Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics

Supplementary material for

Facile and fast detection of bacteria via the detection of exogenous volatile metabolites released by enzymatic hydrolysis.

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1. Synthesis of xerogel monoliths

The xerogel monoliths have a parallelepiped shape. They were produced via the sol-gel process. The chemicals, reagent grade, were purchased and used without further purification. Silicon alkoxyde (TMOS and APTES) precursors were mixed with methanol and deionized water according to the following molar ratio (TMOS/APTES)/MeOH/H2O: (0.97/0.03)/5/4. The reaction is extremely fast due to the alkaline catalysis of APTES and must be carried out at -20°C. 1mL of this sol was immediately poured into a cuvette for spectrophotometry (Fisherbrand disposable cuvette; semimicro; polystyrene; capacity: 1.5mL) to obtain a monolith with 12mm × 5mm × 2mm size. During the drying process, operated slowly at 22°C in humidified atmosphere (55% relative humidity) during 3 days, the cuvettes were sealed with an adhesive microporous film (ABGene Gas permeable adhesive seals).

2. Trapping pNP (p-nitrophenol) in a monolith

2.1 Exposure to gaseous pNP above an aqueous pNP solution

pNP, spectrophotometric grade, was obtained from Fluka. An aqueous solution of 0.11M pNP was prepared. A monolith is placed in a small glass vessel (2.6mL), containing 60µL of the pNP solution. The monolith is not in contact with the solution and is exposed to the vapors (pNP and water) over 48h at 25°C. A UV spectrum of the monolith is recorded with a Cary 300 spectrophotometer before and after exposure. The variation of absorbance at 330 and 385 nm witnesses the trapping of pNP in the monolith, under its protonated and deprotonated form.

2.2 Exposure to gaseous pNP coming from a bacterial culture

The culture medium containing the substrate, targeting β-D-glucuronidase, was optimised to increase the glucuronidase activity. It also contained a buffer at pH 7.33, to keep the ratio pNP/pNP^- constant. The MOPS medium was composed of: MOPS (4-morpholinepropanesulfonic acid sodium salt) 150mM, magnesium sulfate 2.6mM, sodium glucuronate 854µM, methyl β-D-glucuronide 870µM, and 4-nitrophenyl-β-D-glucuronide 113µM. The MES medium has an identical composition, except for the buffer: MOPS 150mM was replaced by MES 150mM (4-morpholineethanesulfonic acid). The resulting pH is 6.1.

After a pre-culture phase overnight (37°C on TSA, Trypcase Soy Agar, bioMérieux), several macro-colonies of *Escherichia coli* ATCC11775 were picked up on TSA and dispersed into Suspension Medium (bioMérieux) to adjust the cell concentration to 0.5McF (measured with a Densicheck, bioMérieux). With the colonies enumeration on agar plates, we measured that the 0.5 McF optical density value corresponds to 10^8 cfu/mL. The enzymatic assay, shown in Figure 5, started at t=0, when the 0.5McF bacteria suspension was diluted (1/500 or 1/1000) with the culture medium containing the enzymatic substrate. The paranitrophenol, released by the microbial culture and present in the gas phase, was trapped in the monolith placed in a spectrophotometric cuvette, under a recipient (Ependorf tip) containing the bacterial culture (see Fig. 5a). The amount of trapped pNP was monitored spectrophotometrically. A first exposure was carried out with *E. coli* inoculated in MOPS medium at 2.10^5 cfu/mL. After 17 hours of incubation, 500µL of the culture were poured in the recipient (Ependorf tip). A cell with a monolith exposed to the laboratory atmosphere was used as a reference (see Fig. 5b). A second exposure was carried out with *E. coli* inoculated in MES at 10^5 cfu/mL. After 9 hours of incubation, 1.5mL of the culture was poured in the recipient. An empty cell was used as a

reference. The UV spectrum of the monolith was monitored with a Cary 300 spectrophotometer overnight (see Fig. 5c).

3. Control experiment without enzymatic substrate

3.1 Exposure of a xerogel monolith to a bacterial culture without pNPG enzymatic substrate

After a pre-culture phase overnight (37°C on TSA), several macro-colonies of *Escherichia coli* ATCC11775 were picked up on TSA and dispersed into Suspension Medium, to adjust the cell concentration to 0.5McF. The UV spectrum of the monolith was recorded with a Cary 300 spectrophotometer before and after exposure. The control experiment started at t=0, when the 0.5McF bacteria suspension was diluted (1/10000) with the culture medium non-supplemented with enzymatic substrate. Two xerogels were placed within the headspace of the bacterial culture and the spectrum was collected after 22 hours of exposure (Figure S1 a).

3.2 Extraction of trapped indole and reaction with DMACA

Two xerogels were ground and mixed with 3mL of methanol to extract the trapped volatile metabolite. After 24h of impregnation at room temperature, the solution was filtered (0.2 µm ∅) and the absorption spectrum of the unknown metabolite was collected (Figure S1 b). The hypothesis that the metabolite is indole was based on the fact that the nutrient medium contains aminoacids such as tryptophane, and *E. coli* strains are known to produce tryptophanase, an enzyme that hydrolyses tryptophane into indole. As a proof, the spectrum of a solution of indole was collected and compared to the metabolite spectrum (Figure S1 b).

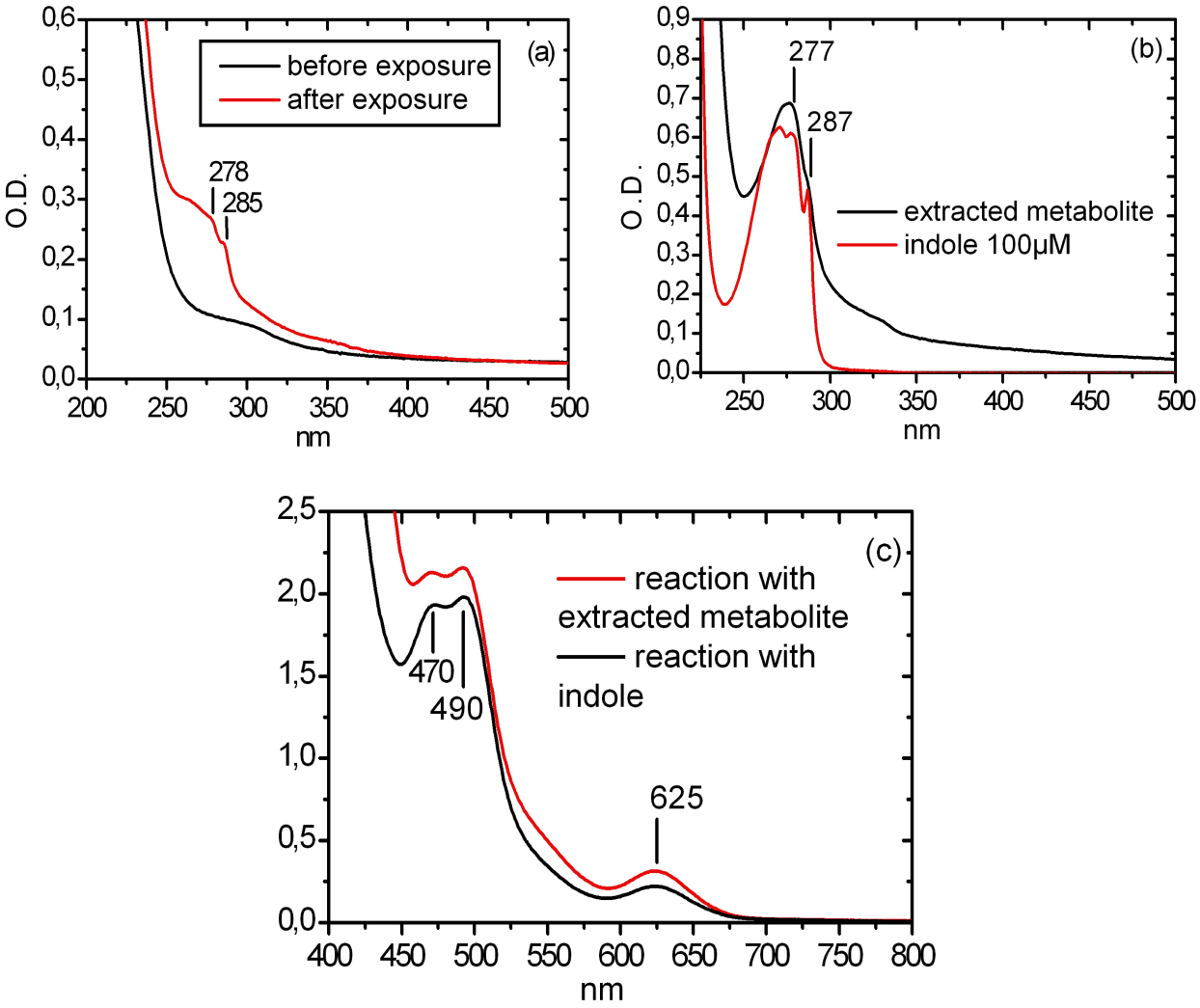

Figure S1. (a) Spectrum of a xerogel monolith collected after 22h of exposure at 37°C to a bacterial culture non-supplemented with enzymatic substrate pNPG. (b) Spectrum of the metabolite in methanol, extracted from ground xerogel. For comparison, the spectrum of a methanolic solution of indole (100 µM) is also plotted. (c) The unknown extracted metabolite is mixed with DMACA under acidic conditions. This reaction is specific to indole. For comparison, the reaction is performed in similar conditions with a methanolic solution of indole.

3.3 Reaction with DMACA

To strengthen the attribution of the metabolite to indole, we achieved the selective condensation of indole with DMACA (4-(dimethylamino)cinnamaldehyde), known to selectively react with indole under acidic conditions to yield the strongly absorbing azafulvenium chloride salt, displaying a high extinction coefficient value at 625 nm ($\varepsilon(625nm) = 97\,000 \pm 13\,000\ M^{-1}.cm^{-1}$, this work).

The following procedure was applied: 500 µL of the methanolic solution of the extracted metabolite was mixed with 75 µL HCl 37% and 925 µL of DMACA 0.0648 M. The final concentrations of the reactants are: [HCl]=0.6 M and [DMACA]=40 mM. The spectrum

collected after the reaction (Figure S1 c) displays the typical absorption band of azafulvenium. As a control, we performed the same DMACA test with 500 µL of a methanolic solution of indole (10 µM). The resulting absorption spectrum (Figure S1 c) being identical to the former one, the unknown volatile metabolite was definitely attributed to indole.

Furthermore, as a blank test, we performed a similar experiment with a culture of bacteria in a medium non-supplemented with pNPG and using a strain that is unable to produce indole. After 22 hours of exposure in similar conditions, both of the xerogels were characterized with UV-vis. Spectrophotometry: no absorption band was found around 280 nm.