5.1 Phage therapy in agriculture and food industry

Slide number and content:

1) This part focuses on the application of bacteriophages as antibacterial agents in agriculture and the food industry.

2) First of all, we will discuss why there is an urgent need for alternative antibacterial agents in these industries.
By 2050, the world population is expected to reach over 9 billion people. This will obviously result in increased demands for food.
Up to 10% of global food production is lost to plant bacterial diseases. Prevention or treatment of these could thus be a solution to partly meet the increasing demands for food in the future.
These pictures show you some examples of crop diseases. Each disease is specifically caused by one bacterial species, like *Agrobacterium radiobacter* biovar 1, which causes crazy roots in tomato or *Xanthomonas campestris* pathovar *campestris*, which causes black rot of cabbage.
Currently, the only option to limit the impact of a bacterial infection is to take preventive measures, like applying a diverse crop rotation or immediately removing any symptomatic plants from the field or greenhouse to limit bacterial spreading. However, due to bacterial leftovers in soil or on plant residues, it is unrealistic to further reduce bacterial contaminations only with these precautions.
Traditionally, copper-based chemicals like copper sulphate or copper oxide were used to eradicate an infection, but these are no longer allowed since they are broad-spectrum and hence kill all bacteria in the soil. Furthermore, any remaining copper residues would be harmful for the environment. Antibiotics could be a possible solution, but these are not allowed because all crops finally enter the food chain and this would eventually help further spreading of bacterial antibiotic resistance.

3) Postharvest, a lot of food is still lost to bacterial spoilage. For example, soft rot of vegetables like tomato and pepper by *Erwinia* species or spoilage of chicken or other meats by *Pseudomonas fluorescens*. This spoilage results in undesirable changes to the sensory characteristics of food, making it unacceptable for human consumption.
Generally, no antibiotics are allowed to prevent these contaminations for the same reason as mentioned before. Therefore, certain chemical preservatives could be added to increase the shelf life of food products. However, consumers more and more expect sustainable products that are contaminant free and of acceptable quality without the use of those artificial preservatives.
Even with other preservation methods and good manufacturing practices, quality control and hygiene, still 25% of the total food produced every year is lost to microbial spoilage. Therefore, alternative preservatives remain crucial to significantly help meeting the ever-increasing demands for food.

4) In addition to the impact of bacteria on the food industry in terms of food spoilage, microbial contamination of food with certain pathogenic bacteria can also pose a serious threat to health and well-being. Examples of these are food poisoning caused by *Salmonella enterica*, *Listeria monocytogenes* or *Escherichia coli* O157:H7. It was estimated in 2011 that approximately 48 million cases of food poisoning occur each year in the United States alone, of which 128,000 result in hospitalization and 3,000 even result in death.

   It should be clear by now that there is an urgent need for an alternative antibacterial agent to reduce crop losses, food spoilage and food poisoning.

5) The application of strictly lytic bacteriophages as a biopesticide, food preservative or biotherapeutic agent is such a valid and biologically sustainable alternative. As a result of the lytic phage infection, host bacteria will lyse and release new phages, which can then infect remaining bacteria.

   A phage therapy has several advantages over conventional chemical pesticides, preservatives and therapeutics. First of all, phages are ecological and environment friendly, since they are biodegradable. Moreover, phages amplify in their host and will in a final step destroy the host to release their progeny. This self-amplification effect is an enormous advantage over other methods, since fewer applications will be needed. Furthermore, phages are very selective against the aimed bacteria or pathogens. This is an advantage, since the phage will have no effect on the treated crop, the other harmless soil bacteria or e.g. on probiotic bacteria present and necessary in the treated food product. However, this high specificity can also be a disadvantage, since a phage might be so specific that it only infects a certain subset of strains of the bacterial species, thus leaving part of the present strains untouched. To solve this problem and to cover a whole bacterial species, phage cocktails of diverse phages, each having a different host strain spectrum, are used. The use of different phages also reduces fast resistance development. Last but not less important, a phage cocktail is economically profitable for the manufacturer, since it is cheaper to produce than conventional pesticides or preservatives.

6) Now lets go to some examples of phage therapy applications in agriculture and in the food industry.

   In recent years, several studies have been published on the use of phages as a biopesticide against a number of important bacterial plant pathogens, like *Dickeya*, *Ralstonia*, *Xanthomonas* and *Pseudomonas*, with many very promising results. If you want to know more about these studies, you can find all references in this table.

   It is important to say that while the infection properties of a given phage may appear to have great potential with *in vitro* studies, this does not necessarily translate into biocontrol potential in the field, so field or greenhouse trials are very important for this research. Once these trials show significant reduction of symptomatic plants after phage administration, the phage product can be registered and approved as a biopesticide by the responsible authorities.

7) Just a few phage biopesticides made it to the market so far. The first one being AgriPhage, a biopesticide from the USA based company OmniLytics, which was registered in 2006. These two phage cocktails are specific for *Xanthomonas campestris* pathovar *vesicatoria* or *Pseudomonas syringae* pathovar *tomato*, and prevent and control bacterial spot or speck of tomato and pepper plants.
   A Hungarian company Enviroinvest was the second company to receive registration for their biopesticide. Erwiphage controls fire blight of apple trees and is specific for *Erwinia amylovora*.
   Both phage products are concentrated liquid products that have to be diluted in the irrigation water and can then be sprayed on the plants in the greenhouses or fields. Since phages are UV sensitive, application should be done several times a week preferably late in the evening or before dawn.

8) In the food industry, there is also great potential for the use of phages as natural antibacterials to control food pathogens and spoilage organisms at the different stages of production: from the decontamination of livestock, to sanitation of equipment and contact surfaces on farms and in industry, biotherapeutic in raw meats and fresh products, and also as natural preservatives in foods to extend product shelf life.
   Again, in recent years, a variety of studies have been published that prove the potential benefits of phages to control and eradicate spoilage organisms and pathogenic bacteria in the food industry. For these, I like to refer you to two review articles by Goodridge and Bisha and by Endersen and colleagues as listed in the references slide.

9) In terms of preservatives to purely extend shelf life, there is currently only one product on the market, which is produced by a Scottish company, APS biocontrol. They developed the phage-based wash solution ‘Biolyse’ for potato tubers. Biolyse is sprayed on the tubers before packaging and is used for prevention of soft rot disease, caused by *Enterobacteriaceae* like *Pectobacterium* during storage.

10) The natural biotherapeutic potential of phages against foodborne diseases is well recognized throughout the world. In 2006, a major milestone was achieved with the approval of the first phage-based product ListShield to control *Listeria monocytogenes* in meat and poultry products. This product, which is also liquid and which can be sprayed on food, is produced by the USA based Intralytix.
    For the moment, Intralytix has two more phage products that have been approved by the FDA and are available on the market: SalmoFresh to control *Salmonella* in poultry, fish, shellfish, and fresh and processed fruits and vegetables and EcoShield to control *E. coli* O157:H7 in red meat that is intended for grinding.
    Very recently, a fourth product, ShigaShield, has received GRAS status by the FDA, to control pathogenic *Shigella* species in food products.

In the Netherlands, there is the company Micreos who also produces two approved phage products under the brand name PhageGuard, that can be applied on ready-to-eat food products to avoid food poisoning. PhageGuard Listex combats *Listeria* while PhageGuard S, previously branded as Salmonelex, attacks *Salmonella*, both without affecting taste, smell or texture.

All these examples confirm that the use of phages is now completely acceptable in agriculture and the food industry and pave the way for the approval of more phage-based products in the near future.