ENUMERATION OF SULPHITE-REDUCING CLOSTRIDIA FROM DRINKING WATER AND RIVERS IN BASRAH CITY USING MODIFIED DRCM MEDIUM

Asaad M. Ridha Al-Taee
Marine Science Centre – Basrah University

ISSN –1817 –2695
(Received 14/11/2006 , Accepted 5/7/2007)

ABSTRACT

The enumeration of sulphite-reducing clostridia from water upon modified DRCM was evaluated against DRCM and RCM using 80 samples of treated drinking waters and river waters with or without heat treatment of the samples.

The recoveries of presumptive sulphite-reducing clostridia were significantly higher on modified DRCM (85.7% on drinking water and 81.25% on rivers), while 48.75% and 28.5% respectively on DRCM, and 31.25% and 21.45% on RCM. Heat treatment of samples to 70 C˚ for 10 minutes prior to filtration reduced both clostridia and background on modified DRCM but did not affect counts on DRCM. Four species were identified as ***Clostridium perfringens, C. baratii, C. ramosum, and C. difficile.***

Key Words: ***RCM, DRCM, m-DRCM, Clostridium***

INTRODUCTION

The sulphite-reducing clostridia are normal inhabitants of the intestinal micro-biota of humans and other mammals. These microorganisms form endospores, which allow the bacteria to survive in almost any habitat, either terrestrial or aquatic, waiting for favorable conditions for growth [1].

The enumeration of sulphite-reducing clostridia is used in assessing drinking water quality in Europe and UK. [2- 8].

Some researchers proposed to use several types of media, which include TSC, MCA and m-CP for recovery of ***C. perfringens*** and other sulphite-reducing clostridia from water and shellfish [9- 12].

In this study the reinforced clostridia agar (RCM) and differential reinforced clostridia agar (DRCM) failed in isolating the species of clostridia, so a new medium was introduced and compared with RCM and DRCM with and without sample pretreatment at 70 C˚ for the enumeration of sulphite-reducing clostridia from rivers and drinking water.

MATERIALS AND METHODS

Samples Field

A total of 80 samples were collected from drinking water (60 samples), Ashar river (10 samples) and Garma river (10 samples). The samples were collected in sterile 250 ml. Nalgene polycarbonate conical flasks to which a solution of 2.5% (W/V) sodium thiosulphate was added as a reducing agent for any residual chlorine (13).

Enumeration Media

Reinforced clostridium medium and DRCM were prepared according to the manufactures instructions and the new medium, which is called modified DRCM (m-DRCM) (Table 1).

Table 1: Comparison between RCM, DRCM, and m-DRCM media.

Components	RCM(Oxoid) (g/l)	DRCM[14] (g/l)	m-DRCM (g/l)
Meet extract		3	9
Yeast extract	3		2.5
Peptone	10	10	7.5
D-Glucose	5		3
Starch	1		1
Sodium acetate	3		4
Sodium chloride	5	5	5
"Lab lemco" powder	10		
L- cystein-HCl	0.5		0.5
Sodium sulphite		0.05	1
Ammonium ferric (III)citrate		0.04	1
Resazurine –Na			0.02
Agar	15	15	15
Polymyxin B			0.02

The media were autoclaved at 1.5 bar for 15 min. A polymyxin was added to the m-DRCM after sterilized by filtration.

Analytical Procedures

All samples were filtered in duplicate through Millipore WCN type membrane filters size 0.45µ (Whatman Corp. Japan). One hundred and fifty milliliters were filtered for each medium, with and without sample heat treatment at 70 C˚ for 10 min.
The plates were incubated anaerobically in gas jar using either Oxoid anaerobic gas generating kits (Code No. BR 38) or Al-Razi anaerobic gas generating kits (B. No. 41317) for 48 h. at 37 C˚.
All black colonies were counted as presumptive sulphite-reducing clostridia. They were subjected to Gram staining and test for motility, nitrate reduction, gelatin liquification and fermentation of lactose, mannose, galactose, mannitol, sorbitol, raffinose, salicin and arabinose. Further more they were identified using API 20 A strips (BioMerrieux).

RESULTS

From Table (2) the isolation of presumptive sulphite-reducing clostridia on m-DRCM was higher than RCM and DRCM for all types of water analyzed.
The counts of presumptive sulphite-reducing clostridia on m-DRCM were higher using unheated samples (Table 3). Heating of the samples to 70C˚ for 10 min. reduced the counts of sulphite-reducing clostridia and the background counts. Both m-DRCM procedures produced higher counts than DRCM and RCM.

Twenty isolates from m-DRCM and 10 from each DRCM and RCM were examined by Gram staining and subjected to biochemical tests and API 20 A strips, only four species were identified as ***Clostridium perfringens*** (8 from m-DRCM, 2 from RCM and 2 from DRCM), ***C. ramosum*** (5 from m-DRCM, 3 from RCM and 2 from DRCM), ***C. baratii*** (2 from m-DRCM, 1 from RCM and 1 from DRCM) and finally ***C. difficile*** (4 from m-DRCM and 1 from DRCM). The remaining one isolate from m-DRCM, four from RCM and four from DRCM could not be identified to species level.

DISCUSSION

In this study two media have been used for isolating clostridia from drinking water and rivers. RCM is based on a basic nutrient medium developed in the 1950s [15]. The use of this medium resulted in non-selective growth of other bacteria (Table 2). Sodium sulphite and ferric citrate are added to RCM to become differential RCM which has been recommended for the detection of sulphite-reducing clostridia in water [14].These two substrates were used as indicator of sulphite reduction by ***Clostridium*** which produces black colonies. However, also this medium failed to decrease the background bacteria in comparison with the new medium m- DRCM which was shown high selectivity for sulphite-reducing clostridia and this may be related to that, the increasing of these substrates (sodium sulphite and ferric citrate) which are incorporated with polymyxin B provident a high degree of selectivity and specificity for these bacteria. This medium also contains starch to promote spore germination and resazurine as a redox indicator.

Some researchers [9, 12] have demonstrated that, the incubation temperature of 45 C˚ appears to be selective for sulphite-reducing clostridia of fecal origin. But in this study the incubation temperature of 37 C˚ was found more selective and specific for these bacteria.

In this study the samples were heated at 70 C˚ for 10 min. to kill vegetative cells and enhance spore germination. From Table (3) heat treatment affects the counts of sulphite-reducing clostridia on m-DRCM but does not affect the counts on DRCM this may be related to that the components of the m-DRCM have some inhibitors which may not enhance the spore germination and this agreed with Warnes and Keevil (2004) who found that the conventional methods for the detection of sulphite-reducing clostridia have incorporated heat killing of vegetative cells of clostridia and contaminating bacteria to identify the presence or quantify the clostridial spores present. This is followed by the use of rich medium to promote spore germination.

We conclude from our study that the incubation of samples without heat treatment on m-DRCM at 37 C˚ for 48 h. may be the best medium for the enumeration of sulphite-reducing clostridia from drinking water and rivers.

Table 2: The percentage of presumptive sulphite-reducing clostridia on RCM, DRCM and m-DRCM.

Sample source	Count per 100 ml upon							
	No. of samples	No. +ve sample	RCM		DRCM		m-DRCM	
			No. +ve samples	(%) +ve samples	No. +ve samples	(%) +ve samples	No. +ve samples	(%) +ve samples
Ashar river	10	10	5	50	6	60	10	100
Garma river	10	8	1	12.5	3	37.5	5	62.5
Drinking water	60	56	12	21.4	1	28.5	48	85.7

Table 3: Impact of heat treatment of samples on recovery of sulphite-reducing clostridia on RCM, DRCM and m-DRCM.

	Count per 100 ml upon					
	RCM		DRCM		m-DRCM	
	Not heated	Heated	Not heated	Heated	Not heated	Heated
Mean (80)	19.4	11.5	50	35.9	131.5	61.7
Range	0-79	0-35	2-101	0-87	6-451	1-255
Standard deviation	21.5	10.2	32.5	28.0	120.5	57.6

REFERENCES

1-S. Robles; J. M. Rodrigues; I. Granados; and M. C. Guerrero. Sulphite-reducing clostridia in the sediment of a high mountain lake (Laguna Grands,Gredos, Spain) as indicators of fecal pollution. Internat. Microbiol. 3: 187-191. (2000).

2- DOE (Department Of the Environment). The bacteriological examination of drinking water supplies. Reports on public health and medical subjects No. 71. HMSO. London. (1983).

3- J. R. Postgate. The Sulphate-Reducing Bacteria. 2ed. Cambridge University Press, Cambridge. (1984).

4- K. M. Emde; D. W. Smith and R. Facey. Initial investigation of Microbially Influenced Corrosion (MIC) in a low temperature water distribution system. Water Res. 26: 169-175. (1992).

5- D. P. Sartory; A. M. Pritchard and P. Holmes. Enumeration of sulphite-reducing clostridia from potable water supplies. Wat. Sci. Tech. 27: 279-282. (1993).

6- J. M. Odom and Jr. R. Singleton. The Sulphate-Reducing Bacteria: Cont-emporary Perspective. Springer Verlag New York, USA. (1993).

7- P. W. Adcock and C. P. Saint. Rapid confirmation of ***Clostridium perfringens*** by using chromogenic and fluorogenic substrates. Appl. Environ. Microbiol. 67: 4382-4384. (2001).

8- Environment Agency. The microbiology of drinking water – Part 6 – Methods for the isolation and enumeration of sulphite-reducing clostridia and ***Clostridium perfringens*** by membrane filtration. Methods for the Examination of Water and Associated Materials. UK. (2002).

9- J. S. Burger; E. M. Nupen and W. O. K. Grabow. Evaluation of four growth media for membrane filtration counting of ***Clostridium perfringens*** in water. Water SA. 10: 185-188. (1984).

10- D. P. Sartory. Membrane filtration enumeration of faecal clostridia and ***Clostridium perfringens*** in water. Water Res. 20: 1255-1260. (1986).

11- T.J. Easterbrook. and P. A. West. Comparison of most probable number and pour plate procedure for isolation and enumeration of sulphite-reducing ***Clostridium*** spores and Group D fecal streptococci from oysters. J. Appl. Bacteriol. 62: 413-419. (1987).

12- D. P. Sartory. Evaluation of two media for the membrane filtration enumeration of ***Clostridium perfringens*** from water. Lett. Appl. Microbiol. 27: 323-327. (1998).

13- APHA. Standard Methods for the Examination of Water and Wastewater. 17th ed. American Public Health Association. Washington D. C. (1989).

14- ISO 6461-2. Detection and enumeration of the spores of sulphite-reducing anaerobes (clostridia) – Part 2. Method by membrane filtration. (1986).

15- Sara L. Warnes and C. W. Keevil. Work package 3, Task 4: Desk studies on feasibility of horizontal standard rapid methods for the detection of ***Clostridium perfringens*** and enterococci in sludges, soil, soil improvers, growing media and biowastes. P: 1-50. University of Southampton. (2004).

الكشف عن بكتريا الكلوستريديا المختزلة للكبريتيت باستخدام وسط m-DRCM من مياه الشرب ومياه الانهار

أسعد محمد رضا الطائي

الخلاصة

استخدم الوسط المحور m-DRCM كوسط جديد وقورن مع الاوساط التقليدية DRCM و RCM للكشف عن بكتريا الكلوستريديا المختزلة للكبريتيت من مياه الشرب ومياه نهري العشار والكرمة في مدينة البصرة. أجريت الدراسة على 80 عينة من المياه قبل معاملتها بالحرارة بدرجة 70 م° وبعدها ولمدة 10 دقائق أظهر العد الافتراضي لبكتريا الكلوستريديا المختزلة للكبريتيت على الوسط m-DRCM 85.7 % لمياه الشرب و 81.25 % لمياه الانهر في حين 48.75 % و 28.5 % على الوسط DRCM و 31.25 % و21.45 % على الوسط RCM على التوالي.

انخفض عدد بكتريا الكلوستريديا المختزلة للكبريتيت على الوسط المحور m-DRCM عند معاملة العينات حراريا" ، في حين لم يتأ ثر على الوسط DRCM .

أظهر التشخيص النهائي باستخدام الاختبارات البيوكيميائية وأ شرطة الاختبارات السريعة API 20 A أربعة أنواع من الكلوستريديا وهي ***Clostridium perfringens*** و ***C. baratii*** و ***C. ramosum*** و ***C. difficile***.