

Supplementary Materials

Article: **Vaginal *Lactobacillus* impair *Candida* dimorphic switching and biofilm formation**

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Figure S1. MTT incubation time optimization. Starting from agar cultures, *Candida* suspensions were prepared in supplemented RPMI at the final concentrations of $1-5 \times 10^7$, $1-5 \times 10^6$ and $1-5 \times 10^5$ CFU/mL and inoculated in flat-bottomed 96-well plates (0.1 mL per well), added with the same volume of MRS medium. Plates were incubated at 35°C in aerobic conditions for 72 h. *Candida* biofilms were assayed with 0.5% MTT as described in the materials and methods and different incubation times were tested: 0.5, 1, 2, 3, 4 and 7 h. The absorbance measured at 570 nm using a GENios Plate Reader are reported (average of at least three replicates \pm SEM). Optimum incubation time was defined for each *Candida* isolate, considering a value of Abs 570nm in the range 0.5-1.5 for $1-5 \times 10^6$ CFU/mL suspension (which corresponded to the starting inoculum in experiments with CFS). Values over the limit of the instrument are arbitrarily indicated as 3 in the figures. Incubation times of 4 h for *C. albicans*, 3 h for *C. glabrata*, 0.5 h for *C. lusitaniae* and *C. parapsilosis*, and 2 h for *C. tropicalis* and *C. krusei* were selected.

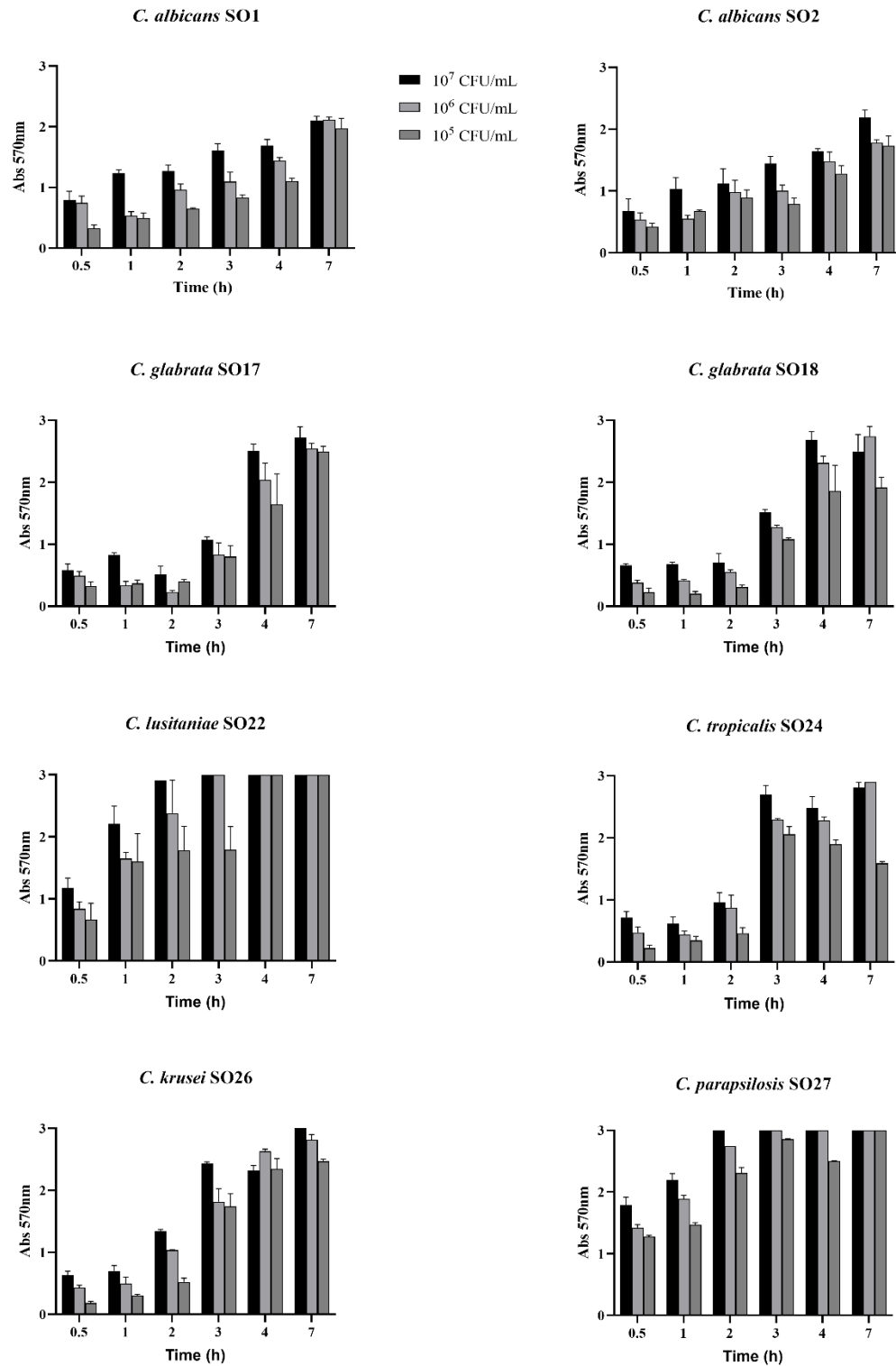




Figure S2. Evaluation of *Candida* cell morphology switching. Images from optical microscope (20X) of *Candida* germ tube/Pseudohyphae/hyphae in presence or not of *L. crispatus* BC3 and *L. gasseri* BC9 pk and bf-CFS. Rows represent different *Candida* strains while columns reported controls and treatments, as indicated above the micrographs.

(A) *C. lusitaniae* SO22. (B) *C. tropicalis* SO24. (C) *C. krusei* SO26. (D) *C. parapsilosis* SO27. Scale bar = 50 μ m.

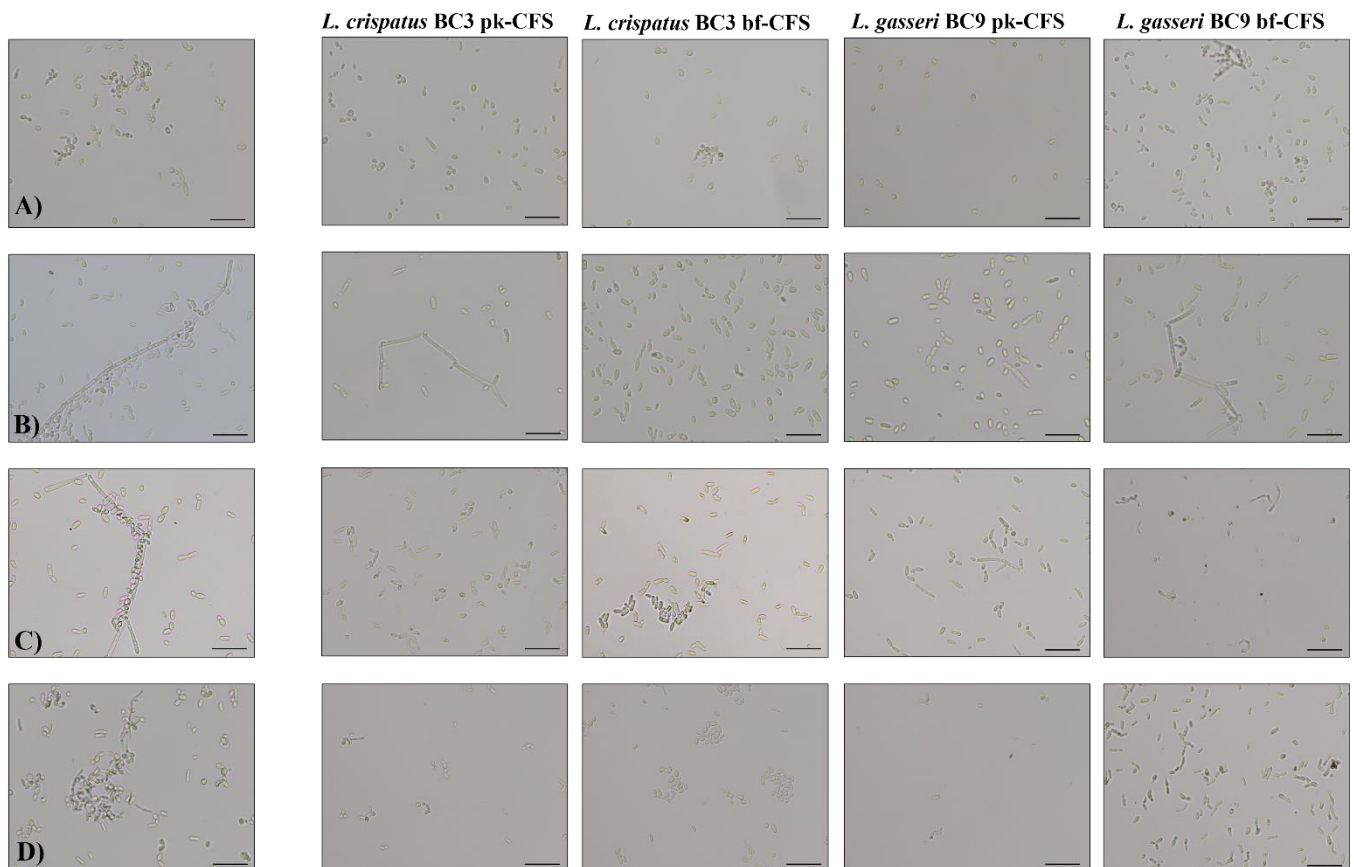


Figure S3. Confocal microscopy analysis of *Candida* biofilm. *Candida* spp. were treated with pk-CFS and bf-CFS from *L. crispatus* BC5 and *L. gasseri* BC12 (second-fifth columns). Biofilms were stained using two fluorescent dyes: thiazole orange (viable cells, green) and Live-or-Dye™ (dead cells, red). (A) *C. glabrata* SO18 biofilm. (B) *C. parapsilosis* SO27 biofilm. Scale bar = 100 µm.

