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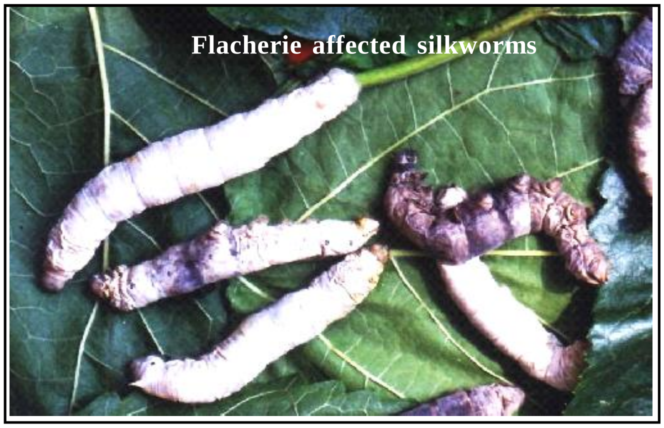
Bacteriophages for controlling bacterial diseases in sericulture

N.R. Singhvi, R.V. Kushwaha[1] **and** Mukund V. Kirsur[2]
Regional Tasar Research Station, BHANDARA (M.S.) INDIA (Email : nrsinghvi@rediffmail.com)
[1]Research Extension Centre, Central Silk Board, Badnera, AMRAVATI (M.S.) INDIA
[2]Central Sericultural Research and Training Institute, Srirampura, MYSORE (KARNATAKA) INDIA


Flacherie affected silkworms

Bacterial diseases in silkworm cause substantial financial loss to the industry, As such, their prevention and control assume greater importance. A parasite of bacteria, Bacteriophage remains the primary cause why bacteria haven't taken total control of the planet yet! It's difficult for bacteria to defend against a phage attack. Hitherto, sericulture industry has not put its efforts on phage therapy for controlling bacterial diseases which is a major reason for crop loss. Phage therapy could well take Indian sericulture to the top, obviously by controlling crop loss.

The word "silk" stands for exclusivity, Royalty, luxury, splendor and grandeur. Sericigenous insect called silkworm secretes silk- the natural lustrous protenious fibre, a product of a unique plant material insect interface.

The production of silk by rearing silkworm is know as *Sericulture* (Latin word *Sericum* meaning silk and French *Culture* means cultivation) Hence, sericulture indeed is an art and science of production of silk.

India has a unique place in the silk map of the world producing all the commercially important silk varieties *viz.* Mulberry, Tasar, Eri and Muga. The golden hued Muga silk and the rougher rugged looking tropical Tasar silk are unique to India as they are produced nowhere else in the world. Tasar, Eri and Muga are known as *Vanya* silks. However, in general parlance silk means mulberry silk. Of all the four types of silk the mulberry silkworm, *Bombyx mori* is of great commercial importance as a foreign exchange earner for many silk producing countries of the world. Realizing importance of sericulture, Research and Development and extension of sericulture has been accorded priority by the governments for providing employment and alleviating poverty especially in the rural areas.

In tropical countries, mulberry silkworm is continuously reared and this makes it highly susceptible to pathogens and hence occurrence of diseases is a major constraint (Samson *et al*., 1998). Four silkworm diseases namely Grasserie (viral), Flacherie (bacterial), Muscardine (fungal) and Pebrine (protozoan) are common in China and India. These diseases significantly reduce the yield of cocoons and cause great loss to the rearers. In India 40 per cent of crop losses occur due to these diseases (Veeranna, 1999). Since silkworm diseases cause substantial financial loss to the industry, their prevention and control assumes utmost importance. Much importance has been given to control of Pebrine and Grasserie diseases. Control of Bacterial diseases also needs equal attention to reduce crop losses and thereby increasing silk production in India.

Bacterial diseases in silkworm: Occurrence of bacterial diseases *viz.*, bacterial flacherie, Septicemia, Sotto disease and Court (Rangi) disease in silkworm cause heavy loss to silkworm crops.

Bacterial flacherie or Gastric injury flacherie: This disease is caused by multiplication of bacteria in the alimentary canal which has become weakened due to bad environmental condition. Various pathogens have been suspected to be the causative organisms. In the initial stages of the disease Streptococci, in the advanced stage approaching death, coli form bacteria and in dead worms Proteus group of bacteria have been isolated from the diseased larvae. The red or brown colour of the dead larva is due to the growth of the proteus bacteria.

Septicemia : Bacterial infection of the haemolymph caused by *Bacillus, Streptococcus* and *Staphylococcus* is the cause for this disease.

Sotto disease : This is a disease due to toxic substances entering into the body. The pathogens *Bacillus thurigensis* or sotio or *Bacillus satta* secrete a toxin which enters the haemolymph from the gut by absorption. The toxin affects the nervous system and causes paralysis to the larva.

Rangi (Court) disease : This disease is called *Rangi* in India because of the colours produced in the dead larva. *Serratia marcescens* (*Bacillus prodigiosis*). This bacterium causes a fatal infection only when inoculated and in harmless when taken orally. Larvae and pupae are infected by this disease. Control of bacterial diseases in Tasar and Muga crops is all the more difficult as the silkworms are reared in the natural conditions. Hygiene maintenance and use of chemical based bed disinfectants are the only measures against these diseases. This calls for development of new therapy and plunge into innovative research so as to develop cost effective ways of controlling bacterial diseases in Sericulture, be it mulberry, muga or tasar. Development of phage therapy can provide a simple and economic approach to prevent / control bacterial diseases in sericulture.

What are phages? : Phage is the shortened form of bacteriophage, a virus that infects bacteria. Bacteriophage is a parasite of bacteria.

Viruses are extremely small, dangerous organisms approximately 15-20 nanometers in diameter, having a set of one or more nucleic acid template molecules, normally encased in a protective coat or coats of protein or lipoprotein, which are capable of organizing their own replication within a suitable host cell. Shortly after invading another life form (called host), they take over the host's survival kit and begin to make copies of themselves. A virus does not eat, drink, excrete, transit or make merry. All it does is reproduce, in a frenzy that is unmatched in the living world. One virus can become million within an hour of infection. In fact, phages are the most numerous organisms on the planet earth; their total weight is more than the weight of all the elephants in the world.

Bacteria are dangerous too, but they are not as focused as viruses. They eat and produce waste, apart from reproducing. Bacteria are clever; they know to evade the antibiotic / chemical missiles we launch at them.

Since ancient times, there have been documented reports of river waters having the ability to cure infectious diseases, such as leprosy. In 1896, Earnest Hanbury Hankin reported that something in the waters of the Ganga and Jamuna rivers in India had marked antibacterial action against Cholera. In 1915, Frederick Twort, a British bacteriologist of the Brown Institution of London, discovered a small agent that infected and killed bacteria.

Independently Frederick W. Twort in Great Britain (1915) and Félix d'Hérelle in France (1917) discovered the viruses that attack / infect bacteria. D'Hérelle coined the term Bacteriophage, meaning "bacteria eater". A bacteriophage therefore is any one of a number of viruses that infect bacteria. Phage is the shortened form of bacteriophage, a virus that infects bacteria.

Phages multiply inside the bacteria, burst open the cell and invade other bacteria, one phage can become a million within an hour of infection. Phages also seem to know a trick or two about bacterial behavior. What can we learn from these soft creatures? How can we get benefited from them? As it is difficult for bacteria to defend against a phage attack, we, can exploit it as a natural enemy of bacteria. Phages seem to be an effective way to tackle the problem of bacteria compared to use of antibiotics /

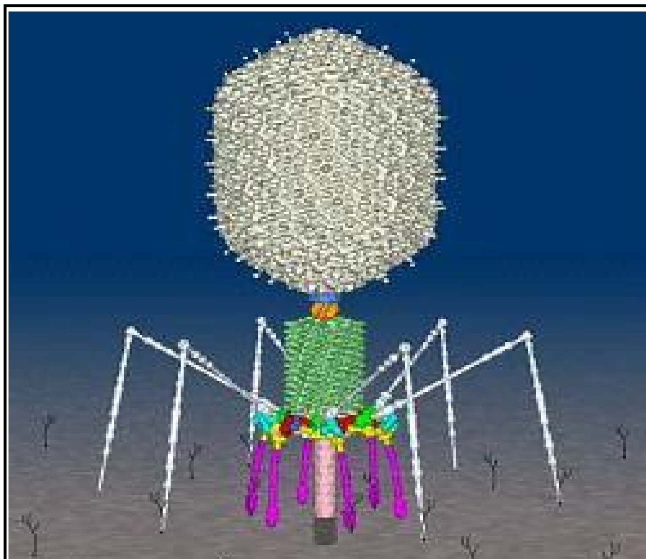


Fig. 1: Structure of T_4 bacteriophage

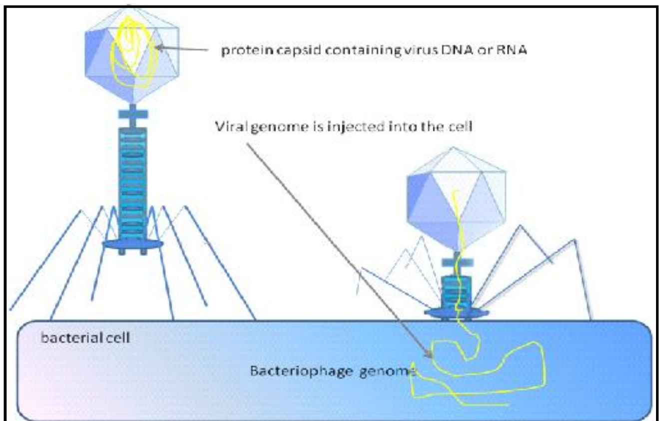


Fig. 2: A bacteriophage injecting its genome into its host bacterial cell

chemical resistance.

Distribution of phages: Phages are the most widely distributed and diverse entities in the biosphere. They are ubiquitous and can be found in all reservoirs populated by bacterial hosts, such as soil or the intestines of animals (silkworm too). One of the richest natural sources for phages and other viruses is sea water, where up to 9×10^8 virions per milliliter have been found in microbial mats at the surface, and up to 70% of marine bacteria may be infected by phages. Preparation of silkworm larvae contained two phages with contractile tails (Myoviridae). One phage was active on *Pseudomonas oaucimobilis* (Ackermann *et al*.

Advantage and disadvantage of bacteriophage therapy	
Advantages	Disadvantages
Very specific; affect the targeted bacterium only; therefore, "dysbiosis" (and chances of developing secondary infections) is avoided.	Because of the high specificity of phages, the disease-causing bacterium has to be identified before the phage therapy is started.
No side effects have been found so far - Able to self-reproduce as long as corresponding host-bacteria are present in the environment. Therefore, the need to repeatedly administer the phage is greatly reduced.	The negative public perception of viruses may also play a role in the reluctance to embrace phage therapy.
Since phage is targeted to receptors on bacterial membrane or capsule, which are important virulence determinants, development of phage-resistance usually means changes in those structures and may, therefore, lead to attenuation of the strains in virulence.	
Phage therapy is generally considered safe.	
Selecting a new phage (e.g., against phage-resistant bacteria) is a rapid process and frequently can be accomplished in days. Evolution drives the rapid emergence of new phages that can destroy bacteria that have become resistant. This means that there should be an 'inexhaustible' supply.	
Production is simple and relatively economical	

(1994) (*Archives of virology*, 137: 185 – 1990). Phages can be separated from diseased leaves/ larvae and soil. Zhu Que Ming (1986) collected 29 bacteriophages thorough out China. Phages can usually be freeze-dried and made into pills without impairing efficiency. In pill form, phages have shown temperature stability up to 55^{0}C and shelf life of 14 months. Application in liquid form is possible by storing preferably in refrigerated vials. Oral administration also works. Topical administration often involves application to gauzes that are laid on the area to be treated.

Phage therapy: Phage therapy is the therapeutic use of bacteriophages to treat pathogenic bacterial infections. Phases have been used for over 60 years as an alternative to antibiotics in the former Soviet Union and Eastern Europe. Recently Georgian doctors successfully used phages to treat wound infections that did not respond to antibiotics. They are seen as a possible therapy against multi drug resistant strains of many bacteria. Phages were used for treating Cholera as early as 1920s in Punjab. In the USA during the 1940s, the large pharmaceutical company, Eli Lilly, undertook commercialization of phage therapy. In 2006, the UK Ministry of Defense took responsibility for a G8-funded Global Partnership Priority Eliava Project as a retrospective study to explore the potential of bacteriophages for the 21st century.

Phage therapy has many potential applications in human medicine; dentistry; veterinary science; and agriculture. As bacteriophages are bacteria specific, they are safe not only to the host (human, animal, or plant; silkworm in sericulture) but also to other beneficial bacteria, such as that of gut flora. They also have a high therapeutic index, that is, phage therapy gives rise to few of any side effects. However, in this field of work also there are challenges, not the least of which is the regulatory regime. Phages therapy seemed promising, but for scientific and business challenges.

Can therapeutic phages be developed against any infection? :

– Therapeutic phages can potentially be developed against any bacterial infection.

– Development and production is faster than antibiotics / chemicals.

Application of phage therapy in agriculture and sericulture : In agriculture, phages were used to fight pathogens like *Campylobacter*, *Escherichia* and *Salmonella* in farm animals, *Lactococcus* and *Vibrio* pathogens in piciculture and *Erwinia* and *Xanthomonas* in plants of agricultural importance. Phages have been investigated as a potential means to eliminate pathogens like *Campylobacter* in raw food and *Listeria* in fresh food or to reduce food spoilage bacteria. Development of phage therapy can provide a simple but economic way to control bacterial diseases in sericulture.

Now, Scientists have created genetically engineered phage, specific to bacterium. Some products have already been developed using phages for tackling diseases in vegetables. It may be possible to develop products that can treat any kind of bacterial infection including serious ones in sericulture.

Of course this is altogether a new field of work! There are many a challenges ahead. But, sericulture scientists have always venture into new realm of research for the benefit of industry! Let us exploit the phage therapy, for victory against bacterial diseases in silkworms off course in a most economic way!

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