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Fun with phages: how do heat and pH affect bacteriophage viability?

Background information for teachers

Salmonella enterica Typhimurium LT2[1] is a nonpathogenic bacterial strain of the phylum Pseudomonadota, the class Gammaproteobacteria, within the family of Enterobacteriaceae, and belonging to the species of *Salmonella enterica* subspecies *Enterica*.[1] It was isolated in the 1940s in Sweden[2] and was used in the first experiments involving phage-mediated transduction.[3]

As a member of *Salmonella enterica*, the cells are Gram-negative bacilli and can be found arranged as single cells or diplobacilli; they are motile,[2] with flagella,[4,5] and are facultative anaerobes.[3] Optimal growth occurs at 37 °C,[3] and they have a great tolerance for acidic pH.[5]

Phage P22[4] is a bacteriophage in the class Caudoviricetes; taxonomically, it currently belongs to the Genus *Lederbergvirus* and, as of the 2022 *International Committee on Taxonomy of Viruses* release,[6] its official species name is *Lederbergvirus P22*. For the sake of simplicity, here *Lederbergvirus P22* is referred to as *Salmonella* phage P22 or its abbreviation, phage P22.

It is a phage composed primarily of six proteins: gp1, gp4, gp5, gp9, gp10, and gp26. Protein gp1 makes up the portal protein, inserted into one of the five vertices of the gp5-composed capsid. Three gp9 proteins make up each 'leg' of the tailspike, and the neck that attaches said tailspike to the capsid is made up of gp4, gp10, and gp26 proteins.[7]

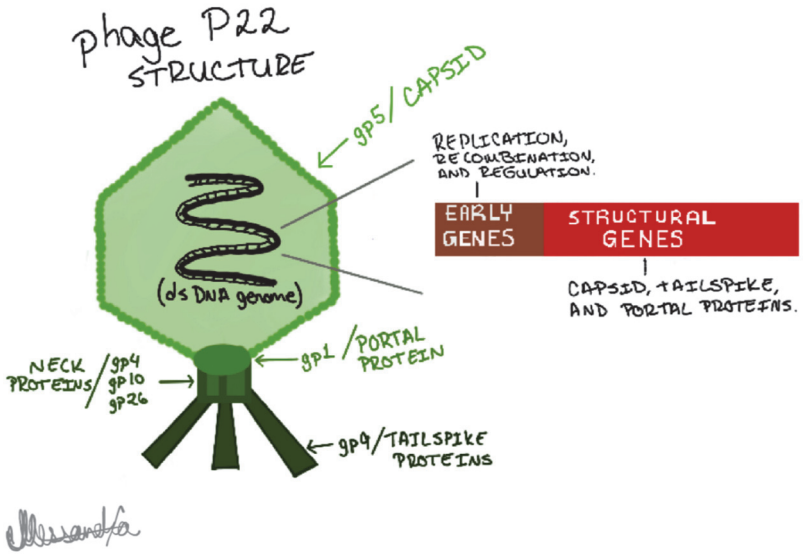

Image courtesy of the author

Phage P22 was first used by Zinder and Lederberg in 1952 to demonstrate generalized transduction and has since become an important molecular biology tool; it has a short tail, a linear double-stranded DNA genome, and an icosahedral capsid.[7] It is a temperate phage, meaning that, after infection, it can enter either a lytic or lysogenic cycle.

Phage P22 is specific to *Salmonella typhimurium*; it enters the host cell through binding of the tailspike trimer to the O-antigen of the lipopolysaccharide. The tailspike trimer cleaves the bond between the galactose and rhamnose[6] saccharides,[7] which allows passage to the outer membrane of the cell, where binding of the base plate to the membrane occurs; the linear genome is then injected into the cell.

In the lytic state, the genome is replicated and transcribed: the linear genome is circularized by recombination and replicated through the rolling-circle mechanism.[7] The first genes transcribed are those involved in the regulation of gene expression, replication, and recombination, as these are clustered together in the chromosome. From here, the structural genes are transcribed and assembly begins. For phage P22, the capsid and the tail are assembled separately and are then joined together to form the active phage.[8]

In the lysogenic state, the viral genome is integrated into the host genome at a specific site, termed *attB*, and the genes responsible for phage development are repressed through the actions of two repressor proteins, *mnt* and *c2*. Once *mnt* is turned off, it activates the protein that turns off *c2*, which allows the phage to enter the lytic cycle.[8]

Salmonella typhimurium LT2 and *Lederbergvirus P22* are the main microorganisms used in the activities, as they are safe organisms with relatively rapid growth and replication times. Other microbes can also be considered for the same experiments, such as *E. coli* and its well-known phage *T4*, and the results should not be significantly different from those observed with the original microbes.

References

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