High-level homogeneous production of fluorescent proteins in E. coli provides a sensitive reporter for antibiotic activity detection

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The uncontrolled use of antibiotics both in the medicinal practice and agriculture demands the development of highly sensitive probes for their preventive detection. Otherwise, this misuse results in the global spread of multidrug-resistant strains. That is particularly critical in the case of gram-negative bacteria which are intrinsically resistant to the majority of novel antimicrobial agents. Recently we presented a concept of deep functional profiling of microbiomes providing a new powerful tool for the detection of biological activity of even minor components of bacterial communities. This concept is based on a combination of microfluidic encapsulation of single cells in droplets of double emulsion (MDE) and fluorescence-activated cell sorting (FACS) isolating phenotype of interest. The stumbling block of this technology is the requirement of a particular fluorescent reporter indicating the desired activity. Moreover, highly sensitive detection of antibiotics in various environments is an urgent task resulted from their uncontrolled use in medicinal practice and agriculture. The present study is dedicated to the development of an *Escherichia coli* strain as a gram-negative reporter. We have tested a number of common laboratory *E. coli* strains for optimal growth conditions, cell morphology, and culture homogeneity. Different bright GFP derivative genes regulated by various strong promoters were examined to gain high levels of production, signal intensity, and narrow fluorescence distribution. Bulk culture fluorescence measurements supplemented with fluorescent microscopy and flow cytometry allowed us to identify a combination of strain, gene, and promoter providing optimal biosensor parameters. We believe our results will be applied for various applications including food safety-related studies, antibiotic pollutant tracking, and antibiotic discovery. This work was supported by RSF grant 19-14-00331.