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DEVELOPMENT OF A PHAGE-BASED BIOSENSOR TO DETECT SALMONELLA IN FOOD STUFF

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EXTENDED ABSTRACT

Salmonella is the causing agent of thousands of deaths per year due to Salmonellosis. The principal source of *Salmonella* for human infection is contaminated food. Therefore the development of a rapid, sensitive, selective and real-time monitoring technique in order to detect this foodborne pathogen is of extreme importance. Until this moment many efforts have been made to detect bacterial presence in food stuff. However, previous detection methods have one or more defective features that make customers to expect improvements in this area. Expensive acquisition costs, expensive non-reusable resources, difficult methods requiring professional skills, long periods of time to give results, high false positive and negative results and non-bacteria-specific devices are only few of them.

In this context, phage-based biosensors have recently emerged as a potential alternative method for pathogen detection.This study aims at developing a phage-based biosensor to detect *Salmonella* in food stuff using a magnetoelastic platform. Different immobilization methods to gold surfaces and magnetoelastic materials respectively will be assessed. Furthermore two types of biosensors will be developed, one that uses the entire phage particle as the sensing agent and another with phage tail proteins.This work involves several fields of knowledge, such as physics, electronics, virology and molecular biology, some of which are occurring at the nano scale level. Thus, the invention consists of a phage ligand sensor device ("PLSD") comprising a magnetoelastic sensor coupled to a binding element. The binding element consists of a surface covered with phages displaying at least one peptide that recognizes and binds a complementary molecule in the bacterial cell wall or membrane. The device allows the detection and characterization of ligands that bind to the binding element. By this manner, the device provides an in vitro assay to detect and examine interactions between ligands and binding elements. The major challenge of this project is the improvement of the sensor´s performance, like the limit of detection, the sensitivity and specificity of the bioelement and (in the future) the possibility to be extended to the detection of other pathogens.

In order to obtain the objective proposed, the work is divided in several parts, namely characterization of three bacteriophages deposited in the phage collection of CEB-IBB (PVP-SE1, PVP-SE2 AND PVP-SE3), immobilization of bacteriophages on biosensor surfaces, evaluation of the performance of different detection platforms with immobilized phages, expression of the phage tail receptor proteins in *E. coli* and construction of a magnetoelastic resonance biosensor with immobilized phage proteins.

The *Salmonella* phages studied in this work belong at two different families: PVP-SE1 (figure 1A) belongs to the Myoviridae family and PVP-SE2(figure 1B) and PVP-SE3(figure 1C) belong to Siphoviridae family.

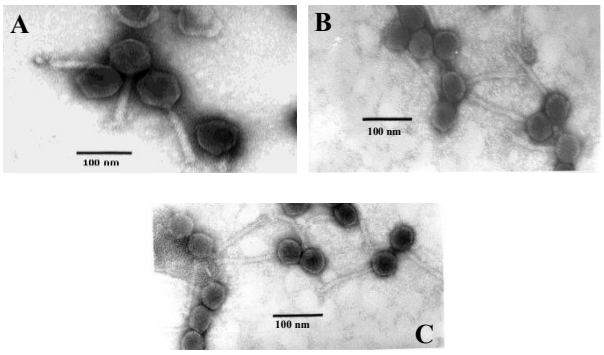

Figure 1- TEM of phage PVP-SE1 (A), PVP-SE2 (B) and PVP-SE3 (C).

These three phages were characterised and their lytic spectra were evaluated against a *Salmonella* isolates (table 1) and against isolated and strains of *E. Coli* (table 2). This allowed the selection of the phage with the broadest lytic spectrum.

The results in Table 1 show that the three tested phages were equally able to infect the Salmonella isolates. However, when the ability of phages was tested against different *Salmonella* subtypes, other bacteria than *Salmonella* (table 1),) and against strains of *E. Coli* (table 2), the PVP-SE1 had the broadest lytic spectrum. The results show that PVP-SE1 have multivalent ability and can be an important feature for therapy, but makes this phage very unspecific as a bioelement.Moreover,as the lytic spectrum of phage PVP-SE2 and PVP-SE3 were very similar it was decided to continue with only one of the phages (PVP-SE2). This phage was sequenced partially and the results showed that this phage have approximately 85 % of similarity with *Salmonella* phage SEPT3. This phage tail fibre proteins were identified and the respective genes cloned and expressed in *E. coli* Further studies are being carried out using PVP-SE2 as a bioelement or its tail fibre proteins

Table 1. Lytic spectra of isolated *Salmonella* phages against *Salmonella* strains and other bacteria.

	Phage phi / Strains	PVP-SE1	PVP-SE2	PVP-SE3
*S.*Enteritidis	EX2	+	+	+
	269	+	+	+
	546	+	+	+
	629B	+	+	+
	657	+	+	+
	821	+	+	+
	855	+	+	+
	AL855	+	+	+
	869	+	+	+
	905	+	+	+
	932	+	+	+
	S1400/94	+	+	+
	9510.85	+	+	+
Salmonella subtypes and other bacteria	*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 CECT 434 (ATCC 25922)	L	-	-
	Enterobacter amnigenes CECT 4078 (ATCC 33072)	L	-	-
	Enterobacter aerogenus CECT 684 (ATCC 13048)	-	-	-
	Klebsiella pseudomonas 11296	-	-	-
	Shigella ATCC 12022	-	-	-

Table 2. Lytic spectra of isolated *Salmonella* phages against isolate and strains of *E.Coli.*

	Phage phi / Strains	PVP-SE1	PVP-SE2	PVP-SE3
E. Coli (Isolate and strains)	n5	+	-	-
	n9	+	-	-
	Eli 1	+	-	-
	Eli 2	+	-	-
	Eli 5	+	-	-
	Eli 6	+	-	-
	Eli 7	+	+	-
	Eli 8	-	-	-
	Eli 9	+	-	-
	Eli 10	+	-	-
	BL21	+	-	-
	K12	+	-	-
	n5	+	-	-

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2002-2007- Integrated Master in Biological Engineering;
2007- Training Research in Application of bateriophages to control of Salmonella;
2007-2008- Research in European Project Phagevet-P (Veterinary phage therapies as alternatives to antibiotics in poultry production, STREP Project no. 2005-7224);
From January 2009- Ph Student in Department of Biological Engineering of University of Minho and in Pathobiology Department in Auburn.