IDENTIFICATION OF RECOMBINANT E. COLI EXPRESSING DIFFERENT PROTEINS USING LIGHT-SCATTERING

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Screening of clonal libraries from recombinant bacteria expressing unique proteins is a difficult task and predominantly relies on a detectable/visual phenotypic change. In this study a recently developed technology for identification of pathogens using light scattering was evaluated for the initial screening of clonal libraries. Light-scattering is a noninvasive method for identification of foodborne pathogens by passing light from a laser (635 nm) through the colony. Bacterial scatter patterns are compared to a known library of images, and identified by comparison of the scatter images. E. coli Top 10 strains were transformed with different plasmids coding for different proteins from various foodborne pathogens, E. coli 101 (pFSP126/invA - Salmonella), E. coli 102 (pFSP127/sipB - Salmonella), E. coli 103 (pFSP128/inlA - Listeria), E. coli 104 (pFSP129/fimY - Salmonella). All bacteria were cultivated in LB broth with Kanamycin at 37°C, overnight. Untransformed E. coli Top 10 was used as a control. Cultures were decimally diluted in MSM broth and dilutions were spread on LB agar plates containing Kanamycin individually, in pairs, and all strains. Plates were incubated at 37°C until colonies reached a diameter of 1 ± 0.2 mm. Colonies were then analyzed using light-scattering and images compared with the control. Individual E. coli strains expressing different proteins had unique scatter patterns. When strains were plated as a mixture, the sensor was able to differentiate the strains. Results were confirmed using PCR. These results suggest light scattering can be used for the selection and discrimination of recombinant colonies expressing different proteins.