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Current Opinion in Biotechnology

Novel strategies to prevent or exploit phages in fermentations, insights from phage–host interactions

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Phages infecting lactic acid bacteria (LAB) provide some of the most advanced model systems for (tailed) phage–host interactions. In particular the identification of receptor molecules of representative lactococcal phages combined with the elucidation of the structure of the receptor-binding protein has permitted crucial insights into the early stages of infection.Dairy and biotechnological fermentations are persistently marred by the destructive activities of phages. Here, we discuss how recent advances in our knowledge on LAB phage–host interactions have provided a basis for the next generation anti-phage strategies. Furthermore, the significant increase in genomic data has furthered our understanding of the genetics of these phages, thereby permitting the exploitation of phage-derived components for food safety and biotechnological applications.

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Current Opinion in Biotechnology 2015, **32**:8–13

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

Edited by **Michiel Kleerebezem** and **Christophe Lacroix**

http://dx.doi.org/10.1016/j.copbio.2014.09.006

Introduction

Bacterio(phages) are a consistent and major threat to food fermentations, particularly dairy fermentations where infection of starter cultures may result in slow vats, low-quality and inconsistent products, and even complete fermentation failure. Since lactococcal phages were first reported as the causative agents of starter failure by Whitehead and Cox in 1935 [1], efforts have been made to improve fermentation systems. These efforts have been epitomized by the development of bacteriophage-insensitive mutants (BIMs), the formulation of blends of phage-unrelated strains and, as a result of extensive research into phage–host interactions and phage-resistance mechanisms, a knowledge-based set of tools to combat phages [2]. Phages infecting *Lactococcus lactis* have dominated dairy phage research since strains of this species are the most widely used starters in dairy fermentations [3]. Consequently, this review will focus on the lessons learned from and models developed for lactococcal strains and phages, highlighting advances made over the past three years in particular, that may be broadly applied to phage–host interactions in other genera with implications for a variety of food and biotechnological fermentations.

Phage–host interactions

Lactococcal phages are currently classified into ten groups based on their morphology and genetic relatedness [3]. Of these, three species, 936, P335 and c2, dominate in terms of persistence and frequency of isolation [4–6]. Members of the 936 and c2 observe a lytic cycle only, while P335 members may propagate lytically or may integrate their genome into that of the host and replicate *in tandem* with the host's chromosome, a scenario during which they are termed lysogenic or temperate phages. The proteinaceous receptor for c2 phages is the membrane-anchored phage infection protein (PIP) [7], a functional analogue of the YueB receptor of *Bacillus subtilis* phage SPP1 [8,9]. The PIP-encoding gene may be altered or deleted from the host without affecting the technological traits of the host, while simultaneously providing resistance against c2 phages [10]. Since selection of c2-resistant derivatives of lactococcal strains probably harbour such alterations/deletions, the c2 phages appear to have become less prevalent in the dairy industry. Receptor-binding proteins (RBP) of the 936 and P335 phages, which are located at the distal end of the phage tail, frequently being part of a larger protein complex termed the base plate, and host-encoded receptors that these phages recognize have been investigated thoroughly over the past decade [11–13,14•,15,16,17•].

Most significantly and after many years of speculation, structural analysis of the RBP of the 936 species phage p2 produced the direct experimental proof of the saccharidic nature of the receptor recognized by these phages [18]. This publication was followed by structural analysis of the RBPs of P335 type phages TP901-1 and Tuc2009, which were also found to possess an affinity and avidity for saccharides [13,19••]. These findings were complemented with biochemical analysis of a novel surface-exposed, cell envelope macromolecule, the so-called pellicle or cell wall polysaccharide (CWPS), of a number of lactococcal strains [14•,16,17•], and with the identification and comparative analysis of the corresponding CWPS biosynthetic operons [20••]. The ultimate outcome from these studies

was the identification of CWPS as the saccharidic receptor of members of the 936 and P335 phage species.

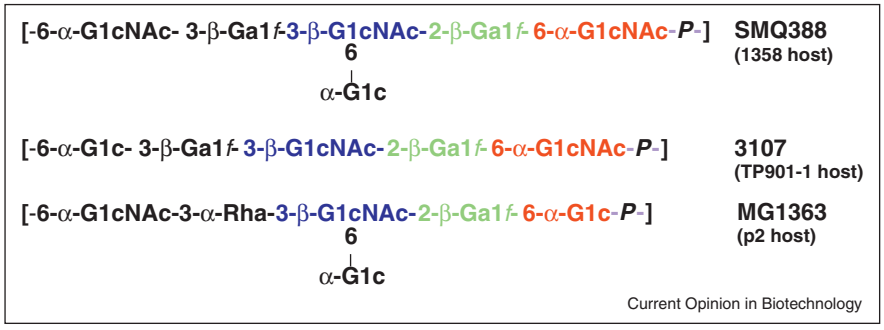
The CWPS of *L. lactis* MG1363, which is host to the 936 species phages p2 and sk1, is composed of repeating units of a phospho-hexasaccharide [16], while that of *L. lactis* 3107, host to the P335 phages TP901-1 and phiLC3, is a phospho-pentasaccharide [17•]. Most recently, the structure of the CWPS of *L. lactis* SMQ-388, host to the namesake of the rarely isolated 1358 lactococcal phage species was defined as repeating subunits of a phospho-hexasaccharide [14•] (Figure 1). While the CWPS of each of these strains contains unique elements, a core trisaccharide exists that probably represents (part of) the target for the saccharide-recognizing phages. The unique aspects of their CWPS biochemical structure explain the specificity and narrow host range of such CWPS-binding phages, which is in major contrast to the generalized PIP protein receptor of the c2 phages. These findings aid in distinguishing phages that recognize proteins from those that recognize saccharides, and such knowledge may be harnessed as a preventive measure if the target carbohydrate or protein is known (see below).

Fighting fire with fire: exploiting phages to prevent infection

In order to develop next generation approaches to counter the phage problem, it is imperative to understand the molecular mechanics of the phage attachment and injection process. Historically, dairy fermentation media were adapted by employing phosphate-containing whey-based media (so-called phage inhibitory media) for bulk starter preparation to 'mop up' calcium and other divalent cations that were believed to be required for phage infection. Phages nevertheless remained problematic, which is now explained by the fact that not all lactococcal phages require divalent cations for infection [19••]: members of the 936 species require calcium to rotate the conformation of their base plate to a cell-facing, infection-ready orientation, whereas many members of the P335 species, including TP901-1, were observed to infect their hosts with equal efficiency in the presence or absence of calcium [19••]. Consistent with this, the baseplate structure of TP901-1 was shown to be in a permanent 'infection-ready' conformation [19••]. Interestingly, some members of the P335 species, such as Tuc2009 and Q33, have been observed to require calcium for efficient infection of their host [19••]. It is clear that observations on the requirement for divalent cations by phages do not apply to all phages, therefore, limiting the effectiveness of the phage inhibitory media approach. In fact, as with the 'hurdle approach' applied in food production and preservation processes, it is probably that a combination of approaches should be employed to ensure limitation of phage problems.

The advances made towards defining lactococcal phage–host interactions have facilitated various novel approaches to prevent or limit phage infection. For example, the distal tail region of the P335 species phages TP901-1 and Tuc2009 is observed as a so-called 'double-disc' baseplate and the structure of the baseplate of both phages has been resolved [15,19••]. In the case of TP901-1, the baseplate is composed of multiple copies of two proteins that comprise the upper (BppU) and lower (BppL) base plate discs, while in the case of Tuc2009, the baseplate is composed of multiple copies of three proteins, namely BppU and BppL and an accessory protein, BppA. Incubation of a heterologously produced, purified phage baseplate complex with cells of the corresponding host (i.e. the base plate is derived from a phage that infects such a host), effectively coating such cells with base plate, specifically inhibits phage adsorption in a dose-dependent manner. In contrast, this complex has no effect on phage adsorption when incubated with non-corresponding (i.e. the base plate is derived from a phage that does not infect the host) host cells (Figure 2) [15]. While the base plate complex of any one phage is unlikely to exclude all phages from attaching and infecting and is, therefore, perhaps not an all-round practical solution for

Figure 1



Schematic representing the biochemical structure of the CWPS repeating subunit of three lactococcal strains: 3107, MG1363 and SMQ-388. Each of these strains represents a host for at least one phage of three distinct species, that is, the P335, 936 and 1358, respectively.