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Innovation in Food Production Standard – Zero Salmonella

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Abstract
Following the request from the Health and Consumer Protection, European Commission, the Panel on Biological Hazards was asked to deliver a scientific opinion on the use and the mode of action of bacteriophages in food production. This action was taken in 2009 and today we have a new standard in food processing: ZERO Salmonella. There are chemical products involved in based on biocidal substances but also a new approach based on non-pathogenic microorganisms such as bacteriophage. The paper presents a study case according to the Panel on Biological Hazards.

Keywords
salmonella, bacteriophage, biological hazards, food production

1. Introduction

The paper presents an interesting and new approach in the safety in food industry.

Modern microbial food safety assurance is based on farm to fork principle. This will involve a lot of standard procedures based on controlling measures in every step of food processing having the same result: to decrease food contamination without increasing the health risks for human consumer. A large range of measures are taken to ensure this: from clean drinking water for animals, antibiotics in food for livestock, clean breeding sheds to clean and safe slaughterhouses and process facilities.

Working with biocidal products based on approved substances is the most common way to inhibit pathogenic microorganisms. Using a natural protection of food seems to be a much better option. Introduced in 1900, bacteriophage therapy was used to combat uncontrolled infectious diseases. In 2009, phage were approved to be use in external decontamination and in 2021 in meat preparation chain.

2. Decontamination

2.1. Physical Decontamination

Physical decontamination includes cold or hot water washing and or rinsing, steam treatments such as pasteurization, steam vacuum etc., high pressure treatments, electromagnetic treatments (such as ultraviolet, infrared etc.), plasma gas treatments, irradiation treatments etc.

2.2. Chemical Decontamination

Chemical decontamination is a must in food processing standards. Different biocides are specific for different applications because the decontamination mechanism is different for every biocide substance. Chemical treatments are using chlorine, clor dioxide, organic acids (lactic, acetic, citric acids etc.), peroxide, trisodium phosphate etc. Some of these substances require a rinsing step after application, some don't.

Each step of these standards has also a standard controlling procedure and national authorities for food safety may intervene to check it. National authorities are working permanently with European ones to describe better different food decontamination treatments and find a better way to inhibit and eradicate pathogenic microorganisms.

All physical and chemical treatments could include potential changes of sensory qualities (due to heat) and potential residues (due to chemical treatment) in the food. The intensity of these treatments can be reduced to limit these risks, but the effectiveness will decrease too. There are other different treatments used in sequence [1] for additive decontaminating effects.

2.2. Treatments Based on Bacteriophage

There are some treatments based on natural antimicrobials: plant extracts, microbial products that are involved in the ecology of the food. Some of them have a protective effect due to the increasing immune system of the livestock, some of these microorganisms inhibit or eradicate pathogenic bacteria or their spores.

Humanity has used microorganisms to produce food such as wine, beer, kefir, yogurt, cheese without having specific knowledge about bacteria. Even without genetic modification is possible to use microorganisms to protect food from unwanted pathogens. The Panel of Biological Hazards made a conclusion: "virulent bacteriophages are the ones of choice for phage-based food decontamination, and some of these, under specific conditions, have been demonstrate to be very effective in the targeted elimination of specific pathogens from foods" [2]. Bacteriophages are viruses which infect bacteria and kill them.

2.3. Parallel Between QACs and Bacteriophage Decontamination Mechanism

Quaternary ammonium compounds (QACs) are membrane-active agents interacting with the cytoplasmic membrane of bacteria and the plasma membrane of yeast. Their hydrophobic activity also makes them effective against lipid-containing viruses. QACs exert their antimicrobial effect by primarily disrupting microbial cell membranes, causing essential components to leak out and leading to cell death. They interact with the cytoplasmic and plasma membranes of bacteria and viruses, and at higher concentrations, they can also interfere with intracellular targets like DNA and the cell division protein FtsZ.

Bacteriophage are a structure built of protein, caring a nucleic acid, a parasite that require a bacterial propagate. They live in salted water, fresh water, soil, milk, vaccines, in digestive tracts and skin of the humans. When a bacteriophage encounters its specific bacterium, it attaches itself to the cell wall of the bacterium using its tail fibres, penetrates the cell wall. The DNA of the bacteriophage is drawn into the bacterium taking over the cell and destroying the bacterium's ability to function or replicate. Like all viruses, bacteriophage invades bacterium and the released toxins kills the bacterium. Finally, bacteriophages destroy the bacteria cells followed by a massive increase of the bacteriophage number.

Figure 1 presents a cocktail of six genetically different types of bacteriophages forming units per ml of solution against a large broad of Salmonella serotypes [3]. It is estimated that in 1 ml of water solution there are 1 million of pathogen bacteria and 10 millions of bacteriophages.

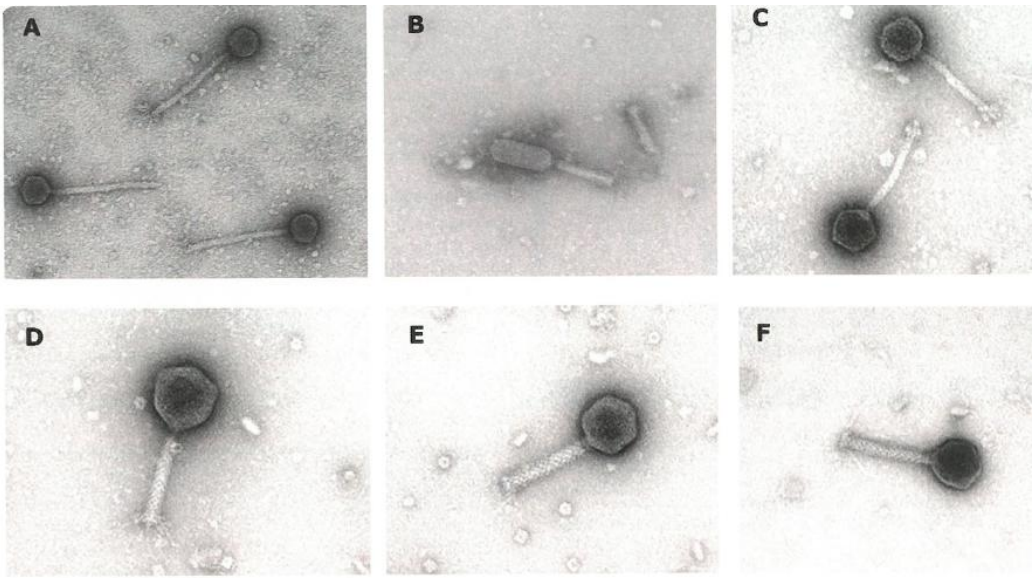

Fig. 1. Six types of bacteriophages composing a cocktail

Spectrum of action of Quaternary ammonium compounds: GRAM+ and GRAM- bacteria (*Mycobacterium Bacillus spores*, *Listeria monocytogenes*, *Antibiotic positive bacteria*), Viruses (enterovirus, Rotavirus, Norovirus, Influenza virus, Hepatitis A virus) and *Protozoa*, *Fungus* and *Algae*.

Bacteriophages provide inhibition and killing GRAM+ bacteria (*Listeria monocytogenes* and *Staphylococcus Aureus*) and Gram- bacteria (Salmonella, E coli).

3. Farm-to-Fork Principle

3.1. Farm-to-Fork Approach

The old approach of the Farm-to-fork principle is focused on control measurements to minimize as much possible the microbial contamination of the food using decontamination treatments. In practice, there is no way to complete eliminate microbial pathogens from food due to surviving pathogens after disinfection steps. The efficacy of the antimicrobial treatment may not be 100%, surviving microflora may remain on treated food or a very high initial microbial load may be present.

A large number of environmental factors could affect the efficacy of disinfection such as: concentration of the disinfection substance, temperature, pH and hardness of the water, duration of the exposure to the biocide, the biofilm formation and the fat and organic material present in the water.

Behind HACCP (Hazard Analysis and Critical Control Points) concept that involves a preventing approach to ensure food safety by identifying potential hazards in food production processes and implementing measures to prevent them, there is system focuses on controlling hazards in critical points named CCP- critical controlling point. All hygiene plans are focused to prevent and solve as much possible food contamination.

"Zero Salmonella Standard" is, by the way, a Salmonella Detection Procedure (EN ISO 6579-1). The International Organization for Standardization (ISO) published the new EN ISO 6579-1 standard in 2017, which specifies a horizontal method for the detection of Salmonella in the food production chain. But a final zero quantity of salmonella is impossible to achieve.

Since bacteriophage behave was studied, they started to be used to the advent of antibiotics and for internal treatment and superficial infections but the results are not conclusive in this direction jet. But also, they could be the cause of failure fermentation due to raw material contamination.

In our days bacteriophages start to be used against Salmonella contamination, especially when they are used in early stage of meat processing. As bonus, the use of bacteriophages has a low environmental impact, do not interfere with good bacteria from wastewater treatment. A large number of studies shows the safety improvement by using bacteriophage. They are natural, safe and specific [4], making them relevant in reducing pathogens.

3.2. Bacteriophage Application Example

The experiment is designed for a chicken meat process factory and is focused on scalding baths. Before and after the scalding bath there are two CCP- critical controlling points.

Necessary information:

- volume of the scalding bath (m^3);
- water consumption of the scalding bath per day (m^3);
- water temperature of the scalding bath (°C);
- retention time per bird in the bath (min);
- type of scalding bath, jet stream, OEM.

Slaughter speed

- chicken per hour;
- operation hours per day.

Salmonella serovars

- Salmonella serovars needed to be isolates from the pre catch available (pretesting is a must to make the right bacteriophage cocktail);
- Living bacteria needed to collect them as samples on an agar plate;
- S2 laboratory permit enclosed.

Chiller

- Where do you find the salmonella? In the water bath or on the carcasses? In the carcases and cut up pieces?
- What method are you using for detection PCR, culture?

- Do you confirm positive PCR findings with culture?
- Specify where is the sample area located on the carcasses: neck skin, the neck skin but also the meat form cut up pieces?

The trial is conducted by organising the service lab for the Salmonella determination (ISO 6579-1 absent in 25 g sample), pretesting of the scalding bath if a Salmonella positive herd is slaughtered >positive result after the herd and Salmonella isolates from pre catch to be sent to lab.

The conclusions of the trial are focused on the scalding bath. The exit from this area contains an important CCP. If Salmonella is present in that point, in the next processing steps – plucking and organs removing by cutting the carcasses – meat contamination will be ensured. The bacteriophage application will prevent the entrance of contamination on the skin and in the meat. Since bacteriophage consists mostly from nucleic acids and proteins, they are non-toxic [5] by releasing bacterial components while killing bacteria in situ, this means in the scalding bath.

4. Conclusions

Bacteriophage must come in contact with their bacterial host. They will survive as long the environment allowed their replication. Dirty, contaminated environments are the best environment for them to replicate.

Bacteriophage can be applied on different steps of food processing, in targeted applications to eliminate pathogens from food and food processing environment.

European Food Safety Authority works to develop new procedures to use bacteriophage as food additives for live stocks and to decrease the quantity of antibiotics in animal feed. Bacteriophage may be present naturally on the surface of food, skin, carcases and meat.

Using bacteriophage prevents food waste by their significant impact on decontamination. This is a major safety-economic advantage.

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