DEVELOPMENT OF A NEW METHOD FOR DETECTION OF ACANTHAMOEBAB KERATITIS

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INTRODUCTION

Acanthamoeba (figure 1) is a free living opportunistic parasite [1]. This pathogen causes a sight threating condition called Acanthamoeba keratitis. Contact lens wearers are at highest risk of this infection and the number of reported cases has increased in the UK due to an increased use of disposable lenses [2]. Detection of this organism relies on traditional methods of culture and results may take up to 14 days [2]. There is a requirement for the development of rapid method for detection.

Phage display is a technology that allows the presentation of peptides and proteins including antibody fragments on the surface of filamentous bacteriophage [3]. The genes encoding the variable domains of antibodies (scFv) and a linker are fused to the g3p gene in the genome of the filamentous phage. In our system the scFv is displayed as a fusion to gene 3 protein(minor coat protein) at the tip of the phage (figure 2).

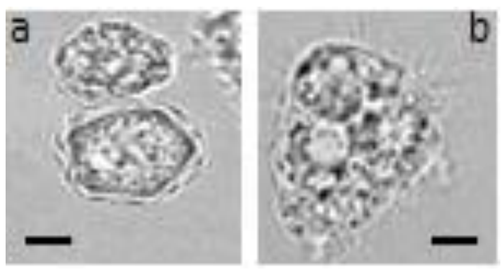

Fig. 1. Image of *Acanthamoeba*(a) cyst,(b) trophozoite . Size bar represents 5µm, [1].

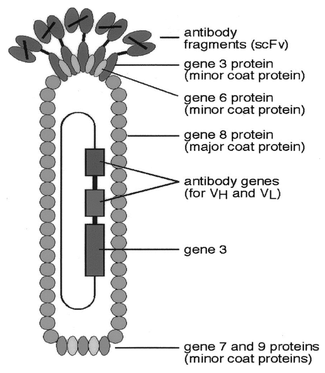

Fig. 2. Phage antibody structure. Antibody genes fused to the phage coat proteins and then the fused genes will display on the top of the phage, [3].

AIM

To generate bacteriophage antibody peptides that specifically bind to *Acanthamoeba* and can be used for identification possibly in vivo.

MATERIALS AND METHODS

Previous work was focused on selection of phage clones from the Christ MRC phage display library by a number of rounds of panning with *Acanthamoeba* (figure 3), followed by primary characterization to isolate desired clones using ELISA, flow cytometry and fluorescence microscopy. PCR for CDR3 length and the whole fragment was performed using specific primers to demonstrate the diversity of bacteriophage clones isolated. PCR fragments amplified directly from bacterial colonies were separated on 5% and 2.5 % (wt/vol) agarose gel stained with Gel Red and visualized under UV light.

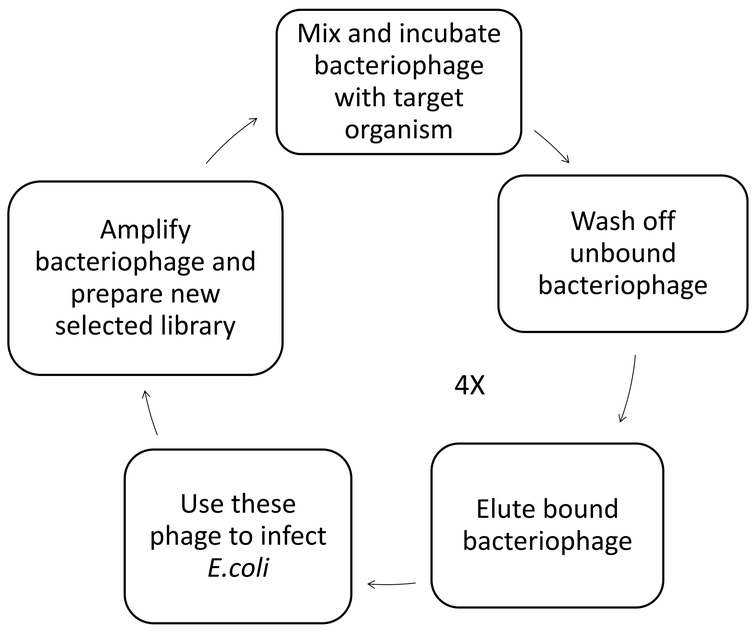

Fig. 3. Summary of bacteriophage selection process. The initial library obtained from the MRC contains bacteriophage expressing 10^{15} different peptides – this is used to initiate the first round of selection – 'panning'. This process is repeated 4 times and after each panning round the new library formed becomes increasing selective.

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RESULTS

Initially, clones were randomly selected and assayed using ELISA. From this selection process we identified 23 phage clones that give high absorbance values (> 0.9). Flow cytometry and fluorescent microscopy were then performed for further characterisation of the clones and these were tested against five *Acanthamoeba* isolates (cysts and trophozoites) and bacteria, fungi and mammalian cells that may be associated with the eye. Nine phage clones were identified from this final screen, table 1.

Table 1. Identification of useful phage clones. The clones have been chosen depending on high absorbance obtained from ELISA for further characterisation using flow cytometry and fluorescence microscopy. Antibody clone's specificity was also assessed. The highlighted rows represent the selected phage clones; (s): strong (significant) signal: (n), no signal: (o) observed signal. Numbers indicate the percentage of binding based on fluorescent shift.

Clones	Flow cytometry analysis										Fluorescence microscopy (antibody specificity)				
	Acanthamoeba isolates														
	SK14-4		SK19-59		SK19-9		PM19		SK19-56		*Acanthamoeba*	*E.coli*	*S.cervisiae*	*C.albicans*	Macrophage
	Troph %	Cyst%	Troph %	Cyst %	Troph %	Cyst %	Troph %	Cyst %	Troph %	Cyst %					
H11	41.25	18.09	12.62	4.51											
G1	63.97	93.13	69.74	58.50	43.44	49.26	14.89	52.47	33.29	41.14	s	n	n	n	n
G12	21.97	34.08	43.46	54.46	39.90	48.69	36.83	44.40	52.77	37.70	s	n	n	o	n
H9	20.88	45.58	60.58	22.04	30.24	22.22	36.18	47.44	42.31	35.30	s	n	n	n	n
C10	22.34	12.04													
F6	39.02	27.69	30.12	34.43	22.79	23.86									
A2	28.30	42.06													
E9	45.42	36.38	48.35	55.72											
A11	21.48	53.21	73.59	53.59	40.94	25.83	30.49	56.43	40.51	22.08	s	n	n	n	n
H6	22.23	18.82													
A8	42.71	26.18													
F12	65.32	92.21	32.85	70.10	46.68	55.68	23.09	37.42	49.87	48.43	s	n	n	o	n
B9	25.76	46.11	11.72	22.86											
F6	33.45	21.60													
A5	91.21	33.23	54.73	50.12	43.05	45.10	28.30	49.83	46.19	32.03	s	n	n	n	n
D11	28.87	35.38													
G6	85.39	90.33	50.66	40.92		49.07	28.80	32.85	54.83	37.46	s	n	n	n	n
A9	7.89	19.66													
C8	9.12	20.13													
F4	12.44	46.30	20.57	60.61	28.80	40.23	42.21	32.96	41.45	38.05	s	n	n	n	n
E12	44.43	66.52	49.11	60.61	36.18	28.80	50.66	60.58	51.33	48.17	s	n	n	n	n
A4	23.77	13.33													
B6	33.45	21.60	44.70	44.68	47.77	25.86									

Figure 4 shows the diversity of selected clones.

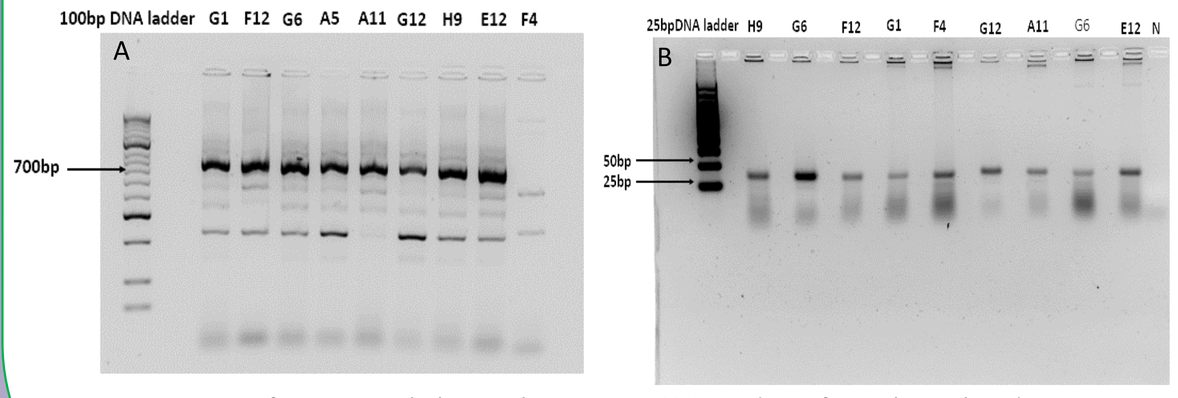

Fig. 4. Image of agarose gel electrophoresis on PCR products from the isolated positive clones(A) Amplification of VH and VL genes (2.5%) gel. (B)) 5% gel electrophoresis represented the diversity in insert sizes.

CONCLUSIONS

From our initial work we have identified 23 clones that show significant binding to *Acanthamoeba* using ELISA. Further work was focused on the characterisation of these clones using flow cytometry and fluorescence microscopy.

We believe that these novel reagents are suitable candidates as potential tools for *Acanthamoeba* diagnosis due to cost effectiveness and ease of production. Such reagents may also have potential for use in targeting therapeutic drugs.

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