Biofilm formation of *E. coli* isolates in vitro: Comparative analysis and synergistic effects

Andreas Reisner[1], Brigitte Hoeller[1], Søren Molin[2] and Ellen L. Zechner[1]
[1] Institut für molekulare Biowissenschaften, Karl-Franzens-Universitaet Graz, Graz, Austria
[2]Center for Biomedical Microbiology, BioCentrum-DTU, Lyngby, Denmark

Background: Our understanding of *E. coli* biofilm formation in vitro is based on studies of laboratory K-12 strains in monoculture grown in standard media. However, natural pathogenic *E. coli* isolates differ substantially in their genetic repertoire from *E. coli* K-12 and are subject to heterogenous environmental conditions, e.g. in the intestinal or urinary tract. *E. coli* also exists in close contact with numerous other species or other *E. coli* clones. Possible synergistic effects on cell-cell interactions due to interfacing membrane components, cell-cell signalling or horizontal gene transfer in mixed *E. coli* biofilms are yet unappreciated. In this study, in vitro biofilm formation of 403 *E. coli* isolates was monitored using a variety of growth conditions in mono-culture and in co-culture with other *E. coli* strains. Results were correlated with factors that are known to promote biofilm formation in *E. coli* K-12.

Methods: Biofilms formed by an *E. coli* reference collection (n = 72) and *E. coli* strains isolated from feces of healthy (n = 105) and diarrhea-afflicted children (n = 68), bacteraemia patients (n = 90), and male UTI patients (n = 68) were quantified after growth on polystyrene microtiter wells as a pure culture in various media (complex and minimal lab media, urine and diluted porcine mucus) and after co-cultivation with *E. coli* MG1655. Biofilm formation of a subset of the strains (n = 79) was evaluated on glass surfaces under continuous flow with SCLM. The prevalence of genes encoding components of IncF conjugative pili and aggregative adherence fimbriae (AAF) among the 403 test strains was assessed by multiplex PCR. Strains exhibiting strong biofilm formation were tested for expression of curli fimbriae on Congo red agar plates. Paired t-tests identified a subset of strains (n = 56) that exhibited significantly stronger biofilm production in co-cultivation. For this subset of strains, the composition of the induced biofilm in the mixed cultures was determined and the transfer of conjugative IncF and IncI plasmid genes to the K-12 strain present in the induced biofilm was investigated by PCR.

Results: Covariance analysis of biofilm data retrieved from monocultures in the 96-well model system revealed a significant dependence on growth media composition ($p<0.05$). No significant effect of strain origin on biofilm formation was discernible. The presence of genes encoding components of IncF conjugative pili or AAF was not positively correlated with biofilm formation capability in monoculture. Curli expression was not sufficient to explain good biofilm formation. In comparison, 189 (47%) of the 403 *E. coli* strains generated a significantly stronger ($p<0.05$) biofilm in the presence of MG1655 as compared to monoculture. In 56 strains (14%), the accumulated biomass in co-culture was significantly higher than the sum of individual monocultures. The proportion of strains positive for IncF conjugation genes was significantly higher in the subset of 56 biofilm-promoting strains (73.2%) than in the remaining test strains (44.6%). In more than 85% of the 56 cases, MG1655 was predominant in the induced biofilms and had acquired conjugative plasmid genes. Strikingly, the effect is not limited to co-cultivation with a K-12 strain since biofilm-promoting isolates were frequently able to form robust biofilms formation in co-cultivation with other clinical isolates.

Conclusions: Biofilm formation of *E. coli* in vitro is variable and dependant on growth conditions. No specific subgroup of strains exhibited significantly stronger biofilm formation than others. Synergistic effects of co-cultivation of *E. coli* strains on biofilm formation in vitro were frequently observed. The mechanisms leading to stimulation of biofilm formation in co-culture have not yet been determined in detail but initial analyses indicate that horizontal gene transfer may play an important role in this phenomenon.