INHIBITOR ACTIVITY OF LACTIPLANTIBACILLUS PLANTARUM LP5 ON THERMOTOLERANT CAMPYLOBACTER WITH DIFFERENT BIOFILM-FORMING CAPACITIES

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Running Header: TC biofilm reduction by *L. plantarum* LP5

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Abstract

Aims

To evaluate the biofilm-forming capacity of thermotolerant *Campylobacter* (TC) strains from poultry production and to analyse the inhibitory capacity of *Lactiplantibacillus plantarum* LP5 against TC on different materials.

Methods and Results

Biofilm-forming capacity by *Campylobacter jejuni* and *Campylobacter coli* was analysed by cell adhesion in polystyrene plates. TC were classified as non-biofilm-forming (NBF, 1.3%), weak biofilm-forming (WBF, 68.4%), moderate biofilm-forming (MBF, 27.6%), and strong biofilm-forming (SBF, 2.7%). The inhibitory capacity of *L. plantarum* LP5 against TC was tested on stainless-steel, nylon, aluminium, and glass disks (treated group) and compared with biofilm-forming TC (control group). *L. plantarum* LP5 was inoculated, and then TC. Biofilm was removed in both experimental groups and TC and LP5 bacterial counts were performed. The *L. plantarum* LP5 presence reduced the formation of TC biofilm ($p<0.001$). The material type and strain category influenced biofilm formation, with stainless-steel and the SBF strain being the material and TC having the highest adhesion ($p<0.001$). *L. plantarum* LP5 formed similar biofilm on all materials ($p=0.823$).

Conclusions

This trial showed very promising results; *L. plantarum* LP5 could be incorporated as a bio-protector of TC on different surfaces.

Significance and Impact of Study

Biofilm in poultry production is a risk factor due to the possible contamination of food with pathogens. The competitive exclusion principle using *L. plantarum* LP5 is a promising approach to colonize surfaces and counteract pathogens.

Introduction

Thermotolerant *Campylobacter* (TC) integrates the microbiota of the gastrointestinal tract of food-producing animals, mainly poultry (Rossler et al., 2019). Poultry production is one of the most important economic activities in the world. Affordable prices and practicality in poultry meat preparation continue to gain market share. However, the transmission of pathogens through the poultry production chain is a crucial food safety concern for this industry. For this reason, the growing production and commercialization have promoted a strict monitoring of animal health and quality control of poultry meat products (Monteiro et al., 2021). *Campylobacter* infections in humans occur directly through human contact with animal faeces, or indirectly through consumption of undercooked animal foods or cross-contamination with ready-to-eat foods (Reeser et al., 2007). Although TC does not survive in atmospheric air for more than a few hours, it is believed that it develops adaptation strategies in these conditions, such as biofilm-forming (Sulaeman et al., 2010). Biofilm and aggregation of TC that form in a microaerobic environment has been shown to allow the bacteria to survive under aerobic conditions (Kim et al., 2021). A biofilm is a structured aggregation of cells in a heterogeneous extracellular polymeric matrix attached to a solid surface. Biofilm can form on different materials such as glass, nylon, aluminium, wood, plastic, stainless-steel, and even on food, and can persist after cleaning and disinfection processes (Cáceres et al, 2019). Biofilm provides protection against sanitizing agents, improves survival, and optimizes microbial growth. It is truly a resilient microbial attribute. TC can form biofilm on animal husbandry equipment, facilities and processing plants (Sharan et al. 2022; Reeser et al., 2007). This impact is even more important for public health since the indiscriminate use of antibiotics in poultry production has motivated the search for natural alternatives to control

these pathogens in feed (Monteiro et al., 2021). The appearance of resistant pathogenic bacteria through the food chain has reinforced the need to investigate various alternatives for the disinfection of surfaces or equipment in the food industry using natural products. Research with lactic acid bacteria (LAB) has shown an inhibitory or reducing effect on microbial consortia of Gram-negative bacteria (Castellano et al., 2017). The inhibition of the growth and adhesion of pathogenic bacteria may be due to the production of bacteriocins, to a combination of factors such as the production of biosurfactants and bacteriocins, and/or to mechanisms of exclusion generating the death of cells embedded in biofilm. The biofilm formed by LAB can be used to reduce pathogens (Gómez et al., 2016). Competition for adhesion sites and nutrients between pathogenic microorganisms and LAB reduces the proliferation and establishment of pathogen biofilm (Jalilsood et al., 2015). The application of a protective biofilm, as a biological control tool based on the competitive exclusion principle, which acts on the main agents of zoonotic diseases in poultry and the food industry becomes a potential tool for the prevention of foodborne diseases. This study aimed to evaluate the biofilm-forming capacity of *Campylobacter coli* and *Campylobacter jejuni* strains from different origins in poultry production and different PFGE profiles, and to analyse the inhibitory ability of *Lactiplantibacillus plantarum* LP5 against TC strains biofilm formation on different materials by a competitive exclusion assay.

Materials and methods

Bacterial strain selection and growth conditions

The TC strains selected to evaluate their biofilm-forming capacities are part of the strain collection of the Food Analysis Laboratory, ICIVET, UNL. A total of 76 strains were used, 40 corresponding to the *C. jejuni* species and 36 to the *C. coli* species. The selection was made considering different PFGE profiles and the same origin (broilers), and the same PFGE

profile and different origins: 82.9% corresponded to the farm (broilers, wild birds like *Passer domesticus*, flies, *Alphitobius diaperinus* larvae and adult, feed, boots and litter), 11.8% to the slaughterhouse (carcass, cecum, evisceration knife, worker's hands, surface of post-chiller area), 3.9% to the human clinical cases and 1.3% to the reference strain (Zbrun et al., 2017; Zbrun et al., 2021). All strains were activated on plates with Charcoal Cefoperazone Desoxycholate Agar medium (CCDA, Oxoid, United Kingdom) for 48 h at 42°C under microaerophilic conditions using an anaerobic jar (Anaero jar 3.5 l HP0011, Oxoid, UK) and mixed gases ($H_2:CO_2:O_2$=85:10:5).

L. plantarum LP5 of porcine origin was previously identified by 16S ribosomal Sanger sequencing and selected for its *in vitro* and *in vivo* antibacterial activity against pathogens involved in foodborne outbreaks, including *C. coli* (Ruiz et al, 2021). *L. plantarum* LP5 has shown biofilm-forming at room temperature (RT) and under refrigeration conditions (Ruiz et al., 2019), and inhibitory capacity against *C. coli* in assays for the formation of *in vitro* biofilm and competitive exclusion (Ruiz et al., 2022). *L. plantarum* LP5 was activated in de Man, Rogosa and Sharpe broth (MRS, Biokar, France) for 24 h at 37°C in aerobiosis.

All strains were preserved in cryoprotective media at -80°C.

Biofilm-forming by TC

The biofilm production quantification was carried out according to Reeser (2007), with some modifications. The TC strains stored at -80°C were cultivated in 3 ml of Muller Hinton (MH) broth for 48 h at 42°C under microaerophilic and static conditions (Ruiz et al., 2022). The cultures were then standardized to an optical density (OD_{600}) of 0.25±0.05. In 96-well polystyrene microtiter plates (Cat 3599 Corning®, USA), 200 µl of MH broth (Biokar, France) and 20 µl of each bacterial suspension in MH (Biokar, France), adjusted to optical density, were placed. The plates were incubated in microaerophilic conditions for 72 h at

42°C. The medium was removed from the wells and the adhered bacteria were fixed with 200 µl of methanol (Anedra, Argentina) for 15 min. The plates were emptied and dried at RT. Each well was added with 200 µl of a 0.2% (w v^{-1}) crystal violet solution (Anedra, Argentina) and kept at RT for 5 min. The dye was removed by gently rinsing with tap water, and the adherent stain cells were released with 200 µl of 33% (v v^{-1}) glacial acetic acid (Anedra, Argentina). Finally, the OD_{600} of each well was measured using a plate reader (CLARIOstar®Plus, BMG LABTECH, Argentina). The negative control was performed in wells containing MH broth (Biokar, France) without TC inoculation. The mean OD value of 3 replicates performed in triplicate was used to establish the different cut-off points, called optical density cut-off (ODc). The results obtained allowed the strains to be classified as four categories: non-biofilm-forming (NBF): OD≤ODc, weak biofilm-forming (WBF): ODc<OD≤2×ODc, moderate biofilm-forming (MBF): 2×ODc<OD≤4×ODc, and strong biofilm-forming (SBF): 4×ODc<OD.

Inhibition of biofilm-forming

The ability of *L. plantarum* LP5 to inhibit biofilm-forming of the four different TC biofilm-forming strains (NBF, WBF, MBF and SBF) was evaluated by competitive exclusion test. The methodology of Gómez et al. (2016) with modifications was used. The quantification of the inhibition of biofilm-forming was estimated by viable bacterial counts. This quantification of viable bacterial counts represents the bacterial adhesion to the inert material that is later detached by mechanical action. In four 24-well polystyrene plates, sterile disks of 12 mm diameter and 1 mm thickness of different materials were placed: stainless-steel, nylon, aluminium and glass. The aluminium and nylon disks were cut with a drilling machine, the glass disks, HDA brand, were provided by the Labdiscount company (CABA, Buenos Aires, Argentina), and the stainless-steel disks were supplied by the Acermel SRL company

(Esperanza, Santa Fe, Argentina). All disks were sterilized at 121°C for 15 min. Each plate was used to analyse one material type and two experimental groups: the control group (wells inoculated only with TC) and the treated group (wells inoculated with *L. plantarum* LP5 and TC). The wells used to evaluate the treatment with *L. plantarum* LP5 were added with 2 ml of MRS broth (Biokar, France) and 200 µl of *L. plantarum* LP5 at OD_{600} of 0.25±0.05. The plates were incubated at 37°C for 72 h. The broths were discarded with a pipette, and each well was washed with 2 ml of sodium chloride (NaCl, Biopack, Argentina) at 0.85% (w v^{-1}) to eliminate planktonic bacteria. Subsequently, 2 ml of MH (Biokar, France) and 200 µl of the suspensions of the different TC strains adjusted to 0.25±0.05 (OD_{600}) were added to each well. The plates were incubated at 42°C for 72 h under microaerophilic conditions. The wells were washed with 2 ml of NaCl solution (Biopack, Argentina) at 0.85% (w v^{-1}). Each disk was removed with sterile forceps and placed in a 50 ml tube with 5 ml of NaCl solution (Biopack, Argentina) at 0.85% (w v^{-1}). The tubes were shaken for 2 h at a speed of 250 rpm in a Shaker (SK L330-Pro, DragonLab, Argentina) at RT. Subsequently, decimal dilutions were made in a NaCl solution (Biopack, Argentina) at 0.85% (w v^{-1}) and spread on CCDA agar (Oxoid, United Kingdom) and MRS agar (Biokar, France) for TC and *L. plantarum* LP5 counts, respectively. After incubation for 48 h at 42°C in microaerophilic conditions and for 72 h at 37°C in aerobiosis, respectively, bacterial counts were performed and expressed as $log_{10}CFUdisk^{-1}$ (Figure 1). In this sense, adhesion to the surface of each material was estimated by bacterial counts that remained attached to the disk and later removed and spread onto the corresponding culture media.

Finally, the results were shown by firstly considering the influence of the biofilm-forming strain category regardless of the type of material, and secondly by considering the type of material regardless of the biofilm-forming strain category.

Statistical analysis

The biofilm-forming inhibition assay was performed three times in triplicate. TC counts in both experimental groups were analysed using a factorial ANOVA with a *post hoc* Tukey Test to determine the influence of *L. plantarum* LP5 and different material surfaces against TC strains. Also, an ANOVA analysis was performed to determine differences between the biofilm formation of *L. plantarum* LP5 on different material types. The differences were considered significant for a value of $p \leq 0.05$.

Results

TC biofilm-forming capacities

Within the classification of four categories of biofilm-forming capacity, the strains were in the following proportions: 1.3% for NBF, 68.4% for WBF, 27.6% for MBF and 2.7% for SBF. The mean DOc value was 0.064. The mean OD values for each TC are shown in Table 1.

TC adhesion to different material surfaces

The TC biofilm count formed on all material surfaces is shown in Table 2.

Regardless of the material used, strains DSPV1214 (SBF) and DSPV354 (NBF) showed higher and lower adhesion, respectively. The DSPV602 (WBF) and DSPV458 (MBF) strains showed intermediate values of biofilm quantification (Table 2).

Regardless of the material used, stainless-steel showed greater TC adhesion than the rest of the materials. On nylon, strains DSPV602 (WBF), DSPV458 (MBF) and DSPV354 (NBF) adhered similarly to each other but showed less adhesion ($p=0.004$) than strain DSPV1214 (SBF). On aluminium, strains DSPV602 (WBF) and DSPV354 (NBF) adhered similarly to each other but showed less adhesion ($p=0.004$) than strains DSPV458 (MBF) and DSPV1214 (SBF). On glass, the strains of the four categories adhered differently among them ($p<0.001$).

L. plantarum LP5 inhibited TC biofilm

The competitive exclusion assay was performed with four TC strains corresponding to each of the biofilm-forming categories. The selected strains were *C. jejuni* DSPV354 as NBF, *C. coli* DSPV602 as WBF, *C. coli* DSPV458 as MBF, and *C. coli* DSPV1214 as SBF.

The results of the competitive exclusion test on the different materials are shown in Figure 2. The adhesion of *C. jejuni* DSPV354, *C. coli* DSPV602, *C. coli* DSPV458, and *C. coli* DSPV1214 on all material types was significantly lower when *L. plantarum* LP5 biofilm was present ($p<0.001$).

Material type influence on TC biofilm-forming and reduction

Both in the treated group and the control group, the material type was found to be a significant factor in the capacity for biofilm-forming by TC ($p<0.001$). In the treated group, the TC strains showed highest adhesion to stainless-steel (5.35 $\log_{10}$CFUdisk^{-1}), followed by nylon (2.47 $\log_{10}$CFUdisk^{-1}), aluminium (1.37 $\log_{10}$CFUdisk^{-1}), and glass (0 $\log_{10}$CFUdisk^{-1}). In the control group, all TC also showed higher adhesion to stainless-steel (8.52 $\log_{10}$CFUdisk^{-1}), but followed by aluminium (7.82 $\log_{10}$CFUdisk^{-1}) and then nylon (7.03 $\log_{10}$CFUdisk^{-1}) and glass (7.07 $\log_{10}$CFUdisk^{-1}) (Table 3).

In the treated group, the TC strains corresponding to the different categories with biofilm-forming capacity (NBF, WBF, MBF and SBF) showed similar adhesion to all types of materials studied ($p=0.82$), showing a range of values between 1.77 $\log_{10}$CFUdisk^{-1} and 2.92 $\log_{10}$CFUdisk^{-1}. Conversely, in the control group, the adhesion of *C. coli* DSPV1214 (SBF category) was significantly higher than the adhesion of the MBF, WBF, and NBF strains on the different material types ($p<0.001$), showing counts of 8.5 $\log_{10}$CFUdisk^{-1} *vs.* 7.6 $\log_{10}$CFUdisk^{-1}, 7.4 $\log_{10}$CFUdisk^{-1}, and 7.0 $\log_{10}$CFUdisk^{-1}, respectively (Table 3).

L. plantarum LP5 adhesion to different material surfaces

The adhesion of *L. plantarum* LP5 in the presence of the different strains of TC, on the disks of the different materials is shown in Figure 2. The adhesion of *L. plantarum* LP5 ranged from 5.67 logCFUdisco^{-1} on glass disks to 8.21 logCFUdisco^{-1} on disks of the same material. The *L. plantarum* LP5 adhesion on the stainless-steel, nylon, aluminium and glass disks did not show differences (p<0.001).

Discussion

Thermotolerant *Campylobacter* is an indigenous inhabitant of poultry gastrointestinal microbiota. A high proportion of broilers contain TC pathogens, which increases the possibility of equipment contamination during the slaughterhouse processes. Contamination of poultry carcasses occurs from the feathers and the gastrointestinal tract during the slaughter process. Compared to many other foodborne diseases-causing pathogens, TC is more sensitive to environmental conditions such as partial pressure of atmospheric oxygen, temperatures below 30°C, and dry environment, among others. These properties make the survival of TC outside the host difficult in natural aerobic environments such as in the agri-food chain. However, TC is widespread in the environment and can be isolated from food, water, and other sources. While it is unclear how TC overcomes these disadvantages to survive in the food chain environment to cause disease, its ability to form biofilm could explain its survival in food processing environments (Teh et al., 2014). Biofilm-forming by TC on abiotic surfaces is one of the least understood factors (Reeser et al., 2007; Ma et al., 2022). However, simple adhesion to surfaces and the presence of biofilm of other microbial species contribute to the survival of TC in food-related environments (Karki et al., 2021). In this study, the highest proportion of TC strains (96%) was categorized as WBF and MBF.

These results differed from those of Sulaeman et al. (2009), who detected mostly NBF (58%), to a lesser extent WBF and MBF (29% and 12%, respectively), and no SBF. Although our study showed that most TC strains form biofilm to a greater or lesser extent, it has been described that this capacity for formation is specific to each strain and, therefore, cannot be predicted only by knowing the species identity (Gunther et al., 2009). In our study, this concept was demonstrated at least for the *C. jejuni* species, within which we found the four categories of biofilm-producing strains. Although the wide variety of origin of the strains studied could explain their ability to adapt to different environments, most of them were classified by the method in both intermediate categories. That is, a similar response was found for most of the biofilm-forming strains, even though they come from a variety of ecosystems. Biofilm-forming capacity is strongly associated with unfavourable environmental conditions. Several investigations have shown that TC could form biofilm on abiotic or inert surfaces, such as glass, stainless-steel, plastic and nitrocellulose (Kalmokof et al., 2006; Gunther et al., 2009). The ability of the *Campylobacter* species to form biofilm on different surfaces has been reported to be variable (Gunther et al., 2009). In general, it is accepted that bacteria adhere more easily to hydrophobic surfaces (Teixeira et al, 2005). Of the three materials studied by Gunther et al. (2009), most of the *Campylobacter* species was found to be able to adhere to stainless-steel surfaces. Stainless-steel also had the advantage of being the roughest of the three surfaces investigated. However, the glass surface, which is the most hydrophilic of the three, supported extremely repeatable adhesion of *Campylobacter*. Plastic, which should have a hydrophobic value between stainless-steel and glass, provided a poor surface for *Campylobacter* attachment. In that study, the four-category model allowed the quantification of the biofilm formed by all the strains, thus being able to discriminate their behaviour on all the surfaces studied. Regardless of the type of material used in our model, the SBF capacity of the TC strain had a great implication in the biofilm formation adhesion to

inert materials. Stainless steel allowed greater adhesion than the rest of the materials. A higher proportion of the strains adhered beyond the biofilm category in which they were classified. However, the adhesion response of the strains to glass was different because it showed a more heterogeneous behaviour, where the different categories are expressed in different ways. Stainless-steel is the most popular food contact material in the food industry since it is chemically inert, easy to clean, and extremely resistant to corrosion over a range of processing temperatures (Carrascosa et al., 2014). Some studies have shown that bacterial adhesion is more likely to occur on rougher surfaces (Tang et al., 2011; Dhowlaghar et al., 2018), while others have found no association between roughness and bacterial adhesion (Jindal et al., 2018). In general, hydrophobic surfaces tend to attract more bacteria, but experiments that have tested the effect of hydrophobicity present opposite results (Gomes et al., 2015, Veluz et al., 2012). Other studies indicate that hydrophilic surfaces allow greater bacterial adhesion than hydrophobic equivalents (Dhowlaghar et al., 2018, Jindal et al., 2018). The diversity of methods, laboratory conditions and bacterial strains used could explain this lack of concrete results regarding bacterial adhesion. All this makes it interesting for future studies on variations of environmental factors.

LAB have great potential for use in bio-preservation because most of them are safe to consume and, during storage, they naturally dominate the microbiota of fermented foods (Castellanos et al., 2008). However, the inhibitory capacity of LAB biofilm against potential TC adhesion has been little studied, and reported studies focus on *C. jejuni* or other foodborne pathogens (Monteiro et al., 2021; Pang et al., 2022). Our study advances the understanding of biofilm formed by both *C. jejuni* and *C. coli* strains on different surface materials that are commonly used by the food industry. The models developed for the use of LAB biofilm in the control of other pathogens involved in foodborne diseases have used zoonotic pathogens such as *Listeria monocytogenes*, *Bacillus cereus*, *Escherichia coli*, *Salmonella* serovar

Typhimurium and *Pseudomonas aeruginosa* (Guerrieri et al., 2009; Gómez et al., 2016; Shokri et al., 2018). Bio-preservation is a relatively recent technology based on the incorporation of live bacteria to the surface of the foods that antagonize the unwanted ones (pathogenic and/or spoilage microorganisms), thus extending their shelf life. *L. plantarum* LP5 was able to form biofilm on glass, nylon, and aluminium surfaces and inhibit TC biofilm-forming by competitive exclusion. Regarding the materials studied, TC presented greater adhesion in stainless-steel in both the control and treated groups. The presence of *L. plantarum* LP5 reduced the biofilm-forming capacity of the TC of the four categories and in all the materials used. The materials studied were not a factor influencing the biofilm-forming capacity of *L. plantarum* LP5.

Regarding the TC strains studied, although the *C. coli* DSPV1214 strain (SBF) presented greater adhesion capacity compared to the other categories, this capacity is reduced in the presence of *L. plantarum* LP5 in the same way as it occurs in the other categories. Stainless-steel favoured the formation of TC biofilm, and the inhibition capacity of *L. plantarum* LP5 was reduced on this surface compared to the other materials. The material was an influential factor in the biofilm-forming capacity of the TC strains, but not for the *L. plantarum* LP5 strain. This fact is interesting because *L. plantarum* LP5 could be used as a competitive exclusion tool on different material surfaces.

This trial showed very promising results; therefore, *L. plantarum* LP5 could be incorporated as a bio-protector of TC on different surfaces, such as carcass surfaces. However, additional studies designed for this purpose should be carried out to test this hypothesis. Although more tests are needed to confirm the resistance capacity of the TC species in hostile environments until they reach the host, this study allows us to further clarify the behaviour of these strains through the formation of biofilm. However, given that our tests were carried out under optimal growth conditions for TC, the results obtained will be the basis for designing new

studies that propose modifications in the intrinsic and extrinsic parameters that can facilitate/inhibit the biofilm formation process. Although refrigeration temperature is a stressor and can negatively influence TC survival during processing and storage, these pathogens can survive and spread through the food chain. Although *C. jejuni* has been shown to be more resistant to cold and other stress factors than *C. coli*, neither the resistance/tolerance of *C. coli* in complex structures, such as biofilm, nor a significant proportion of foodborne diseases caused by *C. coli* is fully understood (Karki et al., 2018). This should encourage researchers and the industry to generate new pre-slaughter strategies to reduce the prevalence and concentration of TC in the digestive tract of birds and new biofilm inactivation processes to decrease the prevalence of TC in poultry carcasses.

Conflict of interest

There is no conflict of interest.

Data Availability Statement

Data available on request.

Funding

Ethical Approval

The study does not imply ethical approval.

Authors' contributions

MJR and LSF designed the experiment; MJR, MAS, CRO, MLW, and FFA carried out the in vitro experiments; NES performed the statistical analyses; MLS and LSF contributed project administration, resources and supervision; MJR and LSF carried out validation, writing, review and editing of manuscript; all authors reviewed this manuscript.

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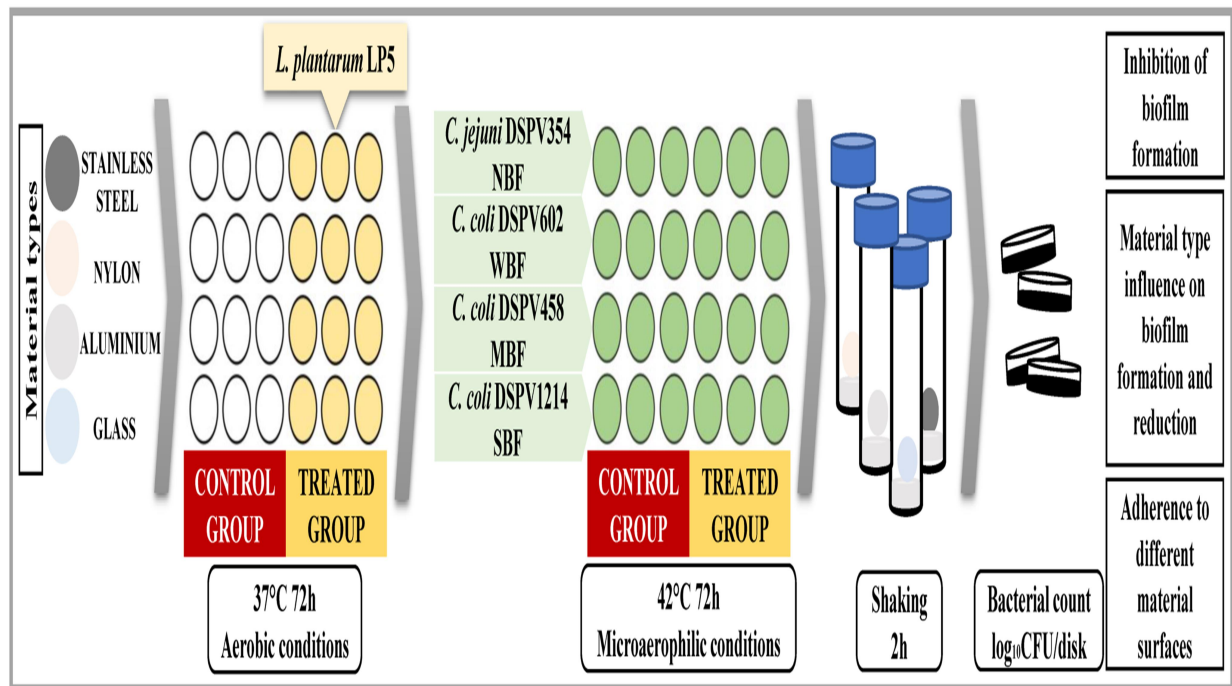
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Figure 1: Experimental design of biofilm-forming inhibition assay

NBF: non-biofilm-forming; WBF: weakly biofilm-forming; MBF: moderately biofilm-forming; SBF: strongly biofilm-forming.
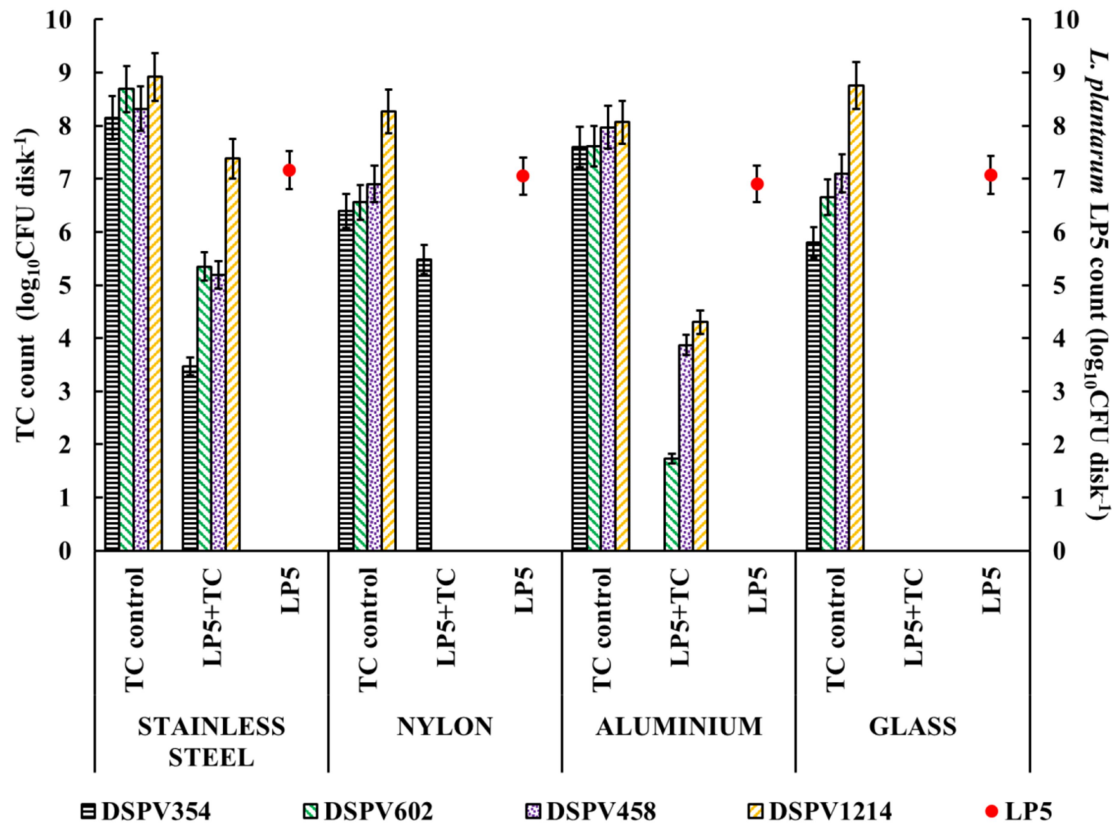
Figure 2: TC quantification on different untreated and *L. plantarum* LP5-treated material surfaces.

NBF: non-biofilm-forming; WBF: weakly biofilm-forming; MBF: moderately biofilm-forming; SBF: strongly biofilm-forming.

Error Bars indicate the standard deviation.

Bars indicate the count of each TC ($\log_{10}$CFUdisk^{-1}) shed from disks inoculated with TC alone (TC Control), and from disks inoculated with TC on *L. plantarum* LP5 biofilm (LP5 + TC) formed on material surfaces.

Dots indicate the count of *L. plantarum* LP5 ($\log_{10}$CFUdisk^{-1}) shed from the *L. plantarum* LP5 biofilm formed on different material surfaces, where each strain of TC (LP5) had also been inoculated.

Table 1: Biofilm-forming capacity by TC strains.

TC species	Strain	PFGE profile*	Source (sample)	Biofilm growth rate (OD$_{600}$±SD)	Biofilm forming category
C. jejuni	DSPV263	c	Farm (*A. diaperinus* larvae)	0.087±0.0040	WBF
C. jejuni	DSPV264	c	Farm (*A. diaperinus* adult)	0.105±0.0100	WBF
C. jejuni	DSPV 272	c	Slaughterhouse (carcass)	0.100±0.0050	WBF
C. jejuni	DSPV 337	c	Farm (broilers)	0.101±0.0035	WBF
C. coli	DSPV 344	j	Farm (broilers)	0.073±0.0030	WBF
C. coli	DSPV 371	j	Slaughterhouse (carcass)	0.081±0.0060	WBF
C. coli	DSPV 380	j	Slaughterhouse (evisceration knife)	0.095±0-0050	WBF
C. coli	DSPV 433	q	Slaughterhouse (cecum)	0.220±0.0100	MBF
C. coli	DSPV 458	q	Farm (fly)	0.155±0.0132	MBF
C. coli	DSPV 464	q	Slaughterhouse (workers' hands)	0.093±0.0171	WBF
C. coli	DSPV 473	q	Farm (broilers)	0.100±0.0120	WBF
C. jejuni	DSPV 476	m	Farm (*P. domesticus*)	0.103±0.0151	WBF
C. jejuni	DSPV 492	m	Farm (boots)	0.102±0.0032	WBF
C. jejuni	DSPV 517	m	Slaughterhouse (carcass)	0.099±0.0115	WBF
C. jejuni	DSPV 527	m	Slaughterhouse (workers' hands)	0.104±0.0076	WBF
C. jejuni	DSPV 534	m	Slaughterhouse (cecum)	0.108±0.0043	WBF
C. coli	DSPV 555	j	Slaughterhouse (cecum)	0.201±0.0090	MBF
C. jejuni	DSPV 586	I	Farm (*P. domesticus*)	0.110±0.0048	WBF
C. jejuni	DSPV 597	D	Farm (litter)	0.120±0.0145	WBF
C. jejuni	DSPV 604	I	Farm (*A. diaperinus* larva)	0.197±0.0080	MBF
C. jejuni	DSPV 619	I	Farm (broilers)	0.187±0.0164	MBF
C. jejuni	DSPV 733	D	Farm (broilers)	0.104±0.0101	WBF
C. jejuni	DSPV 738	D	Farm (*A. diaperinus* larva)	0.195±0.0127	MBF
C. coli	DSPV 1181	H	Farm (broilers)	0.677±0.0539	MBF
C. coli	DSPV 1184	H	Farm (litter)	0.126±0.0071	WBF
C. coli	DSPV 1201	H	Farm (boot)	0.174±0.0156	MBF
C. coli	DSPV 1202	H	Farm (*A. diaperinus* larva)	0.224±0.0123	MBF
C. coli	DSPV 1203	H	Farm (*A. diaperinus* adult)	0.227±0.0176	MBF
C. coli	DSPV 1208	G	Farm (litter)	0.105±0.0171	WBF
C. coli	DSPV 1211	G	Farm (boot)	0.111±0.0162	WBF
C. coli	DSPV 1214	G	Farm (fly)	0.334±0.0110	SBF
C. coli	DSPV 1215	G	Farm (broilers)	0.147±0.0135	WBF
C. coli	DSPV 1216	G	Farm (feed)	0.174±0.0149	MBF
C. jejuni	CH3	c	Human clinic case	0.292±0.0227	SBF
C. jejuni	206	ND	Human clinic case	0.084±0.0250	WBF
C. jejuni	209	ND	Human clinic case	0.108±0.0189	WBF
C. jejuni	DSPV 252	a	Farm (broilers)	0.095±0.0108	WBF
C. jejuni	DSPV 269	c	Farm (broilers)	0.087±0.0089	WBF
C. jejuni	DSPV 270	i	Farm (broilers)	0.115±0.0101	WBF
C. jejuni	DSPV 310	r	Farm (broilers)	0.165±0.0192	MBF
C. coli	DSPV 312	e	Farm (broilers)	0.103±0.0116	WBF
C. coli	DSPV 320	h	Farm (broilers)	0.101±0.0177	WBF
C. coli	DSPV 322	b	Farm (broilers)	0.084±0.0104	WBF
C. coli	DSPV 333	i	Farm (broilers)	0.096±0.0094	WBF
C. coli	DSPV 346	j	Farm (broilers)	0.065±0.0041	WBF
C. jejuni	DSPV 354	d	Farm (broilers)	0.061±0.0096	NBF
C. coli	DSPV 358	k	Farm (broilers)	0.096±0.0161	WBF
C. jejuni	DSPV 383	k	Farm (broilers)	0.106±0.0199	WBF
C. jejuni	DSPV 409	l	Farm (broilers)	0.111±0.0257	WBF
C. coli	DSPV 473	q	Farm (broilers)	0.100±0.0174	WBF
C. coli	DSPV 474	d	Farm (broilers)	0.082±0.0178	WBF
C. jejuni	DSPV 482	f	Farm (broilers)	0.149±0.0097	MBF
C. jejuni	DSPV 485	b	Farm (broilers)	0.084±0.0028	WBF
C. coli	DSPV 493	p	Farm (broilers)	0.080±0.0113	WBF
C. jejuni	DSPV 494	e	Farm (broilers)	0.089±0.0134	WBF
C. jejuni	DSPV 505	h	Farm (broilers)	0.205±0.0179	MBF
C. coli	DSPV 542	n	Farm (broilers)	0.082±0.0118	WBF
C. coli	DSPV 551	l	Farm (broilers)	0.088±0.0230	WBF
C. coli	DSPV 564	m	Farm (broilers)	0.098±0.0157	WBF
C. coli	DSPV 602	I	Farm (broilers)	0.084±0.0042	WBF
C. jejuni	DSPV 611	H	Farm (broilers)	0.105±0.0068	WBF
C. coli	DSPV 613	E	Farm (broilers)	0.086±0.0110	WBF
C. jejuni	DSPV 615	G	Farm (broilers)	0.156±0.0179	MBF
C. coli	DSPV 618	B	Farm (broilers)	0.083±0.0169	WBF

C. coli	DSPV 619	a	Farm (broilers)	0.187±0.0164	MBF
C. coli	DSPV 660	D	Farm (broilers)	0.075±0.0070	WBF
C. coli	DSPV 662	C	Farm (broilers)	0.067±0.0084	WBF
C. jejuni	DSPV 721	I	Farm (broilers)	0.138±0.0135	MBF
C. jejuni	DSPV 732	A	Farm (broilers)	0.112±0.0070	WBF
C. jejuni	DSPV 746	B	Farm (broilers)	0.128±0.0164	MBF
C. jejuni	DSPV 759	D	Farm (broilers)	0.092±0.0115	WBF
C. coli	DSPV 1182	H	Farm (broilers)	0.095±0.0156	WBF
C. jejuni	DSPV 1199	L	Farm (broilers)	0.193±0.0136	MBF
C. coli	DSPV 1215	G	Farm (broilers)	0.146±0.0284	WBF
C. coli	DSPV 1227	J	Farm (broilers)	0.109±0.0109	MBF
C. coli	ATCC33559	ND	Pig	0.126±0,0097	MBF

NBF: non-biofilm-forming; WBF: weak biofilm-forming; MBF: moderate biofilm-forming;

SBF: strong biofilm-forming. ND: no data.

The shaded rows indicate the four selected TC strains corresponding to each category for the

biofilm-forming inhibition assay with *L. plantarum* LP5.

*Capital letters correspond to the PFGE profile used by Zbrun et al. (2017), and lowercase

letters correspond to the PFGE profile used by Zbrun et al. (2021).

Table 2: TC quantification on different material surfaces.

Biofilm-forming categories	TC count ($\log_{10}$CFU/disk^{-1})			
	Material types			
	Stainless-steel	Nylon	Aluminium	Glass
C. jejuni DSPV354 (NBF)	8.15±0.15 [Aa]	6.39±0.06 [Ab]	7.60±0.33 [Ac]	5.80±0.12 [Ad]
C. coli DSPV602 (WBF)	8.69±0.21 [ABa]	6.56±0.75 [Bc]	7.62±0.79 [Ab]	6.65±0.30 [Bc]
C. coli DSPV458 (MBF)	8.32±0.18 [Ba]	6.90±0.13 [Bb]	7.97±0.37 [Ba]	7.10±0.24 [Cb]
C. coli DSPV1214 (SBF)	8.92±0.17 [Ba]	8.27±0.21 [Bb]	8.07±0.47 [Bb]	8.76±0.10 [Da]

NBF: non-biofilm-forming; WBF: weakly biofilm-forming; MBF: moderately biofilm-forming; SBF: strongly biofilm-forming.

Different capital letters within columns indicate differences in adhesion of the different strains in each material ($p < 0.05$).

Different lowercase letters within rows indicate differences in adhesion in the materials for each strain ($p < 0.05$).

Table 3: TC quantification of different biofilm-forming categories in all material types previously treated with *L. plantarum* LP5.

		TC count ($\log_{10}$CFUdisk^{-1})	
		Treated group	Control group
Biofilm formation categories	*C. jejuni* DSPV354 (NBF)	2.24^{Aa}	6.99^{Ba}
	C. coli DSPV 602 (WBF)	1.77^{Aa}	7.38^{Ba}
	C. coli DSPV458 (MBF)	2.27^{Aa}	7.57^{Ba}
	C. coli DSPV1214 (SBF)	2.92^{Aa}	8.51^{Bb}
Material Types	Stainless-steel	5.35^{Ac}	8.52^{Bb}
	Nylon	2.47^{Ab}	7.03^{Ba}
	Aluminium	1.37^{Aab}	7.82^{Bab}
	Glass	0.00^{Aa}	7.07^{Ba}

Means followed by different capital letters within columns of the same row indicate significant differences according to Tukey test (p<0.05).

Means followed by different lowercase letters within row of the same columns indicate significant differences according to Tukey test (p<0.05).

NBF: non-biofilm-forming; WBF: weakly biofilm-forming; MBF: moderately biofilm-forming; SBF: strongly biofilm-forming. Biofilm categories according to the test carried out on polystyrene plates.