E. coli Exploits Co-infection with *Enterococcus faecalis* to Enhance Biofilm Formation and Virulence on Antimicrobial Urinary Catheters

[1]Kidon Sung, [1]Miseon Park, [1]Ohgew Kweon, [2]Alena Savenka, [2]Angel Paredes, [1]Saeed A Khan

[1]Division of Microbiology, National Center for Toxicological Research, FDA, Jefferson, AR; [2]Office of Scientific Coordination, National Center for Toxicological Research, FDA, Jefferson, AR

Abstract

Infections associated with urinary catheters are often caused by biofilms, which are composed of various bacterial species, that form on the surface of the catheters. This study investigates the intricate interplay between *Escherichia coli* and *Enterococcus faecalis* during biofilm formation on urinary catheter segments using a dual-species culture model. We analyzed biofilm formation and global proteomic profiles to understand how these bacteria interact and adapt within a shared environment. Our findings demonstrated dynamic population shifts within the biofilms, with *E. coli* initially thriving in the presence of *E. faecalis*, followed by a decline in its populations during biofilm development, while *E. faecalis* exhibited a rapid decrease in cell numbers by 48 h in both mono- and dual-species biofilms. Interestingly, the composition of the dual-species biofilms was remarkably diverse, with some biofilms predominantly composed of *E. coli*, others primarily of *E. faecalis*, and still others showing a balanced ratio of both species. Notably, elongated *E. faecalis* cells were observed in dual-species biofilms, a novel finding in mixed-species biofilm cultures. Proteomic analysis revealed distinct adaptive strategies employed by *E. coli* and *E. faecalis* within biofilms. *E. coli* exhibited a more proactive response, emphasizing motility, transcription, and protein synthesis for biofilm establishment, whereas *E. faecalis* displayed a more reserved strategy, potentially downregulating metabolic activity, transcription, and translation in response to cohabitation with *E. coli*. Both *E. coli* and *E. faecalis* displayed significant downregulation of virulence-associated proteins when coexisting in dual-species biofilms. By delving deeper into these dynamics, we can gain a more comprehensive understanding of biofilm-associated infections, paving the way for the development of novel strategies to combat these challenging infections.

Introduction

Urinary tract infection (UTI) is a significant global health burden, with estimates suggesting 404.61 million cases and 236,790 deaths annually. The extensive utilization of indwelling urinary catheters during hospital stays poses significant risks for patients, increasing susceptibility to associated complications. Uropathogens possess the capability to colonize the urinary tract and employ specific mechanisms to initiate infection, evade host defenses, and cause cellular damage. Bacterial infiltration into the bladder can transpire during catheter insertion, via the catheter lumen, or along the catheter-urethral interface. Upon insertion, urinary catheters may impair the protective uroepithelial mucosa, thereby exposing new sites for bacterial adhesion. Additionally, the presence of indwelling catheters in the urinary tract disrupts normal mechanical host defenses. Despite efforts to mitigate UTIs through the use of antimicrobial-coated catheters, some have shown diminished efficacy in reducing infections). Notably, catheters coated with silver exhibited minimal impact on the formation of *Escherichia coli* and *Enterococcus faecalis* biofilms. Studies indicate that a majority of urinary catheters harbor three or more bacterial species, including *E. coli* (28%), *Enterococcus* species (17%), *Pseudomonas aeruginosa* (14%), and *Klebsiella* species (8%). However, limited research has delved into interspecies interactions among UTI bacteria, particularly those forming polymicrobial biofilms on urinary catheters. Competition between species within biofilms may amplify virulence potential and/or antimicrobial resistance. In this study, we investigated a dual-species in vitro model to explore the effects of co-cultivating *E. coli* and *E. faecalis* on biofilm formation using a global proteomic approach.

Materials and Methods

Bacterial strain and growth conditions

This study employed *E. coli* strain CFT073 and *E. faecalis* strain ATCC 29212. A single colony from overnight cultures on tryptic soy agar (TSA) plates was inoculated into separate 50-ml conical centrifuge tubes containing 5 ml of brain-heart infusion (BHI) broth. Cultures were incubated overnight at 37°C with shaking (200 rpm) using an Innova 4330 Refrigerated Incubator Shaker. Bacterial cells were harvested by centrifugation at 14,000 rpm for 10 min at 4°C and washed three times with phosphate-buffered saline (PBS). The resulting pellets were resuspended in fresh culture broth and standardized for subsequent experiments.

Protein extraction

Planktonic cells were grown in artificial urine media at 37ºC for 15 h with shaking at 200 rpm. Biofilm cells were grown on catheter segments at 37ºC for 72 h with shaking at 130 rpm. The washed planktonic and biofilm pellets were resuspended in 500 µl of BugBuster Plus Lysonase kit. This suspension was placed in Lysing Matrix B tubes containing 0.1 mm silica spheres. To disrupt the bacterial cells, an FP120 reciprocator was used at speed 6 for 45 sec. Subsequently, the cells were subjected to boiling and vortexing for 5 min and 1 min, respectively. The final protein extract was obtained by centrifuging at 14000 rpm for 20 min at 4ºC.

Ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS)

The protein samples were suspended in 5% SDS, 50 mM Tris-HCl (pH 8.0), 5 mM Tris (2-carboxyethyl) phosphine, and 20 mM 2-chloroacetamide. Protein digestion was achieved using the single-pot solid-phase-enhanced sample preparation method. Liquid chromatography was performed on an EASY-nLC 1200 connected to a Q-Exactive HF-X quadrupole-Orbitrap mass spectrometer. The mass spectrometer was set to acquire by data-dependent acquisition and tandem mass spectra from the top 12 ions in the full scan from *m/z* 350–1,400. MS1-based label-free quantification was employed and peptide peak areas were calculated using OpenMS. Differentially expressed proteins were defined as those exhibiting a fold change of ≥ 2.0 (up) or ≤ 0.5 (down) relative to the control.

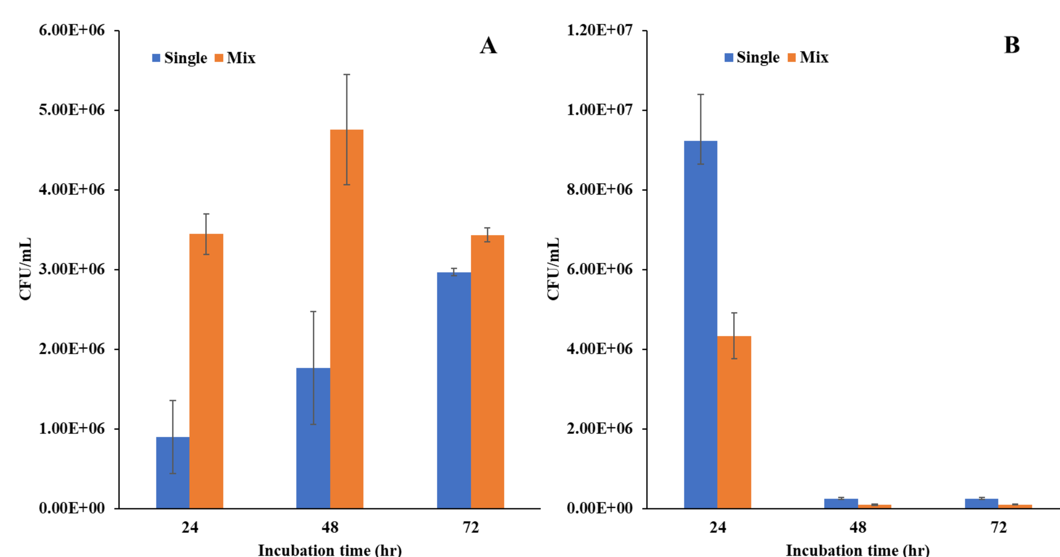

Fig. 1. Biofilm formation in mono- and dual-species model. *E. coli* (A) and *E. faecalis* (B)

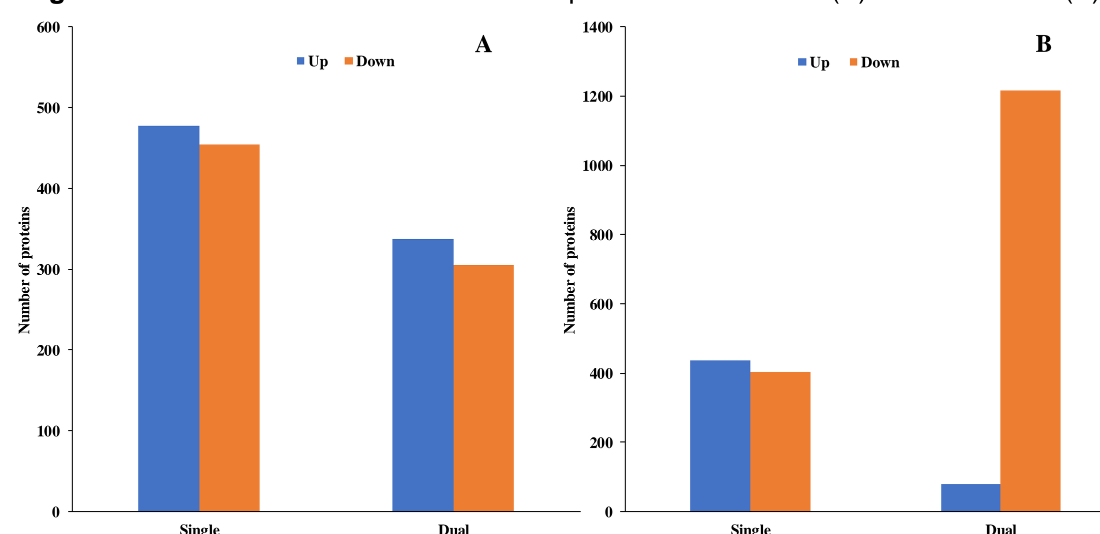

Fig. 2. Differentially expressed proteins identified from *E. coli* (A) and *E. faecalis* (B)

Results

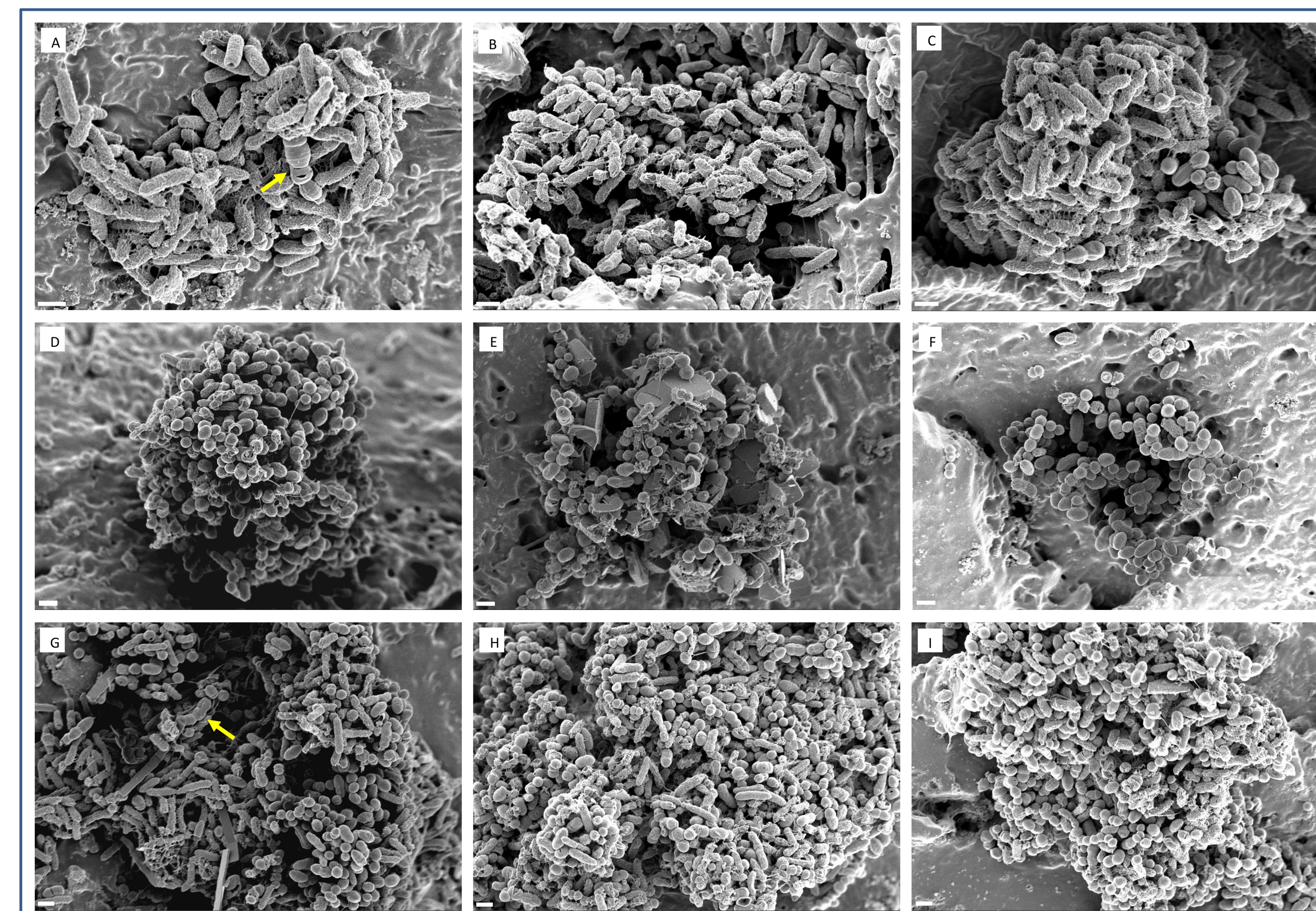

Fig. 3. Images of dual-species biofilms on urinary catheter segments. A-C: Biofilms predominantly composed of *E. coli*. D-E: Biofilms predominantly composed of *E. faecalis*. G-I: Biofilms containing a comparable ratio of both bacteria.

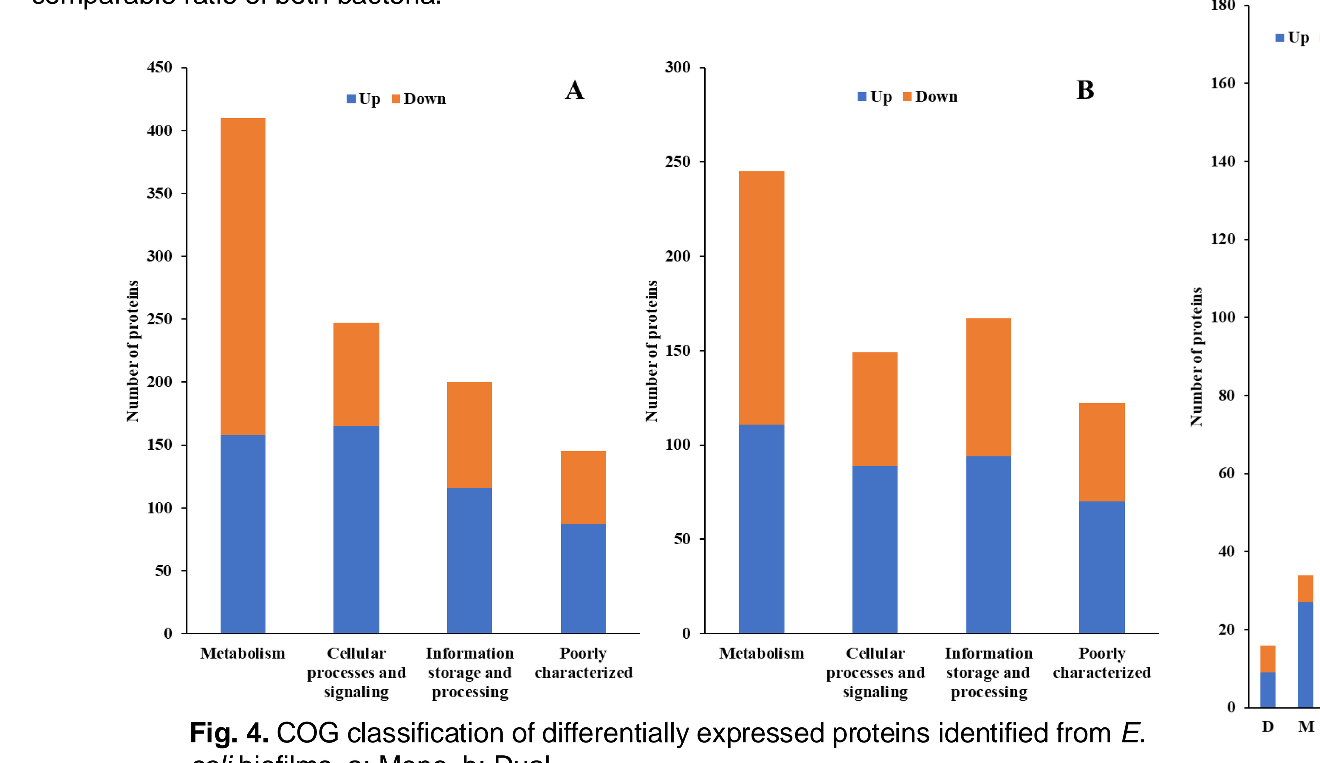

Fig. 4. COG classification of differentially expressed proteins identified from *E. coli* biofilms. a: Mono, b: Dual

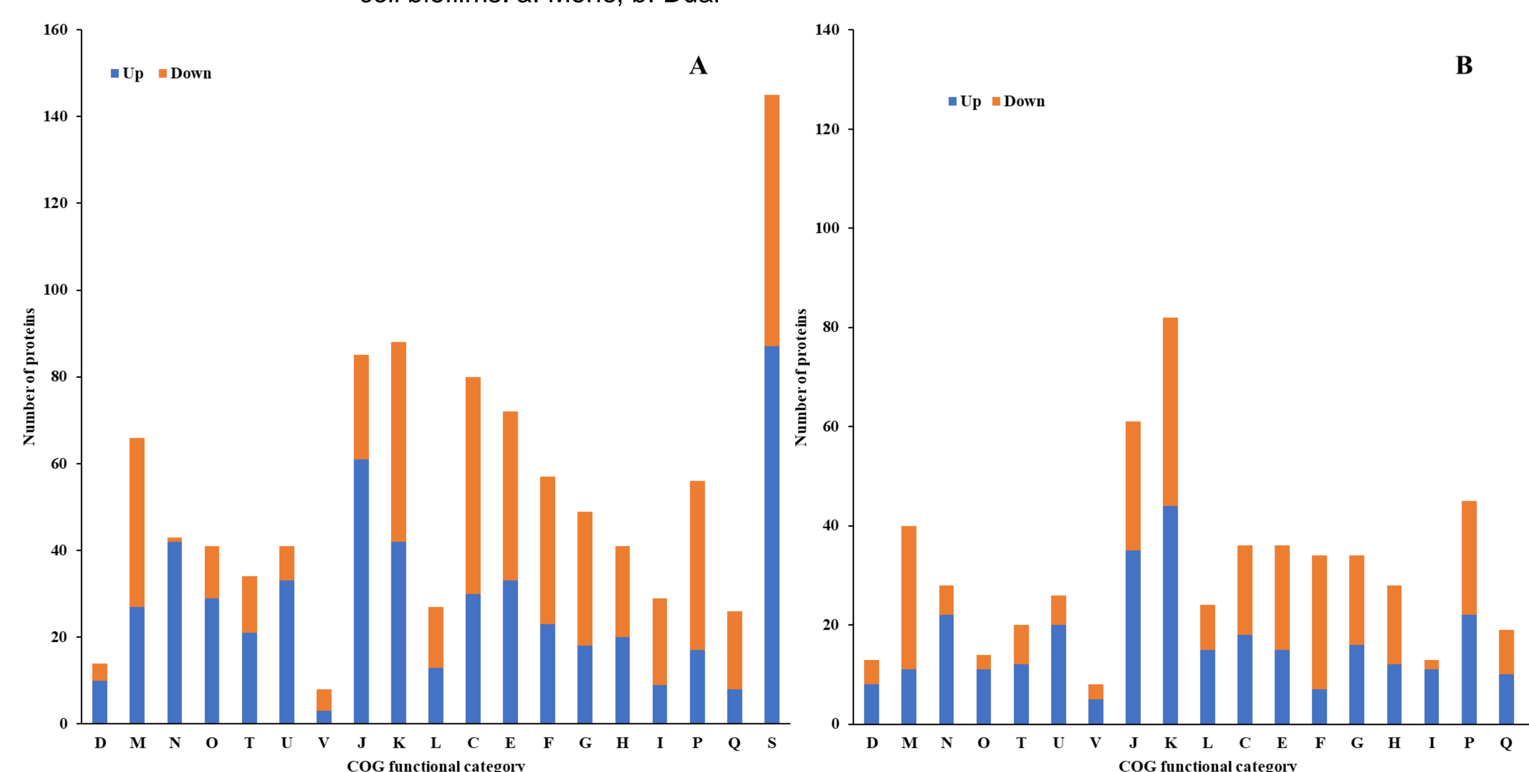

Fig. 5. Detailed COG classification of differentially expressed proteins identified from *E. coli* biofilms. a: Mono, b: Dual

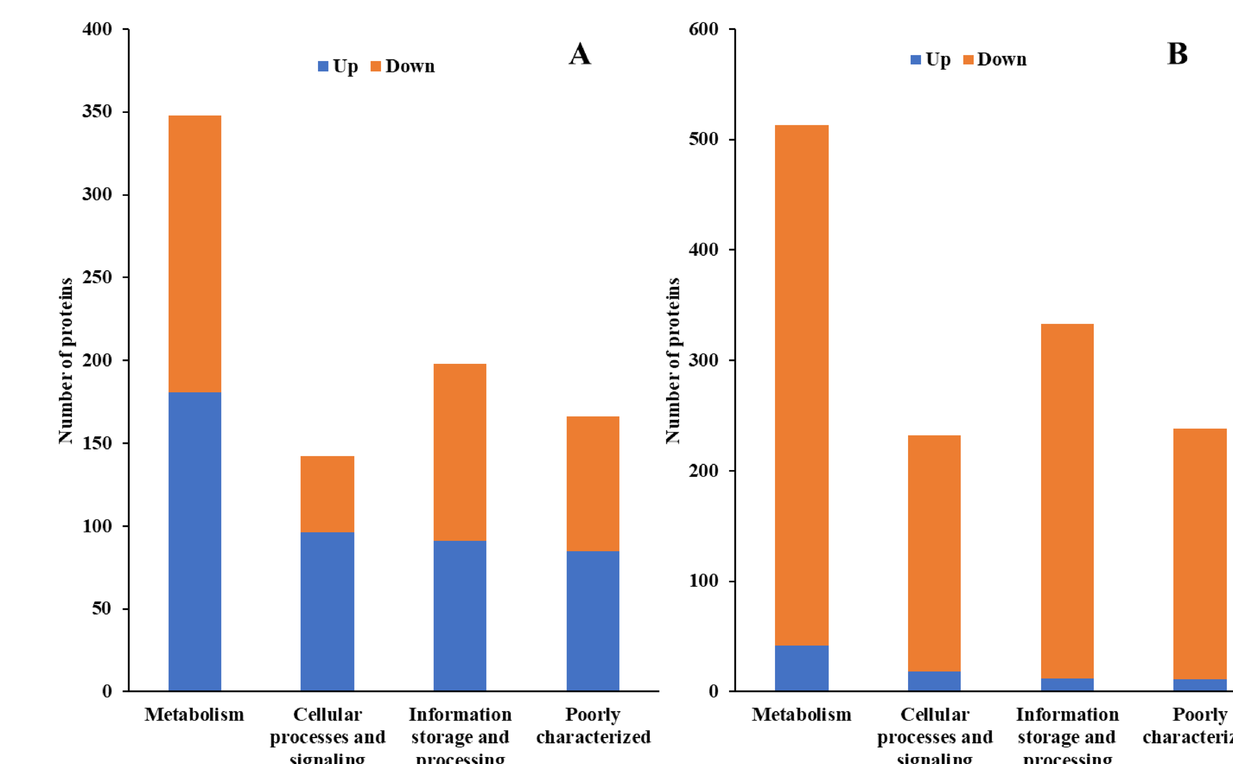

Fig. 6. COG classification of differentially expressed proteins identified from *E. faecalis* biofilms. a: Mono, b: Dual

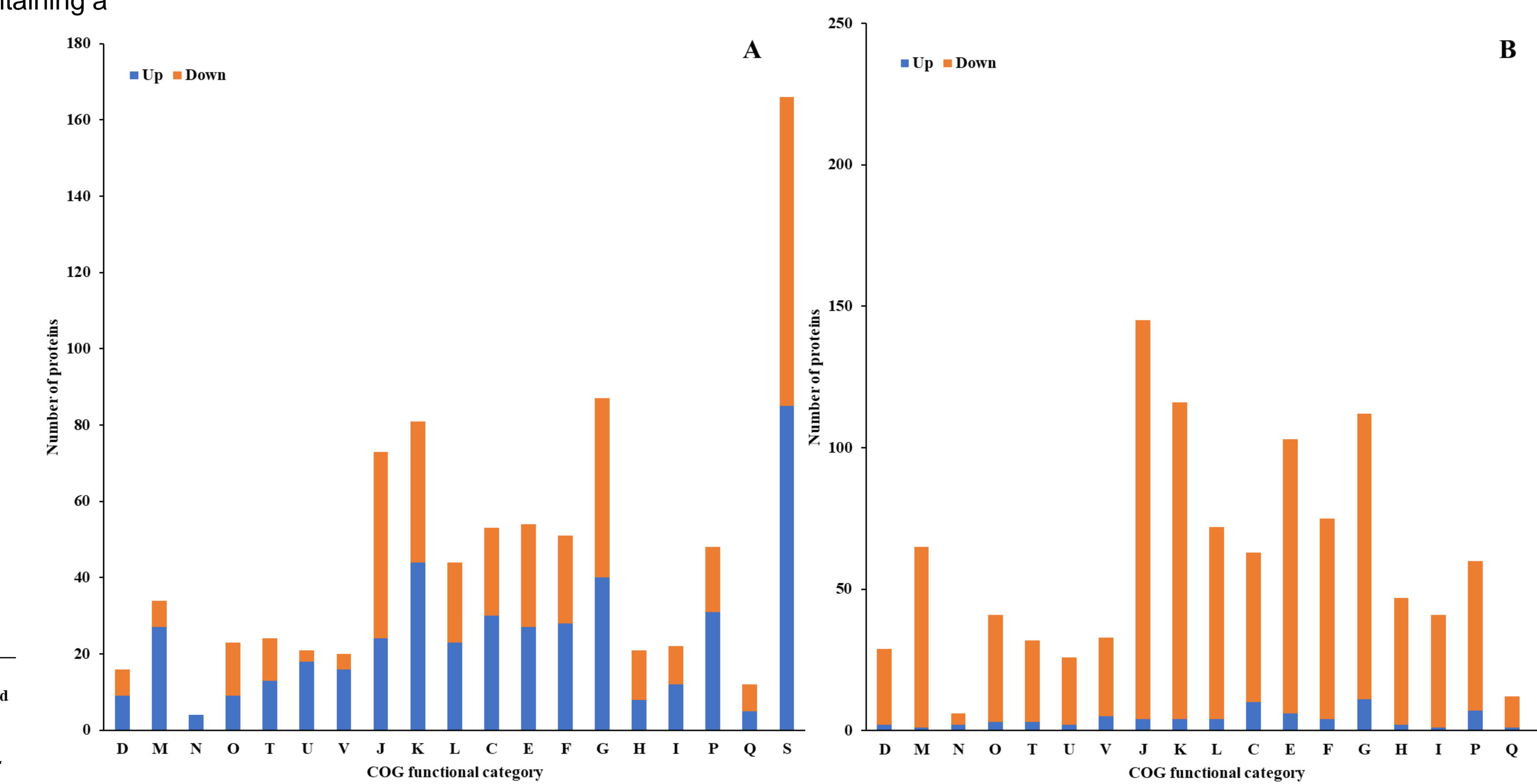

Fig. 7. Detailed COG classification of differentially expressed proteins identified from *E. faecalis* biofilms. a: Mono, b: Dual

Conclusion

This study explores the intricate interplay between *E. coli* and *E. faecalis* in urinary catheter biofilm formation using a dual-species culture model. Results reveal dynamic changes, with initially thriving *E. coli* populations declining during biofilm development, while *E. faecalis* exhibited lower initial growth in dual-species biofilms. The composition of biofilms varied, with some being predominantly *E. coli*, others primarily *E. faecalis*, and some displaying a balanced ratio. Global proteomic analysis uncovered distinct adaptive strategies, with *E. coli* emphasizing motility, transcription, and protein synthesis, and *E. faecalis* exhibiting a conservative approach. Both species downregulated virulence-associated proteins in dual-species biofilms.