Wat. Sci. Tech. Vol. 27, No. 3-4, pp. 257–260, 1993.
Printed in Great Britain.

0273–1223/93 $24·00

THE USE OF MONOCLONAL ANTIBODIES FOR THE DETECTION OF FAECAL BACTERIA IN WATER

R. Kfir, M. du Preez and B. Genthe

Division of Water Technology, CSIR, P.O. Box 395, Pretoria 0001, South Africa

ABSTRACT

Monoclonal antibodies (MAbs) against heat-killed *Escherichia coli* and *Klebsiella oxytoca* originating from wastewater effluent were raised in BALB/C mice. The fusion was highly successful and three hybridomas cloned were selected to study the affinity and specificity pattern of the MAbs. The MAbs were found to react equally well with heat-killed and live bacteria when tested against their original immunogens. The MAbs showed lower specificity towards environmentally isolated faecal bacteria than a pure bacterial strain grown in the laboratory. A commercially obtained MAb showed the same specificity pattern.

KEYWORDS

Monoclonal antibodies, *E.coli*, *Klebsiella*, faecal coliform, specificity.

INTRODUCTION

Limits for faecal coliform counts form the basis of most water quality guidelines and standards world-wide. However, routine methodology based on biochemical and physiological selective culturing techniques for the enumeration of faecal coliforms often fail to detect injured bacteria due to the harsh selective conditions imposed on the stressed microorganisms. The same methods also allow growth of non-faecal bacteria with similar selective biochemical patterns, thus giving a false indication of faecal pollution. The available methods are also time consuming (up to 72h) and the biochemical identification is relatively labour-intensive. Several rapid techniques have been developed in recent years but most of these are lacking in specificity (Joret *et al.*, 1989).

The use of immunoassays, especially with the introduction of the hybridoma technology and the production of monoclonal antibodies (MAbs), offers a means for rapid and specific detection of microorganisms in water. By using hybridoma technology the induction of a specific antibody population can be achieved which can be immortalised to give a continuous source of MAbs with an identical specificity and affinity pattern (Köhler and Milstein, 1975). A number of studies thus far indicated the use of polyclonal and monoclonal antibodies for the detection of faecal bacteria as very promising (Joret *et al.*, 1989; Obst *et al.*, 1989). However, the immunogens used in these were either purified external constitutive protein of *Escherichia coli* outer membrane or the enterobacterial common antigen (ECA).

In this study MAbs raised against heat-killed *E.coli* and *Klebsiella oxytoca* which originated from polluted water were evaluated for their specificity and affinity, and for their ability to recognise and detect faecal bacteria isolated from wastewater, river water, seawater and drinking water.

MATERIALS AND METHODS

Faecal coliform bacteria from a treated effluent (humus tank effluent) were enumerated using the membrane filtration technique and m-FC agar (APHA, AWWA and WPCF, 1989). Typical colonies were isolated and identified using the API 20 E method. Three heat-killed cultures (60 min at 60° C) of identified isolates, including two isolates of *E. coli* (E1 and E2) and one isolate of *K. oxytoca* (KLEB), were used for the immunisation of BALB/C mice (10^6 organisms/mℓ, 0,5 mℓ per mouse). Immunisation and fusion protocols were carried out as described by Kfir *et al.* (1985) but without the use of Freund's adjuvant for immunisation. Hybridomas producing antibodies against either *E. coli* or *K. oxytoca* were identified by ELISA utilising a secondary anti-mouse immunoglobulin conjugated to horse radish peroxidase (Bioclones, South Africa). Positive hybridomas were cloned by limiting dilution and re-tested and grown for large-scale production of antibodies. MAbs were purified by ammonium sulphate precipitation and sub-classes were determined by red cell agglutination with specific antisera (Serotec). The MAbs were tested for their specificity for the original immunogens and faecal coliform bacteria isolated from environmental samples. Cultures of faecal bacteria originating from wastewater, river water, seawater and drinking water were isolated and identified by means of the API 20 E method before being used in specificity studies. A pure laboratory *E.coli* strain was used as a positive control. A commercial MAb preparation (Chemicon International) raised against a pool of four *E. coli* serotypes common to humans was also evaluated. The affinity of the MAbs for live or heat-killed bacterial preparations was studied using ELISA techniques.

RESULTS AND DISCUSSION

Monoclonal antibodies were successfully raised against environmentally isolated *E. coli* and *K.oxytoca* bacteria as seen by the number of positive hybridomas produced (Table 1). The MAbs produced showed a variety of mouse isotypes (Table 2). Three MAbs of the mouse isotype IgG and IgM were studied for their affinity and specificity.

Table 1. Production of Monoclonal Antibodies*

Bacterial immunogen	% Fusion	% Positive hybridomas	% Hybridomas frozen
E. coli-E1	65	62	52
E. coli-E2	61	46	70
Klebsiella	89	27	96

* all screening was done using heat-killed bacteria

Some of these MAbs showed higher specificity towards the original bacteria used as immunogen while some recognised other *E.coli* or *K.oxytoca* isolates with higher affinity. The Kleb 1 clone showed higher affinity and specificity to its original immunogen than the anti-*E.coli* MAbs. Recognition of bacteria other than immunogens such as *Salmonella* and *Pseudomonas* was limited for all three MAbs studied (Table 3). Live and heat-killed bacteria were recognised equally when the original immunogens were studied (Table 4). Other studies indicated that MAbs produced against heat-killed bacteria have lower specificity and sensitivity to live bacteria (Aydintug *et al.*, 1989).

Table 2. Cloning and Determination of Isotypes

Bacterial immunogen	Cloned hybridoma (x2)*	MAb isotope
E. coli -1	E1a	IgG_2b
	E1b	IgM
	E1c	IgA
K. oxytoca	Kleb1	IgG_1
	Kleb2	IgM

* all hybridomas were cloned twice

Table 3. Evaluation of MAbs Specificity*

MAbs	Bacterial strain				
	Ps**	*E.coli*-1	*E.coli*-2	Kleb***	Sal****
E1a	0,75	1,00	1,65	2,00	1,55
E1b	2,15	2,90	4,50	3,10	2,85
Kleb1	2,00	1,50	2,25	4,20	2,00

* Ratio to control, control- no antibodies added; ** - *Pseudomonas aeruginosa*; *** - *Klebsiella*; **** - *Salmonella* (poly I, Group)

Table 4. Determination of Affinity of the MAbs to Live and Heat-killed Bacteria*

Bacteria	Monoclonal antibodies		
	Anti-E1	Anti-E2	Anti-Kleb
Kleb	1,70	0,60	**1,00**
E1	1,70	**1,20**	0,63
E2	**1,00**	1,00	1,00

* - optical density ratio (O.D.) = O.D.live bacteria/O.D.heat-killed bacteria

The MAbs produced in this study showed a specificity pattern similar to the commercially available MAb. Only limited specificity and affinity were observed for environmentally isolated *E.coli* and other faecal bacteria were recognised by the MAbs produced in this study as well as the commercially available MAb. The MAbs produced in this study showed higher affinity and specificity for their original immunogens. Recognition and cross-reactivity of antibodies produced against *E.coli* with *Salmonella* was previously reported (Pollack *et al.*, 1989).

Due to the limited affinity and specificity of the MAbs obtained in this study no further studies on the production of a rapid detection kit were carried out. For such a detection kit a MAb with high affinity and specificity to environmental isolates of *E.coli* is needed. The production of MAb with high specificity only to *E.coli* has been challenged in reported literature. A panel of 8 murine MAbs produced against heat-killed *E.coli* was shown to react with up to 20 heterologous Gram-negative bacteria. It is suggested that antibodies produced against heat-killed bacteria target antigenic determinants which may be cryptic in live bacteria, and that these antigenic determinants may only be exposed after heat-treatment. Another explanation given is that the specificity expressed for lipopolysaccharides on heat-killed bacteria differs from that of live bacteria (Aydintug *et al.*, 1989). Although specificity of the MAbs produced in this study was limited, equal recognition of heat-killed and live bacteria was observed.

Table 5. Evaluation of MAbs Affinity and Specificity to Environmental Bacterial Isolates of Faecal Coliforms

Environmental samples	Isolated bacteria	Monoclonal antibodies		
		Kleb1	E1a	Com. MAb*
Wastewater effluent	*E.coli*	+	+	+
	K.pneumonia	+	+	+
River water	*E.coli*	+	+	+
	K.pneumonia	+	+	+
Seawater	*E.coli*	+	+	+
	E.coli	+	+	+
Potable water	*K.pneumonia*	+	+	+
	Ent.agglomerans	+	+	+
Original immunogen	*E.coli*	+++	++	++
	K.oxytoca	+++	++	++

* - commercial anti-*E.coli*, raised against a pool of 4 common human serotypes

CONCLUSIONS

The use of MAbs as a rapid tool for the detection of faecal coliform bacteria in water with special reference to *E.coli* identification is questionable, unless antibodies with high affinity and specificity towards *E.coli* become available. The production of such specific antibodies is questioned in the literature.

REFERENCES

APHA, AWWA and WPCF (1989). Standard methods for the examination of water and wastewater, 17th Edition, L.S.Clesceri, A.E. Greenberg and R.R. Trussel (Eds). APHA, NY.

Aydintug, M.K., Inzana, T.J., Letonja, T., Davis, W.C. and Corbeil, L.B. (1989). Cross-reactivity of monoclonal antibodies to *Esherichia coli* J5 with heterologous Gram-negative bacteria and extracted lipopolysaccharides. *J. Infect. Diseases,* **160,** 846-857.

Joret, J.C.,Cervantes, P., Levi, Y., Dumoutier, N., Cognet, L., Hasley, C., Husson, M.O. and Leclerc, H. (1989). Rapid detection of *E.coli* in water using monoclonal antibodies. *Wat. Sci. Tech.* **21** (3), 161-167.

Kfir, R., Botes, D.P. and Osthoff, G. (1985). Preparation and characterisation of monoclonal antibodies for *Naja nivea* cardiotoxin V"1. *Toxicon,* **23**, 135-144.

Köhler, G. and Milstein, C.(1975). Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* **227**, 680-685.

Obst, U., Hubner, I., Wecker, M. and Bitter-Suermann, D. (1989). Immunological method using monoclonal antibodies to detect enterobacteriaceae in drinking water. *Aqua,* **38,** 136-142.

Pollack, M., Chia, J.K.S., Koles, N.L., Miller, M. and Guelde, G. (1989). Specificity and cross-reactivity of monoclonal antibodies reactive with the core lipid A region of bacterial lipopolysaccharide. *J. Infect. Diseases,* **159**, 168-188.