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Table 1. Relative metabolite amounts in the biofilms of *E. faecalis* or *C. albicans*.

Metabolites	Ef-Ca/Ca	Ef-Ca/Ef	Pathways
Tricarboxylic acid cycle			
Citric acid	0.09*** ↓	3.95** ↑	Carbohydrate Metabolism
Fumaric acid	0.49* ↓	1.58** ↑	
Lactic Acid	0.38** ↓	-	
Malic acid	2.1** ↑	-	
Ketoglutaric Acid	5.42** ↑	-	
Oxalic Acid	0.4** ↓	-	
Pyruvic Acid	-	-	
Succinate	0.45* ↓	0.3** ↓	
Maltose	0.49* ↓	5.11** ↑	
Glucose	0.29** ↓	-	
Leucrose	0.49* ↓	5.43** ↑	
Amino acid metabolism			
Cysteine	0.21** ↓	0.44* ↓	Amino Acid Metabolism
Serine	0.15*** ↓	0.4** ↓	
Threonine	-	0.33** ↓	
Aspartate	2.05** ↑	-	
Glutamic Acid	2.37** ↑	1.63** ↑	
Proline	0.40** ↓	0.41** ↓	
Tyrosine	0.06*** ↓	-	
Valine	-	0.36** ↓	
Leucine	-	0.33** ↓	
Alanine	0.44** ↓	0.45* ↓	
Glycine	-	0.40** ↓	
Methionine	-	0.37** ↓	
Lysine	-	-	
Tryptofan	-	-	
Phenylalanine	-	0.43** ↓	
Metabolism of nitrogen containing compounds			
Urea	-	0.45* ↓	Nitrogen Metabolism
Ornithine	8.74*** ↑	-	
Ornithine-Arginine	7.33*** ↑	-	
Creatine	-	0.43** ↓	
Other Metabolisms			
Putrescine	9.99*** ↑	3.38*** ↑	Polyamine metabolism
Pipecolic Acid	24.2*** ↑	14.10*** ↑	
Ethanolamine	-	3.09** ↑	Lipid metabolism
Glycerol-1-phosphate	-	9.37*** ↑	
Glycerol	-	2.53** ↑	

*Compared to dual-species biofilm, the metabolite level was significantly changed in single-species biofilm (*P < 0.5, **P < 0.05, ***P < 0.001)

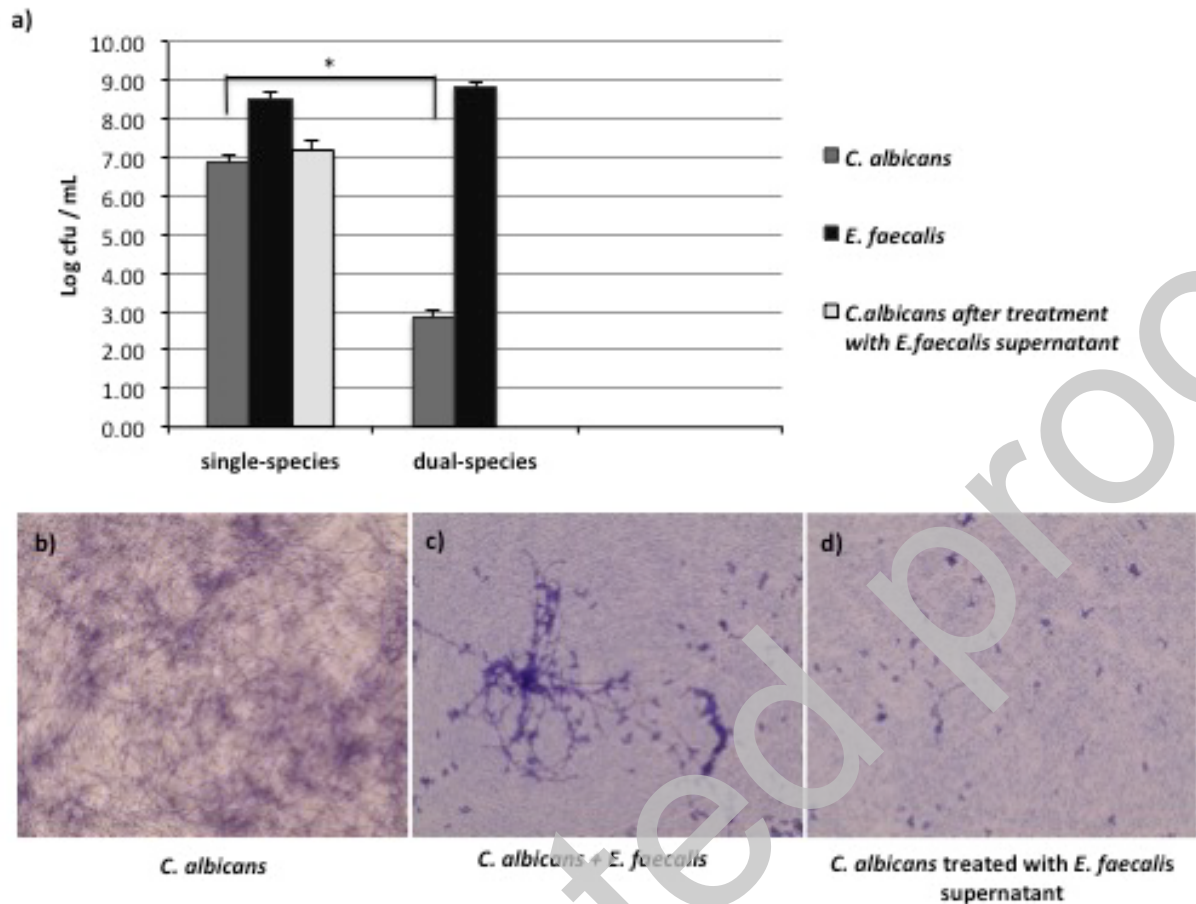


Figure 1. *E. faecalis* biofilm cells prevent the proliferation and hyphal development of *C. albicans*. a) The proliferation of cells in single and dual biofilms (cfu/mL). Compared to *C. albicans* single biofilm, *E. faecalis* prevented the proliferation of *C. albicans* cells in dual biofilm (*, $P < 0.05$). Optical microscope images of b) *C. albicans* biofilm cells formed in 6 well cell culture plate, c) *C. albicans* biofilms in the existence of *E. faecalis* cells and d) *C. albicans* biofilms exposed to the supernatant of biofilm culture of *E. faecalis*.

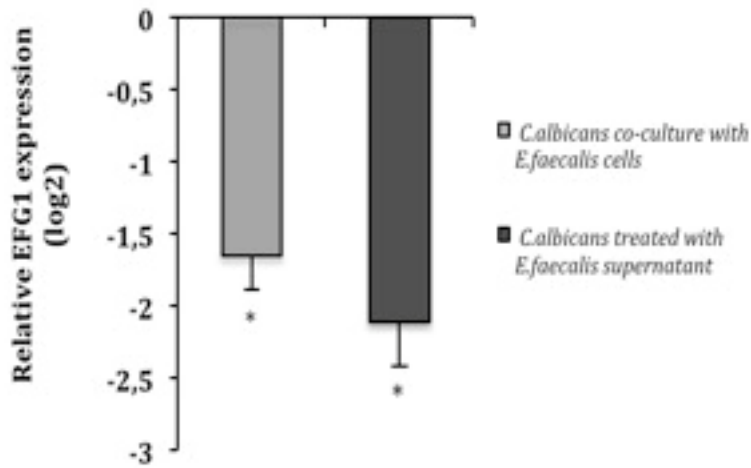


Figure 2. The expression of *Efg1* gene in *C. albicans*. It was significantly downregulated both in the existence of *E. faecalis* cells and in treatment with biofilm culture supernatant of *E. faecalis*. The statistical significance (*, $P < 0.05$) relative to untreated *C. albicans* single biofilm cells.

Discrimination of metabolomic profiles

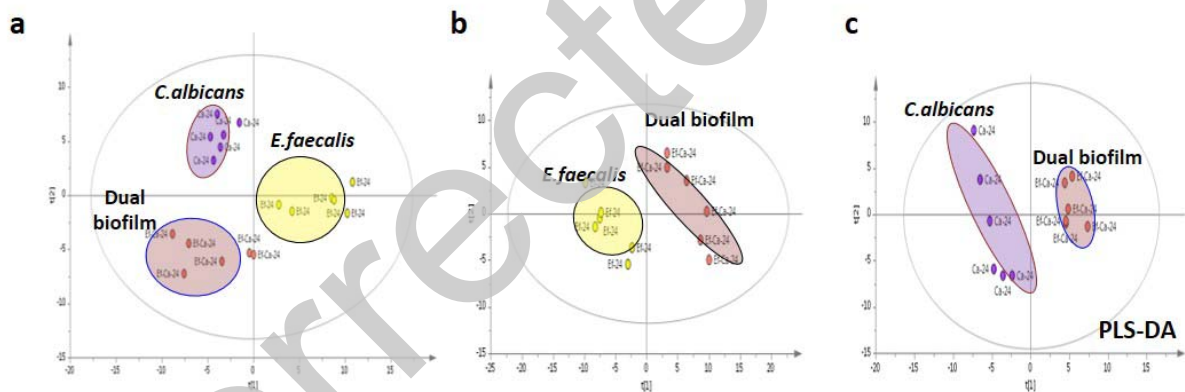


Figure 3. a) PLS-DA score graphs of single and dual biofilm of *C. albicans*. for metabolomic profile comparison. b) PLS-DA score plots show clear separation between *E. faecalis* and its dual-species biofilm. c) PLS-DA score plots demonstrate apparent distinction with *C. albicans* and its dual biofilm. Each circle represents the sharp metabolomic distinction in the biofilms.

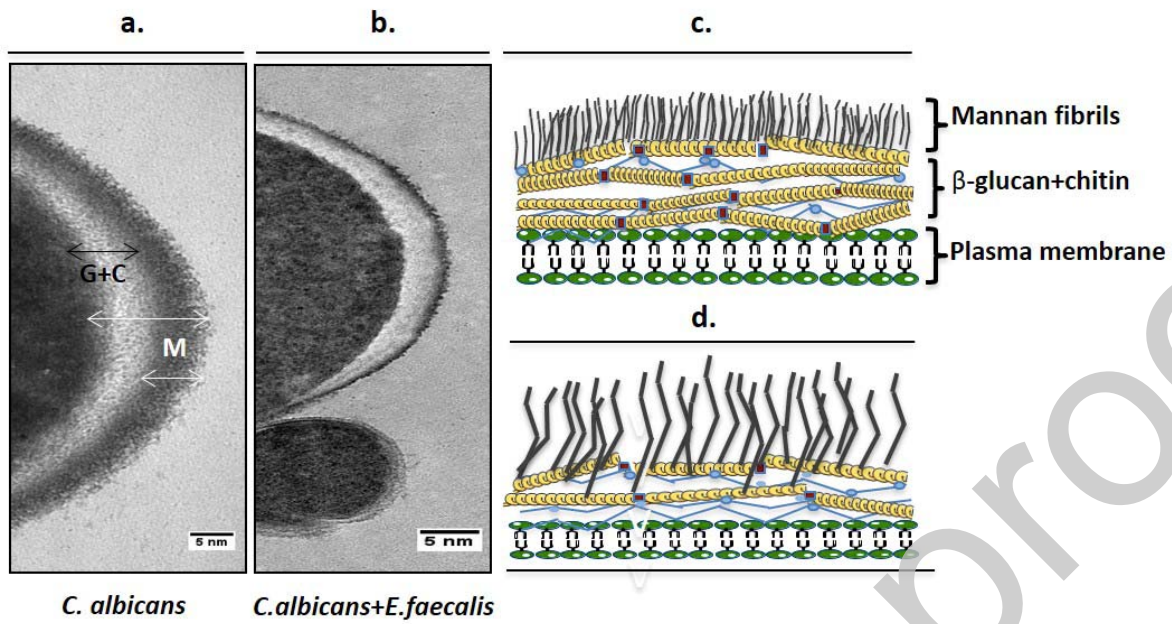


Figure 4. The visualization of *C. albicans* cell walls grown in the single (a) and dual biofilms (b). (Presented figures were consisted of ≈ 100 cells images), Bar, 5 nm. G+C, β -glucan and chitin; M, mannan. Drawings representing the possible structural changes are shown in c (for the cell wall of *C. albicans* in dual biofilm) and d (for the cell wall of *C. albicans* in single biofilm).

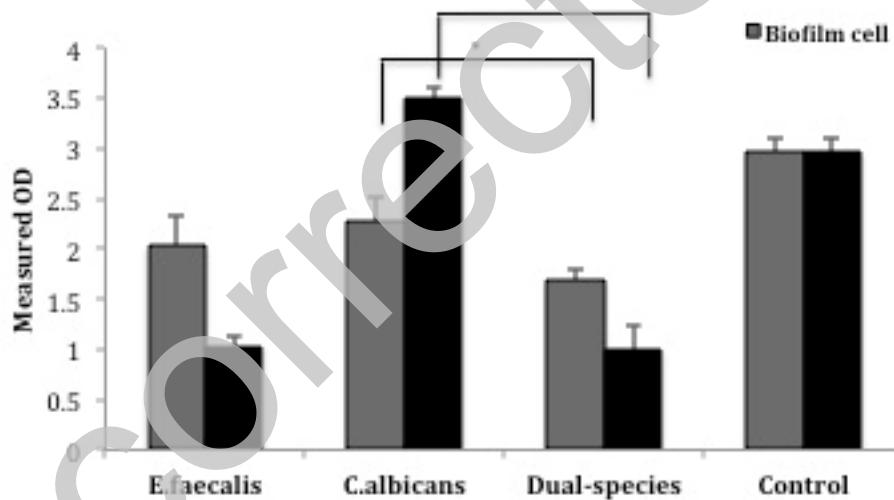


Figure 5. Quantitative measurement of violacein in both single and dual biofilms. Asterisks indicate the statistical significance ($P < 0.05$). A statistically significant decrease was shown for all test conditions compared to the control.