

DISCRIMINATION OF *E. COLI* B^E BL21(DE3) PORIN MUTANTS BY ATR-FTIR SPECTROSCOPY

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Introduction

Attenuated total reflectance Fourier-transformed infrared (ATR-FTIR) spectroscopy measures the changes that occur in a totally internally reflected infrared beam when the evanescent wave it produces penetrates only a few micrometers (0.5-5µm) into the sample, deposited on top of an optically dense crystal (Figure 1) [1]. Both conventional FTIR and ATR-FTIR have been successfully used for bacteria discrimination and classification, as each species presents an unique spectral "fingerprint", making it a promising, fast and reliable screening method for pathogen identification in a clinic setting [2-3].

In this study, ATR-FTIR is used in an attempt to discriminate between *Escherichia (E.) coli* B^E BL21(DE3) and a derived mutant lacking the major *E. coli* porins OmpF and OmpC (Table 1). Porins are transmembrane proteins located in the outer membrane of Gram-negative bacteria such as *E. coli* that allow the passage of various solutes, including antibiotics, playing therefore an important role in the development of antibiotic resistance. The ability to effectively distinguish between strains differing only in the porin profile would mean more than just information on structural differences, but also an initial indication on resistance mechanisms [4].

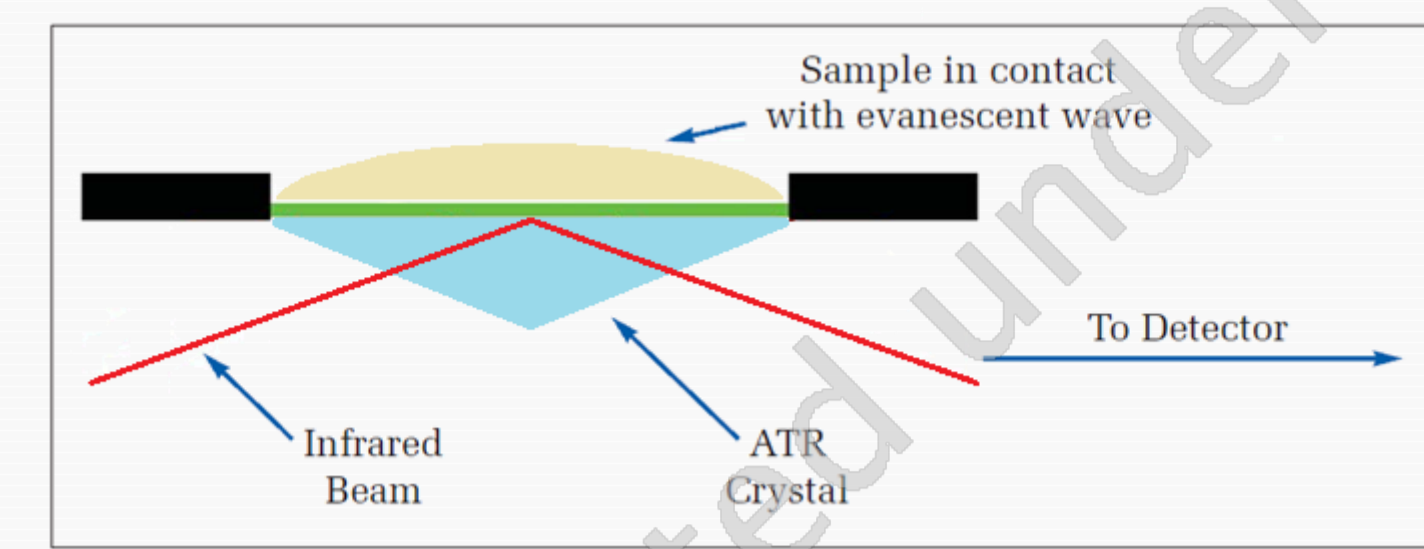


Figure 1 – A single reflection ATR system [adapted from 1].

Table 1 – *E. coli* strains used in this study [5].

Strain	Relevant genotype
BL21(DE3)	F, ompT hsdS _B (r _B m _B) gal dem (DE3)
BL21(DE3)omp8	BL21(DE3), ΔlamB ompF::Tn5 ΔompA ΔompC

Results

Bacterial cells were harvested from solid medium 12 and 24 hours after inoculation (cultures in exponential and stationary phase, respectively) and applied directly onto the diamond crystal of a PIKE Technologies GladiATR accessory. After a few minutes of air-drying (to allow the formation of a film of cells on the crystal), spectra were obtained with a PerkinElmer Spectrum BX FTIR Spectrophotometer at 4 cm⁻¹ resolution and as an average of 32 consecutive interferograms. Cultures were grown in triplicate and three replicates of each strain/growth phase combination totals 36 spectra in this pilot study.

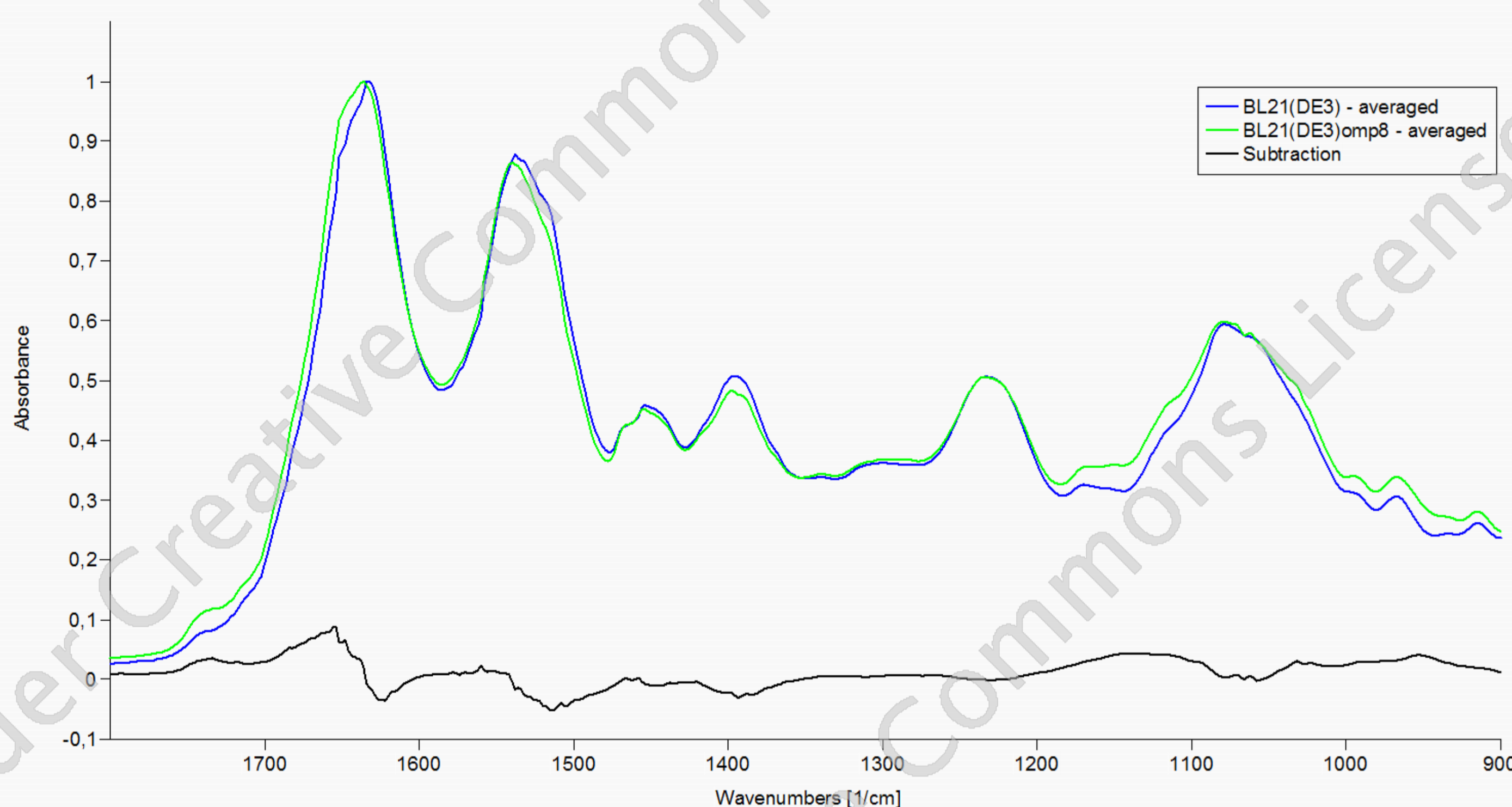


Figure 2 – Averaged normalized ATR-FTIR spectra for stationary phase *E. coli* BL21(DE3) (blue) and BL21(DE3)omp8 (green) in the 1800-900 cm⁻¹ region. Difference spectrum in black.

After a preliminary analysis, *E. coli* BL21(DE3) and BL21(DE3)omp8 seem to show some relevant differences (Figure 2), namely in the amide I (1600-1690 cm⁻¹) and amide II (1480-1575 cm⁻¹) regions, the two most prominent vibrational bands characteristic of polypeptides and proteins [6]. Principal component analysis (PCA) confirmed that BL21(DE3) spectra cluster in a different region of the PCA map than BL21(DE3)omp8, and shows that differentiation is clearer when the cells are harvested in their fully-expressed phenotype stationary phase rather than in their adaptive exponential phase (Figure 3).

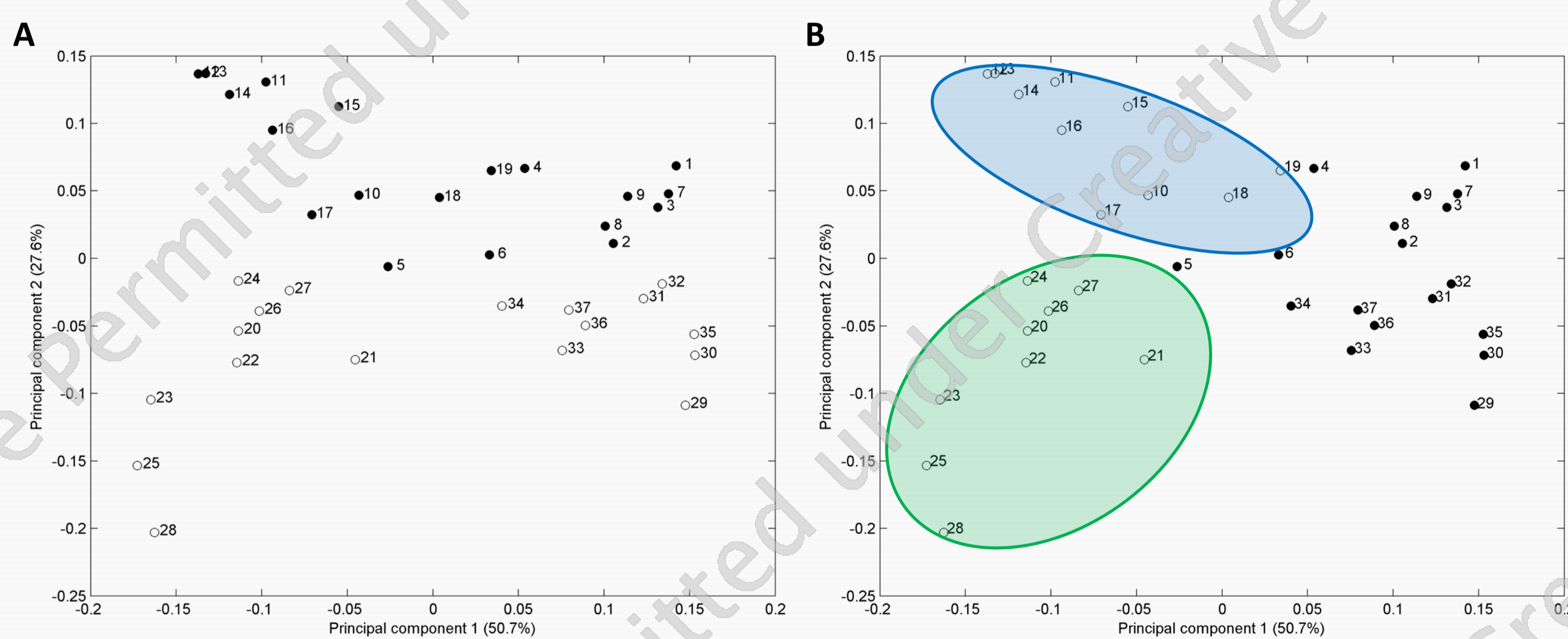


Figure 3 – PCA maps of the 36 spectra measured. (A) shows BL21(DE3) in filled dots and BL21(DE3)omp8 in blank dots. (B) shows exponential phase in filled dots and stationary phase in blank dots and also displays the stationary phase BL21(DE3) cluster in blue and the same for BL21(DE3)omp8 in green.

Conclusions

Screening bacteria by means of ATR-FTIR spectroscopy seems to be possible and allowed the discrimination of *E. coli* strains that differ solely in their outer membrane porin profile. This points towards incomparable advantages because, as can be perceived from the necessary strain validation steps, molecular analysis is complex and time-consuming. Being able to conclude on the bacterial phenotype after straightforward and quick procedures is, therefore, something worth pursuing using ATR-FTIR spectroscopy. Further studies using single knockouts of OmpF and OmpC will allow to test the true specificity of this technique.

In the meantime, the study of cells grown in liquid cultures is also something to carry out. Cultures harvested from solid medium tend to be less homogeneous (different metabolic states according to cell location within the colony) and may account for the rather wide clusters obtained after PCA analysis.

Molecular Analysis

Prior to the ATR-FTIR studies, the strains were subject of molecular analysis in order to ascertain whether the most relevant mutations described were indeed present. Conventional and reverse transcriptase polymerase chain reaction (PCR and RT-PCR) tests were conducted so as to check if both the genomic DNA and the RNA transcripts of the bacteria coded the desired information (data summed up in Table 2).

A novel immunoprecipitation lab-on-a-chip technique [7] was also optimised and applied to determine the presence or absence of OmpF and OmpC porins. The target proteins were isolated by pull down using magnetic beads (Invitrogen Dynabeads[®] Protein A) previously coupled to anti-OmpF or anti-OmpC antibodies; the resultant immunocomplexes were then eluted and the antigen-enriched sample was analysed in an Agilent High Sensitivity Protein 250 chip using an appropriate Agilent 2100 Bioanalyser (Figure 4).

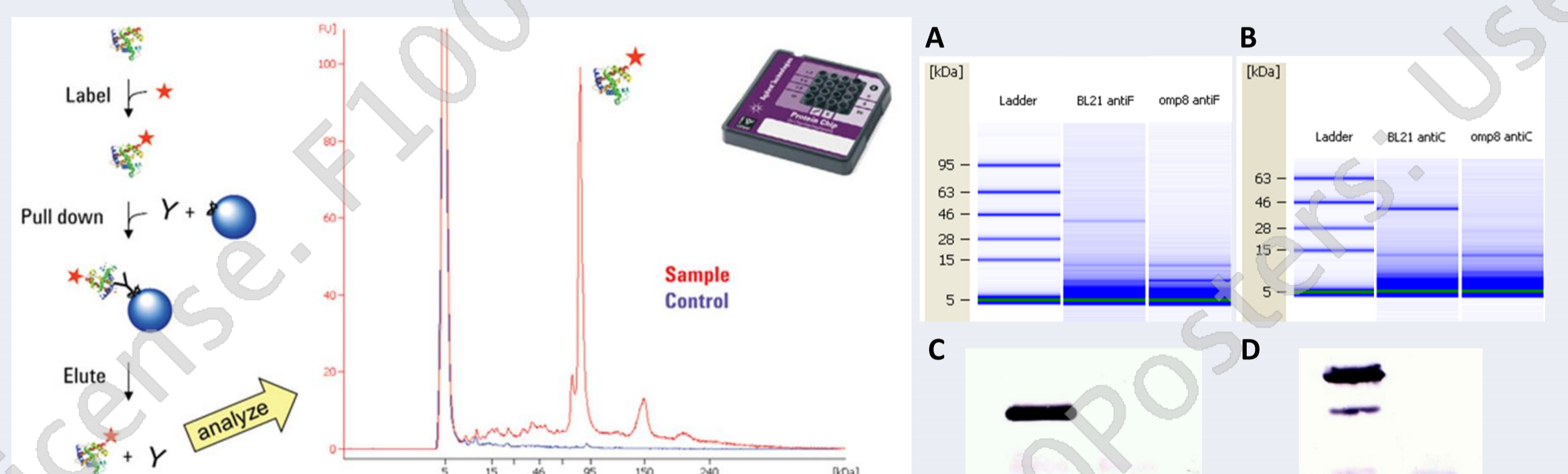


Figure 4 – Immunoprecipitation lab-on-a-chip protocol [7].

Figure 5 – *E. coli* BL21(DE3) and BL21(DE3)omp8 lab-on-a-chip results with anti-OmpF (A) and anti-OmpC (B) against conventional western blotting using the same antibodies (C and D).

Figures 5-A and 5-B depict the gel-like results obtained with this innovative technique, showing that BL21(DE3) expresses OmpF and OmpC and BL21(DE3)omp8 lacks both porins. The western blots (Figures 5-C and 5-D) point to the same conclusions, but in comparison the lab-on-a-chip allows a more accurate identification of the protein bands (OmpF/OmpC monomers in the 37-40 kDa region as expected) and seems to be somewhat more specific, as the crosstalk of anti-OmpC antibody with OmpA (supposedly) is not perceived, though it is clear on the western blotting image.

Table 2 gathers all the results of this molecular analysis. BL21(DE3) exhibits the expected profile, but in the BL21(DE3)omp8 the allegedly absent ompC gene can still be detected (Table 1), suggesting that the supposed deletion was most likely a mutation that impaired this gene's transcription. lamB transcripts were also surprisingly found in this thought to be ΔlamB strain, meaning that the malto-porin is expressed after all. Nonetheless, and even though the actual genotype and phenotype of BL21(DE3)omp8 deviate from the previously published results [5], it is clear this isolate indeed lacks the major *E. coli* porins, proving it suitable for the subsequent ATR-FTIR experiments.

Table 2 – Summary of the molecular analysis performed on *E. coli* BL21(DE3) and BL21(DE3)omp8 regarding four targets: lamB, ompA, ompC and ompF.

	lamB		ompA		ompC		ompF		
	RNA	DNA	RNA	DNA	RNA	Prot	DNA	RNA	Prot
BL21(DE3)	+	+	+	+	+	+	+	+	+
BL21(DE3)omp8	+	-	-	+	-	-	-	-	-

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