Section 1. Biology

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METHODS OF MICROBIAL DECONTAMINATION OF INSECT SURFACE

Abstract. The use of molecular genetic methods to study the composition of insect microbiome faces the problem of differentiating microorganisms that contaminate the surface structures of insects (adults, nymphs, chrysalis and eggs) and microorganisms that make up the intestinal microbiome. The article presents modern methods used in the food industry and scientific research to carry out microbial decontamination of food and insect surfaces.

Keywords: microbiota, insects, microbial decontamination.

Introduction

Today, to study the microbial communities of insects, researchers use the whole range of known molecular genetics methods – "meta-omics" [1], which include such methods as meta-genomics, meta-transcriptomics, meta-proteomics and metabolomics. These methods, in addition to providing knowledge about the taxonomic composition of insect bacterial communities, reveal their functional and metabolic capabilities. This information is essential for understanding the role of bacterial communities in interacting with insect hosts and for possible applications in biotechnology.

The small size of insects and the used sample preparation methods do not allow differentiating microorganisms contaminating the insect surface from representatives of the intestinal microbiota. This leads to an erroneous idea of the qualitative composition of the intestinal microbiota. Such differentiation is necessary for diagnosing the ways and mechanisms of infection transmission when conducting epidemiological studies and monitoring studies of the translocation of pathogenic bacteria into natural ecosystems. Therefore, the development and application of effective methods for decontamination of the surface of insects is one of the urgent tasks facing researchers.

All known methods of decontamination of surfaces (food, insects …) can be divided into disinfection using disinfectant solutions, exposure to temperature and the use of non-thermal food sterilization methods. Heat treatment is a traditional and effective microbial decontamination method that is still used effectively to kill pathogens. The two main convection thermal processes are pasteurization and sterilization. Pasteurization is a heating process in the temperature range of 60–80 °C. The result is cytolysis of microorganisms and inactivation of enzymes. Sterilization is carried out at temperatures above 100 °C to kill spores or spore-forming bacteria. Pasteurization mainly destroys vegetative cells, but not spores. Perhaps these methods will be effective in reducing microbial contamination of the surface of

insects, but as a result, the microorganisms that make up the intestinal microbiome of insects will also die, which is not desirable.

Insects are rich in nutrients and moisture and provide a favorable environment for the growth of microorganisms [2]. Therefore, for insects eaten, immersion in hot water of 80–100 °C or frying is recommended.

So, eaten in African countries – *Gonimbrasia belina (Westwood, 1849) (Lepidoptera: Saturniidae)*, also known as the mopani worm, traditionally goes through a 24-hour fasting period (the number of microorganisms in the intestine decreases), blanching 15–30 minutes and from one to three days of drying in the sun before eating [3]. Fresh insects have a high bacterial load of Enterobacteriaceae and spore-forming bacteria. Microwave blanching kills vegetative cells but not spores [4].

A number of studies [5] have shown that blanching (10 min for *Tenebrio molitor* or 5 min for *Acheta domesticus*) effectively destroys bacteria. In addition, the bacterial load of the intestines of edible insects may be higher than the bacterial load of their surface [6]. Thermal treatment of insects for 10–15 minutes in a water bath at 90 °C significantly reduced the total number of aerobic bacteria on the surface of the insects, but did not affect the spore-forming bacteria and mycelium. There are no standardized protocols for handling insects for food [7].

Freeze drying can be considered as an effective method of microbial decontamination of insect surfaces. However, this method mainly inactivates the vegetative forms of the bacteria. Freeze drying is not effective against spore-forming bacteria and mycelium.

Sterilization of insects by autoclaving has proven to be more effective than blanching and freeze-drying. Insects were ground in a universal blender and inoculated into 5% NaCl solution. The solution was autoclaved for 16 min at 120 °C. This mode of autoclaving contributed to the reduction of microbial contamination of insects up to 93%. It is possible that additional treatment with acetic acid solution can reduce the bacterial load [8].

Recently, several non-thermal methods of food preservation have been proposed that can also be used for microbial decontamination of the surface of insects – high pressure processing [9], ultrasound [10], pulsed electric field [11], ultraviolet light [12], high-intensity pulsed light [13], gamma irradiation [14], and cold plasma [15].

While these non-thermal food preservation methods have minimal impact on food taste, nutrients, aroma, and freshness, they do not have a 100% bactericidal effect on bacteria that contaminate food surfaces.

The high pressure processing (HPP) is one of the promising methods of disinfection in agriculture and the food industry. Thus, high pressure processing (HPP) of spores of *Fusarium graminearum*, a pathogenic microorganism that causes wheat fusarium wilt, in the mode (380 MPa, 60 °C, 30 minutes) completely inactivates spores [16]. When high pressure processing (HPP) spores of *Aspergillus niger* and *Penicillium exspansum*, in the mode (300 MPa, 60 °C, 30 minutes), they were completely inactivated. This method can be used in the food industry, both to inactivate spores on foodstuffs and on the surface of edible insects. Although high pressure processing (HPP) can be used to decontaminate insect surfaces, how it will affect the gut microbiome remains unknown.

Another non-thermal food preservation method is high intensity ultrasound (HIU). High intensity ultrasound (HIU) locally creates a high pressure and temperature gradient with power (20–100 kHz). It destroys cell membranes and DNA, which causes a cytolytic effect that helps reduce the number of bacteria [17]. The mechanism of action of ultrasound is based on the phenomenon of cavitation. Low-intensity ultrasound may not have a lytic effect on the bacterial cell due to poor formation of cavitation bubbles [18]. In most studies using high intensity ultrasound (HIU), cells inoculated in liquid were used to create a cytolytic effect, which is of fundamental importance for the cytolysis of a microbial cell. The cytolytic effect of ultrasound (42 kHz) on *Escherichia coli* cells increased with increasing exposure time:

60 min (99.6%), 75 min (99.7%), 90 min (99.8%) [19]. Sonication (20 and 40 kHz) of *Escherichia coli* and *Klebsiella pneumonia* inoculated in phosphate buffered saline (PBS) reduced the number of living cells [20]. However, the use of ultrasound at a higher frequency of 580 kHz did not affect the destruction of cell membranes, but promoted cell deagglomeration. The use of high intensity ultrasound (HIU) in the range of 20–40 kHz significantly reduced the number of viable gram-negative bacteria *Salmonella spp*., *Escherichia coli*, but to a lesser extent, ultrasound had an effect on *S. aureus* cells. At the same time, the duration of exposure to ultrasound on bacterial cells was more important than its intensity. High intensity ultrasound (HIU) is being considered for use in the food industry to sterilize liquid foods.

Irradiation with ultraviolet light at a wavelength of 254 nm is a powerful method for disinfecting surfaces. Light in the wavelength range of 250–260 nm is lethal to microorganisms [21]. The mechanism of action of ultraviolet light is based on its ability to damage the DNA of microorganisms, forming thymine dimers. They block DNA replication, which causes the death of microorganisms. UV irradiation reduced the amount of *Escherichia coli* in beef by (1.0 ± 0.2) $\log_{10}$ CFU/mL after 5 minutes of exposure. In chicken and pork, the amount of Escherichia coli decreased by (1.6 ± 0.7) $\log_{10}$ CFU/mL and (1.6 ± 0.4) $\log_{10}$ CFU/mL after 4 and 10 min of irradiation, respectively [22]. These results indicate that irradiating food or insect surfaces with ultraviolet light will kill microorganisms only on the surface without penetrating deeply. The presence of folds or furrows on the surface, which is characteristic of the cuticle surface of beetles, will significantly reduce the effectiveness of UV light treatment. Therefore, surface texture will have a significant impact on the effectiveness of microbial decontamination of insect surfaces with ultraviolet light.

Another of the modern non-thermal methods of food preservation is the processing of food at low temperatures and a short time interval, the so-called cold plasma technique (CP) [23]. Plasma is an ionized gas containing reactive oxygen species (ROS: O, O_2, ozone (O) and OH), reactive nitrogen species (RNS: NO, NO_2 and NO_x), ultraviolet radiation (UV), free radicals and charged particles. Typically, plasma is generated when electrical energy is applied to a gas present or flowing between two electrodes with a high electrical potential difference causing the gas to ionize due to the collision of free electrons with these gas molecules.

The cold plasma (CP) method is used in the food industry to reduce microbial contamination, inactivate toxins, allergens and enzymes. This method has proven to be effective for surface decontamination and can be used for microbial decontamination of insect surfaces.

In work [24], 11 protocols for surface disinfection of *Alphitobius diaperinus (Panzer) (Coleoptera: Tenebrionidae)* beetles were studied. The effect of a combination of substances (hydrogen peroxide (H_2O_2), 95% ethanol (EtOH) and sodium hypochlorite (NaOCl) on microbial decontamination of the beetle surface was studied. For each protocol, the tube into which the beetle was immersed was covered with a barrier film, inverted three times and sonicated in for 2 minutes (40 kHz). Sonication was used to ensure standardization of bacterial load to compare protocols. As a result of the experiments performed, it was shown that the most effective surface treatment protocol for beetles was a protocol that included a primary surface treatment with 95% EtOH (ethanol) and further treatment with 20% H_2O_2 or 7.35% H_2O_2 /0.23% acetic acid. This protocol resulted in 100% microbial decontamination of the beetle surface. A possible explanation for the effectiveness of the 95% EtOH (ethanol) surface pretreatment is that the outside of the cuticle is covered by a thin layer of epicuticle which serves as to minimize water loss from the beetle's body. The epicuticle consists of several layers – a superficial waxy or lipid layer of long-chain hydrocarbons and esters of fatty acids and alcohols. It inhibits surface drying and reduces the effectiveness of H_2O_2 and NaOCl surface disinfection. EtOH pre-treatment improved the efficiency of H_2O_2 and NaOCl disinfection.

In the study of the mechanisms of infection of adults and larvae of beetles, it is often necessary to establish the place of translocation of the pathogenic microorganism on the surface of the beetle or in the intestine of the insect. Therefore, to study the translocation of bacteria and conduct monitoring studies of the duration of microbial contamination of the surface of beetles, an important condition is the complete sterilization of the surface of adults, larvae and eggs of beetles.

In addition, it must be taken into account that the number of bacteria carried by each individual beetle before disinfection varies greatly. The bacterial load depends on many factors, but sometimes bacteria are not sown from the surface of the beetle, despite the fact that it has been in the same cage with other beetles for a long time. Perhaps this is due to the individual features of the structure of the beetle cuticle and its self-purification. In addition, surface disinfection of beetles with EtOH or NaOCl is detrimental to beetles, but after treatment with H_2O_2, beetles usually survive.

Conclusions

An analysis of the methods of microbial decontamination of the surface of insects and food products shows that there is no single highly effective method that would inactivate microorganisms in 100% of cases. The effectiveness of microbial decontamination of the surface of insects and food products is associated with the use of a set of methods that allow decontamination of certain groups of microorganisms (bacteria, fungi, spore-forming bacteria, viruses) at each stage. For example, sonicating the surface of insects will promote the destruction of bacterial cells and may increase the effectiveness of disinfection solutions. Treatment of the surface of insects with multicomponent solutions, stages of treatment (using a group of solutions with different physical and chemical properties + surfactants) and treatment time can be the most effective way of microbial decontamination of the surface of insects. However, this method of processing will undoubtedly reduce their survival rate.

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