Rapid Detection of *Clostridium perfringens* by a New Chromogenic Media

Selective chromogenic media for isolation and enumeration of Clostridium perfringens in water samples using membrane filtration

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ABSTRACT

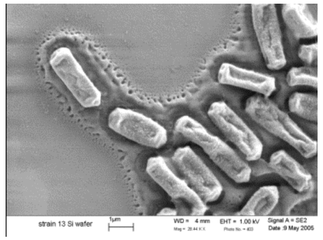
CP *ChromoSelect* Agar is a selective chromogenic media for isolation and enumeration of *Clostridium perfringens* in water samples using membrane filtration. *C. perfringens* is an anaerobic, Gram-positive, spore-forming rod-shaped bacteria. It is widespread in the environment and also found in the digestive systems of humans, and domestic and feral animals. Perfringens poisoning, usually from ingesting under-cooked food, especially meat, is one of the most commonly reported foodborne illnesses. Early detection of *Clostridium* in food and water is important to control outbreaks. To facilitate detection, Sigma-Aldrich, with its Fluka brand, has developed a new chromogenic medium, the CP *ChromoSelect* Agar, for enumeration and differentiation of *Clostridium* sp., in particular *Clostridium perfringens*, in aqueous samples. This new agar is more reliable and easier to handle than m-CP and TSC agars. The color does not diffuse in the agar and confirmation is not required since the green coloration is specific for *C. perfringens*.

INTRODUCTION

Clostridium perfringens is found in under-cooked or improperly sterilized canned foods (germination of endospores) and in water (surface water). The natural contamination source is human and animal faeces mainly transmitted into food by water. *C. perfringens* is an anaerobic, Gram-positive, spore-forming rod-shaped (cf. Figure 1) bacteria (1,2). *C. perfringens* produces an extensive range of invasins and exotoxins. The enterotoxins cause the undesirable, mostly meat-associated, food poisoning, and wound and surgical infections that lead to gas gangrene.

C. perfringens plays a subsidiary role in water examination (3). Clostridia are spore builders and are resistant to heating, chlorination and other stress factors.

Figure 1. Scanning Electron Micrograph of *C. perfringens* grown on a silicon wafer (source: S. Melville, Department of Biological Sciences, Virginia Tech University)



In contrast to vegetative cells like coliforms (*E. coli,* enterococci), which are less resistant, *C. perfringens* has the advantage of surviving longer (4). Therefore, while fecal contamination is detected mostly by coliforms as an indicator, which could disappear after a processing step, *C. perfringens* remains present. The organism is not a hazard in water; rather, it is problematic when the water comes in contact with food.

In consideration of the aforementioned facts, it is obvious that detection and identification of *C. perfringens* is an important step toward the control and eradication of this potent pathogen. Some characteristic enzymes of *C. perfringens* are: hemolysins (β -hemolysis), lecithinase, extracellular proteases, lipases (phospholipase-C), collagenase, hyaluronidase, saccharolytic, and enzymes to reduce sulphite to sulphide. These enzymes are also used as detection and differentiation targets. It is also notable that *C. perfringens* is a non-motile bacterium, and it is the most important of the sulphite reducing clostridia.

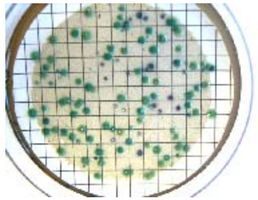
C. perfringens normally grows at 44 °C, whereas some other clostridia are inhibited at this temperature. This property is used in ISO methods to give the medium more selectivity (5).

RESULTS AND DISCUSSION

Early detection of *Clostridium* in food is important to control outbreaks. To facilitate detection, we have introduced a new chromogenic media, CP *ChromoSelect* Agar, for enumeration and differentiation of *Clostridium* sp., in particular *Clostridium perfringens*, in aqueous samples.

In the present study, three media types (mCP,TSCF and CP *ChromoSelect* Agar) were evaluated for recovery of *C. perfringens* in different surface water samples. Using a membrane filtration technique on 139 water samples, 131 samples (94.2%) were found positive for *C. perfringens* in at least one of the culture media. Green colored colonies on CP *ChromoSelect* Agar (cf. Figure 2) were counted as presumptive *C. perfringens* isolates.

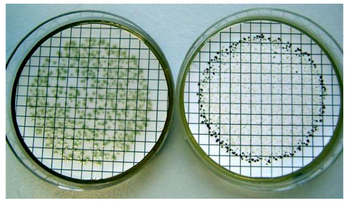
Figure 2. Drinking water sample cultured on CP *ChromoSelect* Agar. *C. perfringens* appears as distinct green colonies



For detection of *C. perfringens,* mCP and TSC agar have been recommended (3,6). However, there are problems associated with each of these media.

CP *ChromoSelect* Agar is more reliable and easier to handle than m-CP and TSC agars. The color does not diffuse in the agar and confirmation is not required since the green coloration is specific for *C. perfringens*. In addition, the recovery of *C. perfringens* was rejected by ISO in favor of methods based on TSC agar (4,7). CP *ChromoSelect* Agar also eliminates the excessive and variable blackening of the peripheral colonies encountered with TSC agar, which makes colony counting at lower dilutions difficult and leads to false positives. It is also more reliable at high bacteria counts, where the TSC agar can produce false negatives because of interference with the other enzymatic mechanisms from acid production and oxygen contact (cf. Figure 3). TSC detects all sulphite-reducing clostridia, and not only *C. perfringens*.

Figure 3. *C. perfringens* ATCC 10873 on CP *ChromoSelect* Agar (left) **and TSC agar** (right)
(Note the false negatives on the TSC agar).



Out of 483 green colonies on CP *ChromoSelect* Agar, 96.3% (465 strains, indole negative) were identified as *C. perfringens*, 15 strains (3.1%) were indole positive and were identified as *C. sordelli, C. bifermentans* or *C. tetani.* Only 3 strains (0.6 %) gave false positive results and were identified *as C. fallax, C. botulinum,* and *C. tertium* (cf. Table 1). Variance analysis of the obtained data showed statistically no significant differences in the counts obtained between media used in this work (cf. Figure 5).

In general, the identification of typical and atypical colonies isolated from all media demonstrated that CP *ChromoSelect* Agar was the most useful medium for *C. perfringens* recovery in water samples (2).

CP *ChromoSelect* Agar avoids the disadvantages of m-CP agar, such as, the presence of ammonia that prevents subculturing the *C. perfringens* colonies, the too-selective nature of m-CP agar, and the evanescence of the red color of colonies after the addition of ammonia, which makes further confirmation impossible (cf. Figure 4).

Figure 4. *C. perfringens* on mCP agar

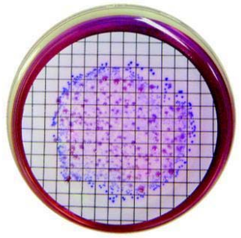


Figure 5. Comparison between TSCF agar and the mCP and CPC (CP *ChromoSelect* Agar) media for enumerating strains of *C. perfringens* in water samples

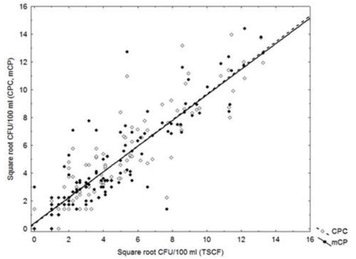


Table 1. All green colonies isolated from CP *Chromo-Select* Agar and identified with API system (n=483)

Strains	Indole reaction	n
C. Perfringens	-	465 (96.3%)
C. Tertium	-	1 (0.2%)
C. Botulinum	-	1 (0.2%)
C. Fallax	-	1 (0.2%)
C. Bifermentans	+	2 (0.4%)
C. Sordelli	+	12 (2.5%)
C. tetani	+	1 (0.2%)

Besides its advantages over m-CP and TSC agars, CP *ChromoSelect* Agar is an ideal growth media. It contains only vegetable peptones and, together with yeast extract, it is an excellent source of nitrogen, carbon, amino acids and vitamin B complex. Sucrose acts as the fermentable carbohydrate, and reducing agents lower the redox potential of the media. Diverse salts provide the required ions for enzymatic reactions. Buffering agents stabilize the pH within the ideal growth range. Inhibitors D-cycloserine and polymyxin B give the medium its selectivity, while further selectivity is achieved by incubation under anaerobic conditions at 44 °C. Various promoters and substrates protect injured cells to improve recovery rate and enhance growth. The chromogenic enzyme substrates in the CP *ChromoSelect* Agar provide the differentiation, for *C. perfringens* in particular (Table 2). A negative indol reaction (Kovac's Reagent) is confirmatory for *C. perfringens*.

Table 2. *Clostridium sp.* cultural characteristics in CP *ChromoSelect* Agar

Organisms (ATCC)	Growth	Colony appearance
Clostridium perfringens (13124)	+++	Green
Clostridium bifermentans (638)	+++*	Dark blue with violet halo
Clostridium sporogenes (8534)	-	-
Clostridium sordelli (9714)	++	Dark green with halo (change to yellow with Kovac's Reagent)
Enterococcus faecalis (29212)	++	Violet
Escherichia coli (25922)	-	-
Pseudomonas aeruginosa (27853)		Colorless
Staphylococcus aureus (25923)	-	-
Bacillus subtilis (6051)	-	-
Salmonella typhimurium (DSM 554)	++	Violet

*** Growth at 40 °C, but no growth at 44 °C.**

CONCLUSION

- CP *ChromoSelect* Agar was the most useful medium for *C perfringens* recovery in water samples.
- CP *ChromoSelect* Agar is more reliable and easier to handle than m-CP and TSC agars. The color does not diffuse in the agar and confirmation is not required since the green coloration is specific for *C. perfringens*.

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