**Supplementary File**

Article title: Systematic analysis of the ability of Nitric Oxide donors to dislodge biofilms formed by *Salmonella enterica* and *Escherichia coli* O157:H7

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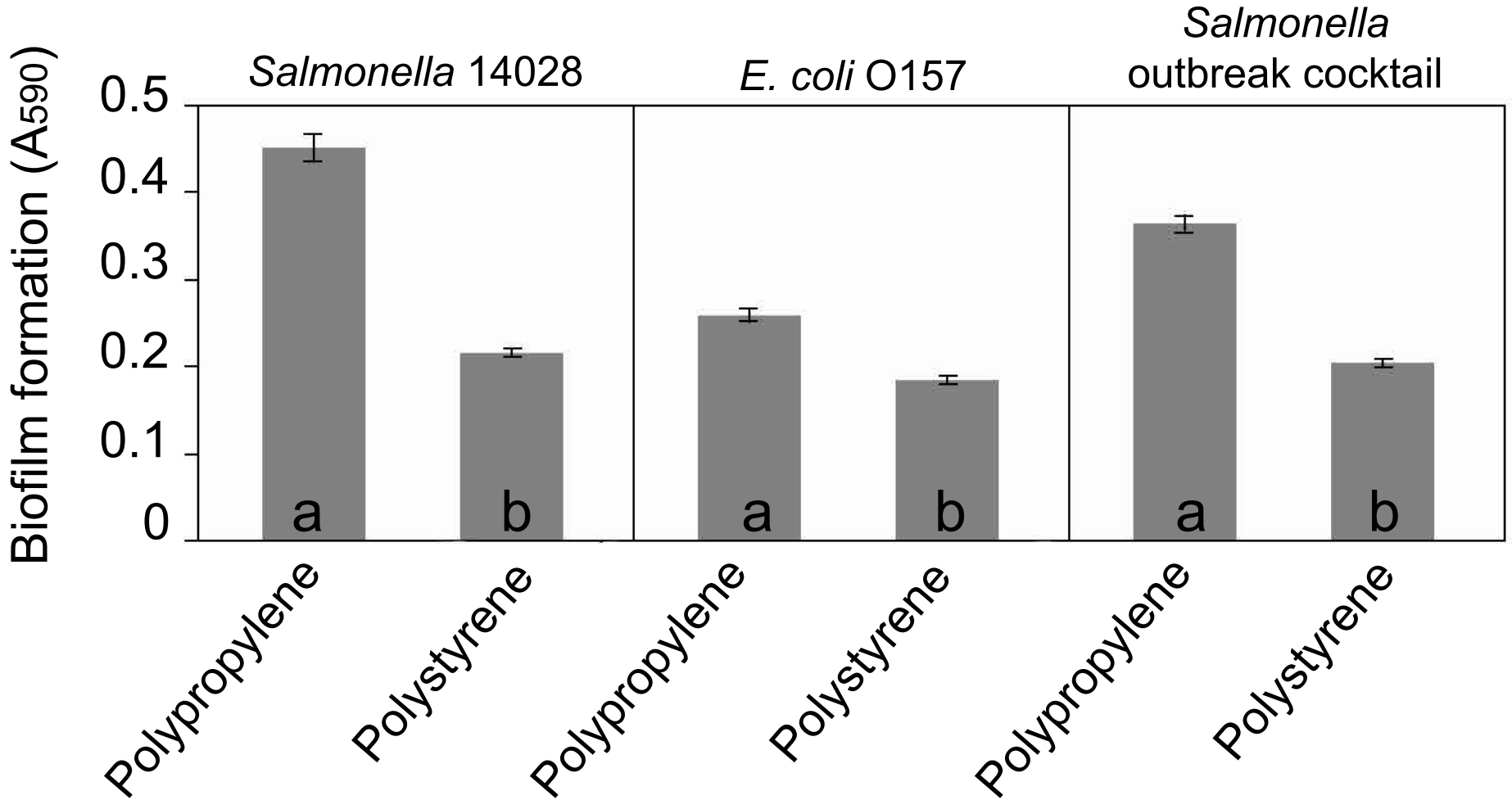
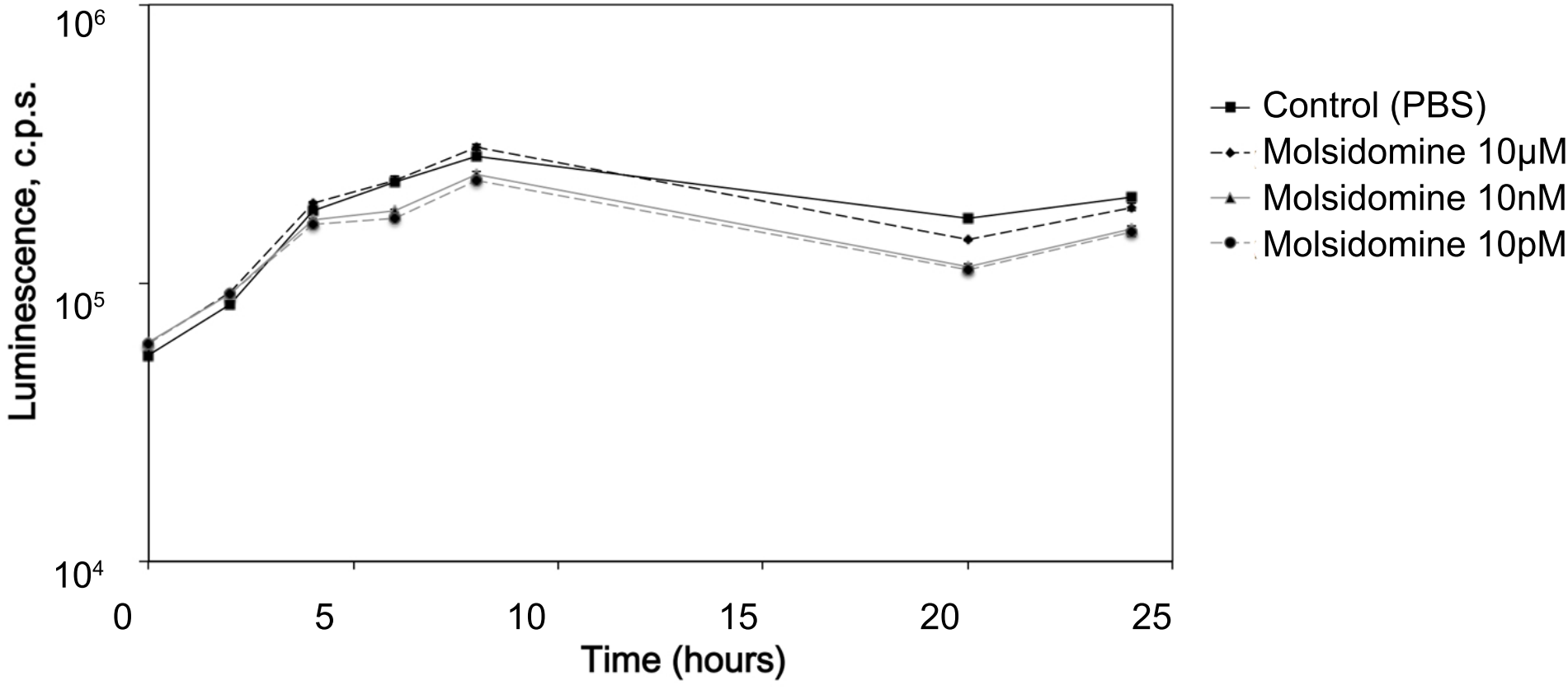
# Biofilm formation on different plastics by different pathogens



**Supplemental Material - Figure S1. Biofilm formation on different plastics by *Salmonella* and *E. coli* O157:H7.** Biofilms were established on polypropylene and polystyrene surfaces in CFA medium for 24 hrs and stained with 1 % crystal violet and then washed. The absorbed crystal violet and the biofilm were dissolved in 33% acetic acid and A590 was measured with a spectrophotometer. Error bars represent standard error. Significant different means are displayed with different letters.

# Luminescence of *Salmonella* 14028 pTIM2442 after molsidomine treatment



**Supplemental Material – Figure S2. Luminescence of *Salmonella* 14028 pTIM2442 upon exposure to molsidomine.** General metabolic state of the cells was assessed using the redox-coupled $FMNH_2$/Luciferase produced by a *S.* Typhimurium ATCC14028 strain harboring high copy number plasmid in which the *luxCDABE* operon is under the phage λ promoter (pTIM2442). Concentrations of molsidomine to which cultures of *Salmonella* 14028 pTIM2442 were exposed are listed on the figure. Error bars represent the standard error of 12 replicas.

**Supplementary Table S1. Fluorescence of the *Salmonella* cells detached by molsidomine treatment**

Measurement of *Salmonella* 14028 planktonic cells detached from preformed biofilm during treatment with molsidomine. polypropylene

| Conc. | Log (Fluorescence 485nm/535nm) | | | P<0.05 |
|---|---|---|---|---|
| | 0 hours | 3 hours | 6 hours | |
| *Biofilms formed on polypropylene* | | | | |
| 0 | 4.335 ± 0.065 | n.a. | 4.450 ± 0.177 | 0.5492 |
| 10 μM | 4.307 ± 0.067 | 4.846 ± 0.054 | 4.466 ± 0.080 | <.0001* |
| 10 nM | 4.275 ± 0.055 | 4.780 ± 0.050 | 4.753 ± 0.075 | <.0001* |
| 10 pM | 4.355 ± 0.064 | 4.815 ± 0.125 | 5.002 ± 0.144 | 0.0024* |

*Statistically significant effects ($p<0.05$) of molsidomine treatment.

**Supplementary Table S2.** The complete set of experiments on the effect of the NO donors on preformed biofilm after 24 hours of contact time.

| NOD | Material | Strain | Incubation temperature | Prob>F (p=0.05) |
|---|---|---|---|---|
| D184 | Polypropylene | *Salmonella* 14028 | 4°C | <0.0001 |
| D184 | Polypropylene | *Salmonella* 14028 | 22°C | n.s. |
| D184 | Polypropylene | *E.coli* O157:H7 | 4°C | n.s. |
| D184 | Polypropylene | *E.coli* O157:H7 | 22°C | n.s. |
| D184 | Polypropylene | *Salmonella* cocktail | 4°C | n.s. |
| D184 | Polypropylene | *Salmonella* cocktail | 22°C | <0.0001 |
| D5431 | Polypropylene | *Salmonella* 14028 | 4°C | <0.0001 |
| D5431 | Polypropylene | *Salmonella* 14028 | 22°C | <0.0001 |
| D5431 | Polypropylene | *E.coli* O157:H7 | 4°C | 0.0004 |
| D5431 | Polypropylene | *E.coli* O157:H7 | 22°C | <0.0001 |
| D5431 | Polypropylene | *Salmonella* cocktail | 4°C | <0.0001 |
| D5431 | Polypropylene | *Salmonella* cocktail | 22°C | <0.0001 |
| M1555 | Polypropylene | *Salmonella* 14028 | 4°C | n.s. |
| M1555 | Polypropylene | *Salmonella* 14028 | 22°C | 0.0049 |
| M1555 | Polypropylene | *E.coli* O157:H7 | 4°C | n.s. |
| M1555 | Polypropylene | *E.coli* O157:H7 | 22°C | <0.0001 |
| M1555 | Polypropylene | *Salmonella* cocktail | 4°C | 0.0185 |
| M1555 | Polypropylene | *Salmonella* cocktail | 22°C | <0.0001 |
| MOL | Polypropylene | *Salmonella* 14028 | 4°C | <0.0001 |
| MOL | Polypropylene | *Salmonella* 14028 | 22°C | <0.0001 |
| MOL | Polypropylene | *E.coli* O157:H7 | 4°C | <0.0001 |
| MOL | Polypropylene | *E.coli* O157:H7 | 22°C | n.s. |
| MOL | Polypropylene | *Salmonella* cocktail | 4°C | 0.0001 |
| MOL | Polypropylene | *Salmonella* cocktail | 22°C | <0.0001 |
| D184 | Polystyrene | *Salmonella* 14028 | 4°C | n.s. |
| D184 | Polystyrene | *Salmonella* 14028 | 22°C | n.s. |
| D184 | Polystyrene | *E.coli* O157:H7 | 4°C | n.s. |
| D184 | Polystyrene | *E.coli* O157:H7 | 22°C | n.s. |
| D184 | Polystyrene | *Salmonella* cocktail | 4°C | <0.0001 |
| D184 | Polystyrene | *Salmonella* cocktail | 22°C | <0.0001 |
| D184 | Polystyrene | *Salmonella* 14028 | 4°C | <0.0001 |
| D5431 | Polystyrene | *Salmonella* 14028 | 22°C | 0.0006 |
| D5431 | Polystyrene | *E.coli* O157:H7 | 4°C | 0.0001 |
| D5431 | Polystyrene | *E.coli* O157:H7 | 22°C | n.s. |
| D5431 | Polystyrene | *Salmonella* cocktail | 4°C | <0.0001 |
| D5431 | Polystyrene | *Salmonella* cocktail | 22°C | <0.0001 |
| M1555 | Polystyrene | *Salmonella* 14028 | 4°C | 0.0229 |
| M1555 | Polystyrene | *Salmonella* 14028 | 22°C | <0.0001 |
| M1555 | Polystyrene | *E.coli* O157:H7 | 4°C | n.s. |
| M1555 | Polystyrene | *E.coli* O157:H7 | 22°C | n.s. |
| M1555 | Polystyrene | *Salmonella* cocktail | 4°C | <0.0001 |

| | | | | |
|---|---|---|---|---|
| M1555 | Polystyrene | *Salmonella* cocktail | 22°C | <0.0001 |
| MOL | Polystyrene | *Salmonella* 14028 | 4°C | 0.0465 |
| MOL | Polystyrene | *Salmonella* 14028 | 22°C | 0.0078 |
| MOL | Polystyrene | *E.coli* O157:H7 | 4°C | n.s. |
| MOL | Polystyrene | *E.coli* O157:H7 | 22°C | n.s. |
| MOL | Polystyrene | *Salmonella* cocktail | 4°C | <0.0001 |
| MOL | Polystyrene | *Salmonella* cocktail | 22°C | <0.0001 |

* Statistically significant effects ($p<0.05$) of the NO donor treatments. n.s., not statistically significant biofilm dispersion. MOL, Molsidomine; M1555, MAHMA NONOate; S150, SPERMINE NONOate; D5431, diethylamine NONOate diethylammonium salt; D184, diethylamine NONOate sodium; S8432 sulfo NONOate disodium salt.

**Supplementary Table S3.** The complete set of experiments on the effect of the NO donors on preformed biofilm after 6 hours of contact time.

| NO donor | Material | Strain | Incubation temperature | Prob>F* |
|---|---|---|---|---|
| D184 | Polypropylene | *Salmonella* 14028 | 4°C | <0.0001 |
| D184 | Polypropylene | *Salmonella* 14028 | 22°C | n.s. |
| D184 | Polypropylene | *E.coli* O157:H7 | 4°C | n.s. |
| D184 | Polypropylene | *E.coli* O157:H7 | 22°C | 0.0551 |
| D184 | Polypropylene | *Salmonella* cocktail | 4°C | n.s. |
| D184 | Polypropylene | *Salmonella* cocktail | 22°C | <0.0001 |
| D5431 | Polypropylene | *Salmonella* 14028 | 4°C | <0.0001 |
| D5431 | Polypropylene | *Salmonella* 14028 | 22°C | <0.0001 |
| D5431 | Polypropylene | *E.coli* O157:H7 | 4°C | 0.0004 |
| D5431 | Polypropylene | *E.coli* O157:H7 | 22°C | <0.0001 |
| D5431 | Polypropylene | *Salmonella* cocktail | 4°C | <0.0001 |
| D5431 | Polypropylene | *Salmonella* cocktail | 22°C | <0.0001 |
| M1555 | Polypropylene | *Salmonella* 14028 | 4°C | n.s. |
| M1555 | Polypropylene | *Salmonella* 14028 | 22°C | 0.0049 |
| M1555 | Polypropylene | *E.coli* O157:H7 | 4°C | n.s. |
| M1555 | Polypropylene | *E.coli* O157:H7 | 22°C | <0.0001 |
| M1555 | Polypropylene | *Salmonella* cocktail | 4°C | n.s. |
| M1555 | Polypropylene | *Salmonella* cocktail | 22°C | <0.0001 |
| MOL | Polypropylene | *Salmonella* 14028 | 4°C | <0.0001 |
| MOL | Polypropylene | *Salmonella* 14028 | 22°C | <0.0001 |
| MOL | Polypropylene | *E.coli* O157:H7 | 4°C | <0.0001 |
| MOL | Polypropylene | *E.coli* O157:H7 | 22°C | n.s. |
| MOL | Polypropylene | *Salmonella* cocktail | 4°C | 0.0001 |
| MOL | Polypropylene | *Salmonella* cocktail | 22°C | <0.0001 |
| S150 | Polypropylene | *Salmonella* 14028 | 4°C | n.s. |
| S150 | Polypropylene | *Salmonella* 14028 | 22°C | n.s. |
| S150 | Polypropylene | *E.coli* O157:H7 | 4°C | 0.0273 |
| S150 | Polypropylene | *E.coli* O157:H7 | 22°C | 0.0486 |
| S150 | Polypropylene | *Salmonella* cocktail | 4°C | 0.0036 |
| S150 | Polypropylene | *Salmonella* cocktail | 22°C | 0.0003 |
| D184 | Polystyrene | *Salmonella* 14028 | 4°C | n.s. |
| D184 | Polystyrene | *Salmonella* 14028 | 22°C | n.s. |
| D184 | Polystyrene | *E.coli* O157:H7 | 4°C | n.s. |
| D184 | Polystyrene | *E.coli* O157:H7 | 22°C | n.s. |
| D184 | Polystyrene | *Salmonella* cocktail | 4°C | <0.0001 |
| D184 | Polystyrene | *Salmonella* cocktail | 22°C | <0.0001 |
| D5431 | Polystyrene | *Salmonella* 14028 | 4°C | <0.0001 |
| D5431 | Polystyrene | *Salmonella* 14028 | 22°C | 0.0006 |
| D5431 | Polystyrene | *E.coli* O157:H7 | 4°C | 0.0001 |
| D5431 | Polystyrene | *E.coli* O157:H7 | 22°C | n.s. |

| | | | | |
|---|---|---|---|---|
| D5431 | Polystyrene | *Salmonella* cocktail | 4°C | <0.0001 |
| D5431 | Polystyrene | *Salmonella* cocktail | 22°C | <0.0001 |
| M1555 | Polystyrene | *Salmonella* 14028 | 4°C | 0.0229 |
| M1555 | Polystyrene | *Salmonella* 14028 | 22°C | <0.0001 |
| M1555 | Polystyrene | *E.coli* O157:H7 | 4°C | n.s. |
| M1555 | Polystyrene | *E.coli* O157:H7 | 22°C | n.s. |
| M1555 | Polystyrene | *Salmonella* cocktail | 4°C | 0.0184 |
| M1555 | Polystyrene | *Salmonella* cocktail | 22°C | <0.0001 |
| MOL | Polystyrene | *Salmonella* 14028 | 4°C | <0.0001 |
| MOL | Polystyrene | *Salmonella* 14028 | 22°C | 0.0078 |
| MOL | Polystyrene | *E.coli* O157:H7 | 4°C | n.s. |
| MOL | Polystyrene | *E.coli* O157:H7 | 22°C | n.s. |
| MOL | Polystyrene | *Salmonella* cocktail | 4°C | <0.0001 |
| MOL | Polystyrene | *Salmonella* cocktail | 22°C | <0.0001 |
| S150 | Polystyrene | *Salmonella* 14028 | 4°C | 0.0418 |
| S150 | Polystyrene | *Salmonella* 14028 | 22°C | n.s. |
| S150 | Polystyrene | *E.coli* O157:H7 | 4°C | n.s. |
| S150 | Polystyrene | *E.coli* O157:H7 | 22°C | n.s. |
| S150 | Polystyrene | *Salmonella* cocktail | 4°C | n.s. |
| S150 | Polystyrene | *Salmonella* cocktail | 22°C | 0.0183 |

* Statistically significant effects ($p<0.05$) of the NO donor treatments. n.s., not statistically significant biofilm dispersion. MOL, Molsidomine; M1555, MAHMA NONOate; S150, SPERMINE NONOate; D5431, diethylamine NONOate diethylammonium salt; D184, diethylamine NONOate sodium; S8432 sulfo NONOate disodium salt.