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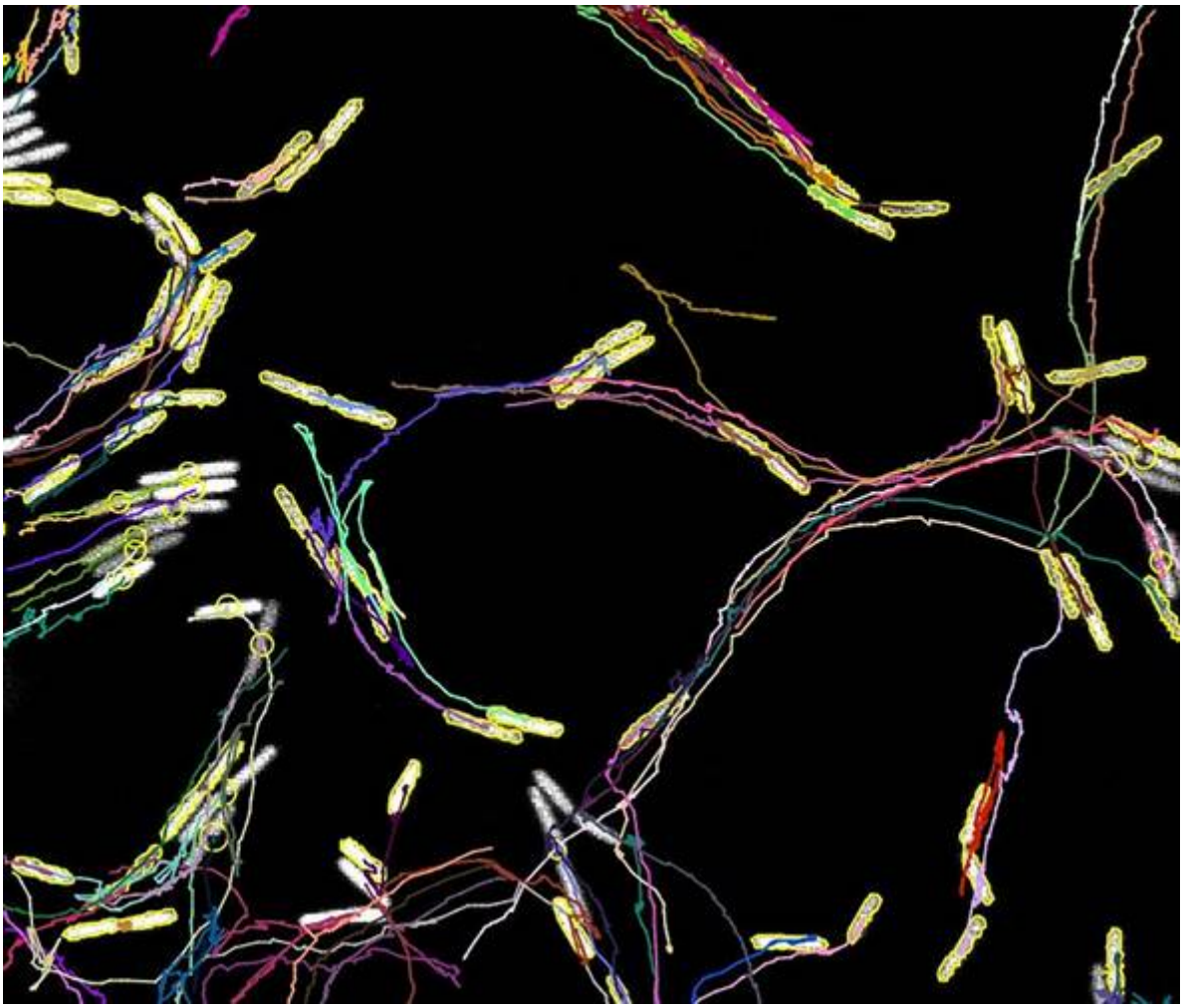

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

# *Enterococcus faecalis* AND *Escherichia coli*: DIABETIC FOOT ULCER ISOLATES INTERACTING IN POLYMICROBIAL BIOFILMS

Costilla, Celeste Rocio - Galvan, Estela Maria

Centro de Estudios Biomédicos, Básicos, Aplicados y Desarrollo, Universidad Maimónides - Ciudad Autónoma de Buenos Aires - Argentina
*Contacto: costilla.celeste@maimonides.edu*

Diabetic foot ulcer (DFU) infections are usually polymicrobial. A high prevalence of Enterococcus faecalis co-isolated with Escherichia coli has been reported, forming biofilms in these wounds. Within polymicrobial biofilms, bacteria are able to interact with each other, potentially affecting infection development. The goal of this study was to explore the dynamics of E. faecalis (Ef) and E. coli (Ec) clinical DFU strains growing in dual-species biofilms in comparison to single-species and planktonic cultures. To this aim, biofilms were grown on multi-well plates in Lubbock-glucose medium (emulating the conditions of diabetic foot ulcer) at 37°C for up to 24 h. Biomass was measured by crystal violet staining and cellular viability by colony-forming-units (CFU) counting in selective media; Student´s t test and one-way ANOVA statistical analysis of data were performed. Species distribution at the adhesion stage was observed after Gram staining by optical microscopy. Adhesion stage results evidenced that Ec in dual-species cultures was at a significant disadvantage compared to single-species (6.17±0.07 vs 7.08±0.12 Log10 CFU/cm², respectively, $p<0.05$). Ec cells observed through optical microscopy showed different adhesion patterns in single- and dual-species cultures. While Ec both formed aggregates and were found dispersed on the surface in single-species cultures, smaller Ec aggregates and an absence of dispersed cells were observed in dual-species. For Ef cultures, cells adhered to the surface in pairs or as short chains in both conditions, but also surrounded the Ec aggregates in dual-species. Crystal violet staining assays showed that the biomass of mixed biofilms at the adhesion stage remained similar to that of Ef single-species biofilms (A595nm 0.064±0.018 vs 0.034±0.014, respectively) and both were significantly lower than Ec single-species biomass (A595nm 0.365±0.070, $p<0.0001$). After adhesion, Ec in dual-species biofilms remained at a disadvantage compared to single-species, with lower cell counts up to 24 h growth (7.11±0.34 vs 8.06±0.13 Log10 CFU/cm², respectively, $p<0.0001$). On the contrary, Ef showed no significant differences in CFU counts when growing for 24 h in single- or dual-species biofilms (Ef: 7.05±0.23; Ef-dual: 7.29±0.41 Log10 CFU/cm²). The biomass of all biofilms increased significantly up to 24 h growth (A595nm of Ec: 9.296±2.463; Ef: 7.517±1.205; dual: 8.927±1.187), with no significant differences among them. Evaluation of single- and dual-species growth dynamics in planktonic cultures indicated that both species and conditions reached similar CFU counts at 24 h (approximately 8.83 Log10 CFU/ml). Altogether, the results presented suggest

that Ec and Ef interact within polymicrobial biofilms in DFU and compete with each other, with Ec being at a disadvantage from the adhesion stage. These novel findings could contribute to a better understanding of this type of polymicrobial infections.

Palabras clave: E. faecalis - E. coli - Biofilms - Interactions - Competition