Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Biotechnology

Phages as friends and enemies in food processing

Alessandra Gonçalves de Melo[1], Sébastien Levesque[1] and Sylvain Moineau[1,2]

Phages can infect all bacterial genera on the planet leading to their abundance in ecosystems. While these bacterial viruses may represent a risk to industrial fermentations, they may also be valuable tools to control foodborne pathogens. Here we review these two sides of phage replication. As bacterial fermentations are constantly under threat of phage attacks, we will discuss phage diversity and industrial strategies employed to reduce their impact on food processing. Furthermore, we will explore the use of pathogen-infecting phages to reduce the risks associated with foodborne diseases.

Addresses
[1] Département de biochimie, de microbiologie, et de bioinformatique, Faculté des sciences et de génie, Groupe de recherche en écologie buccale, Faculté de médecine dentaire, Université Laval, Québec City, QC G1V 0A6, Canada
[2] Félix d'Hérelle Reference Center for Bacterial Viruses, Université Laval, Québec City, QC G1V 0A6, Canada

Corresponding author: Moineau, Sylvain (Sylvain.Moineau@bcm.ulaval.ca)

Current Opinion in Biotechnology 2018, **49**:185–190

This review comes from a themed issue on **Food biotechnology**

Edited by **Maria Marco** and **Eddy Smid**

http://dx.doi.org/10.1016/j.copbio.2017.09.004

Introduction

Bacteriophages or phages are recognized as the most abundant biological entities on our planet, outnumbering bacteria by an estimated 10-fold [1]. They are ubiquitous and, as such, can be found in natural and man-made environments, especially those in which their bacterial host thrives [2]. Many fermentation processes rely on the metabolic capabilities of bacterial cells to transform substrates, which make phage contamination a major concern in the food industry [3]. Sensitive host encounters with lytic phages can result in fermentation arrest or delay, leading to low-quality products and at worst, production loss [4]. Although phages are usually acknowledged as a menace, they can also be considered allies against pathogenic bacteria that represent a persistent food safety hazard. This review will highlight the character of phages as the enemies of food manufacturing, using dairy phages as examples. Furthermore, we will also focus on the role of phages as biocontrol agents in several steps of the food chain and the advances made for the application of phage cocktails to control bacterial pathogens.

Phages as a threat in food processing

Owing to the nature of food fermentation processes, performed at large scale, under non-sterile conditions [5] and often relying on the same bacterial cultures in successive batches [4], it is not surprising that virulent phages can cause fermentation failures [5]. The food industry has adopted several antiphage strategies as countermeasures to prevent and diminish contamination. Example strategies include developing bacteriophage insensitive mutants (BIMs), establishing bacterial strain rotations as well as using chemical and physical treatments to decontaminate the factory and the substrates [5,6]. The state of knowledge on phage–host interaction to prevent viral contamination in food processing [7] as well as standard phage control strategies have been extensively reviewed lately [4,6]. Only very recent developments will be highlighted here.

Heat is a primary measure used to inactivate undesirable microorganisms found in raw milk [8]. However, several dairy phages are thermal-stable, surviving, for example, pasteurization [9]. Moreover, whey proteins can provide an additional protective effect to phages [10•]. Because thermal inactivation of phages require higher temperatures and lead to protein denaturation, other alternatives, such as membrane filtration, are been investigated as a mean to reduce phage levels without changing protein structure [11].

Several biocides have proven to be effective antivirals to clean industrial environs [8,9,12•]. However, increasing resistance to chemical compounds has been observed, leading to phage persistence in manufacturing sites [12•]. For example, some members of the highly prevalent *Lactococcus lactis* phage genus Sk1virus (formerly 936) are broadly tolerant to multiple classes of compounds found in commonly used sanitizers [12•]. A similar conclusion was made in another study, in which the virulent lactococcal phages CB13 (isolated in Canada) and P1532 (isolated in Germany) were less sensitive to biocides than other phages [8]. These observations, and the phylogenetic separation between resistant and non-resistant phages, suggest that biocide resistance is due to the intrinsic robustness of the phage, rather than to the biocide's mechanism of action [12•]. Therefore, rotation

of sanitizers may be a recommended precautionary measure to avoid selection of increasingly resistant viruses [8,9].

Phage diversity

Dairy phages are by far the best-characterized food-related phages due to their global impact on milk fermentation processes. Carefully selected strains of lactic acid bacteria (LAB), for example, *L. lactis*, *Lactobacillus* sp., *Leuconostoc* sp., and *Streptococcus thermophilus*, are repetitively added to milk to manufacture yogurt, fermented milks, and various cheeses on artisanal and industrial scales and, therefore, are ideal targets for virulent phages [6]. In recent years, several studies have focused on phage biodiversity, including genomic characterization of the most predominant phage groups as well as rarely isolated phage types [13], new emerging groups [14,15•], prophages in dairy strains [16] and also other LAB phages [17].

The understanding of phage evolution and diversity at food processing sites can shed light on phage population dynamics to ensure more efficient strategies to control them [15•]. For instance, a novel emerging group of *S. thermophilus* phages, the 987 phages, was recently discovered [15•] and compared to the three other known *S. thermophilus* phage groups [18]. Interestingly, genomic relatedness of 987-type phages to the morphogenesis and replication modules from some lactococcal phages (P335 group) and *S. thermophilus* phages, respectively, suggests genetic exchanges that resulted in a new mosaic architecture and emerging phage group. Additionally, adsorption of the 987 phages to both *S. thermophilus* and *L. lactis* indicated that cell surface molecules might be shared between both bacterial species [15•]. The recent industrial practice of mixing *L. lactis* and *S. thermophilus* strains in starter cultures may have led to the rise of this new phage group. Another example involves recent lactococcal phage isolates of the Sk1virus genus that are able to cross-infect various groups of *L. lactis* strains, thereby displaying a more dynamic host range than previously described for this group of phages [19]. Clearly, phage populations are dynamic in industrial environments and they need to be constantly monitored in order to adapt the antiviral strategies, particularly for bacterial strain rotation.

A comparative genomic analysis of ninety siphophages has established the core genome and variable elements of the Sk1virus genus [20••]. Interestingly, a probable hotspot for gene rearrangements was identified surrounding the major tail protein-encoding gene (*mtp*), including the presence or absence of a gene encoding the neck passage structure (NPS) and/or a gene encoding a tail extension protein (*tepX*). For the latter phenotype, MTP-TepX was arranged as a spiral structure on the phage tail [20••]. Both structures were also observed on phage persisting over several years in whey powders, suggesting that they might be beneficial for phage structural stability in harsh conditions [17].

The arms race of phage and bacteria

Whenever they encounter each other, bacteria and phages are continuously outcompeting in an antagonistic co-evolution process. In this arms race, bacterial derivatives acquire resistance to phages using several strategies (frequently through receptor mutations), whereas phages counter-attack to overcome antiviral barriers in order to infect and propagate [21]. Bacteria can also harbor phage-resistance mechanisms, such as restriction modification systems, abortive infections, CRISPR-Cas systems and others. These mechanisms have been extensively reviewed elsewhere as well as the strategies used by phages to respond to these challenges (see [21,22]).

Of particular interest in the past decade, the CRISPR-Cas system allows the 'immunization' of bacterial strains against several phages. While this can be observed by challenging bacteria with virulent phages, the system can also be 'programmed' via a plasmid to protect bacteria against multiple phages in a single assay [23]. This programming was described for *S. thermophilus*, using a high copy plasmid with highly conserved protospacers and specific protospacer adjacent motifs targeting the greatest number of virulent phages [24••]. This strategy was very efficient at conferring resistance to multiple phages at once and offers the possibility of rapidly developing specific BIMs in strains carrying active CRISPR-Cas systems.

This natural coevolution can sometimes be directly observed when analyzing mixed starter cultures [25]. Food fermentations can be performed using a few numbers of defined bacterial strains as indicated above but also with mixed starter cultures. The latter are composed of undefined numbers and ratios of bacterial strains that will drive the fermentation process. These mixed cultures will also coexist with diverse phages leading to a complex dynamic of phage–host interactions. It is usually not recommended to use both defined and mixed cultures in the same industrial environment.

Beneficial applications of bacteriophages in the food industry

Phages are also prospective antimicrobial alternatives to prevent contamination by bacterial pathogens at different stages of the commercial food chain. Diseases caused by foodborne bacteria affect millions of people annually on a global scale [26,27]. While the intensive use of antibiotics led to the emergence of resistant bacteria and increased the likelihood of untreatable infections [28•,29], phages are highly specific in their bacterial targets and will only replicate if available hosts are in their surroundings (auto-dosing) [30]. Consequently, phages are also been investigated for treatment of several bacterial infections [31].