Effects of parabens exposure on drinking water bacteria

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Abstract Parabens are considered emerging contaminants that are frequently detected in water sources. Besides being detected in drinking water (DW) at residual concentrations, their effects on DW microbial quality and safety have been disregarded so far. This work assesses for the first time the impact of methylparaben (MP), propylparaben (PP), and butylparaben (BP) on selected bacteria isolated from DW: *Acinetobacter calcoaceticus* and *Stenotrophomonas maltophilia*. Although the minimum inhibitory concentrations (MICs) values obtained were found to be much higher (200 - 400 mg/L) than concentrations of parabens found in the environment, parabens induce bacterial membrane modifications at environmental concentrations (15 µg/L). Overall, parabens caused an increase in the total surface hydrophilicity of both bacteria, being this effect more pronounced for MP and BP on *S. maltophilia*. Dual-species biofilms grown on polypropylene (PPL) and high-density polyethylene (HDPE) and exposed to MP (15 µg/L) were found to be more metabolically active (198% and 98%, respectively) than non-exposed biofilms. Even though, MP (15 µg/L) was able to reduce the cell growth rate of *A. calcoaceticus* from dual-species biofilms. The overall results show that parabens induce modifications in bacterial community characteristics and behaviour, which may compromise the safety of DW.

Keywords: drinking water, parabens, bacterial community, biofilms

1. Introduction

Bacterial contamination, particularly in the biofilm state is frequently reported in drinking water distribution systems and may endanger consumers' health as well as the safety and quality of drinking water (DW) (Gomes et al., 2020). Aside from microbiological problems, the existence of emerging contaminants (ECs) also affects DW quality. Parabens are an example of these contaminants and are used as antibacterial compound and preservatives in personal care products (PCPs), pharmaceuticals, and food (Wei et al., 2021). These compounds have drawn more attention to their pervasiveness in water sources, where they have been found in DW at residual concentrations (ng/L - µg/L). However, there is few research on the impact of parabens on the DW microbiome and biofilms, with only one recent study exploring this problem (Pereira et al., 2023). Environmental concentrations of parabens can alter biofilm culturability, density, and membrane integrity, as well as affect DW bacterial virulence factors and motility (Pereira et al., 2023). More and deeply research is needed to understand the interaction between parabens and DW bacteria and their mode of action. This work assesses the impact of parabens (methylparaben – MP, propylparaben – PP and butylparaben – BP and a triple combination of all - MIX) on DW bacteria (*Acinetobacter calcoaceticus* and *Stenotrophomonas maltophilia*) in terms of surface cell modifications, metabolic activity and cell growth.

2. Methods

2.1. Minimum inhibitory concentration (MIC) assessment

A. calcoaceticus and *S. maltophilia* were used as model bacteria of DW bacteria. Bacteria cultures were prepared in R2A broth medium overnight at 25 °C with 160 rpm of agitation.. The minimum inhibitory concentration (MIC) of each paraben (MP, PP and BP) for both bacteria was assessed as explained by McBain et al. (2004). Different paraben concentrations were prepared as described by Pereira et al., (2023) and tested(100, 150, 200, 250, 300, 350, 400 and 450 mg/L). Ultrapure water or acetone at 0.005% (v/v) were used as solvent control. The MIC was determined as the lowest concentration of parabens at which an inhibition growth is detected.

2.2. Characterization of parabens-exposed bacteria cell envelope

The contact angles of unexposed and exposed bacteria to parabens solutions were measured by the sessile drop contact angle technique as described by Pereira et al. (2022) with some modifications. For that, bacteria (1×10^8

CFU/mL in sterile tap water - STW) and exposed to MP, PP, BP isolated and in a mixture of the three parabens - MIX at 15 µg/L in a total volume of 30 mL for 24 hours. Afterwards, samples were filtered using a 0.45 µm cellulose nitrate filter. For each sample, contact angles data were obtained from at least 15 determinations accordingly to Pereira et al., (2022).Results are presented as surface tension components and the total surface hydrophobicity values ($\Delta G_{iwi}, mJ/m^2$).

2.3. Consequences of parabens exposure on DW bacteria from dual-species biofilm

2.3.1. Parabens effects on the metabolic activity of bacteria from DW biofilms

7-days-old dual-species biofilms were formed on high-density-polyethylene (HDPE) and polypropylene (PPL) coupons, exposed to MP at 15 µg/L and unexposed as described by Pereira et al. (2023). The metabolic activity of MP-exposed and unexposed bacteria from dual-species biofilms was evaluated by the Alamar blue assay according to Arruda et al. (2022). Coupons with biofilms were washed with STW to remove non-adherent or weakly adhered bacterial cells. After that, 900 µL of fresh R2A and 100 µL of Alamar blue solution at 0.1 mg/mL were added to each coupon inside the well sequentially. Then, the fluorescence intensity was measured in a microplate reader at 560 nm excitation wavelength and 600 nm emission wavelength for 8 hours with intervals of 30 min to assess the differences in the metabolic activity of non-exposed and exposed biofilms. The percentage of potentiation of biofilm metabolic activity was given by:

$$\%BP = \frac{FI_{MP} - FI_C}{FI_C} \times 100 \quad (1)$$

Where $\%\ BP$ is the percentage of biofilm potentiation, FI_{MP} is the fluorescence intensity value for biofilms exposed to MP and FI_C is the fluorescence intensity of biofilms non-exposed (control).

2.3.2. Parabens effects on cell replication of bacteria from DW biofilms

The impact on cell replication of MP exposure at 15 µg/L to both bacteria isolated from 7-day-old dual-species biofilms were based on the measurement of optical density at 610 nm (OD_{610}) as described by Pinto et al. (2023). The absorbance-based growth kinetics of MP non-exposed (control) and exposed bacteria were adjusted to Gompertz model, as described by Fernandes et al. (2020).

3. Results and discussion

3.1. Minimum inhibitory concentration (MIC) assessment

The MICs values obtained for parabens towards both bacteria were found to be much higher (in the range of 200 to 400 mg/L) than concentrations of parabens found in the environment (15 µg/L) (Table 1). Consequently, at 15 µg/L, parabens are expected to not compromise the viability of DW bacteria.

Table 1. Minimum inhibitory concentrations (MICs) (mg/L) for selected parabens against both bacteria tested.

		A. calcoaceticus	*S. maltophilia*
Parabens	**MP**	400	400
	PP	300	250
	BP	200	200

3.2. Characterization of parabens-exposed bacteria

Physicochemical surface properties of bacteria, in particular the total surface hydrophobicity, were evaluated to assess the effects of the selected parabens on the cell envelope. Table 2 presents the surface tension parameters and hydrophobicity of non-exposed and exposed bacteria to each selected paraben at 15 µg/L (maximum concentration found in DW). Based on the free energy of interaction, both bacteria were classified as hydrophilic ($\Delta G_{iwi} > 0\ mJ/m^2$) and had a strong ability to donate electrons (γ^-). *A. calcoaceticus* was not predisposed to physicochemical changes in surface tension parameters when exposed to paraben solutions ($P > 0.05$) (Table 2). Therefore, parabens did not affect the total surface hydrophobicity of *A. calcoaceticus* at trace concentrations. However, *S. maltophilia* revealed some changes in physicochemical surface parameters after exposure to MP and BP at 15 µg/L ($P < 0.05$). Both MP and BP promoted an increase (superior to 20%) in the total surface hydrophilicity values (ΔG_{iwi}) when compared to bacteria non-exposed to those parabens ($P < 0.05$). This increase in the total hydrophilicity was mainly due to the contribution of the polar component of the free energy with an increase of $\Delta G_{iwi}{}^{AB}$ ($P < 0.05$). Hence, at trace concentrations found in the environment, parabens can change the structure and conformation of the bacterial cell envelope.

Table 2. Apolar (γ^{LW}) and polar (γ^{AB}) components of the surface tension (mJ/m^2), and hydrophobicity (ΔG_{iwi}) (mJ/m^2) unexposed (control) and exposed bacteria to different parabens at 15 µg/L. The values are the means ± SDs of three independent experiments.* corresponds to P < 0.05 in comparison to control (STW) and ** corresponds to P < 0.05 in comparison to control (Ac).

		Surface tension parameters (mJ/m^2)				Hydrophobicity (mJ/m^2)		
		γ^{LW}	γ^{AB}	γ^{+}	γ^{-}	ΔG_{iwi}	ΔG_{iwi}^{LW}	ΔG_{iwi}^{AB}
A. calcoaceticus	Control (STW)	33 ± 2.2	21 ± 3.1	2.0 ± 0.6	56 ± 5.4	29 ± 0.80	-2.0 ± 0.5	31 ± 0.63
	Control (Ac)	33 ± 1.5	20 ± 2.0	2.0 ± 0.3	53 ± 2.03	31 ± 2.02	-2.0 ± 0.5	33 ± 1.5
	MP	34 ± 0.83	21 ± 1.1	2.0 ± 0.3	53 ± 2.1	29 ± 2.6	-3.0 ± 0.3	32 ± 2.9
	PP	33 ± 1.5	21 ± 2.9	2.0 ± 0.6	52± 0.64	29± 0.69	-2.0 ± 0.6	32 ± 1.3
	BP	34 ± 1.4	20 ± 2.2	2.0 ± 0.4	53 ± 4.9	30 ± 5.5	-3.0 ± 0.6	33 ± 4.9
	MIX	33 ± 0.97	21 ± 3.2	2.0 ± 0.7	52 ± 3.3	29 ± 4.3	-3.0 ± 0.4	32 ± 4.4
S. maltophilia	Control (STW)	29 ± 4.6	27 ± 6.4	4.0 ± 2.0	52 ± 2.0	26 ± 4.0	-1.0 ± 1.0	27 ± 4.9
	Control (Ac)	36 ± 2.6	17 ± 3.4	1.0 ± 0.5	54 ± 2.1	32 ± 3.3	-4.0 ± 1.2	35 ± 4.5
	MP	**38 ± 2.03***	**16 ± 1.5***	**1.0 ± 0.2***	54 ± 0.78	**32 ± 0.94***	**-4.0 ± 1.0***	**37 ± 1.8***
	PP	29 ± 6.1	24 ± 10	3.0 ± 3.0	51± 2.03	27± 6.01	-2.0 ± 1.0	29 ± 7.3
	BP	34 ± 1.5	14 ± 1.9	1.0 ± 0.2	**59 ± 2.1****	**40 ± 2.9****	-3.0 ± 0.6	**43 ± 2.3****
	MIX	34 ± 1.4	19 ± 2.9	2.0 ± 0.6	53 ± 2.7	30 ± 4.2	-3.0 ± 0.6	33 ± 4.2

If ΔG_{iwi} > 0 mJ/m^2, hydrophilic and if ΔG_{iwi} < 0 mJ/ m^2, hydrophobic; γ^{+}- electron-acceptor parameter; γ^{-}- electron-donor parameter; LW - Liftshitz-van der Waals; AB - Lewis acid-base.

3.3. Parabens impact of DW microbial community: metabolic activity and cell replication

The assessment of metabolic activity of dual-species biofilms exposed and non-exposed biofilms was also evaluated. It was possible to observe that the exposure of MP at 15 µg/L potentiated the metabolic activity of these biofilms formed in different substrate materials (HDPE and PPL) in comparison to non-exposed biofilms (P < 0.05) (Figure 1). At 2.5 hours (representative of the exponential phase of metabolic activity), MP-exposed dual-species biofilms at 15 µg/L formed on PPL and HDPE were 198% and 98% more active than non-exposed counterparts, respectively (P < 0.05). Comparing both materials, PPL favoured the formation of biofilms more metabolically active when exposed to MP, increasing the concern about its use on DW plumbing systems.

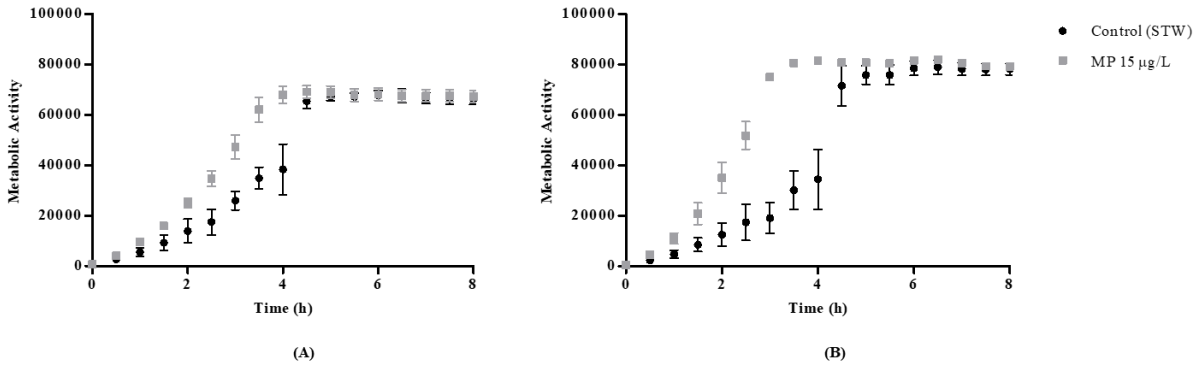

Figure 1. Metabolic activity of 7-days old dual-species biofilms formed in HDPE (A) and PPL (B).

The impact of MP on bacteria from dual-species biofilms was also analyzed in terms of cell growth replication. It was found that MP at 15 µg/L significantly affected *A. calcoaceticus* cell replication by reducing to half the maximum specific growth rate, μ_m (h^{-1})(Figure 2 and Table 3).

Table 3. Kinetic parameters obtained from the Gompertz model for both unexposed and 15 µg/L MP-exposed bacteria.

	A. calcoaceticus		*S. maltophilia*	
	Control	MP	Control	MP
A_{max}	0.9±0.01	1.0±0.02	0.9±0.01	0.9±0.008
μ_m (h^{-1})	0.1±0.006*	0.05±0.002*	0.1±0.006	0.1±0.005
λ (h)	0.2±0.2	0.50±0.3	1.9±0.2	1.9±0.2
r^2	0.9271	0.9667	0.9445	0.9686
t_d (h)	13	14	6.0	6.0

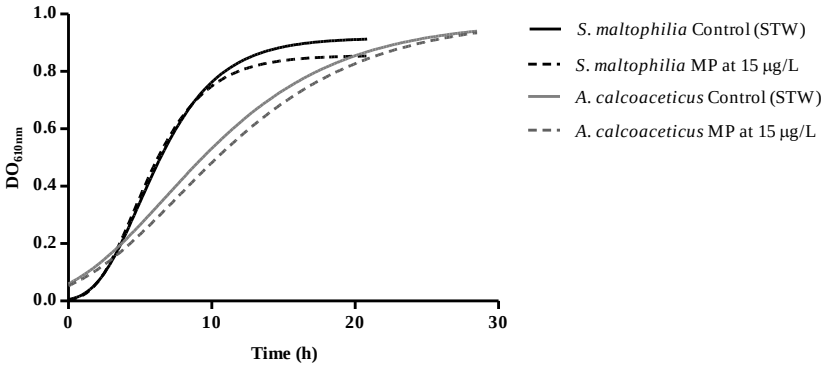

Figure 2. Planktonic growth curves adjusted to the Gompertz model of MP unexposed (control) and MP-exposed bacteria cells from dual-species biofilms.

4. Conclusions

The exposure to parabens affected the cell envelope (increasing the hydrophilicity) of DW bacteria, even at residual concentrations (15 µg/L). Among the different parabens tested, MP and BP were the most relevant in inducing modifications in the DW bacteria. In addition, bacteria from MP-exposed biofilms had a slower growth rate. Moreover, MP increased the metabolic activity of bacteria from DW dual-species biofilms formed on HDPE and PPL, being this increase more pronounced on PPL. Therefore, PPL is the most critical material to be used in DW plumbing systems contaminated with parabens.

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