# Parabens from personal care products compromise drinking water disinfection

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## Background

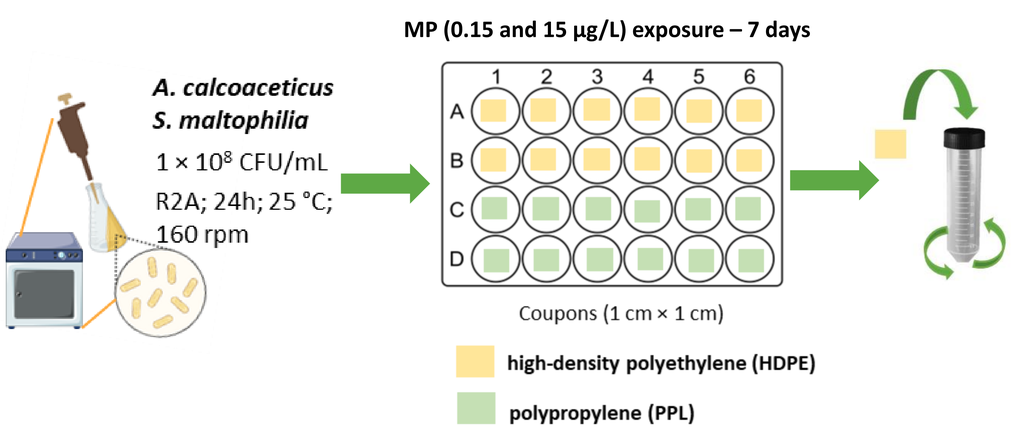
Drinking water distribution systems are known to harbor biofilms, even after disinfection, which constitute a source of microorganisms that may remain in the drinking water (DW) delivered through the consumer's tap[1]. The presence of parabens (an anthropogenic contaminant) in DW is another problem that may affect microbial characteristics and the susceptibility to chlorine, compromising DW disinfection and quality[2].

**Goal: Evaluate the effects of methylparaben (MP) at environmental concentrations on dual-species biofilms (*Acinetobacter calcoaceticus* + *Stenotrophomonas maltophilia*) from DW.**

✓ Biofilms characteristics; Susceptibility to disinfection; Virulence factors production

## Methodology

### 1 Dual-species biofilm formation and MP exposure[3]

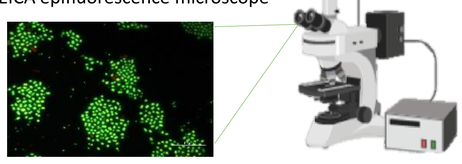



### 2 Biofilm characterization

✓ **Culturability –** Drop plate method

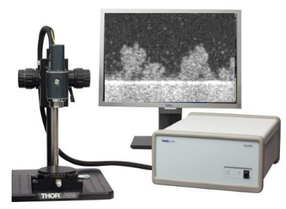

✓ **Cellular density and viability**

- **Live/Dead BacLight kit**
- 250 µL of SYTO 9TM + 50 µL of PI
- LEICA epifluorescence microscope



✓ **Biofilm structure –** Optical Coherence Tomography (OCT)

- Thorlabs Ganymede instrument
- **Thickness (µm)**



✓ **Quantification of Extracellular Polymeric Substances (EPS)**

- **Extraction** – 4 h at 400 rpm and 4 °C
- **Quantification of Proteins** - Total Protein Kit, Micro Lowry, Peterson's Modification
- **Quantification of Polysaccharides** - Phenol-sulphuric acid method

### 3 Virulence Factors Production

✓ Swimming (0.3% agar)
✓ Swarming (0.7% agar)
✓ Twitching (1.5% agar)
(15 µL) 72 h; 25 °C

✓ Siderophores production (20 µL – orange halo)
✓ Gelatinase activity (10 µL – transparent halo)
48 h; 25 °C

✓ Protease Production (10 µL – transparent halo)
✓ Lipase activity (15 µL – light-yellow halo)
72 h; 25 °C

### 4 Biofilm susceptibility to chlorine disinfection

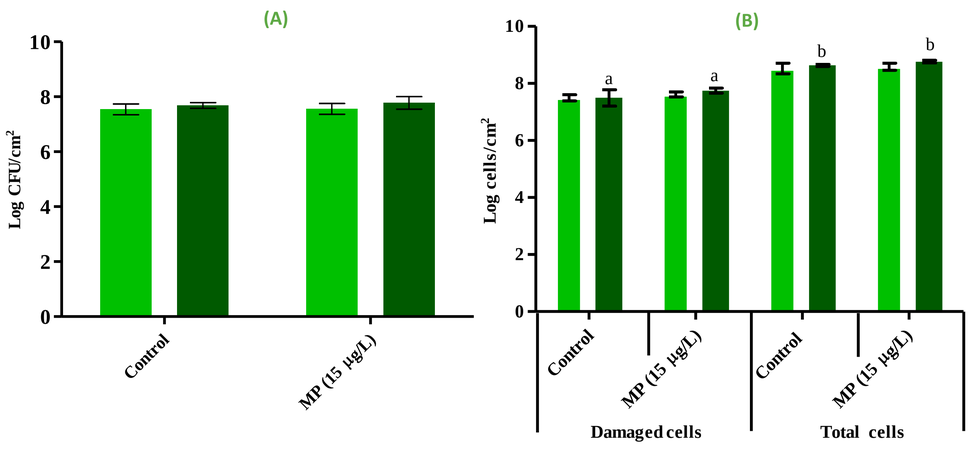
✓ Biofilms exposed and non-exposed to MP were treated with **sodium hypochlorite** using **free chlorine** at **5 mg/L** prepared through the **N, N-diethyl p-phenylenediamine (DPD)** method.

**30 min**

**Neutralization:** sodium thiosulphate (0.5% w/v)

## Results

### Biofilm characterization




(A) Culturability of biofilm cells (Log CFU/cm²) and (B) cellular density (Log cells/cm²) of dual-species biofilms after growing for 7 days. [a, b] - samples were statistically different from unexposed bacterial biofilms (t-test, P < 0.05).

Biofilms formed on **HDPE**
Biofilms formed on **PPL**

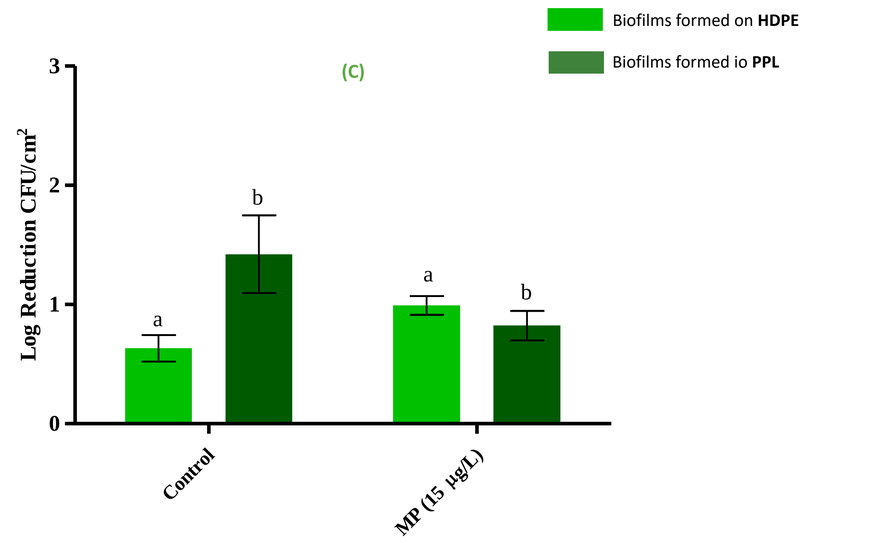
✓ **Culturability:** Both biofilms formed on HDPE and PPL were not affected by MP exposure

✓ **Density and viability:**

**HDPE:** Biofilms were not affected by MP exposure

**PPL:** MP-exposure potentiated the proliferation of biofilms, resulting in an increase of **63%** in the number of **total cells** in relation to non-exposed biofilms and caused an increase of **36%** in the number of **bacterial cells with damaged membrane**.

### Biofilm susceptibility to chlorine disinfection




(C) Logarithmic reduction of culturable cells of biofilms. [a, b, c, d] - corresponds to conditions that have statistically significant differences from each other (t-test, P < 0.05).

✓ MP-exposed dual-species biofilms seem to be more tolerant to chlorine disinfection

### Biofilm thickness (µm)

% increase (↑) in biofilm thickness for MP-exposed biofilms in relation to non-exposed

| | HDPE | PPL |
|---|---|---|
| Control | 24.5±2.0 | 22.6±2.8 |
| MP (15 µg/L) | - | ↑ 45% |

### EPS content

% increase (↑) and decrease (↓) in EPS content for MP-exposed biofilms in relation to non-exposed

| | | HDPE | PPL |
|---|---|---|---|
| **Polysaccharides (µg/cm²)** | Control | 8.0±1.0 | 9.0±3.5 |
| | MP (15 µg/L) | - | ↓ 43% |
| **Proteins (µg/cm²)** | Control | 11±6.7 | 11±5.2 |
| | MP (15 µg/L) | ↑ 120% | - |

### Virulence factors production

Increase of virulence factors activity of MP-exposed *S. maltophilia*.

| *S. maltophilia* | MP (150 ng/L) |
|---|---|
| Siderophores | / |
| Protease | ++ |
| Gelatinase | + |
| Lipase | / |
| Swimming | +++ |
| Swarmming | / |
| Twitching | / |

**Legend:**
**+ ( < 50%)**
**++ (50 – 100%)**
**+++ ( > 100%)**
**/ (not statistically different from control – non-exposed *S. maltophilia*)**

## Conclusions

✓ **MP exposure induces the proliferation of biofilm cells and affects biofilm structure and composition**
✓ **MP presence may compromise chlorination efficacy**
✓ **MP exposure may increase *S. maltophilia* virulence (protease and lipase activity, and swimming motility)**

## Acknowledgments

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## References

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