A flow cytometry method for quantitative measurement and molecular investigation of the adhesion of bacteria to yeast cells.

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Supplementary Information
Supplementary Figures

Figure S1: Yeast singlets isolation. Single yeast population with similar cell size and granularity was selected based on the FSC-Area vs FSC-Height plot

Figure S1 (Schiavone-Dagkesamanskaya et al.**)**

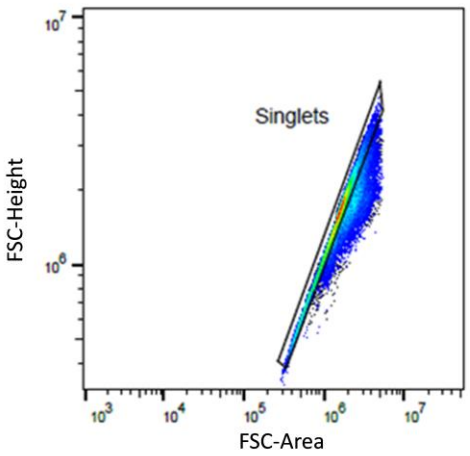

Figure S2: Effect of washing and preincubation time on adhesion of bacteria to yeast –(A). Different amount of fluorescent E22 cells were mixed and incubated with yeast BY4741 10^8 cells for 60 min in PBS at 37°C. Successive washings were done with 1 ml of PBS, cells were centrifuged and then resuspended in PBS and fluorescence was measured after each washing. (B) 10^9 cells of bacteria E22 fluorescent strain were mixed with yeast BY4741 10^8cells and incubated at 37°C for different time period. Fluorescence was measured after 2 washes in PBS.

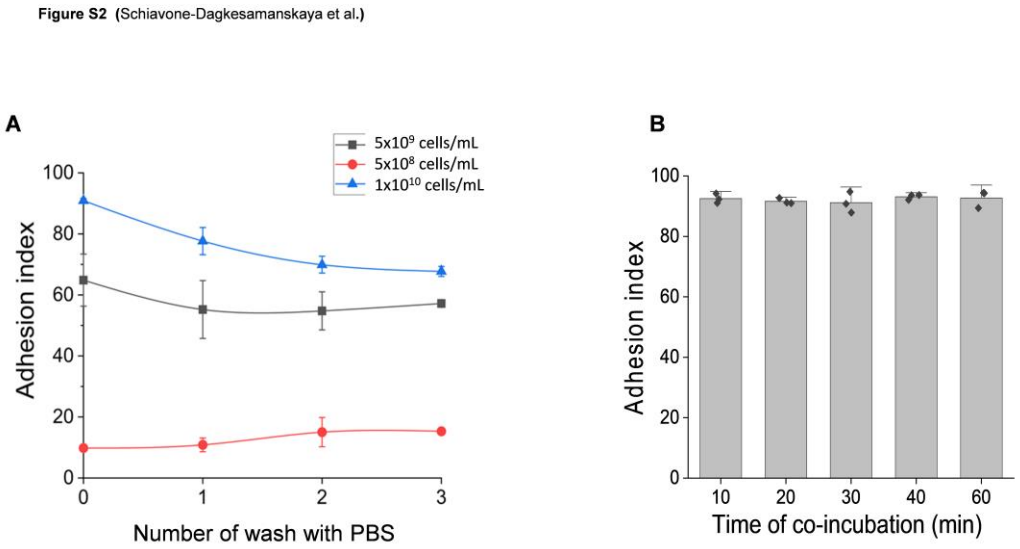

Figure S3: Importance of yeast cell aggregates removing for quantification of bacteria adhesion. Flow cytometer plots(A) and calculated AI(B) of *E. coli* interaction with sonicated and non-sonicated yeast *gas1* mutant cells. The mean ± sdv and individual results of 3 independent experiments are shown.

*Shows significance difference at p <0.05 by One-way ANOVA with Fischer post-test compared to the wildtype strain BY4741 (WT).

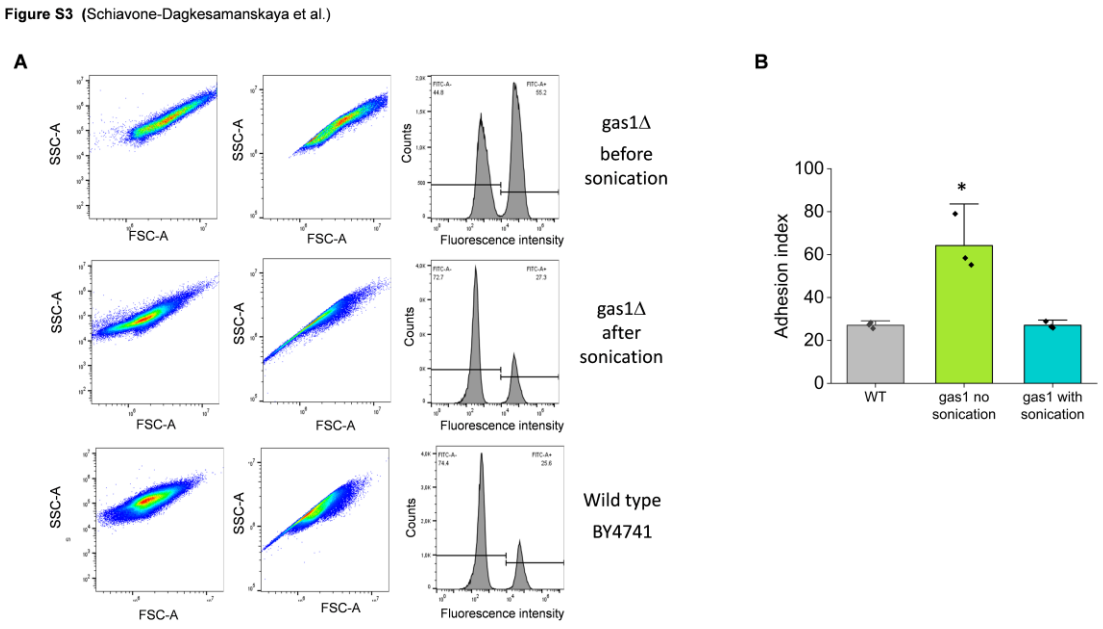

Figure S4. The adhesion of *E. coli* to yeast is barely affected by pH, temperature and ionic strength. Adhesion index for yeast-*E. coli* at different pH (A), different temperature(B) and different salt concentration(C). For the temperature and pH comparison experiments (A and B), $1x10^8$ fluorescent *E. coli* E22 cells were mixed with 10^8 BY4741 yeast cells in 1 ml for 90 min at 37°C. For salt concentration studies, 10^9 cells of E22 strain were mixed with 10^8 yeast cells. The mean ± sdv and individual results of 3 independent experiments are shown. P-values are indicated.

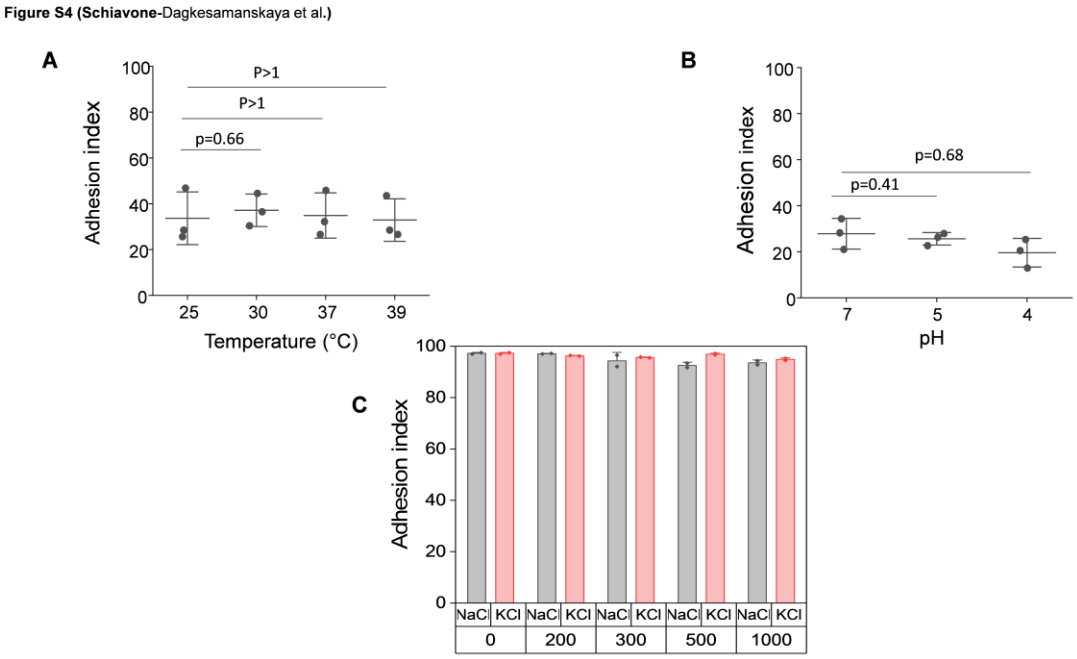

Figure S5. Low adhesion of *fimA, fimH* mutants is not stable. Adhesion of control strain was carried out with a mix of 10^7 cells fluorescent BW25113 with 10^8 cells yeast BY4741. In case of fimH *and fimA* mutants 10^9 cells were added for interaction(Ai 1,3,5 columns) Additional wash with PBS after the first measurement removed completely bound mutant cells and didn't affect the WT (2,4,6).

Figure S5 (Schiavone-Dagkesamanskya et al.**)**

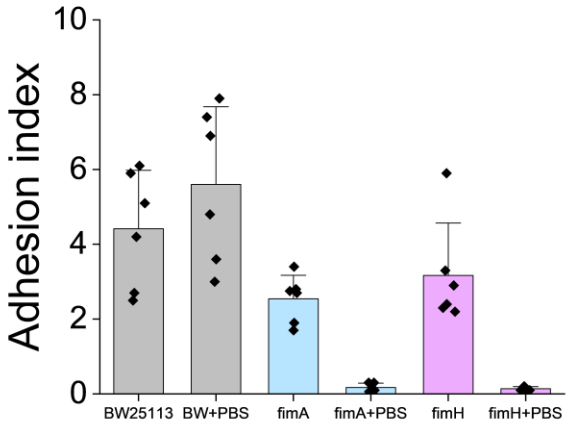

Figure S6: Effect of addition of excess of mannosyl-sugars to the adhesion of bacteria to yeast cells

Adhesion was carried out with a mix of 10^9 fluorescent E. coli E22 cells/ml with 10^8 yeast BY4741/ml incubated for 90 min at 37°C. After two quick washes with 1 ml of PBS, the mix was centrifuged and then the pellet was resuspended either in 1 ml PBS or in 1 ml of PBS containing 1 M Methyl mannose (MeMan) for 30 min at 37°C prior to be analyzed for the fluorescence by flow cytometry. Results shown are the mean of 2 independent experiments.

Figure S6 (Schiavone-Dagkesamanskaya et al.**)**