Development of a combined preparation of bacteriophages for the prevention and treatment of salmonellosis in poultry

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Introduction

Salmonella is one of the most common zoonotic diseases in the modern world. Some strains of Salmonella are pathogenic for both animals and humans.

Salmonellosis accounts greatly for economic losses in the poultry industry. Moreover, salmonella is often transmitted to human beings through meat and eggs and causes serious health issues. Pathogenic salmonella strains are usually resistant to antibiotics. Ineffectiveness of antibiotics induces the search for alternative means of combating salmonellosis. One of such alternative solutions is the use of bacteriophages.

In Russia, various applications of phage therapy are set as a priority for the “Research and Production Centre “MicroWorld”. The scientists of MicroWorld have developed various phage preparations that are successfully introduced into agriculture, veterinary and medicine. One of the major assets of MicroWorld is its vast bacteriophage collection. As of today MicroWorld’s bacteriophage museum numbers over 400 highly lytic phages and is registered in World Federation for Culture Collection (WFCC), reg. № 986.

In this study, MicroWorld presents an effective multi-component phage preparation for prevention and treatment of salmonellosis in birds.

Research methods

At the initial stage an intensive microbiological analysis of the microbial landscape of the birds intestine and other pertinent biomaterials was carried out with both traditional methods of identification and the MALDI TOF mass-spectrometry method.

Phages for prospective preparation were isolated by the scientists of MicroWorld from natural sources on the basis of a proprietary methodology and technique. Various phage combinations were tested on the Salmonella strains (Salmonella enteritidis, Salmonella typhimurium, Salmonella infantis) collected from a number of poultry farms across Russia.

Phage candidates were screened by thorough analysis of their biological, immuno-chemical and physico-chemical characteristics. Phages characteristics were examined in accordance with the recommendations of the International Committee on Taxonomy of Viruses (Ackermann, Dubow, 1987).

Each phage candidate was scrutinized with the respect to safety considerations vis-a-vis the normal chicken flora, the phage-cell interaction was monitored by a fluorimetry method in the presence of a membrane-acting probe of ANS (Zhilenkov et al., 1998).

Results.

A. An effective composition of phages has been selected taking into account the following criteria:

- Each phage candidate is to have a broad lytic spectrum vis-a-vis various strains of Salmonella serotype.
- The phage candidate is not to affect microorganisms of the chicken normal flora.
- The phage candidate is to rapidly lyse the pathogenic cell with high yield of the offsprings.
- The phage candidate is to be reasonably sustainable to a wide variation of physical and chemical factors (heat, pH variation, etc.).
- The target multi-component phage preparation is to include phages, having different adsorption mechanism to the host cell. This criterion essentially provides for the elimination of phage-resistant forms to appear in the population of Salmonella.

Table 1 - Phage titers in organs of chicken after administration per os

S. enteritidis phage	Phage morphotype	Phage titre, PFU/g		
		blood	liver	intestine (faeces)
SPZ1	A2	$0{,}2 \cdot 10^2$	$1{,}7 \cdot 10^2$	$0{,}7 \cdot 10^2$
SPZ3	C1	$1{,}5 \cdot 10^3$	$0{,}7 \cdot 10^3$	$5 \cdot 10^4$
SPZ7	B1	$1{,}0 \cdot 10^3$	$1{,}0 \cdot 10^2$	$1{,}0 \cdot 10^4$

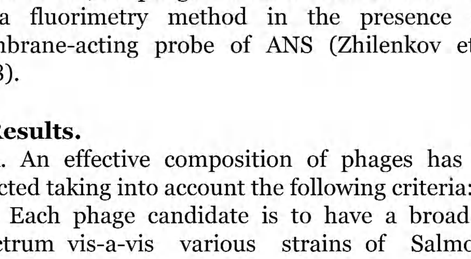

Figure 2. Interaction of phages with a Salmonella cell. Scale: 50 nm.

The eventual Preparation contains 9 phages (3 different phages for each of Salmonella species: Salmonella enteritidis, Salmonella typhimurium, Salmonella infantis). Selected phages belong to morphotypes A, B and C (Fig. 1, 2).

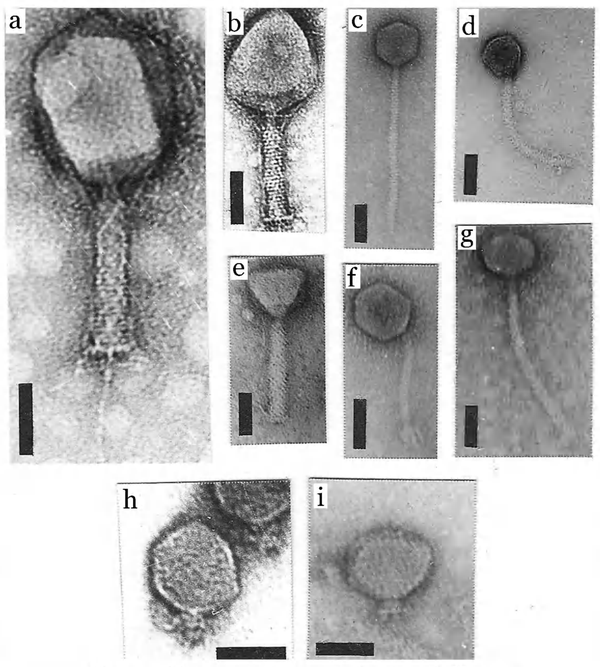

Figure 1. Electron micrograph images of phages with a lytic activity towards: a, f, i - Salmonella enteritidis; b, c, h - Salmonella typhimurium; d, e, g - Salmonella infantis. Scale: 50 nm.

B. Safety tests of the Preparation vis-a-vis the chicken normal flora.

In safety tests chicken normal flora was represented by the following bacteria isolated from the intestines of healthy chickens:

Alcaligenes faecalis ssp faecalis, Alcaligenes faecalis ssp phenolicus, Alcaligenes faecalis ssp paraphaecalis, Arthrobacter creatinolyticus, Arthrobacter parietis, Arthrobacter pigmentis, Arthrobacter roseus, Arthrobacter sulfureus, Arthrobacter woluwensis, Bacteriodes fragilis, Bacteriodes caccae, Bacteriodes vulgatus, Bifidobacterium ssp, Bifidobacterium infantis, Bifidobacterium longum , Brachybacterium faecium, Corynebacterium stationis, Corynebacterium confusum, Corynebacterium flavescens, Corynebacterium stationis, Clostridium bifermentas, Enterococcus faecium, Enterococcus faelalis, Enterococcus avium, Enterobacter cloacae, Echerichia coli (5 strains), Lactobacillus delbrueckii, Lactobacillus fructivorans, Lactobacillus malefermentas, Lactobacillus paracasei, Lysinibacillus fusiformis, Lysinibacillus sphaericus, Providencia rettgeri, Streptococcus salivarius.

The interaction between the phage and the bacterial cell membrane was monitored by measuring the fluorescence intensity in the system of ANS-cell-phage. The curve A on the diagram (Fig. 3) clearly shows positive results of the control. In all tests no change of Iem was registered, that proves the absence of even non-specific adsorption of 9 bacterial viruses on cells (version B in the diagram).

C. Pharmacokinetics of Salmonella phages.

A single dose of preparation (0,5 ml) containing three types of phages (1:1:1) was introduced to 10 chickens (5 days old). Concentration of each phage was $0{,}5.10^7$PFU/ml.

The subsequent analysis (after 60 min.) detected the presence of phages in birds' intestine, bloodstream and liver and importantly the phages retained their lytic activity in vivo (Table 1).

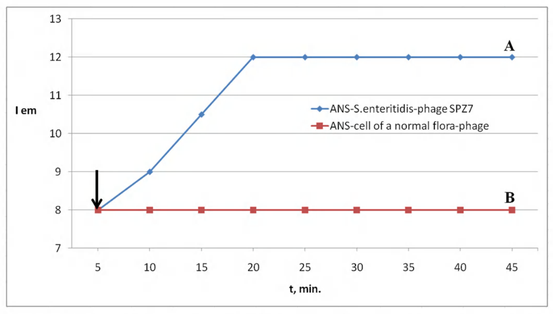

Figure 3. Fluorescent response in the system of ANS-microorganism-phage. Iem - fluorescence intensity of a probe in relative units. The arrow points to the introduction of the phage.

D. Efficacy of the developed Preparation in treatment of chickens with an experimental Salmonella infection.

Test and control groups of chickens (of 20 chickens each) were infected per os with Salmonella enteritidis in 10^7CFU/g. One hour later test group was given per os 0,5 ml. of the phage Preparation with concentration of 10^8PFU/ml. The treatments were repeated 3 times with 24hr interval. Consequently the Preparation was added into drinking water (1:100), and the test group was watered during 10 days. Chickens of the control group did not receive phages. 14 days later, the bacteriological study of parenchymatous organs of the test group revealed no Salmonella enteritidis therein. Data from the control group showed the presence of Salmonella enteritidis in the intestine, liver and spleen of every chicken.

E. Efficacy of the developed Preparation in treatment of salmonellosis in pigeons.

The experiment was carried out in a pigeon loft (150 birds), in the city of Dmitrovsk, Orel Region of the Russian Federation. Salmonella typhimurium isolated from the birds droppings proved to be resistant to a number of antibiotics. Pigeons in the test group were given per os 0.5 ml of the Preparation containing 10^8PFU of each phage. In 12 hours, the procedure was repeated one more time. During 12 subsequent days the Preparation was being added to drinking non-chlorinated water (1:100). Daily analysis of the droppings showed gradual decrease in the concentration of the pathogen in the litter. After 12 days no Salmonella was detected (Fig. 4). The test group also clearly demonstrated improvement in the pigeons’ behavior (activity, restoration of appetite, etc.).

F. Application of the developed Preparation in poultry farming.

Developed Preparation was successfully tested in several poultry farms in Russia to prevent of salmonellosis.

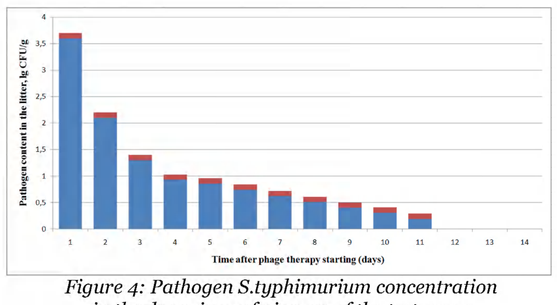

Figure 4: Pathogen S.typhimurium concentration in the droppings of pigeons of the test group

Conclusion.

1. A multi-component phage preparation for treatment and prevention of salmonellosis in birds was developed.
2. The Preparation demonstrated strong evidence to be safe vis-a-vis the normal flora of the birds intestine.
3. Phages demonstrated their ability to penetrate into various birds organs and retain their lytic activity therein.
4. The Preparation efficacy tested and proved on a number concrete examples.

"MicroWorld" is interested in expanding the practical application of the developed multi-component Preparation against Salmonellesis and is available to partner any interested party with a goal to develop similar products targeted at the prevention and treatment of any bacterial diseases of birds.

REFERENCES

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