Food Microbiology 62 (2017) 32–38

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Tolerance of *Clostridium perfringens* biofilms to disinfectants commonly used in the food industry

Audrey Charlebois, Mario Jacques, Martine Boulianne, Marie Archambault*

Faculté de Médecine Vétérinaire, Département de Pathologie et Microbiologie, Centre de Recherche en Infectiologie Porcine et Aviaire (CRIPA), Université de Montréal, Saint-Hyacinthe, Canada

ARTICLE INFO

Article history:
Received 22 October 2015
Accepted 14 September 2016
Available online 15 September 2016

Keywords:
Clostridium perfringens
Biofilms
Disinfectants
Food microbiology

ABSTRACT

Clostridium perfringens is an opportunistic pathogen that can cause food poisoning in humans and various enterotoxemia in animal species. Recently, it was shown to form mono-species biofilms, a structured community of bacterial cells enclosed in a self-produced extracellular matrix. Biofilms have been associated with tolerance to antibiotics, disinfectants, and physical and environmental stresses. Very little is known about the tolerance of *C. perfringens* biofilm toward disinfectants. In the present study, susceptibilities of *C. perfringens* biofilms to five types of commonly used disinfectants on farms and in food processing environments were analysed. In this paper, we show that *C. perfringens* mono-species biofilms can protect the bacterial cells from the action of potassium monopersulfate, quaternary ammonium chloride, hydrogen peroxide and glutaraldehyde solutions. However, sodium hypochlorite solution was shown to be effective on *C. perfringens* biofilms. Our investigation of dual-species biofilms of *C. perfringens* with the addition of *Staphylococcus aureus* or *Escherichia coli* demonstrated that overall, the mono-species biofilm of *C. perfringens* was more tolerant to all disinfectants than the dual-species biofilms. For the anaerobic grown biofilms, the mono-species biofilm of *C. perfringens* was more tolerant to sodium hypochlorite and quaternary ammonium chloride than the dual-species biofilms of *C. perfringens* with *S. aureus* or *E. coli*. This study demonstrates that *C. perfringens* biofilm is an effective protection mechanism to disinfectants commonly used on farms and in food processing environments.

1. Introduction

Clostridium perfringens is a Gram-positive, aerotolerant anaerobic spore-forming bacterium that causes a wide variety of diseases in humans and animals, primarily as a result of its ability to produce many different toxins (Markey et al., 2013). In humans, *C. perfringens* is responsible for gas gangrene, enteritis necroticans, food poisoning, and antibiotic-associated diarrheas (Myers et al., 2006). Currently, *C. perfringens* type A food poisoning ranks as the second most commonly reported foodborne illness in Canada (Thomas et al., 2013). In poultry, avian-specific *C. perfringens* strains cause necrotic enteritis, an economically significant poultry disease that costs the global industry over $2 billion annually in losses and control measures (Stanley et al., 2014). In some countries, this disease appears to be on the rise because of removal of antibiotic growth promoters (Stanley et al., 2014). *C. perfringens* is also a cause of various enterotoxemia in other animal species. Isolates of animal origin constitute a risk for transmission to humans through the food chain.

In order to persist in the environment, many bacteria have evolved the ability to form biofilms (Davey and O'Toole, 2000; Jefferson, 2004). In fact, the predominant organizational state of bacteria in nature is biofilms (Costerton, 1999). Important features of cells in biofilms include: aggregation in suspension or on solid surfaces, increased antibiotic tolerance, and resistance to physical and environmental stresses (Davey and O'Toole, 2000; Davies, 2003; Hall-Stoodley and Stoodley, 2009). It is now generally accepted that the biofilm growth mode induces bacterial tolerance to disinfection that can lead to substantial economic and health concerns (Bridier et al., 2011). Although the precise mechanism of such tolerance remains unclear, a review has recently discussed the subject as a multifactorial process involving the spatial organization of the biofilm (Bridier et al., 2011). More recently, we, and others, have described the formation of biofilms in *C. perfringens*

* Corresponding author.
E-mail addresses: audrey.charlebois@umontreal.ca (A. Charlebois), mario.jacques@umontreal.ca (M. Jacques), martine.boulianne@umontreal.ca (M. Boulianne), marie.archambault@umontreal.ca (M. Archambault).

http://dx.doi.org/10.1016/j.fm.2016.09.009
0740-0020/

(Charlebois et al., 2014; Varga et al., 2006). We demonstrated that the biofilm formed by *C. perfringens* could protect the cells from an exposure to atmospheric oxygen and to high concentrations of antibiotics and anticoccidial agents (Charlebois et al., 2014). It has also been observed that the biofilm formed by *C. perfringens* could protect the cells from an exposure to 10 mM of hydrogen peroxide even though this bacterium is catalase-negative (Varga et al., 2006). The capacity of *C. perfringens* to be part of dual- or multi-species biofilm has recently been reviewed (Pantaleon et al., 2014) and *C. perfringens* biofilm was detected in many types of multi-species biofilm including biliary stents (Leung et al., 2000; Pantaleon et al., 2014). However, susceptibilities of *C. perfringens* mono- and dual-species biofilms exposed to most disinfectants are currently unknown. This study was undertaken to investigate the tolerance of *C. perfringens* mono- and dual-species biofilms to disinfectants used in farms and food processing environments.

2. Materials and methods

2.1. Bacterial strains

C. perfringens strains used in this study are described in Table 1. Commensal strains of *C. perfringens* ($n = 15$) from poultry were recovered from the normal intestinal microbiota of animals taken at five processing plants located in the province of Québec, Canada and are part of our *C. perfringens* culture collection. Strains of clinical origin ($n = 15$) were from cases of necrotic enteritis from chickens. These strains were kindly provided by Dr Martine Boulianne and the Clinical Laboratory of Molecular Diagnostic of the Université de Montréal (St-Hyacinthe, Québec, Canada). Thawed strains were grown on Columbia agar with 5% sheep blood (Oxoid, Nepean, Ontario, Canada) and then incubated in an anaerobic chamber at 35 °C. *Escherichia coli* EcL 17606 and *Staphylococcus aureus* E452b3 were kindly provided by the Clinical Bacteriology Laboratories of the Université de Montreal.

2.2. Disinfectants

Five types of disinfectants commonly used on farms and in the food processing industry were tested in this study at concentrations suggested by the manufacturers: a quaternary ammonium chloride–based disinfectant (1%) (Aseptol 2000, S.E.C. Repro Inc., Ange-Gardien-de-Rouville, Québec, Canada), a potassium monopersulfate solution (1%) (Virkon, Vétoquinol N.-A. Inc., Lavaltrie, Québec, Canada), a sodium hypochlorite solution (0.27%) (Lavo Pro6, Lavo Inc., Montréal, Québec, Canada), a hydrogen peroxide (H_2O_2) solution (10%) (Sigma, Oakville, Ontario, Canada), and a glutaraldehyde-based disinfectant (2%) (Gluterate, Germiphene corp., Brantford, Ontario, Canada). Solutions were prepared following the manufacturer's instructions in sterile distilled water. Hydrogen peroxide solution was prepared fresh daily in sterile distilled water from a 30% H_2O_2 stock solution. The glutaraldehyde-based disinfectant was supplied as a ready-to-use solution.

2.3. Mono- and dual-species biofilms

Mono-species biofilms were cultured as previously described (Charlebois et al., 2014) to obtain optimal biofilm growth of

Table 1
Bacterial strains used in this study.

Bacteria	Origin	Strains	Type	Mean optical density values (OD)	Biofilm formation[a]	Source
Clostridium perfringens	Commensal	c2614_B	A	0.026	Weak	(Charlebois et al., 2014)
	Commensal	c2628_A	A	0.022	Weak	(Charlebois et al., 2014)
	Commensal	c2643_A	A	0.052	Weak	(Charlebois et al., 2014)
	Commensal	c2645_A	A	0.036	Weak	(Charlebois et al., 2014)
	Commensal	c2647_A	A	0.032	Weak	(Charlebois et al., 2014)
	Commensal	c2649_A	A	0.028	Weak	(Charlebois et al., 2014)
	Commensal	c2650_A	A	0.090	Weak	(Charlebois et al., 2014)
	Commensal	c2651_A	A	0.033	Weak	(Charlebois et al., 2014)
	Commensal	c2652_A	A	0.044	Weak	(Charlebois et al., 2014)
	Commensal	c2654_A	A	0.038	Weak	(Charlebois et al., 2014)
	Commensal	c2662_A	A	0.068	Weak	(Charlebois et al., 2014)
	Commensal	c2725_A	A	0.072	Weak	(Charlebois et al., 2014)
	Commensal	C3039_A	A	0.075	Weak	(Charlebois et al., 2014)
	Commensal	C3080_A	A	0.009	Weak	(Charlebois et al., 2014)
	Commensal	C3143_A	A	0	No	(Charlebois et al., 2014)
	Clinical	DUR-109-L469	A	0.231	Moderate	This study
	Clinical	DUR-109-B468	A	0.228	Moderate	This study
	Clinical	DUR-106-E170	A	0.078	Weak	This study
	Clinical	DUR-106-D170	A	0.090	Weak	This study
	Clinical	DUR-106-C170	A	0.078	Weak	This study
	Clinical	DUR-106-B170	A	0.076	Weak	This study
	Clinical	DUR-106-A170	A	0.084	Weak	This study
	Clinical	FBC-3200A-5	A	0.588	High	This study
	Clinical	FBC-3200A-3	A	0.110	Weak	This study
	Clinical	FBC-3200L-410	A	0.084	Weak	This study
	Clinical	FBC-3200K-410	A	0.100	Weak	This study
	Clinical	FBC-3200J-410	A	0.096	Weak	This study
	Clinical	FBC-3200E-176	A	0.093	Weak	This study
	Clinical	FBC-3200D	A	0.353	High	This study
	Clinical	FBC-3200B	A	0.298	Moderate	This study
	Clinical	ATCC13124	A	0.024	Weak	This study
Escherichia coli	Clinical	EcL 17606	N/A	0.218	Moderate	This study
Staphylococcus aureus	Commensal	E452b3	N/A	0.969	High	This study

[a] Strains are divided into four categories (no biofilm producer, weak biofilm producer, moderate biofilm producer and strong biofilm producer) based upon the previously calculated optical density (OD) values measured at 570 nm: OD ≤ ODc = no biofilm producer; ODc < OD ≤ 2X ODc = weak biofilm producer; 2X ODc < OD ≤ 4X ODc = moderate biofilm producer; 4X ODc < OD = strong biofilm producer. ODc is defined as three standard deviations (SD) above the mean OD of the negative control.