Colicins’ regulation, ecology and evolution were primarily studied in planktonic cultures, yet it is well known that *Escherichia coli* form complex biofilms in natural environments. *E. coli* cells residing in a biofilm express genes and interacts differently than planktonic populations. The aim of this study was to investigate the differential expression of colicins in biofilm and planktonic cultures of *E. coli*.

Reporter strains marking the DNA degrading colicins E2 and E7 were used to monitor colicin expression at a single cell level. The planktonic cells were grown in suspension, and the biofilms were cultivated on eight slides mounted in a polypropylene flow cell. Laser scanning confocal microscopy images of the cells were taken from each culture at intervals and the percentages of colicins producers within the total population were quantified by automated image analysis.

The results suggest that colicinogenic bacteria residing in a biofilm produce more colicins. *E. coli* enhanced the production of colicin E7 in a biofilm compared to planktonic environment (8.5±0.6% and 3.14±0.1%, respectively). Likewise, the production of colicin E2 in the biofilm was augmented compared to planktonic environment (12.1±2.8% and 3.3±1.7%, respectively).

We showed that colicin production is enhanced in a biofilm setting by ~3 folds. This suggests that the role bacteriocin plays in biofilms, where competition for available resources is fierce, may be more pronounced than in planktonic environments.