International Journal of Food Microbiology 171 (2014) 41–47

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Inactivation of dairy bacteriophages by commercial sanitizers and disinfectants

Céline Campagna [a,1], Manuela Villion [a,2], Simon J. Labrie [a], Caroline Duchaine [a,b], Sylvain Moineau [a,*]

[a] Département de biochimie, microbiologie et bio-informatique, Faculté des sciences et de génie, Groupe de recherche en écologie buccale, Faculté de médecine dentaire, Université Laval, Québec G1V 0A6, Canada
[b] Centre de recherche de l'Institut universitaire de cardiologie et de pneumologie de Québec, 2725 chemin Sainte-Foy, Québec, QC G1V 4G5, Canada

ARTICLE INFO

Article history:
Received 7 June 2013
Received in revised form 1 November 2013
Accepted 11 November 2013
Available online 19 November 2013

Keywords:
Bacteriophage
Phage
Lactic acid bacteria
Inactivation
Sanitizer
Disinfectant

ABSTRACT

Many commercial sanitizers and disinfectants have been used over the years to control microbial contamination but their efficacy on phages is often unknown. Here, 23 commercial chemical products, including 21 food-grade sanitizers were tested against virulent dairy phages. These food-grade chemicals included oxidizing agents, halogenated agents, alcohols, quaternary ammonium compounds, anionic acids, iodine-based acids, and an amphoteric chemical. Phage P008 was first exposed to each sanitizer for 2 and 15 min at room temperature and at two different concentrations, namely the lowest and highest no-rinse sanitizing concentrations. Organic matter (whey or milk) was also added to the testing solutions. At the end of the exposure period, the test solution was neutralized and the number of infectious phages was determined by plaque assays. The five most efficient sanitizers against phage P008 (<4 log of inactivation) were then tested against virulent lactococcal phages P008, CB13, AF6, P1532 of the 936 group, P001 (c2), Q54, and 1358 as well as *Lactobacillus plantarum* phage B1 and *Streptococcus thermophilus* phage 2972 using the same protocol. The oxidizing agents and the quaternary ammonium compounds were the most efficient against all phages although phages CB13 and P1532 were less sensitive to these chemicals than the other phages. This study may help in the selection of appropriate chemicals for controlling phage contamination in industrial factories and research laboratories.

1. Introduction

Virulent phages infecting lactic acid bacteria (LAB) still represent a significant risk for milk fermentation failures during the production of cheeses and a variety of other fermented dairy products. These phages can also reduce product quality (Coffey and Ross, 2002; Émond and Moineau, 2007). Strains of *Lactococcus lactis*, *Streptococcus thermophilus*, and *Lactobacillus* sp. are the most important LAB used by the dairy industry (Hols et al., 2005).

Many antiphage strategies have been devised to control lactic phage populations (Samson and Moineau, 2013). These include, among others, the use of starter culture rotation as well as phage-resistant strains (Émond and Moineau, 2007; Labrie et al., 2010). Others have also proposed reducing the number of bacterial strains to limit phage biodiversity within any given cheese factory (Quiberoni et al., 2006). These approaches have been successfully used for reducing phage contamination in large-scale industrial fermentations (Émond and Moineau, 2007). However, these selective pressures also led to the emergence of novel phages (Mahony et al., 2012; Rousseau and Moineau, 2009).

In dairy processing plants, novel LAB phages can be introduced and dispersed through various sources (Émond and Moineau, 2007; Briggiler Marcó et al., 2012a,b; Verreault et al., 2011): i) raw milk in which they are found; ii) ingredients added to the milk, iii) re-used dairy by-products such as whey protein concentrates; iv) movement of employees within the plant; v) ineffective cleaning of the equipments; vi) water used for rinsing equipment or for the dilution of cleaners and disinfectants; and vii) ambient air.

Heat is the primary treatment used to inactivate most microorganisms traditionally encountered in raw milk. However, the majority of virulent phages infecting LAB can resist pasteurization (Guglielmotti et al., 2011; Murphy et al., 2013). High-pressure treatments have also been suggested but some LAB phage species can resist pressures up to 100 MPa (Capra et al., 2009; Mercanti et al., 2012). Numerous commercial chemical products are also used in food processing plants for disinfecting and sanitizing contact surfaces. To be approved by health authorities, food contact sanitizers must meet several criteria, such as

* Corresponding author at: Groupe de recherche en écologie buccale, Faculté de médecine dentaire, Université Laval, Quebec City, Quebec G1V 0A6, Canada. Tel.: +1 418 656 3712; fax: +1 418 656 2861.
E-mail address: Sylvain.Moineau@bcm.ulaval.ca (S. Moineau).
[1] Present address: Direction de la santé environnementale et de la toxicologie, Institut national de santé publique, 945 avenue Wolfe, Québec G1V 5B3, Canada.
[2] Centre d'expertise en analyse environnementale du Québec, Ministère du Développement durable, de l'Environnement, de la Faune et des Parcs, 2700, rue Einstein, Québec (Québec) G1P 3W8, Canada.

0168-1605/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijfoodmicro.2013.11.012

minimum residue levels, low human toxicity and antimicrobial efficacy (minimum of 3 log reduction of specific bacteria or viruses in 5 min, or, for a sanitizer with a disinfectant claim, 5 log reduction in 30 s (Gaulin et al., 2011).

In dairy processing plants, cleaning in place procedures (CIP) are used on equipment and surfaces (including floors) as the first step of a sanitization program to physically and chemically remove organic and microbiological contamination (Cords et al., 2001). This step is important since organic matter (such as milk or whey residues) may inactivate or lead to decreased effectiveness of sanitizers (Gaulin et al., 2011; Gelinas and Goulet, 1983). A food contact sanitizer is then applied to the equipment to properly sanitize or disinfect the surfaces. In Canada, for example, approved food contact sanitizers include chlorine compounds (e.g., bleach), peroxide and peroxyacid mixtures, carboxylic acids, quaternary ammonium compounds, anionic acids, and iodine compounds (Gaulin et al., 2011). For the sanitizing step of the CIP treatment, the US FDA has approved over 40 different compounds for the food industry (US FDA, 2012). Although food contact sanitizers with disinfecting claims are effective in reducing or eliminating food microorganisms (including viruses) linked to human diseases, little is known about their efficiency in inactivating LAB phages. In Europe, such LAB phage reduction claims exist and must provide a 4 log reduction of the number of viable units (or plaque forming units in case of phages) in an established time (European Committee for Standardization (CEN), 2002).

In the past decade, a few studies have attempted to evaluate the efficiency of biocides on a few LAB phages. In the case of phages of *Lactobacillus helveticus*, *Lactobacillus casei*, *Lactobacillus delbrueckii*, and *Lactobacillus paracasei*, the efficiency of chemical biocides (peracetic acid, sodium hypochlorite, ethanol, isopropanol) varied and was phage- or formulation-dependent [(Capra et al., 2004; Ebrecht et al., 2010; Quiberoni et al., 2003; Quiberoni et al., 1999), reviewed in Guglielmotti et al. (2011) and Mercanti et al. (2012)]. In general, as shown with *L. lactis* phages, peracetic acid (0.15% (v/v)) is an efficient sanitizer while sodium hypochlorite requires prolonged contact time and alcohols are not efficient (Suárez and Reinheimer, 2002; Murphy et al., 2013). Taken together, it is rather difficult to compare the effectiveness of these products since the methodologies vary between the studies. Factors influencing the efficacy of disinfectants that are realistically found in the processing plant environment, such as organic matter and hard water, are not always included in these phage inactivation protocols.

The aim of this study was, therefore, to measure the efficiency of traditional and commercial food contact sanitizers on representative LAB phages (infecting *L. lactis*, *Lactobacillus* or *S. thermophilus*) in a worst-case scenario of a dairy plant environment (i.e., in the presence of organic contamination and hardened water), using a standardized protocol. Our underlying goal was to determine the most efficient sanitizers against phages for the food industry and research laboratories.

2. Materials and methods

2.1. Strains, phages and growth conditions

The virulent lactococcal phages P008, CB13, AF6, P1532, P001, 1358 and Q54 as well as *Lactobacillus plantarum* phage B1 and *S. thermophilus* phage 2972 were obtained from the Félix d'Hérelle Reference Center for Bacterial Viruses (www.phage.ulaval.ca). The bacterial hosts used to amplify them were *L. lactis* IL1403, *L. lactis* SMQ-404, *L. lactis* SMQ-1001, *L. lactis* HER1142, *L. lactis* SMQ-388, *L. lactis* SMQ-562, *L. plantarum* ATCC8014 and *S. thermophilus* DGCC7710, respectively. Phage P008 (Loof et al., 1983) was selected as a representative of the lactococcal 936 group, which is the most predominant group in cheese factories worldwide (Mahony et al., 2012; Rousseau and Moineau, 2009), and is also suggested in European standards (European Committee for Standardization (CEN), 2002). Lactococcal phages CB13 and AF6, belonging to 936 group, were recently isolated from whey samples from a Canadian cheese plant (Moisan and Moineau, 2012; Rousseau and Moineau, 2009) and phage CB13 was found to be persistent for over one year in the same cheese factory (Rousseau and Moineau, 2009). Phage P1532 (936 group) was selected because it was shown to be highly resistant to heat treatment (Atamer et al., 2009). Phage P001 was selected as a representative of the lactococcal phage group c2, and is also a reference virus in the European standards (Braun et al., 1989; European Committee for Standardization (CEN), 2002). Phages 1358 and Q54 belongs to lactococcal phage groups rarely encountered in milk fermentation facilities (Jarvis, 1984; Fortier et al., 2006; Deveau et al., 2006). Virulent phage 2972 (Lévesque et al., 2005) was used as a reference for streptococcal phages since it represents one of the two main groups of *S. thermophilus* phages (Le Marrec et al., 1997; Quiberoni et al., 2010) encountered in dairy environments and phage B1 was selected as the representative of *Lactobacillus* phages (Briggiler Marcó et al., 2012a,b).

Bacterial strains were cultured in M17 (Oxoid) supplemented with either 0.5% glucose (GM17) or 0.5% lactose (LM17) at 30 °C for the lactococcal strains or with LM17 at 42 °C for the streptococcal strain. *Lactobacillus* strains were cultured in MRS (Difco) at 37 °C. When propagating phages, 10 mM $CaCl_2$ was added to the medium. For the plaque assays, an aliquot of phage solution was mixed with an appropriate volume of an overnight culture of the host strain in soft agar at 45–50 °C using the appropriate medium for the bacterial host strain (GM17, LM17 or MRS) supplemented with 0.75% agar and 10 mM $CaCl_2$. The inoculated soft agar was then poured over a 1% agar medium (of the same composition) in a Petri dish. The plates were incubated overnight at the appropriate temperature for the bacterial host strain.

2.2. Sanitizers

Five different chemical companies accredited by the Canadian Food Inspection Agency to sell sanitizing products to the Canadian dairy industry provided samples of commercial sanitizers (between 1 and 20 L). These sanitizers were chosen on the basis of their relevance to the food and dairy industries and were certified by their respective companies to be effective for the inactivation of enteric and environmental microorganisms. Different sanitizers ($n = 21$) were chosen among the following chemical families: chlorinated agents, peroxide and peroxyacid (PPA) mixtures, amphoteric compounds, quaternary ammonium compounds (QAC; benzalkonium chloride-based), anionic acids (phosphoric acid-based), and iodine compounds (iodine-based acids). As traditional disinfectants, ethanol and isopropanol were also included. Each sanitizer is described in Table 1, as per the Material Safety Data Sheets (MSDS) provided by the respective chemical companies. Note that the lists of active ingredients composing the different sanitizers listed in Table 1 may be incomplete, since only toxic ingredients are listed in the MSDS.

The chemical concentrations used for the phage inactivation assays were determined according to the recommended concentration interval for a sanitizing procedure described in the technical sheet of each sanitizer. Concentration 1 was selected as the lowest sanitizing concentration not requiring water rinse, and concentration 2 was either the highest no-rinse sanitizing concentration or the disinfecting concentration, depending on the product, since some companies did not specify a range of sanitizing concentrations for the product. Although not approved as contact sanitizers per se, we also tested two other chemicals, sodium dichloro-S-triazinetrione and bromochlorodimethylhydantoin (BCDMH), which are both solid tabs used in water treatment systems in food industries and commonly used in drains (wastewater). All concentrated sanitizers were diluted in hardened water (1.26 mM $MgCl_2$, 2.52 mM $CaCl_2$, 3.36 mM $NaHCO_3$, pH 7.0; for a desired concentration of 300 mg/kg $CaCO_3$).