S. boulardii fails to hold its cell wall integrity against non-pathogenic *E. coli*: are probiotic yeasts losing out the battle?

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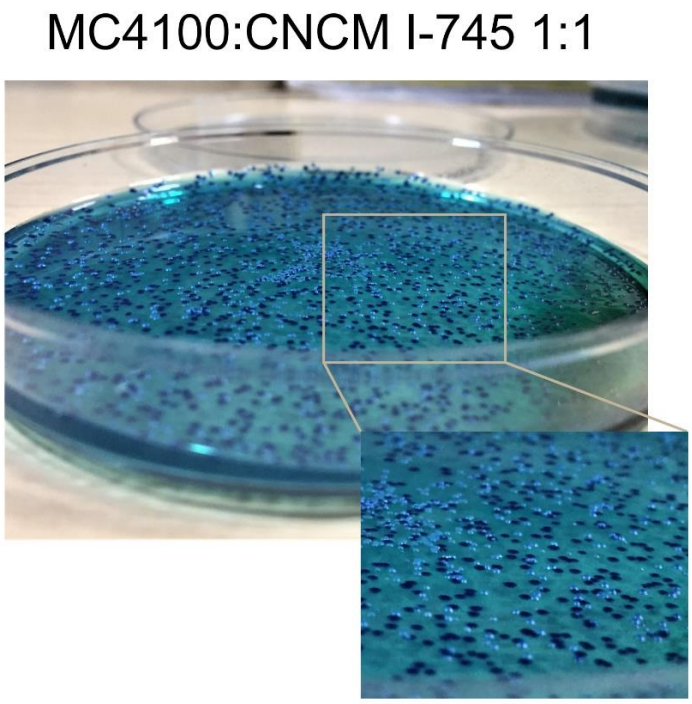

Figure S1. MB-supplemented agar plate assay image of *S. boulardii* CNCM I-745: MC4100 *E. coli* 1:1 co-incubated sample (at 0 hr). Area inside white rectangular box is further zoomed to clearly show differences in appearance of light blue and dark blue colonies. The light blue colonies are larger and raised from the agar plate level while dark blue colonies are at the plate level.

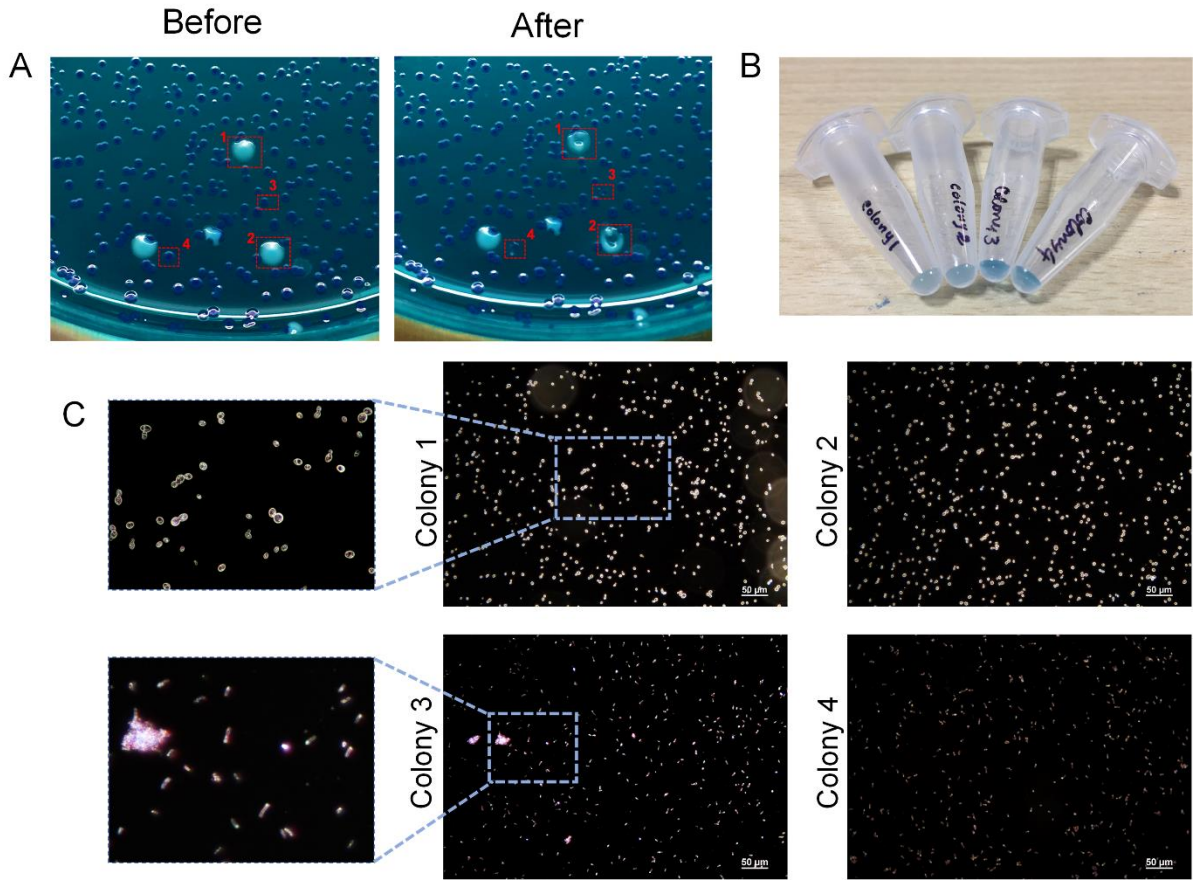

Figure S2. Examination of co-plate colony compositions using microscopy. (A) Portions of several random isolated light blue and dark blue colonies were scooped from the co-plates. Two of each type of colony are shown for representation purpose. **(B)** Each scooped portion was suspended in small volume sterile phosphate buffer. The colony numbers written on the tubes correspond to the colony numbers indicated in (A). **(C)** Dark field images of colony suspension samples.

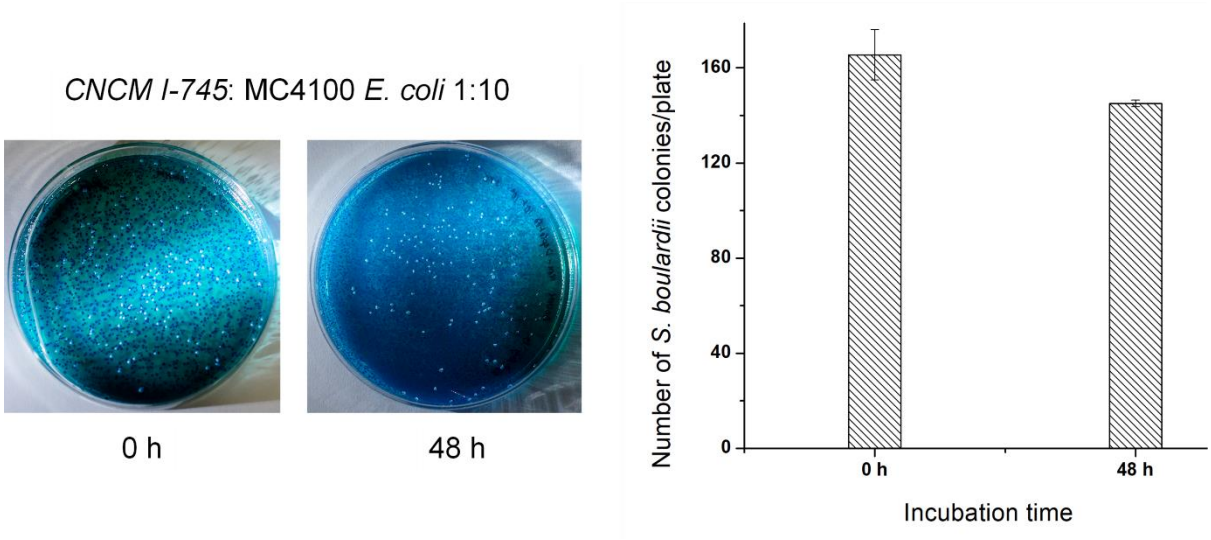

Figure S3. Number of probiotic *S. boulardii* CNCM I-745 colonies decrease (white colonies) at 48 h when aliquots from *sample-1* (*S. boulardii* CNCM I-745: MC4100 *E. coli* 1:10) are plated on MB-supplemented agar plates after 0 h and 48 h of co-incubation. Left: representative agar plate images. The images are captured from an angle to illuminate all colonies for counting. Right: plot of number of *S. boulardii* colonies against incubation time after which sample aliquot was spreaded on agar plate. Error bars denote standard deviations in determining average number of colonies, which are obtained from two independent experiments. The aliquots were 100 times diluted prior to spreading on agar plates.

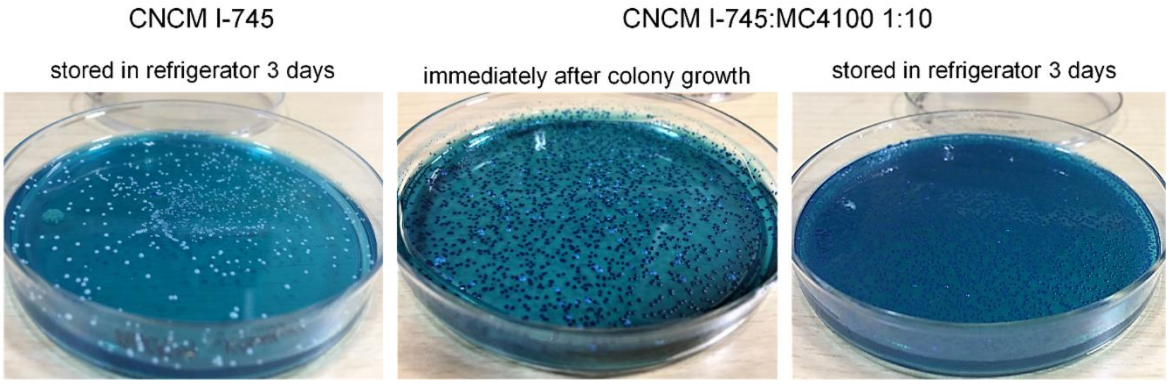

Figure S4. **MC4100 *E. coli* induced cell wall damage of *S. boulardii* increases with time.** Probiotic *S. boulardii* CNCM I-745 colonies, when left in refrigerator for 3 days after capturing 48 h photographs, remain the same. But, in presence of *E. coli* in sample, the colonies turn blue like *E. coli* colonies after 3 days.

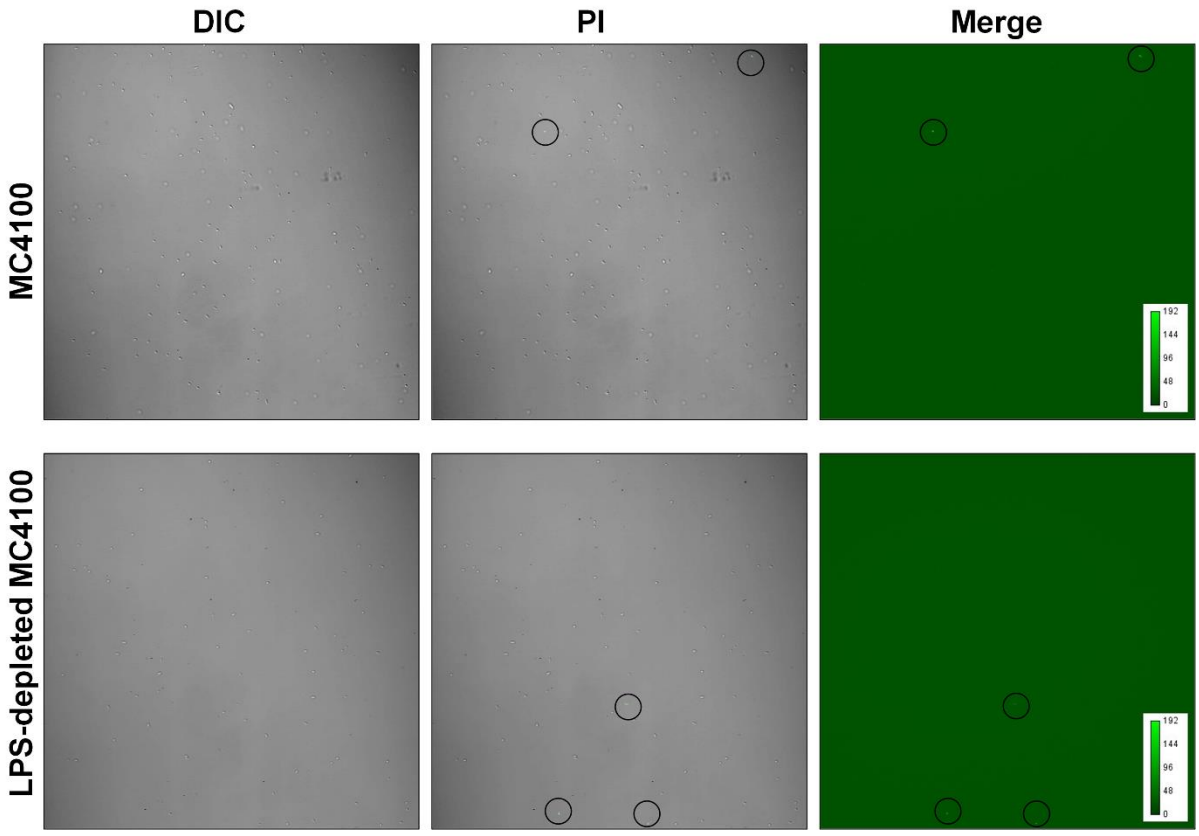

Figure S5. *E. coli* cell viability assayed after LPS-depletion. After EDTA-treatment, *E. coli* cells are washed with PBS and stained with propidium iodide. DIC, PI fluorescence and merged images are shown side-by-side for comparison. Scale bar presented in the first image (MC4100, DIC) is applicable for all images shown in this figure. The frames shown contain ~ 200 cells, and only ≤ 3% cells show minor PI fluorescence. Comparison of control MC4100 and LPS-depleted MC4100 confocal images suggest, EDTA-treatment we used for LPS-depletion from MC4100 *E. coli* cells has caused no viability change.

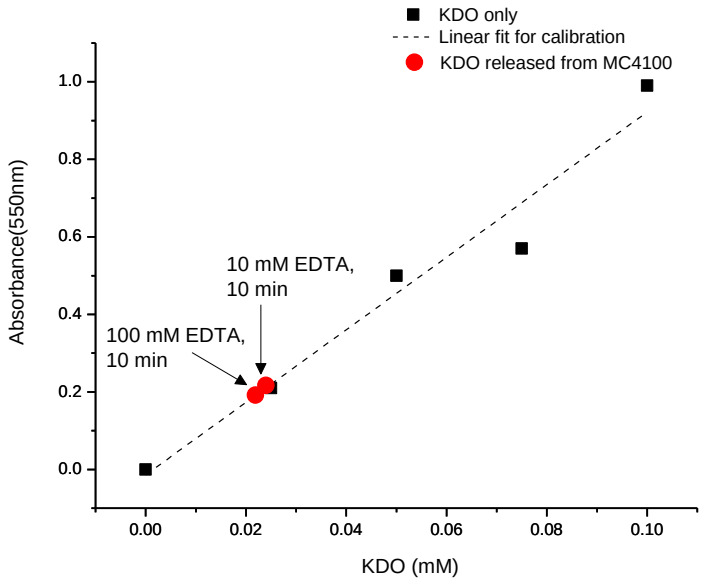

Figure S6. **Quantification of KDO released from *E. coli* MC4100 after EDTA treatment**. Calibration curve was obtained from known concentrations of pure KDO using colorimetry assay as described in methods. After LPS-depletion of *E. coli* MC4100 cells using short EDTA treatment (described in methods), supernatants were subjected to the KDO assay (described in methods). KDO released in supernatants were determined from the absorbances measured at 550 nm using the calibration curve.

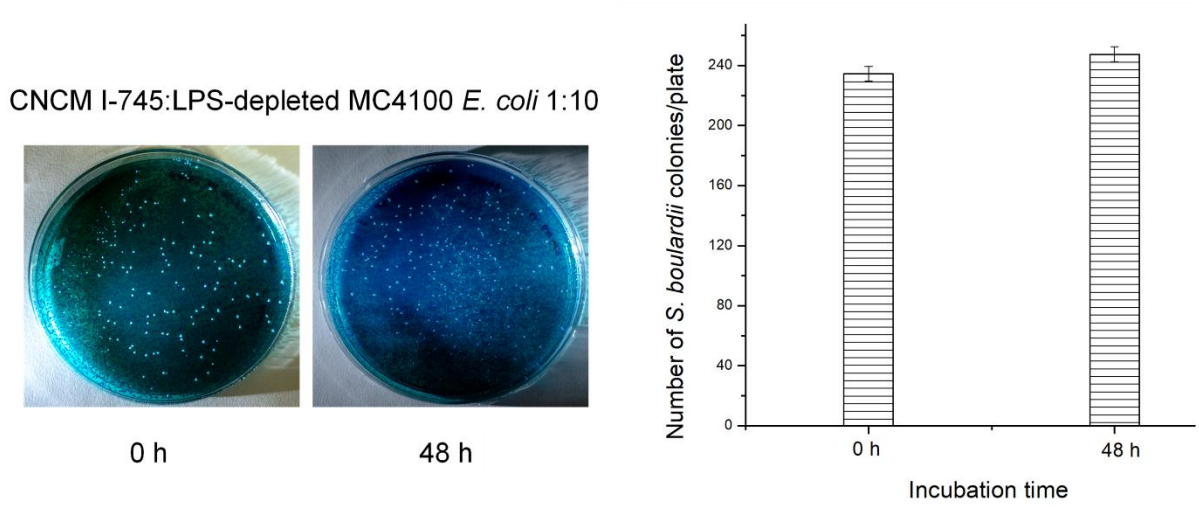

Figure S7. Number of *S. boulardii* colonies do not change (white colonies) when aliquots from *sample-1* (*S. boulardii* CNCM I-745: LPS-depleted MC4100 *E. coli* 1:10) are plated on MB-supplemented agar plates after 0 h and 48 h of co-incubation. Left: representative agar plate images. The images are captured from an angle to illuminate all colonies for counting. Right: plot of number of *S. boulardii* colonies against incubation time after which sample aliquot was spreaded on agar plate. Error bars denote standard deviations in determining average number of colonies, which are obtained from two independent experiments. The aliquots were 100 times diluted prior to spreading on agar plates.

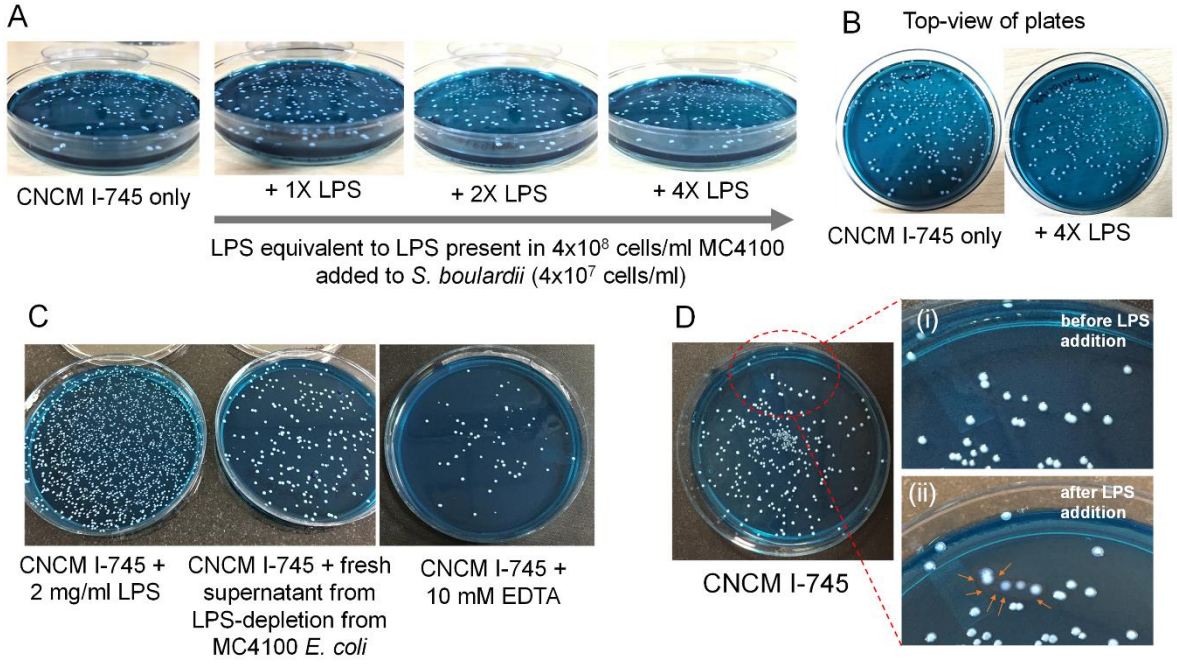

Figure S8. Effect of LPS on *S. boulardii* viability and survivability in absence of MC4100 *E. coli*. **(A)** Variable concentrations of only LPS (from O111:B4 *E. coli*, Sigma) are incubated with *S. boulardii* (4 x10^7 cells/ml) and plated on MB-supplemented YPD agar plate (at 0 hr). Plates are incubated at 37 °C and photographs after 48 hours are shown. Photographs in **(A)** show no colour change of LPS-incubated colonies with respect to control *S. boulardii* colonies. **(B)** Top view of plates containing only *S. boulardii* (control) and *S. boulardii* incubated with 4-fold of LPS that could be present in 4x10^8 cells/ml MC4100 *E. coli* (side-view of same plates shown in **(A)**). The colony counts are clearly similar (~240 on each plate). **(C)** 3000-fold LPS and fresh supernatant from LPS-depletion are also incubated with *S. boulardii* and plated for assessing colony viability. These two samples are only 10-times diluted prior to plating whereas all other samples shown in Figure S7 are 100-times diluted before plating. *S. boulardii* are also incubated with 10 mM EDTA as control of the supernatant sample (100-times diluted before plating). Photographs show no change in viability. Reduction in colony number for supernatant-incubated sample clearly seems to be EDTA-effect. **(D)** LPS (from O111:B4 *E.*

coli, sigma) 2mg/ml drop-casted on fully grown control *S. boulardii* colonies marked in **D (ii)** (mark shown by arrows). Appearance of colonies change after LPS addition.

List of Supporting Video files and their captions:

Video S1: Interactions between *S. boulardii* and MC4100 *E. coli* are slow and no damage is seen from adherence within 1 hour

Video S2: Cytosol leaks from *S. boulardii* that trap *E. coli*

Video S3: Cellular material leaks out from *E. coli*-attached regions of *S. boulardii* surfaces

Video S4: *E. coli* localize themselves at the leaked cellular materials of damaged *S. boulardii*

Video S5: *E. coli* length increases in presence of damaged yeasts and become comparable to *E. coli* length in culture medium (LB broth)

Video acquisition and processing:

All videos were captured using Nikon Eclipse Ti-2E inverted microscope. The samples were illuminated by broadband white light from a halogen light source. A dry dark field condenser (Nikon, numerical aperture (NA) = 0.80-0.95), oriented normal to glass surface, was used to focus the white light onto the cell sample between microscope slide and coverslip. Scattered light from individual particle was collected using a dry objective (60x, 0.7 NA). The videos were acquired with 100 frames per second. Fiji (ImageJ) software was used for selecting regions of interests and annotation files were further compressed by Fiji at 100 frames per second. Final threading and annotations were done using *Adobe Premiere Pro* CC 2015. The time displayed in all Videos are the real-time of the cellular interactions.

All the images, photos, and videos were taken by one or more of the authors.