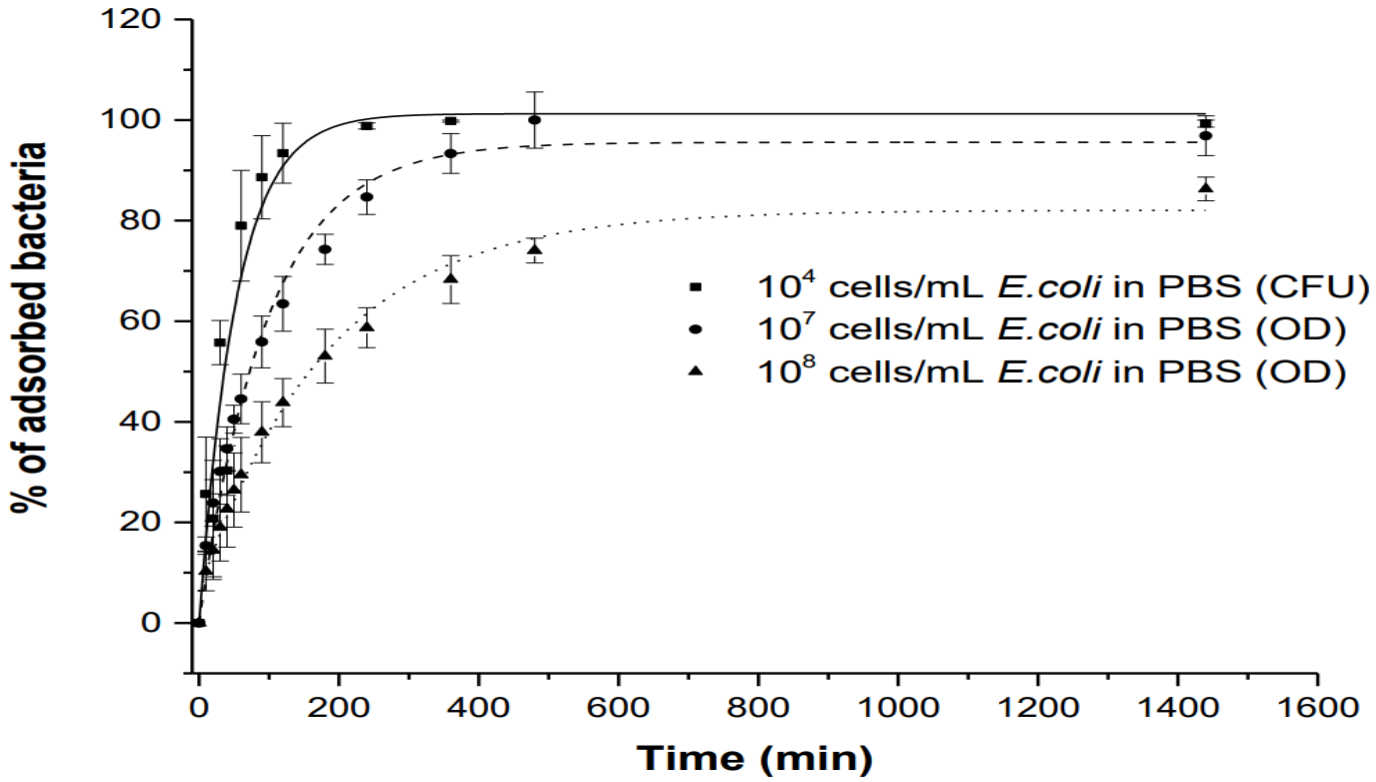


(b)

SEM image of the P(HEMA-AEM) cryogel before and after incubation with bacteria showing its 3D microporous structure and clearly evidencing the binding of bacteria after 6 h of incubation.

Results and Discussion

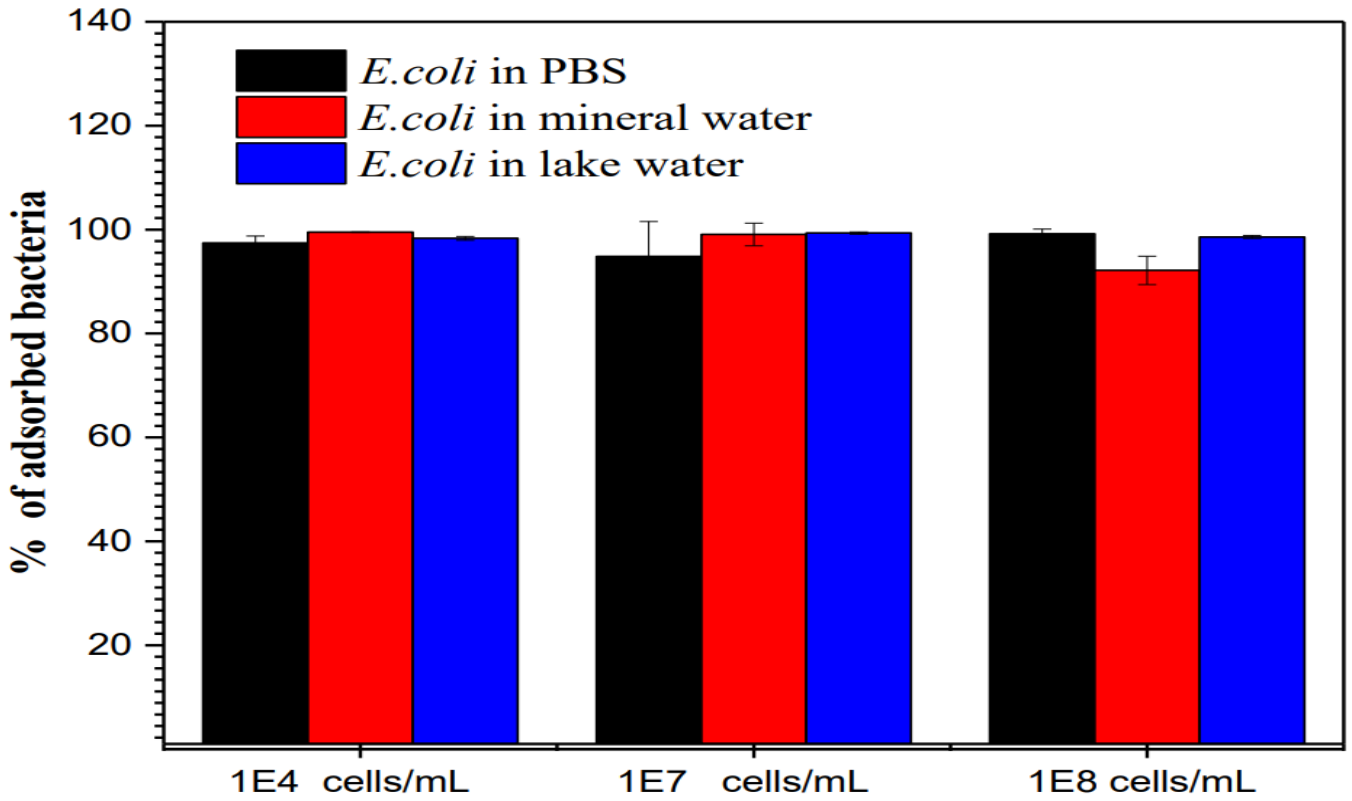
2. Adsorption of Bacteria in PBS and Spiked Water Samples



Adsorption kinetics of *E. coli* (initial concentration = 10^4, 10^7 and 10^8 cells mL-1) onto P(HEMA-AEM) cryogel in PBS.

Results and Discussion

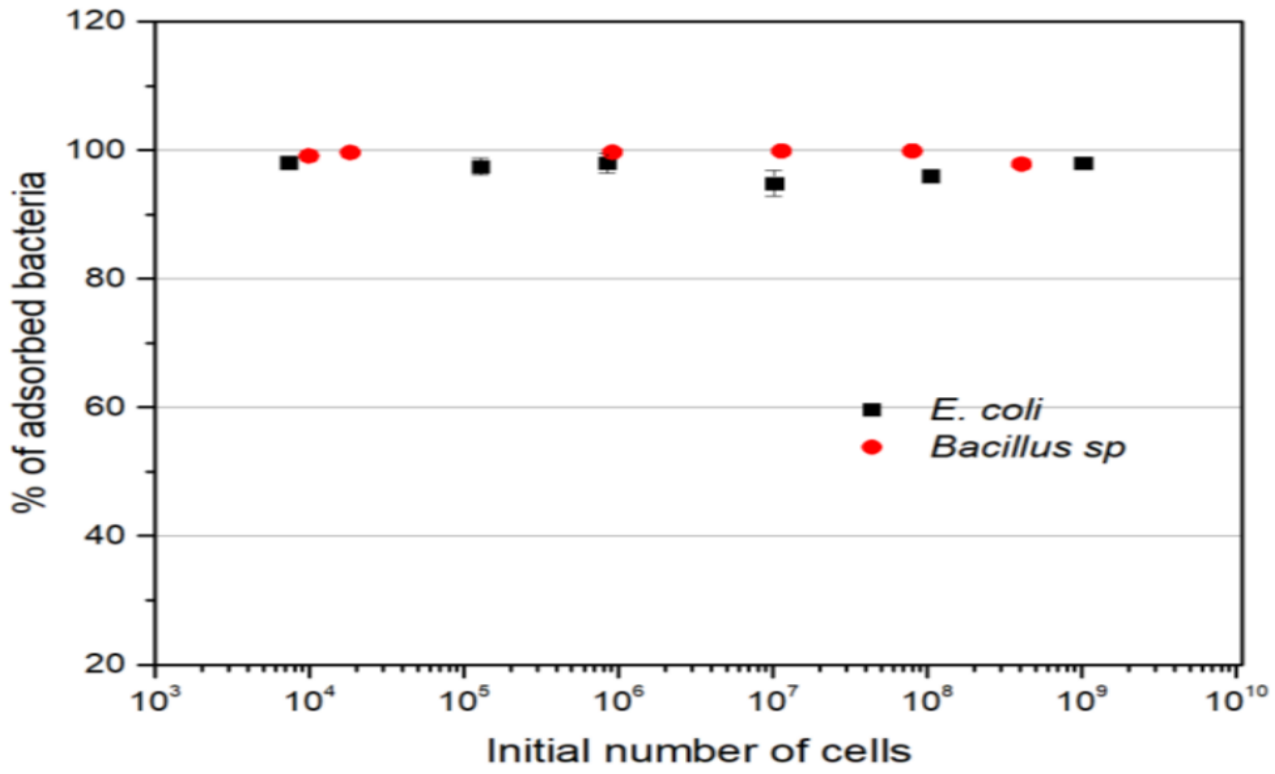
2. Adsorption of Bacteria in PBS and Spiked Water Samples



- Comparative adsorption of E. coli bacteria at the concentration of 10^4, 10^6 and 10^8 cells mL-1 in PBS, in lake water and in commercial mineral water.
- 45 mg of dried cryogel were suspended in 4 mL of bacteria suspension

Results and Discussion

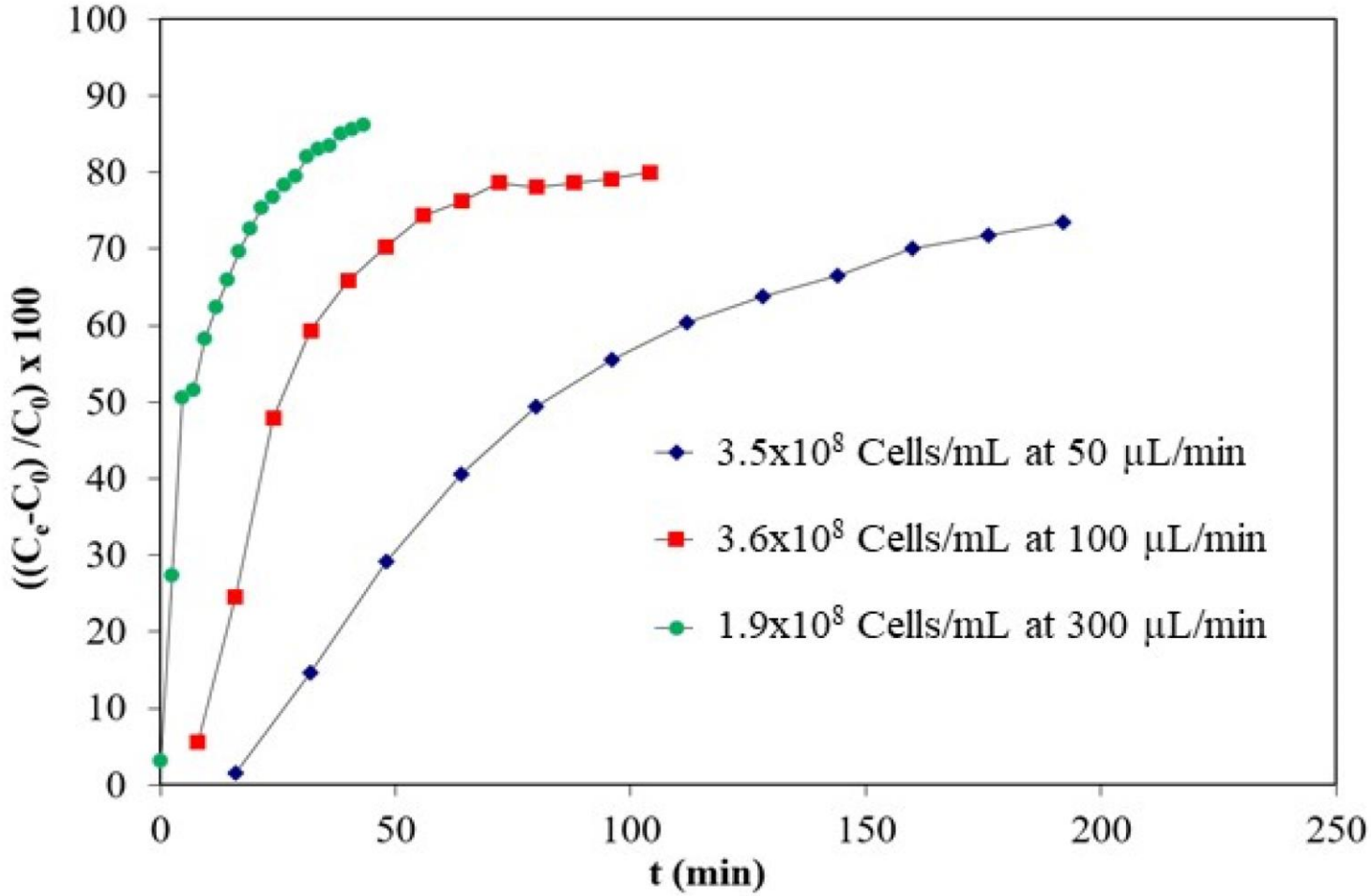
2. Adsorption of Bacteria in PBS and Spiked Water Samples



- Adsorption of bacteria ranging from 10^4 to 10^8 cells mL-1 *E. coli* and *Bacillus sp*. bacteria keeping constant the ratio of cryogel and volume of bacteria to 45 mg per 4 mL

Results and Discussion

2. Adsorption of Bacteria in PBS and Spiked Water Samples



Results and Discussion

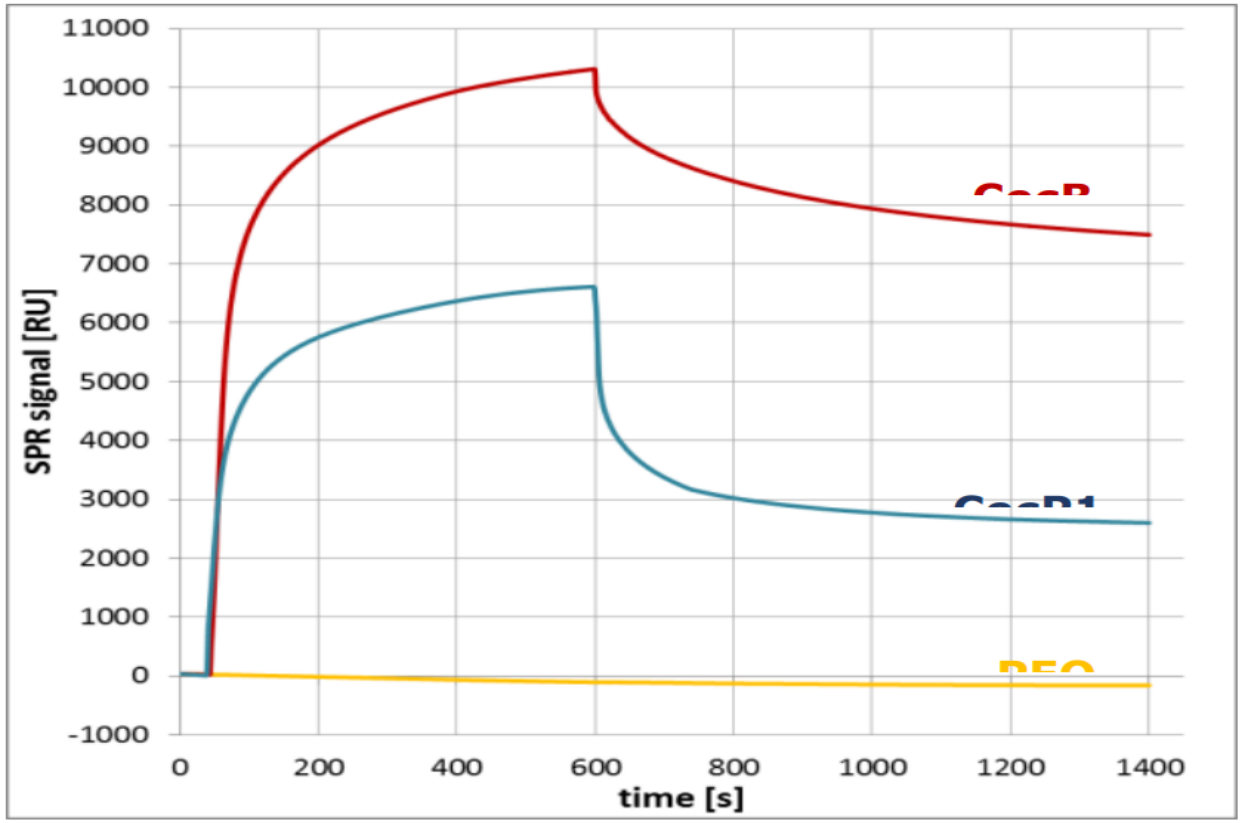
3. Elution of Adsorbed Bacteria from P(HEMA-AEM) Cryogel

- ✓For the desorption, the same cryogels were suspended in a water solution containing 1 M NaCl, which enabled the release of **45% of the *E. coli*** bacterial cells attached on the cryogel within 1 h**.** (data not shown)
- ✓The use of **0.01 M phosphate buffer at pH 12** was more effective; enabling the release of **45% of *Bacillus sp***. from the cryogel**.**
- ✓capability to entrap and elute large amounts of bacteria makes the cryogel very efficient in harvesting pathogens
- ✓adapting the cryogel to a solid phase extraction (SPE) disk holder, for bacteria harvesting directly in situ

Results and Discussion

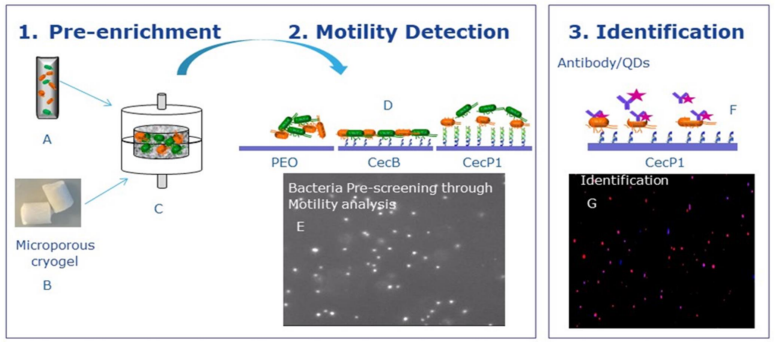
4. Detection and Identification of Bacteria

►Bioreceptor Immobilization



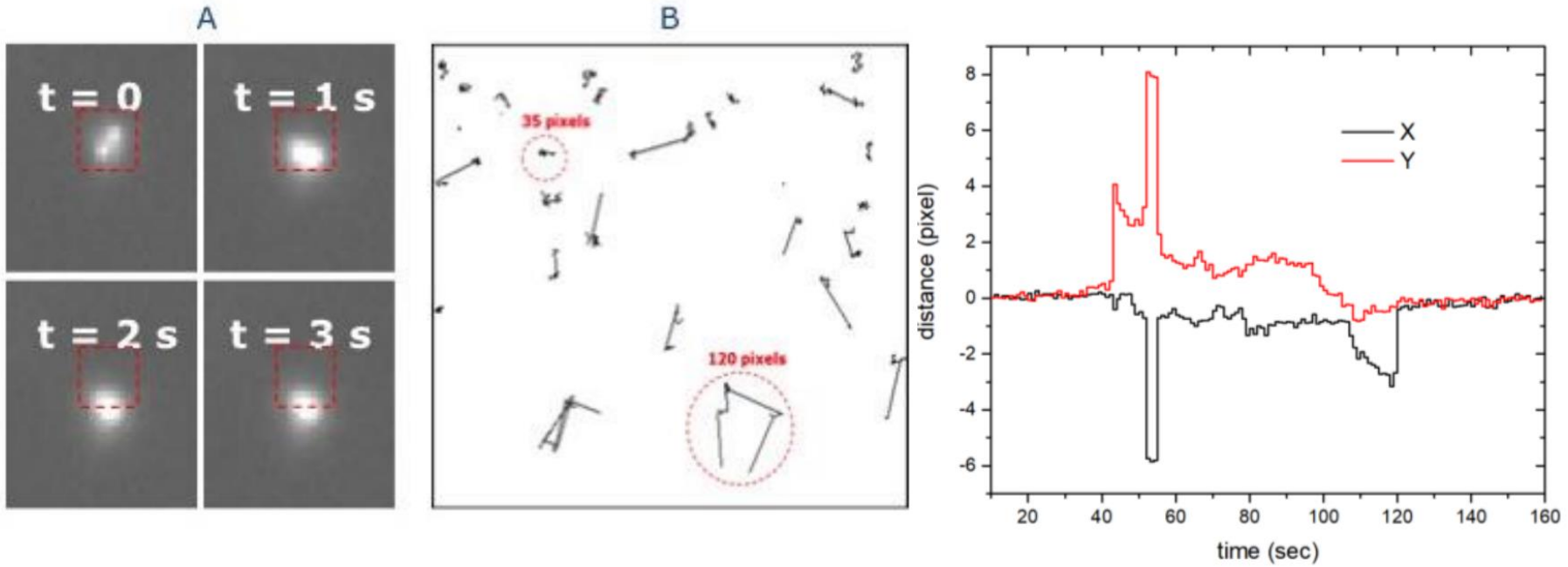
- PEO Surface functionalization with Cecropin P1 (CecP1) and Cecropin B (CecB) monitored by SPR.

Results and Discussion



4. Detection and Identification of Bacteria

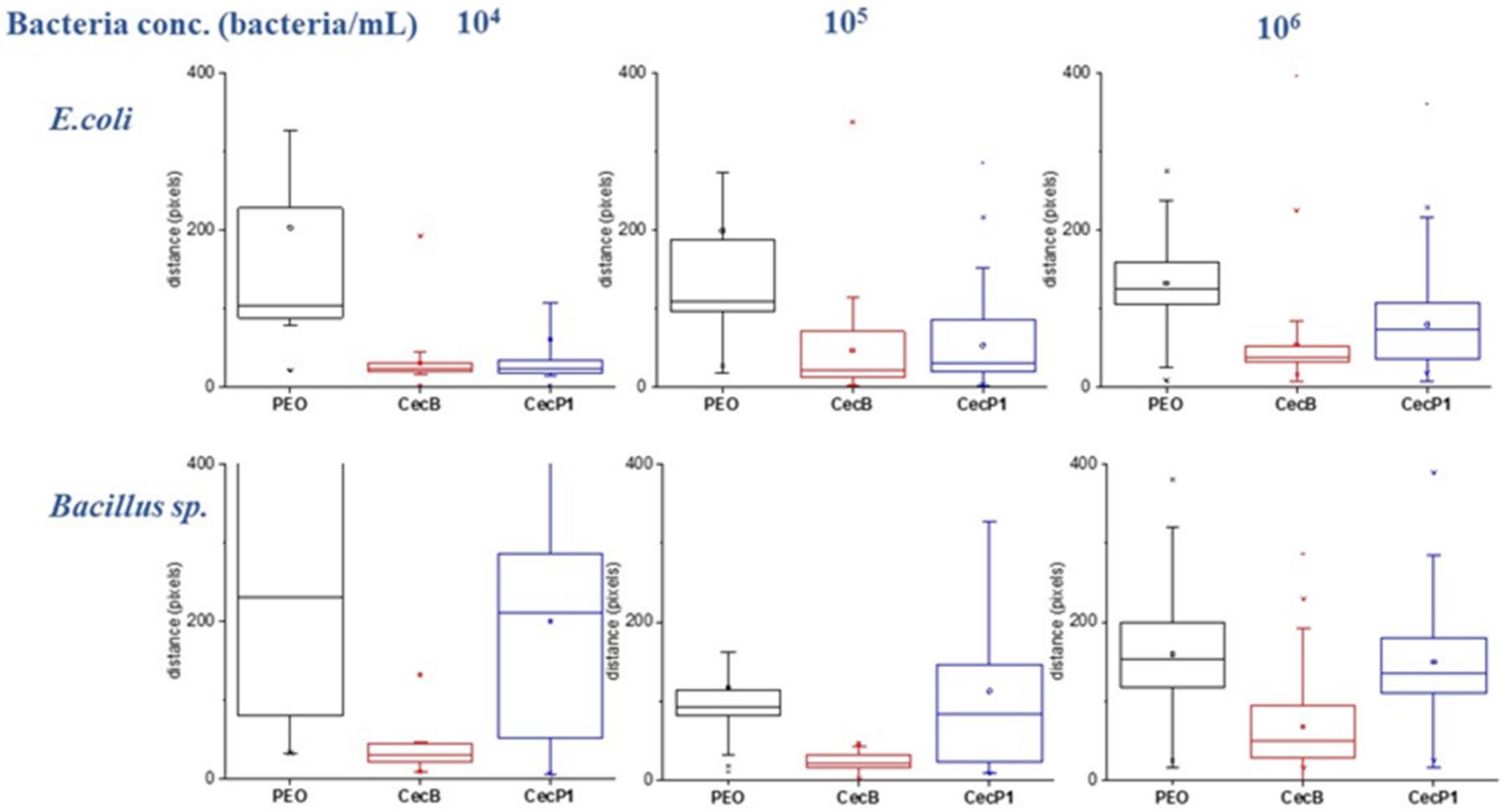
►Determination of Bacteria Affinity toward AMPs by Motility Measurement



- Cell motility is presented as (a) shifting of cell position regarding the geometrical centre at time zero, (b) vectorial travelled distance of the cell along the 160 frames and (c) variation of the X and Y position regarding the time zero (starting position).

Results and Discussion

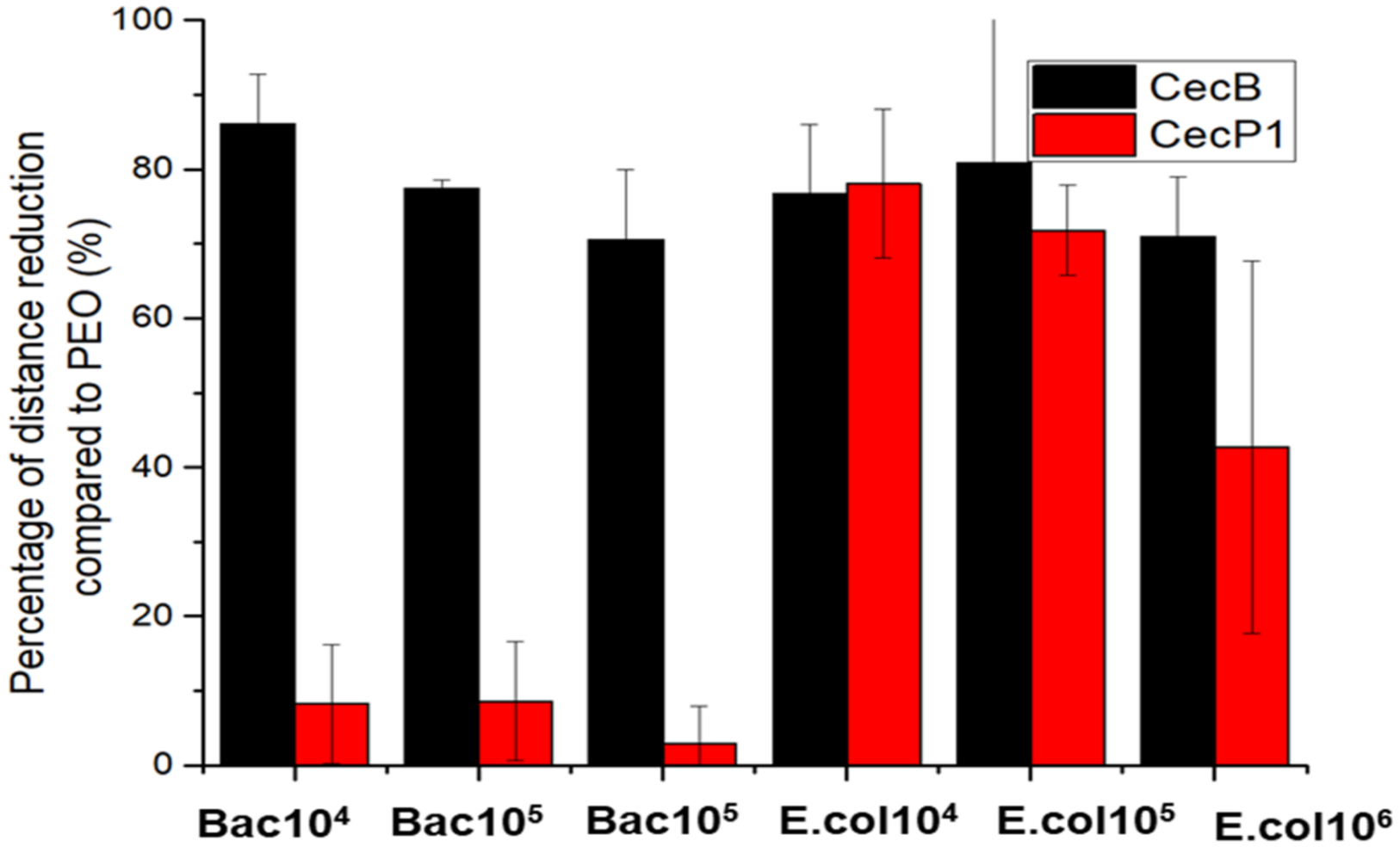
5. Bacterial Motility Measurements



- Figure shows the motility measurements on the bare PEO and AMPs functionalized PEO (average distance travelled by the counted bacteria) for different bacteria concentrations of *E. coli* and *Bacillus sp*.

Results and Discussion

5. Bacterial Motility Measurements



Summary of the motility analysis of *E. coli* and *Bacillus sp.* on PEO, CecB and CecP1 at different concentrations of bacteria. It shows the percentage of reduction in motility compared with the motility of bacteria on PEO.

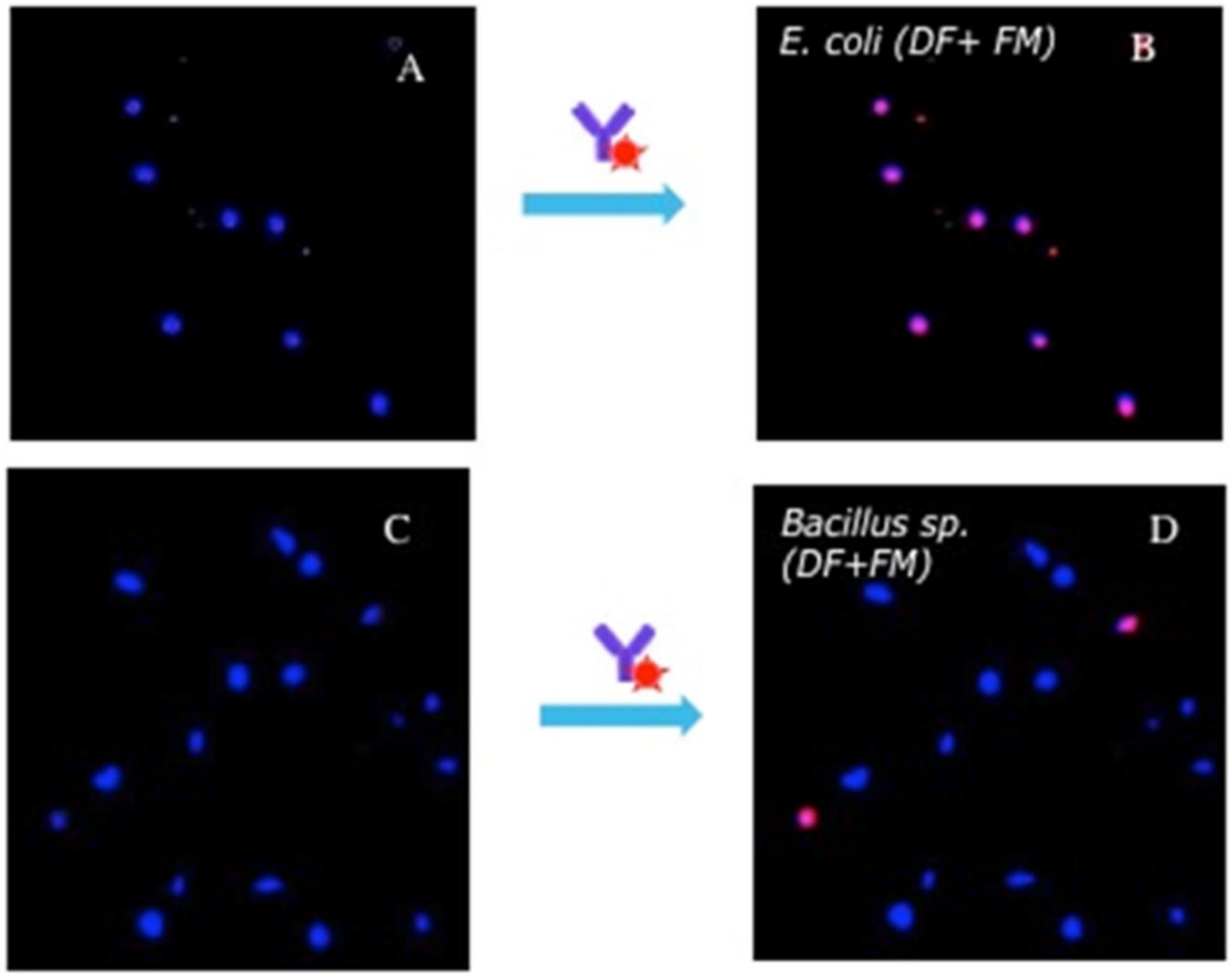
Results and Discussion

5. Bacterial Motility Measurements

- The obtained results are in agreement with the literature and show that **CecB AMPs have a high-affinity for both Gram (+) and Gram (−) bacteria**, while the **CecP1 only shows a high-affinity for the Gram (−) bacteria.**

Results and Discussion

6. Bacteria Identification by Labelled Antibodies



(**A**) DF image of *E. coli* bacteria immobilized on cecropin B AMPs.

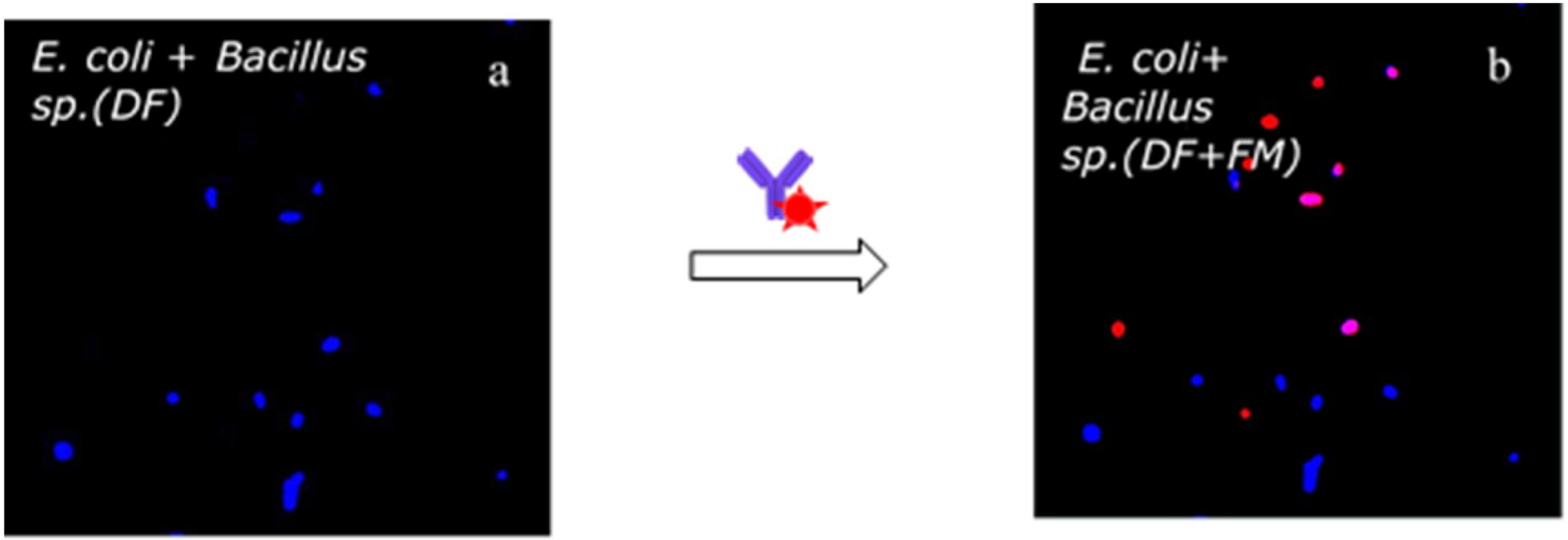
(**B**) Overlapping of DF and fluorescent images of the same area (fluorescent bacteria in pink).

(**C**) DF image of *Bacillus sp*. bacteria immobilized on cecropin B AMPs (bacteria in blue).

(**D**) Overlapping of DF and fluorescent images in the same area (fluorescent bacteria in pink).

Results and Discussion

6. Bacteria Identification by Labelled Antibodies



(a) DF image of *E. coli* and *Bacillus sp.* bacteria immobilized on cecropin B AMPs (bacteria in blue).

(b) Overlapping of DF and fluorescent images of the same area (fluorescent bacteria in pink).

Conclusions

⁂ P(HEMA-AEM) microporous cryogel is a material of choice to enrich bacteria concentration from different samples .

⁂ The advantages of this approach:

(i) the use of a **label-free method** for detecting the presence of possible pathogenic bacteria in water samples;

(ii) **fast response** in detecting the bacteria since the screening is carried out **in 30 min**; and

(iii) the use of dark field and optical microscopy as detection methods, which is often available as laboratory equipment and also does not need specialized personnel.

Recap…

►this study shows that **motility monitoring**, i.e., affinity towards a small set of AMPs, allows us to differentiate bacteria families by looking at *Escherichia coli* and *Bacillus sp.* **as models** for Gram-negative and Gram-positive bacteria, respectively.

►The use of **AMPs** with broad specificity combined with labelled antibodies enabled the detection and potential categorization of a large spectrum of unknown or unexpected bacteria.

►The ability of the **biosensor** to detect targeted pathogens in low concentrations among several other bacterial species and cells needs to be further addressed

Thank you for your attention ☺