Detection of bacterial pathogens by phage antibody display

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1. Introduction

Vibrio parahaemolyticus is a halophilic Gram-negative facultative anaerobe commonly found in estuarine waters and in seafood like shellfish. It has been implicated in outbreaks of gastro-enteritis in several countries [1]. Food borne illness caused by *V. parahaemolyticus* results chiefly from the consumption of insufficiently heated or raw seafood, especially in the summer months. The standard method for detection of *V. parahaemolyticus* (Bacteriological Analytical Manual procedure) is a culture-based procedure which can take up to four days for positive identification.

Recent outbreaks of *V. parahaemolyticus* related illnesses have heightened the need to develop a rapid and reliable method to detect this pathogen in shellfish. PCR methods, based on the amplification of the toxin producing genes, unfortunately give false postive results with other Vibrio species [2]. In our studies we have used phage antibody display to differentiate between pathogenic *V. parahaemolyticus* and other pathogenic and non-pathogenic *Vibrio* sp.

2. Methods

2.1. Bacterial cultures

Cultures of *V. parahaemolyticus* and other *Vibrio* sp. were obtained from the National Collection of Type Cultures, London and the ATCC.

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2.2. Panning

The human synthetic scFv (NISSIM) library was used as a source of antibody fragments of different specificities (greater than 10^8) [3].

Five rounds of panning were used to select for antibodies specific for surface markers on *V. parahaemolyticus*. Pooled cultures of *V. parahaemolyticus* (cell density $= 8 \times 10^8$ cells/ml at A_{600}) were used in 4 rounds of positive panning. One round of negative panning was carried out using pooled cultures of non-parahaemolyticus Vibrios (cell density $= 8 \times 10^8$ cells/ml at A_{600}) to try and remove all cross-reactive phage.

The negative panning step was carried out after the first round of positive panning. The titre of eluted phage was checked after each round of panning.

2.3. ELISA

ELISA was used to determine binding specificity of phage clones. Phage clones after the 5th (and last) round of panning were grown up in 96-well round bottom ELISA plates overnight.

Flat bottom 96-well ELISA plates were coated with 10^8 cells/well of different strains of *V. parahaemolyticus* and strains of other non-parahaemolyticus sp. overnight. Phage supernatant was obtained by growing up individual phage clones overnight at 30 °C and then spinning the cells at 2500 rpm for 10 minutes. Supernatant containing phage antibody was added to the wells and the phage were allowed to bind for 2 hours. Excess unbound phage was then washed off, and the bound phage detected by adding anti-M13 HRP conjugated antibody for 1 hour followed by the addition of tetramethylbenzidine (TMB) substrate. The absorbance was measured at 450 nm using an ELISA reader (MRX microplate reader, Dynex technologies). Irrelevant phage (anti-NIP) was used as a negative control.

Table 1
ELISA (Absorbance at 450nm) of phage clones binding to different strains of *Vibrio* sp

	1	2	3	4	5	6	7	8
A	*	*	*		*	*	*	
B	*	*	*		*	◇	*	
C	*	*	*	*	△	*	△	△
D		*	*	*				
E		*	*			*	*	
F	*	*	*	△		*	*	

Key: Readings greater than 1.000 at A_{450} have been considered positive.
◇ = *Vibrio parahaemolyticus* NCTC 10884.
* = *Vibrio parahaemolyticus* NCTC 10903 + NCTC 10884 (1 : 1 ratio).
△ = *Vibrio mimicus* NCTC 11346 and *Vibrio harveyi* NCTC 11435 (1 : 1 ratio) (cultures used for negative panning).

Table 2
Binding percentages of selected phage antibodies against *Vibrio* sp. (Percentage binding of anti-NIP was used as blank)

Clone	*Vibrio parahaemolyticus* NCTC 10903	*Vibrio parahaemolyticus* NCTC 10884
A1	11.3%	7.7%
B3	21.3%	21.9%
B5	27.6%	27.8%
C2	33%	30.5%
E3	16.6%	12.5%
F7	16.9%	8.15%

2.4. FACS analysis

Repeatedly strong binding clones were selected for screening their binding properties using a FACSCalibur flow cytometer. Single cultures of *V. parahaemolyticus* as well as pooled cultures were used for analysis.

3. Results

After the final round of panning, 48 clones were picked for screening by ELISA. ELISA was carried out using individual cultures of *V. parahaemolyticus* NCTC 10903 and *V. parahaemolytiucs* NCTC 10884 as well as pooled cultures mixed in a ratio of $1:1$. Cell density was maintained at 10^8 cells/well. Out of 48 clones, 28 clones showed repeated strong binding to both strains of *V. parahaemolyticus*. Preliminary results have been shown in Table 1. Clone B6 was the only clone that was positive with *V. parahaemolyticus* NCTC 10884 and negative with NCTC 10903. It may therefore represent a clone that is strain specific.

ELISA was also carried out using pooled cultures of *V. mimicus* NCTC 11346 and *V. harveyi* NCTC 11435 in a $1:1$ ratio. Only four clones of the 28 were cross reactive with the above cultures.

CDR3 insert size was determined using PCR as previously described [3]. BstN1 restriction digestion of a PCR fragment covering the whole scFv region was also carried out as previously described [4].

Six phage clones out of the 28 were picked at random and were analyzed for their binding specificities using flow cytometry. All 6 clones were positive for *V. parahaemolyticus* on FACS analysis. The percentage binding to each strain was also determined (Table 2).

4. Discussion

Using phage display, we have been able to isolate antibodies that bind to *V. parahaemolyticus*. We are now in the process of screening these antibodies against a broad range of *Vibrio* and other related sp., from different sources, to determine their specificity.

References

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