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PRODUCTION OF THE BROAD HOST RANGE *SALMONELLA* PHAGE PVP-SE1 WITH SAFETY CONCERNS

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EXTENDED ABSTRACT

Salmonella enterica has long been recognised as an important zoonotic pathogen of economic significance in animals and humans and remains the primary cause of reported food poisoning worldwide with massive outbreaks occurring in recent years. The most commonly serovars reported in human infections have been Enteritidis and Typhimurium, comprising respectively 76% and 14% of the cases in the EU while other serovars caused each 1% or less of the cases. Although many efforts have been done to prevent and control *Salmonella* Enteritidis, it is still a major cause of gastrointestinal disease in the EU and USA. Investigations of outbreaks and sporadic cases have repeatedly pointed out that the most common sources of S. Enteritidis infection are poultry and poultry derivatives. For this reason, controlling *Salmonella* infections has become an important goal for the poultry industry.

The use and misuse of antimicrobials in both humans and animals have given rise to the emergence of infectious bacteria displaying resistance toward many, and in some cases all, effective antimicrobials, turning difficult the treatment of both animals and humans. This has become a critical problem that limits the use of once widely used drugs. In recent years, an increasing number of *Salmonella*e isolates resistant to multiple antimicrobial agents has been identified. Thus, as many authors agree and pursue, development of alternatives to chemotherapy is imperative and a critical priority. The use of bacteriophages (phages), viruses that specifically infect and lyse bacteria, as a therapeutic agent (phage therapy) is one possible option for controlling pathogenic bacteria. Phages as bio-control agents for *Salmonella*e and other pathogens have already been tested with success showing some advantages over antibiotics. However, due to the high specificity of phages, they can only be produced in their natural hosts. Their replication in pathogenic hosts causes the release of cell debris and large quantities of both endotoxins and exotoxins are found in the crude phage lysate.

The use of a non-pathogenic host in the production process would eliminate the risk of accidentally administering a pathogen and the commitment of 100% efficiency in contaminants removal. Nevertheless, this tends to be a difficult or even impossible approach since ambivalent phages, especially those infecting both *Salmonella* and *E. coli*, are rare.

In this work, the previously isolated *Salmonella* phage PVP-SE1 (figure 1) belonging to the Myoviridae family showed the broadest lytic spectrum among all tested phages (table 1).

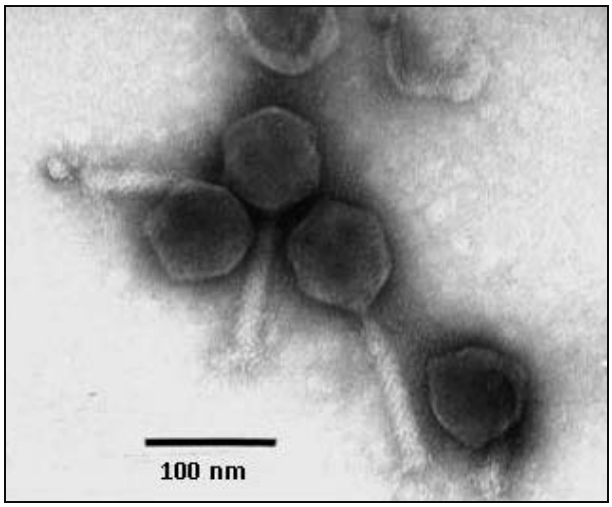

Figure 1: TEM of phage PVP-SE1

Furthermore, besides being able to lyse several *Salmonella* isolates, it was also able to infect two isolates of *E. coli* (table 1) and thus phi PVP-SE1 can be considered a polyvalent phage. This characteristic is particularly fascinating since only a few phages with a relatively broad host range (with a host range over species borders) are known and such phages would be useful for therapeutic purposes. Moreover, this phage was able to infect more strains than Felix 01, which is known for its wide lytic spectrum among *Salmonella* being able to lyse up to 99.5% of *Salmonella* strains.

Due to its ability to lyse *E. coli* BL21 strain we study the possibility of producing PVP-SE1 in this non-pathogenic alternative host. Although, the genome of a bacterial virus is always subject to host-controlled variation, a general phenomenon in which DNA may become modified when it is synthesized in one

cytoplasm and then undergo restriction upon entering another cytoplasm. Thus, the genotype of the host in which a virus reproduces affects the phenotype of the new formed virus progeny. In contrast to mutation, this phenomenon may be applied simultaneously to almost all the members of a developing phage population and generally is determined only by the nature of the last host in which the phage was replicated being independent of the previous phage history.

Table 1: Lytic spectra of isolated *Salmonella* phages against the different *Salmonella* subtypes and other bacteria than *Salmonella*.

Phage phi PVP-SE	**1**	**2**	**3**
Strains			
Salmonella Typhimurium NCTC 12416 - subsp. I	+	-	-
Salmonella NCTC 13349 - subsp. I	+	+	+
Salmonella spp. SGSC 3047 - subsp. II	+	-	-
Salmonella spp. SGSC 3039 - subsp. II	+	-	-
Salmonella Arizonae SGSC 3063 - IIIa	L	-	-
Salmonella Arizonae 83 (isolate) - IIIa	-	-	-
Salmonella spp. SGSC 3069 - subsp. IIIb	+	-	-
Salmonella spp. SGSC 3068 - subsp. IIIb	+	-	-
Salmonella spp. SGSC 3086 - subsp. IV	L	-	-
Salmonella spp. SGSC 3074 - subsp. IV	+	-	-
Salmonella Bongori SGSC 3103 - subsp. V	+	-	-
Salmonella Bongori SGSC 3100 - subsp. V	+	-	-
Salmonella spp. SGSC 3118 - subsp. VI	+	-	-
Salmonella spp. SGSC 3116 - subsp. VI	+	-	-
Salmonella spp. SGSC 3121 - subsp. VII	+	-	-
Salmonella spp. SGSC 3120 - subsp. VII	+	-	-
Escherichia coli N9	L	-	-
Escherichia coli N5	L	-	-
Escherichia coli CECT 434 (ATCC 25922)	L	-	-
Escherichia coli BL21	+	-	-
Escherichia coli K12	+	-	-
Enterobacter amnigenes CECT 4078 (ATCC 33072)	L	-	-
Enterobacter aerogenus CECT 684 (ATCC 13048)	-	-	-
Klebsiella pseudomonas 11296	-	-	-
Shigella ATCC 12022	-	-	-

Absence of phage halo and phage plaques (-); Presence of phage halo and phage plaques (+); Presence of phage halo and absence of phage plaques (L-Lysis from without).

In the present study the production of PVP-SE1 in the BL21 strain did not modify its lytic spectrum neither the phage DNA restriction profile (figure 2).

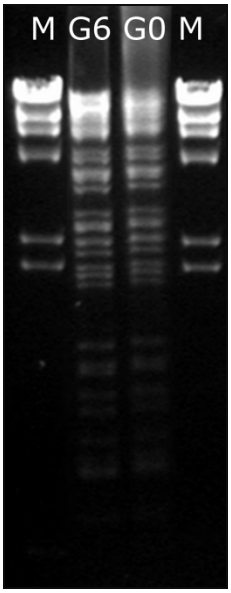

Figure 2: Restriction profile of DNA of phage phi PVP-SE1 produced in its natural host *Salmonella* Enteritidis (G0) and produced in the alternative host *E. coli* BL21 (G6) digested with EcoRV. M (Marker) is a HindIII digest of lambda DNA.

These facts suggest that modifications were not induced in phage phi PVP-SE1 upon replication in a different host which supports the possibility of producing the phage in a non-pathogenic host contributing for the safety of a product based on this *Salmonella* biocontrol agent assuring this way its stability when used in phage therapy. Furthermore, the use of this non-pathogenic *E. coli* in phi PVP-SE1 production will surely facilitate the production and purification processes by eliminating the risk of introducing a phage resistant pathogenic bacterium. The consequent reduction in costs and increased safety of the phage preparations will lead to an easier and faster approval of phage products for a wide diversity of commercial applications.

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