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Biotechnological applications of bacteriophages: State of the art

Liliam K. Harada[a], Erica C. Silva[a], Welida F. Campos[a], Fernando S. Del Fiol[a], Marta Vila[a], Krystyna Dąbrowska[b], Victor N. Krylov[c], Victor M. Balcão[a,d,*]

[a] PhageLab – Laboratory of Biofilms and Bacteriophages, i(bs)^2 – Intelligent Biosensing and Biomolecule Stabilization Research Group, University of Sorocaba, Sorocaba, SP, Brazil
[b] Bacteriophage Laboratory, Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland
[c] State Institute for Genetics and Selection of Industrial Microorganisms, Laboratory for Genetics of Bacteriophages, Mechnikov Research Institute for Vaccines and Sera, Russian Academy of Medical Sciences, 1st Dorozhnii Proezd 1, 113545 Moscow, Russia
[d] CEB – Centre of Biological Engineering, University of Minho, Braga, Portugal

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ABSTRACT

Bacteriophage particles are the most abundant biological entities on our planet, infecting specific bacterial hosts in every known environment and being major drivers of bacterial adaptive evolution. The study of bacteriophage particles potentially sheds light on the development of new biotechnology products. Bacteriophage therapy, although not new, makes use of strictly lytic phage particles as an alternative in the antimicrobial treatment of resistant bacterial infections and is being rediscovered as a safe method due to the fact that these biological entities devoid of any metabolic machinery do not have affinity to eukaryotic cells. Furthermore, bacteriophage-based vaccination is emerging as one of the most promising preventive strategies. This review paper discusses the biological nature of bacteriophage particles, their mode(s) of action and potential exploitation in modern biotechnology. Topics covered in detail include the potential of bacteriophage particles in human infections (bacteriophage therapy), nanocages for gene delivery, food biopreservation and safety, biocontrol of plant pathogens, phage display, bacterial biosensing devices, vaccines and vaccine carriers, biofilm and bacterial growth control, surface disinfection, corrosion control, together with structural and functional stabilization issues.

1. Introduction

Bacteriophages (or phages, viruses that infect bacteria) are the most abundant entities on our planet, being harmless for all organisms including humans except for their target bacterial hosts, infecting every type of bacterium in every known environment and being among the major drivers of bacterial adaptive evolution (Santos et al., 2014; Brussow and Kutter, 2005; Chibani-Chennoufi et al., 2004; Brussow and Hendrix, 2002; Bergh et al., 1989; McCallin et al., 2013; Abedon, 2015; Pirnay et al., 2015). Due to both the vast number of bacteriophages and the even larger number of unexplored genes that these metabolically inert particles carry, research is of utmost importance to fully understand and benefit from the biology and biotechnological potential of such entities. Bacteriophages are highly diverse and infect essentially all bacteria on earth (Catalão et al., 2013). Their genomes encode proteins that have been useful for biotechnology applications including food safety diagnostics, antibiotherapy of infections caused by antibiotic-resistant bacterial strains, DNA delivery vehicles and many more relevant technologies. Phages infect one bacterial cell, replicate, and then new virions are released and infect another cell. As a consequence, phages control bacterial population numbers, and on the other hand they contribute to moving genes from one bacterium to another. The study of bacteriophage particles provides insights into the evolution of genomes, bacterial adaptative evolution, and the way DNA is expressed and copied, and potentially sheds light on the development of new biotechnology products. Worldwide, the unstoppable increase of bacterial resistance to conventional chemical antibiotics is becoming a renewed driving force for bacteriophage (or phage) therapy, making use of lytic phage particles, since these biological entities devoid of any metabolic machinery do not possess affinity to eukaryotic cells (Chan and Abedon, 2012; Dąbrowska et al., 2005). Additionally, the utilization of bacteriophage particles in phage-based vaccination is emerging as one of the most promising preventive strategies, together with plant and animal gene transfer methods, phage display-based selection for biological affinity molecules, bacterial biosensing devices, gene delivery, food biopreservation and safety, biocontrol of plant pathogens, biofilm control, surface disinfection, and corrosion control. The issue of structural and functional stabilization acquires a special relevance in

* Corresponding author at: PhageLab – Laboratory of Biofilms and Bacteriophages, University of Sorocaba, Campus Cidade Universitária Prof. Aldo Vannucchi, Rodovia Raposo Tavares km 92.5, CEP 18023-000 Sorocaba, SP, Brazil.
E-mail address: victor.balcao@prof.uniso.br (V.M. Balcão).

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the context of phage particles. Since these are of proteinaceous nature, stabilization from both structural and functional points of view is directly related to rigidification of their three-dimensional structure (Balcão and Vila, 2015). This is a critical feature that will be discussed further below in this review paper.

1.1. Bacteriophage particles

Bacteriophages are viruses that infect solely bacterial cells, being biological entities known for over a century. To answer the question "what is life?", bacteriophages were elected as the simplest and most logical biological systems, with research based on them becoming the cradle of molecular biology (Chibani-Chennoufi et al., 2004). Despite all the basic biological research involving bacteriophages, a special interest in bacteriophages has now re-emerged, viewing them as potential alternatives and/or complements to conventional chemical antibiotherapy mainly because of their high-specificity and unique properties to fight multi-resistant bacterial strains (Summers, 2012; Rios et al., 2016). Bacteriophages are (biological) entities totally devoid of any metabolic machinery, thus being obligate intracellular parasites requiring a bacterium for their replication *via* their genetic material, taking over the biochemical machinery of the bacterial cells (Hyman and Abedon, 2010; Hermoso et al., 2007; Skurnik and Strauch, 2006; Kokjohn et al., 1991). Due to the important role that bacteriophages play in influencing the evolution of bacterial genomes, also inducing the development of bacterial pathogenicity, these metabolically inert particles may provide potential tools to face the current antibiotic resistance crisis (Chibani-Chennoufi et al., 2004). Over the last few years, a clear shift from the reductionist approach focusing on selected bacteriophages in carefully controlled laboratory conditions, towards the study of many different bacteriophages in the complexity of real-life situations can now be perceived. Indeed, bacteriophages harness the potential to be utilized in many different biotechnological applications, ranging from human antibiotherapy to environment disinfection. The vast majority of bacteriophages discovered so far interact with bacterial cells that express specific membrane surface receptors. However, if a bacterial cell does not expose a specific receptor for a particular bacteriophage at its surface, then the bacteriophage cannot infect it, which demonstrates the naturally high specificity of a bacteriophage to a particular bacterial host. Estimates suggest about ten different bacteriophages for every bacterial cell, some of which are highly specific for their bacterial host (either monophages (recognizing only one type of receptor) or polyphages (displaying a broader host range and recognizing more than one type of receptor)) (Skurnik and Strauch, 2006; Hyman and Abedon, 2010; Chan and Abedon, 2012). From the morphological point of view, bacteriophage particles exhibit a well-defined three-dimensional structure, the vast majority presenting an icosahedral protein capsid enclosing the genetic material in its core, a spiral contractile sheath (or tail) (surrounding a core pipe) and, usually, six tail fibers connected to a baseplate containing the receptor-binding proteins responsible for recognizing specific molecules at the surface of the bacterial membrane, as graphically illustrated in Fig. 1.

In relation to the type of genetic material they harness within the capsid's core, bacteriophage particles can be divided into four major groups (see Fig. 2): single stranded DNA phages (ssDNA), double stranded DNA phages (dsDNA), single stranded RNA phages (ssRNA), and double stranded RNA phages (dsRNA).

Over the last fifty years, more than 5100 bacteriophages have been identified and studied, with more than 90% of them possessing tails and belonging to the *Myoviridae*, *Siphoviridae* and *Podoviridae* families (Wittebole et al., 2013; Ackermann, 2007; Hanlon, 2007; Dąbrowska et al., 2005) (see Table 1). More than 2200 complete bacteriophage genomes can be found in the NCBI Genome database (as verified on October 7th 2017).

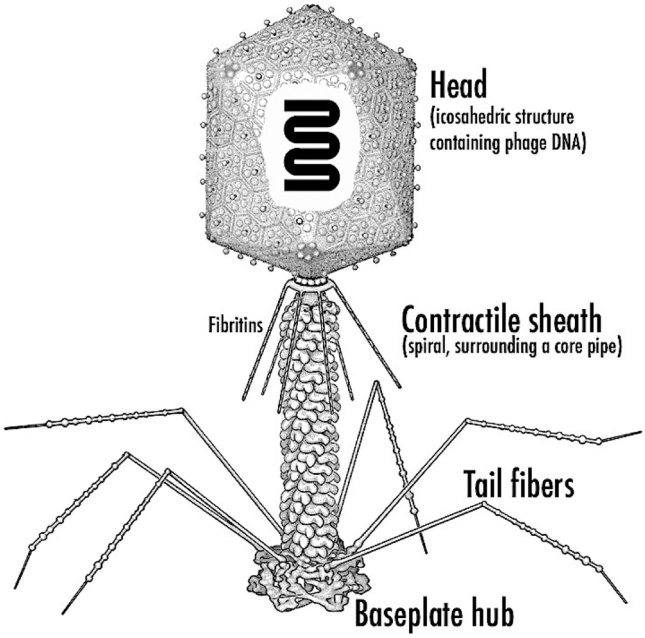

Fig. 1. Schematic representation of a prototypical bacteriophage particle. The bacteriophage DNA is protected by the icosahedral capsid, which is attached to the contractile sheath, a highly specialized and extremely efficient phage component required for infecting its host. The hexagonally shaped baseplate is situated at the distal end of the contractile sheath, and coordinates the movement of the tail fibers that initially sense the presence of the host, the short tail fibers that unfold from underneath the baseplate to firmly anchor on its bacterial host surface, and the spiral contractile sheath surrounding a core pipe that contracts, ejecting DNA into the bacterial host.
Adapted from Rossmann et al. (2005).

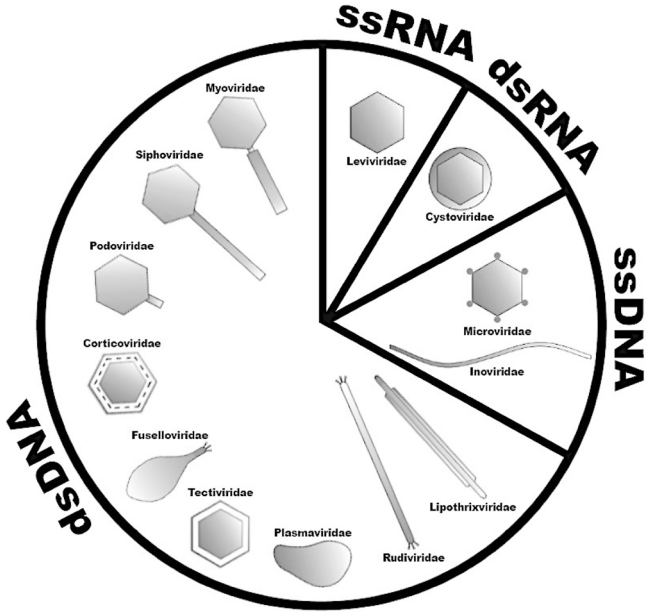

Fig. 2. Classification of bacteriophages according to their morphology, genetic material and major characteristics.
Adapted from Ackermann (2007) and Hanlon (2007).

1.1.1. Bacteriophage-bacterial host surface interactions

The baseplate in the bacteriophage three-dimensional structure coordinates both bacterial host recognition and attachment, with tail sheath contraction (if applicable), a movement initiated at the baseplate and propagated through the entire sheath in a wave-like fashion.