Electronic Supplementary Material (ESI) for ChemComm.
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Supplementary Information

Microbial Separation from a Complex Matrix by a Hand-Held Microfluidic Device

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Materials. Tris (2-carboxyethyl) phosphine hydrochloride (TCEP) and sodium chloride were obtained from Sigma-Aldrich; sodium hydroxide was obtained from Fisher Chemical and hydrochloric acid 50% (v/v) was from Ricca Chemical, USA. Standard glass microscope slides were purchased from Thermo Fisher Scientific, USA. Nanopure water (resistivity ~18.2 MΩ.cm) from a SpectraPure Lab Grade Type 1 DI system (SpectraPure Inc., USA) was used to prepare all aqueous solutions and culture media. The water, all glass and plasticware and culture media were autoclaved before use.

Preparation of the working suspensions of microbial cultures. *Lactobacillus delbrueckii subsp. bulgaricus* (ATCC® 11842™), *Escherichia coli,* Serotype O6, Biotype 1 (ATCC® 25922™), and *Saccharomyces cerevisiae* (*Saccharomyces boulardii*) (ATCC® MYA-796™) were purchased from the American Type Culture Collection (ATCC), USA. *Ceratocystis fagacearum* was obtained from the USDA Forest Service, Northern Research Station (NRS-16), Saint Paul, Minnesota, USA. Gram-positive (*L. delbrueckii*) and gram-negative (*E. coli*) bacteria were maintained in MRS and Luria Bertani broth respectively at 4°C until used. Culture aliquots were transferred to 50 mL sterile MRS and trypticase soy broth (TSB) media respectively and were incubated overnight at 37°C. A non-filamentous (*S. boulardii*) fungus was maintained in yeast mold broth (YM) at 4°C. The fungal culture was transferred to 50 mL sterile YM medium and incubated overnight at 26°C or 37°C. Colonies of the filamentous fungus *C. fagacearum* were obtained according to Yang[1] by culturing the single pieces of sapwood of the infected

branches and stem samples of oak tree onto acidified potato dextrose agar plates. The plates were incubated at room temperature under ambient lighting and the pure cultures of *C. fagacearum* were identified in 7–14 days by the presence of gray to olive-green colonies and a characteristic fruity odor. Fungal mycelium was collected via spreading and gentle swirling of water over the colonies in the plates. Before use, the cells from all microbial cultures were harvested by centrifugation (5,000 x g, 10 min) and the pellet was washed three times with water. The obtained cell suspensions were diluted in Nanopure water to prepare working samples with the optical density of 0.1–1.5 measured at a wavelength of 600 nm (UV-visible spectrophotometer, UV-1800, Shimadzu Corporation, Kyoto, Japan).

Microbial separation and concentration experiments.

Reduction of microbial Dsbc. Cell suspensions of the intact *L. delbrueckii, E. coli, S. boulardii*, and *C. fagacearum* cultures were individually suspended in 2 mM TCEP for 10 min (during incubation, the reaction tubes were protected from light with aluminum foil). The final concentration of the microbial cells in the reaction was kept similar for all microorganisms taken for the reduction. After incubation, the cells were used directly without removing TCEP from the reaction medium. The microbial suspensions were studied individually on a single gold-coated slide and *L. delbrueckii* was also studied for the capture with a microfluidic device. The studies with the single gold-coated slides were designed to confirm the ability of the microbial thiol groups to react with a flat gold surface. To study that, the aliquot of each treated microorganism was drop-casted on a gold-coated slide (suspension volume 100 μL) and after incubation for 1 min, the slide was rinsed with water to remove the unbound material. The slide was analyzed by SEM and the corresponding images are presented and discussed in the manuscript main text (see Fig. 2).

The effect of the reducing agent on the cells' viability was studied for cultures of *S. boulardii* and *E. coli* treated with 2 mM TCEP for 10 min followed by inoculation into growth media. In particular, reduced and non-reduced *S. boulardii* cultures both with a volume of 1.72 mL were added to 10 mL of YM broth and incubated at 37°C for 24 h on a rotator. For the *E. coli* culture, suspensions of reduced and non-reduced cells with the volume of 1 mL were added to 10 mL of TSB medium and incubated at 37°C

for 16 h on a rotator. The growth was followed by determining the optical density (OD) of the suspensions by photometric measurements of the absorption at 600 nm. The obtained values (**Table S1**) demonstrated that the reducing agent used to activate cell surface moieties and enable their attachment to gold does not affect the microbial viability as the optical density of the cultured bacteria and fungi, both reduced and non-reduced, reached similar values after the same incubation time. The microscopy (with the use of light microscope) done for the reduced and non-reduced *E. coli* at the same concentration (OD=0.3) did not reveal changes in the cells' size and shape.

Table S1. *Cell viability studies for S. boulardii and E. coli treated with TCEP in comparison with untreated cells*

Microorganism	Cells after treatment with TCEP/untreated cells, before inoculating the growth medium, OD	Growth (24 h[1] and 16 h[2] respectively), OD	Volume of the inoculum and growth medium, mL
S. boulardii, reduced[1]	0.344	1.66	1.72 and 10
S. boulardii, non-reduced[1]	0.091	1.59	1.72 and 10
E. coli, reduced[2]	0.140	1.63	1.0 and 10
E. coli, non-reduced[2]	0.306	1.27	1.0 and 10

An analogous comparison was performed for the cells of *S. boulardii* used in the capture experiments with a microfluidic device (**Table S2**). In particular, 5.2 mL of the sample (*S. boulardii* treated with TCEP) were injected in the device followed by the incubation of the sample in the device chamber for 1 h. The elution of the cells from the gold-coated slides after the capture was performed by soaking the slides in 100 mM NaCl for 10 min. The resulting solution was collected and centrifuged at 11,700 rpm for 20 min to remove NaCl. The supernatant was discarded while the pellet (approximately 200 μL) was collected for further analysis (measurements of the elution efficiency) or used to inoculate the growth medium.

Table S2. *Capture of S. boulardii treated with TCEP in the experiment with a microfluidic device in comparison with the untreated cell sample*

Microorganism	Cells after treatment with TCEP/untreated cells, before passing through the device, OD	Capture efficiency of the device (%)	Cells after elution from the slides (OD)/elution efficiency (%)	Growth of the eluted cells (24 h) , OD	Volume of the inoculum and YM medium, mL
S. boulardii, reduced	0.344	86.34	0.014/5.52	1.57	1.72 and 10
S. boulardii, non-reduced	0.468	33.33	0.002/1.45	1.01	1.72 and 10
S. boulardii, non-reduced (was not passed through the device)	0.091	n/a	n/a	1.59	1.72 and 10

n/a – not applicable

As it is seen from Table S2, treated *S. boulardii* was captured with almost three-fold higher capture efficiency compared to the untreated cells that suggests a significant impact of the reduction procedure on the efficiency of converting the disulfide bonds on the fungal surface to the chemically reactive thiol groups. At the same time, the untreated cells demonstrated a possibility to be captured by the gold surface at the surprisingly high rate that we explain by the presence of high number of exposed functional moieties on the fungal cell surface that have high affinity to gold and can spontaneously interact with it (e.g., protein thiols, amine groups). As it can be observed, the bonds between gold and such moieties do not deteriorate after multiple washings of the slides with water and after elution with NaCl since the elution efficiency measured for the untreated cells was insignificant and lower compared to the elution efficiency found for the treated cells (1.45% vs. 5.52%). Together with that, high growth rate was observed for the eluted cells that were both previously treated and untreated with TCEP, revealing that after capture in the device the studied cells can be retrieved alive. Elution of the captured cells was also studied for *Lactobacillus gasseri* (ATCC® 33323™) with use of 0.5 M NaOH. Bacterial cells were passed through the device after standard reduction procedure and incubated in the device chamber for 1 h. For the elution of the captured cells, the slides were soaked in 0.5 M NaOH for 10 min followed by centrifugation of the resulting solution to remove NaOH. The elution efficiency of the procedure applied was determined as 30.4% (SD 1.85%, n=3). However, when the eluted cells were transferred to the growth medium (MRS), no cell growth was observed in the media after 24 h and 42 h suggesting that

during treatment with NaOH the cells were irreversibly damages (lysed). The performed investigations yielded the following conclusions and recommendations for the practical application: (1) higher elution efficiency can be obtained with the use of 0.5 M NaOH (exposure time 10 min), however the retrieved cells cannot be cultured; (2) after capture experiments, live cells can be eluted from the slides with the use of 100 mM NaCl (exposure time 10 min), however the elution efficiency of the method can be lower in comparison to the NaOH-based protocol.

Deacetylation of the fungal chitin. For deacetylation, different variants of the experimental protocol were used: (1) Cell suspensions of the intact *S. boulardii* and *C. fagacearum* cultures were re-suspended in NaOH (25%, w/w) for 30 min at room temperature. During incubation, the reaction tubes were kept under constant mixing conditions. Afterwards, NaOH was removed by multiple washings in water and centrifugation (10,000 rpm, 5 min) until the pH of the sample reached pH 6.5–7.0. (2) Cell suspensions of the intact *S. boulardii* and *C. fagacearum* cultures were re-suspended in NaOH (4.8%, w/w) and incubated for 10 min at room temperature followed by neutralization of the alkali with hydrochloric acid (the resulting pH of the sample was around 8). (3) Cell suspensions of the intact S. boulardii and C. fagacearum cultures were re-suspended in NaOH (9.6%, w/w) and incubated for 10 min at room temperature followed by neutralization of the alkali with hydrochloric acid (the resulting pH of the sample was around 8). The microbial suspensions prepared in the ways described above were studied separately during the experiments with gold-coated slides and a microfluidic device. The cells prepared by the protocol (1) were used in the experiment with a single gold-coated slide to confirm the ability of the fungal chitosan (obtained through the deacetylation protocol) to react with a flat gold surface. To test this reaction, aliquots of the treated *S. boulardii* and *C. fagacearum* were drop-casted on the gold-coated slides (suspension volume 100 μL) and after incubation for 1 min, the slides were rinsed with water to remove the unbound material. Corresponding SEM images for the deacetylated fungi studied individually can be found in the manuscript main text (see Fig. 2).

The effect of NaOH at a concentration of 4.8% (w/w) and 9.6% (w/w) on the cells' viability was studied for the cultures of *S. boulardii* treated with NaOH according to protocols (2) and (3) and further

inoculated into the growth media. In particular, 0.6 mL of deacetylated *S. boulardii* was added to 10 mL of YM broth and incubated at 37°C for 24 h on a rotator. Measurements of the optical density of the growth media (**Table S3**) showed a damaging effect of the alkali on the fungi (at least at the applied concentrations) as there was practically no growth observed. An analogous comparison was performed for the cells of *S. boulardii* used in the capture experiments with a microfluidic device (**Table S4**). In particular, approximately 6 mL of the sample of *S. boulardii* treated with NaOH according to the protocols (2) and (3) was injected in the device followed by incubation of the sample in the device chamber for 1 h. The elution of the cells from the gold-coated slides after the capture was performed by soaking the slides in 100 mM NaCl for 10 min. The resulting solution was collected and centrifuged at 11,700 rpm for 20 min to remove NaCl. The supernatant was discarded while the pellet (approximately 200 μL) was collected for further analysis (measurements of the elution efficiency) or used to inoculate the growth medium.

Table S3. *Cell viability studies for S. boulardii treated with NaOH at different concentration*

Sample treatment for deacetylation	Cells after treatment with NaOH and titration with HCl, before inoculating the growth medium, OD	Growth (24 h), OD	Volume of the inoculum and YM medium, mL
S. boulardii with 4.8% NaOH	0.378	0.019	0.6 and 10
S. boulardii with 9.6% NaOH	0.228	0.014	0.6 and 10

Table S4. *Capture of S. boulardii treated with NaOH in the experiment with a microfluidic device*

Sample treatment for deacetylation	Cells after treatment with NaOH and titration with HCl, before inoculating the growth medium, OD	Capture efficiency of the device (%)	Cells after elution from the slides (OD)/elution efficiency (%)	Growth of the eluted cells, OD	Volume of the inoculum and YM medium, mL
S. boulardii with 4.8% NaOH	0.378	61.64	0.001/0.43	0.95	1.4 and 10

S. boulardii with 9.6% NaOH	0.228	56.14	0.00/0.00	0.00	1.4 and 10

As it can be observed from Table S4, deacetylation with 4.8% NaOH contributed to the higher capture efficiency of the device towards *S. boulardii* and allowed for the retrieval of viable cells after treating the slides with sodium chloride in comparison with deacetylation procedure based on 9.6% NaOH. Use of 25% NaOH in our preliminary studies for deacetylation of the fungal chitin was also found to be unacceptable as no growth was observed after the cells were treated with 25% NaOH. Based on these observations, we may presume that 4.8% NaOH could be the highest possible concentration of alkali that can be used for deacetylation of fungal chitin and that allows, at the same time, retrieval of viable cells after their capture on gold surfaces.

Microscopic analysis performed throughout the study was done with field emission gun-scanning electron microscope FEG-SEM-JEOL 6500 (JEOL Ltd., Tokyo, Japan) and optical microscope Leica DM4000M (Leica Microsystems, IL, USA).

Microbial separation and concentration from complex matrices. After confirming the possibility to separate microorganisms on gold slides from model cultures, the microfluidic device was used to separate the microbial agents from complex matrices. These been namely "Yoplait Original" yogurt (Yoplait USA, Inc.) and wood shavings (obtained from USDA Forest Service, Northern Research Station, NRS-16, St. Paul, MN). Each studied sample was diluted using Nanopure water with a 1:3 dilution factor and tested both in its original form and after artificial inoculation with bacterial and/or fungal agents. The detailed information and results of the experiments with the real-world samples are discussed in the manuscript main text, and supported with **Figure S1** given below.

Figure S1

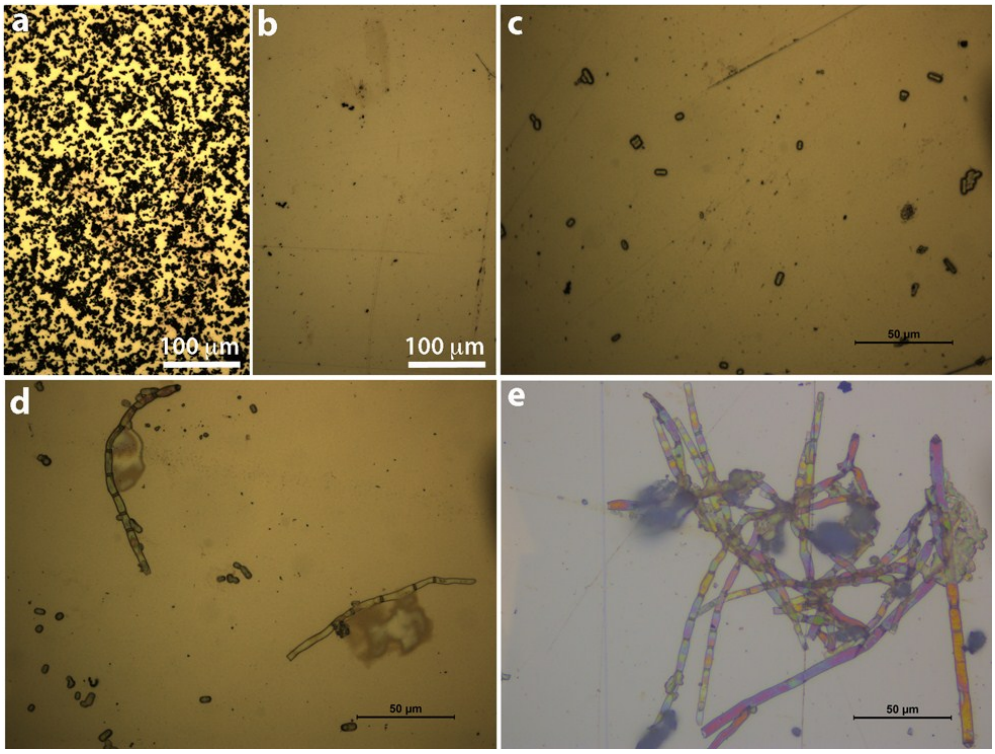

Figure S1. *Microbial separation from real-world samples using a microfluidic device.* ***(a)*** *Optical images of captured bacteria from yogurt sample after reduction.* ***(b)*** *Optical image of yogurt sample after deacetylation.* ***(c)*** *Optical image of yogurt sample inoculated with S. boulardii after deacetylation.* ***(d)*** *Optical image of yogurt sample inoculated with C. fagacearum after deacetylation.* ***(e)*** *Optical image of captured C. fagacearum from oak wood sample after deacetylation.*

References

1. Yang, A.; Juzwik, J., Use of Nested and Real-Time PCR for the Detection of Ceratocystis fagacearum in the Sapwood of Diseased Oak Species in Minnesota. *Plant Disease* **2017,** *101* (3), 480-486.