Research Article

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PHAGE DETECTION OF PATHOGEN MICROORGANISMS IN AGRICULTURAL ECOSYSTEMS' MONITORING AS PART OF SECTORAL FORESIGHT

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ABSTRACT

The purpose of the agricultural ecosystems lies in supplying people with the outputs of crop production and livestock farming. The main feature is a purposeful or unintentional anthropogenic change of the living conditions of cultivated plants and domestic animals. The aim of study is working out the methods of practical application of the bacteriophages for the detection of the pathogenic microorganisms as part of sectoral foresight. The activity of the target phages was evaluated by their ability to lyse a bacterial culture in liquid and solid growth medium and represented by maximal dilution. Four bacteriophages *Listeria* – L2A, L4A *(*SRI NRIVVaMR RAAS, Pokrov, Russia*)*, P100 *(*Listex™, Netherlands*)*, Lm1 *(*IRCMiB, Ulyanovsk, Russia*)* were tested and found that phages L 2A, L 4A and P100 are genus-specific, the phage Lm1 is species-specific.

Keywords: Detection, bacteriophage, pathogen microorganisms, monitoring, agricultural foresight.

INTRODUCTION

Unfavorable changes in agricultural ecosystems can have negative consequences*:* can become a cause of the diseases of plants *(*epiphytotics*)*, animals *(*epizooty*)* and human beings *(*epidemics*)*. Thus one of the priority directions is the system of agricultural foresight and microbiological monitoring that help to detect the degree of potential pathogenic microorganism's contamination.

The majority of the soil microorganisms are nonpathogenic saprophytes, but there also exist such saprophytic bacteria that are pathogenic for human beings and animals. Bacteria of *Listeria* Genus belong to these microorganisms. *Listeria* monocytogenes – the listeriosis agent of human beings and animals – is highly stable in external environment, it grows in a wide range of temperature *(*from 1 to 45°C*)* and pH *(*from 4 to 10*)* and can multiply within subzero temperatures.

In many countries adopted national food control system for products bearing the risk of *Listeria* infection, developed standards for finished food products, taking into account the possibility of their contamination during storage in refrigerators, rules and technological standards in the food industry, in transport, storage and sale products.

Biopreparation based on phages are not alien for human beings and their microflora because of the selectivity of their action. As a result of their interaction with bacteria, they maintain bacteriological balance by restricting the number of bacteria and preventing them from multiplying. This enables the preparations to act as a biological protector against opportunistic pathogenic bacteria [3-5].

The key characteristics of the biopreparations based on phages are the following: the specificity of action, the quickness and validity of detection and the simplicity of performance.

The aim of the project is working out the methods of practical application of the bacteriophages for the detection of the pathogenic microorganisms**.**

MATERIALS AND METHODS

Four bacteriophages *Listeria* – L2A, L4A (SRI NRIVVaMR RAAS, Pokrov, Russia), P100 (Listex™, Netherlands), Lm1 (IRCMiB, Ulyanovsk, Russia) were the objects of the research. 50 bacteria strains were studied: 33 strains of *Listeria* (17 – *L.monocytogenes*, 10 – *L.innocua*, 2 – *L.seeligeri*, 3 – *L.welshimeri*, 4 – *L.grayi*, 3 – *L.ivanovii*) and reference strains of other genera stored in the museum of Department of Microbiology, Virology, Epizootology and Veterinary-Sanitary Evaluation of Food Products of Ulyanovsk State Agricultural Academy named after P.A. Stolypin (1 strain of *Erysipelothrix bacteria*, 2 – *Jonesia* and 6 strains of *Staphylococcus bacteria*). The bacteria had qualities typical of such genera and species. The research of the biological properties of the phages was performed with the methods suggested by M.R.J. Clokie, A.M. Kropinski [1], E.V. Suldina, E.N. Kovaleva, D.A. Vasilyev, S.N. Zolotukhin [3].

RESULTS

The morphology of the phage plaques was studied within phage inoculation by the agar-layer technique of Gragia. The results were checked after 20 hours of incubation at 28ºC.

The plaques formed by the phages had a similar morphology: round plaques with even edges, from 0,7 to 2 mm in diameter, transparent, without secondary growth and a zone of incomplete lysis.

The activity of the phages was evaluated by their ability to lyse a bacterial culture in liquid and solid growth medium and represented by maximal dilution, in which the studied phage showed its lytic activity. The lytic activity of the phages was detected by Apelmann method (method of serial cultivation in liquid growth media) and Gragia method (agar-layer technique on solid growth media).

The research showed that the studied phages had different lytic activity from 10^{-2} to 10^{-8} according to Apelmann method and from 2×10^3 to 1×10^9 phage corpuscles per 1 ml according to Gragia method (Table 1).

Table 1: Lytic Activity of Listeria Phages

Lytic Activity	Bacteriophages			
	L 2A	L 4A	P100	Lm1
According to Apelmann method	10^{-6}	10^{-5}	10^{-8}	10^{-2}
According to Gragia method	1×10^7	2×10^6	1×10^9	2×10^3

Lytic spectrum is a specific characteristic of a phage strain. Lytic spectrum detection was performed by phage application on bacterial lawn. The experiments showed that the lytic spectrum of homologous microorganisms was within 9 % – 100 % of the number of the studied strains (Table 2).

Table 2: Lytic Spectrum and Specificity of Listeria Phages

Bacteria species	Number of studied strains	Bacteriophages			
		L 2A	L 4A	P100	Lm1
		Lysed cultures, %			
L.monocytogenes	33	78,8	9	100	100
L.innocua	10	–	40	100	–
L.ivanovii	3	–	66,6	100	–
L.grayi	4	–	–	–	–
L.welshimeri	3	33,3	33,3	66,6	–
L.seeligeri	2	–	50	–	–
Erysipelothrix insidiosa	1	–	–	–	–
Jonesia dentrificans	2	–	50	–	–
Staphylococcus	6	–	–	–	–

*Note: «–» - absence of lysis

Specificity is one of the main characteristics of the phage that determines the possibility of its application for the identification of the agent culture. Specificity of the studied phages was studied with the method of phage application on the heterologous bacterial lawn. As the result of study phages L 2A, L 4A and P100 against the representatives of other genera (*Erysipelothrix*, *Jonesia*, *Staphylococcus*) and species of Listeria Genus (*L.innocua*, *L.seeligeri*, *L.welshimeri*, *L.grayi*, *L.ivanovii*) it was detected that the given phages lysed most of the tested cultures (Table 2).

CONCLUSION

The research results give grounds to the conclusion that phages L 2A, L 4A and P100 are genus-specific, the phage Lm1 is species-specific. Thus using specific phages one can successfully perform the monitoring of the pathogenic microorganisms contamination in the agricultural ecosystems.

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