Foods 2023
The 4th International Electronic Conference on Foods
15–30 October | Online

Investigating the inhibitory effect of lactic acid on biofilm production by raw chicken *Campylobacter* spp. isolates in pure and mixed cultures

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Introduction & Study Aim

***Campylobacter* spp.** are the main cause of foodborne gastroenteritis worldwide with broiler chickens and their products being their main reservoirs. An important role in the survival and eventual dominance of these bacteria against other pathogens is believed to be played by their ability to attach to food-related surfaces and be included in multi-species **biofilms**.

During food animal processing organic acids such as **lactic acid (LA)** may be used to remove pathogens from carcasses and decrease their microbial load.

- **The purpose of this study was to investigate the inhibitory effect of LA against planktonic and biofilm growth of *Campylobacter* raw chicken isolates. Biofilm growth was tested in both mono-cultures and mixed-cultures.**

Methods

- Initially, the **minimum inhibitory and minimum bactericidal concentrations (MICs** and **MBCs**, respectively) of LA were determined against each planktonic *Campylobacter* isolate (**Table 1**), through the broth microdilution and agar spot assays, respectively.
  - For this purpose, two different nutrient broths were used (i.e., **Muller – Hinton (MH) broth with or without supplementation with laked horse blood (HB)**).
  - For each broth, seven different concentrations of LA were tested ranging from **4,096 to 64 μg/mL** (two-fold dilutions).
- Subsequently, **the minimum biofilm inhibitory concentrations (MBICs)** of LA against the biofilm growth of each **individual isolate** and three different **mixed *Campylobacter* cultures (consortia)**, each composed of three isolates (**Table 2**) were determined, through the **crystal violet assay on 96-well polystyrene (PS) microtiter plates**.
  - Biofilm growth was tested in MH broth supplement with 5% v/v chicken juice (CJ)
  - Nine different concentrations of LA were tested ranging from **16,384 to 64 μg/mL** (two-fold dilutions).
- Lastly, the **inhibitory effect** of LA on the development of a **mixed-culture biofilm** composed of three isolates (i.e., CAMP_130-083-048; CONS1) was determined using 6-well PS microplates and stainless steel (SS) coupons as the abiotic substrata.
  - Four different concentrations of LA were tested ranging from **4,096 to 512 μg/mL**.
  - The quantification of planktonic and biofilm cells at each LA treatment was done by plate counting (the colonies of each isolate differed macroscopically) (**Figure 1**).

Table 1. *Campylobacter* raw chicken isolates used in this study and their relevant info

Isolate code	Species	Other Information	Poultry isolation origin
CAMP[1]_005	*C. coli*	strong BP[2], MDR[3]	wings
CAMP_022	*C. jejuni*	strong BP, MDR	minced meat
CAMP_025	*C. coli*	strong BP, MDR	neck
CAMP_048	*C. jejuni*	strong BP	souvlaki
CAMP_083	*C. coli*	weak BP, high resistance to ERY[4]	thigh
CAMP_091	*C. jejuni*	weak BP, high resistance to ERY	wings
CAMP_114	*C. jejuni*	moderate BP, MDR	neck
CAMP_130	*C. jejuni*	weak BP, MDR	wings

[1] *Campylobacter*; [2] Biofilm producer; [3] Multidrug resistance; [4] Erythromycin

Table 2. The three different *Campylobacter* consortia, each composed of three isolates. The six different isolates of these consortia were divided into three different groups (A-C) depending on their drug resistance and biofilm-forming phenotypes.

Consortium code	Group A[1]	Group B[2]	Group C[3]
CONS1	CAMP_130	CAMP_083	CAMP_048
CONS2	CAMP_130	CAMP_091	CAMP_022
CONS3	CAMP_130	CAMP_083	CAMP_005

[1] MDR; [2] No MDR, with high-level resistance to ERY; [3] Strong biofilm producing capacity

Figure 1. Image of the colonies on MH-HB agar of the three *Campylobacter* isolates (CAMP_130, CAMP_083, and CAMP_048) that were used for the development of CONS1 mixed-culture biofilm.

Results

Table 3 presents the results of MIC/MBC/MBIC determination for each one of the eight *Campylobacter* isolates.

For almost all the *Campylobacter* isolates, the MICs of LA were equal to the MBCs, indicating its strong bactericidal action.

- The **MBIC results** do not reveal any relationship between biofilm-forming capacity (weak, moderate, strong) of a given isolate and LA biofilm-inhibitory action against it. However, these denote the favouring effect of strain interactions on the ability of mixed-cultures to develop biofilms in the presence of LA ($MBIC_{consortium} > MBIC_{ind.\ Isolate}$).

Figure 1 presents the biofilm populations (Log10CFU/mL) for each one of the three isolates (CAMP_130, CAMP_083 and CAMP_048) of the mixed *Campylobacter* culture CONS1, on either PS (6-well microplates) or SS (coupons) surfaces.

- For both surfaces CAMP_130 and CAMP_083 isolates appeared to dominate over CAMP_048 isolate at the two highest LA concentrations that were applied (2,048 and 4,096 μg/mL), probably due to the higher MBIC of LA against them.

Table 3. MIC, MBC and MBIC values of LA against the eight *Campylobacter* isolates and the three different consortia.

Campylobacter /Consortium code	Species/Isolates	MIC[2] in MH[5]	MBC[3] in MH[5]	MIC μg/mL in MH -HB[6]	MBC μg/mL in MH -HB[6]	MBIC[4] in MH -CJ[7]
CAMP[1]_005	*C. coli*	1,024	1,024	2,048	2,048	1,024
CAMP_022	*C. jejuni*	1,024	1,024	2,048	2,048	1,024
CAMP_025	*C. coli*	1,024	1,024	1,024	1,024	1,024
CAMP_048	*C. jejuni*	2,048	2,048	2,048	2,048	1,024
CAMP_083	*C. coli*	1,024	2,048	2,048	2,048	2,048
CAMP_091	*C. jejuni*	2,048	2,048	2,048	2048	1,024
CAMP_114	*C. jejuni*	1,024	1,024	2,048	2,048	1,024
CAMP_130	*C. jejuni*	1,024	1,024	2,048	2,048	2,048
CONS1	CAMP_048/083/130					4,096
CONS2	CAMP_022/091/130					4,096
CONS3	CAMP_005/083/130					4,096

[1] *Campylobacter*; [2] Minimum Inhibitory Concentration; [3] Minimum Bactericidal Concentration; [4] Minimum Biofilm Inhibitory Concentration; [5] Muller – Hinton broth; [6] MH with 5% v/v laked horse blood; [7] MH broth with 5% v/v chicken juice.

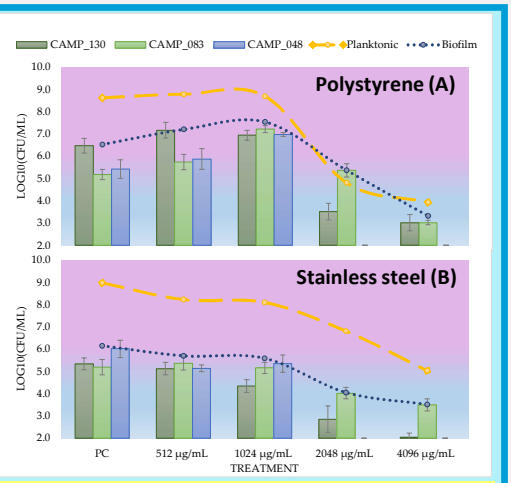

Figure 2. Biofilm populations (log_{10} CFU/mL) for each isolate of the mixed *Campylobacter* culture (two *C. jejuni* isolates, i.e., CAMP_130 and CAMP_048, and one *C. coli* isolate, i.e., CAMP_083) on the PS surface of the 6-well microplates **(A)** and the SS surface of the coupons **(B)**, in the presence of four different LA concentrations (two-fold dilutions ranging from 4,096 to 512 μg/mL). The biofilm populations of the positive control (PC; without LA treatment) are also shown. The total biofilm populations for each treatment are also shown as blue dots (dotted curved line), while the total planktonic populations found in the wells/tubes at the time of sampling (48 h) are also shown for each treatment (as yellow dotted curved lines).

Conclusion

Overall, the results of this work offer insight into biofilm control of a pathogen of public health importance.

Acknowledgements: Support for this study was provided by the research program "AGRICA II: AGrifood Research and Innovation Network of ExCellence of the Aegean" (MIS 5046750), ESPA 2014-2020, In the context of the call of the Operational Program "Competitiveness, Entrepre-neurship and Innovation", Action "Support of Regional Excellence".