CHAPTER ONE

INTRODUCTION

1.1 Research Background

Clostridium perfringens (CP), an anaerobic, spore-forming and gram positive bacteria, is no stranger among clinical pathogens. Although being notorious as the "flesh-eating anaerobe" (Shimizu *et al*., 2002; Schlapp *et al*., 1995), it however has a beneficial application in environmental pollution studies. Since first suggested by Klein and Houston back in 1899 (Bisson and Cabelli, 1978), CP has long been serving as a faecal pollution indicator. The main characteristics of CP that make it a good indicator include being a universal gastrointestinal inhabitants (Tonooka *et al*., 2005; Gholamiandekhordi *et al*., 2006; Wise and Siragusa, 2005; Alkhaldi *et al*., 2004a; Hughes *et al*., 2007), ubiquitous in the environment (Johansson *et al*., 2006; Fung *et al*., 2008; Barbour *et al*., 2004; Hang'ombe *et al*., 2000; Plancherel and Cowen, 2007; Fujioka, 2001; Mar Gamboa *et al*., 2005), and forming spores that are resistant under harsh environments (Dahlen *et al*., 2006; Casteel *et al*., 2006; Touron *et al*., 2007; Garcia-Alvarado *et al*., 1992; de Jong *et al*., 2004). Throughout the last millennium, other prominent faecal pollution indicators have emerged too, notably *E. coli* and coliforms. Nevertheless, studies in recent decades have provided more evidence on the usefulness of CP as an indicator for faecal pollution. CP is now categorized into five toxinotype, Type A to Type E based on the alpha, beta, epsilon and iota toxin genes combinations (Hatheway, 1990; Al-Khaldi *et al*., 2004b) as shown in Table 1.1. The CPE (*Clostridium perfringens* enterotoxin) gene and its expression are also of current interest due to the links to food poisoning and antibiotic-associated-diarrhea (Grant *et al*., 2008; Camacho *et al*., 2008; Efuntoye and Adetosoye, 2004; Modi and Wilcox, 2001).

Table 1.1 *Clostridium perfringens* toxinotype categorization

Clostridium perfringens toxinotype	Toxin(s)
Type A	alpha toxin gene only
Type B	alpha, beta and epsilon toxin genes
Type C	alpha and beta toxin genes
Type D	alpha and epsilon toxin genes
Type E	alpha and iota toxin genes

It is interesting to ask "Is the ubiquity of CP in the environment linked to its universal presence in intestinal tracts?" While there is no exact answer to this question, it is important to understand that CP from the environment will enter the food chain and the cycle goes on. Matches *et al.* (1974) had long realized that CP density in guts of fish caught from polluted coastal area were higher compared to CP in sediments of the same area. Thus, it could be implied that the same is also happening in terrestrial food chain. Temperature abuse and improper food handling tend to cause CP food poisoning whereby CP spores thrive and multiply (Paredes-Sabja *et al*., 2008a; Brynestad and Granum, 2002; Sarker *et al*., 2000; Nakamura *et al*., 2004). But how did the food get the initial amount of CP in vegetative cells or spores? The answer is quite obvious that it was from the food chain, and the source can be traced back to the environment that sustain the food production.

Water supply demand in the Selangor State and Federal Territory of Kuala Lumpur is projected to be 5000 million litres per day (MLD) in 2010. Sungai Selangor is the main river fulfilling the current water demand. However, with an annual 6% increase, water demand is due to reach 9000 MLD in 2050. Therefore, the federal government is seeking alternative water sources from neighbouring states to circumvent a possible water shortage in the coming decades (Syarikat Pengeluar Air Sungai Selangor Sdn Bhd, Water facts : Magnitude of water problem). Located between the Selangor and Perak state, Sungai Bernam is one of the options. Therefore, it is of utmost importance to protect these rivers from water quality deterioration that would cause further water scarcity or increase the treatment burden of procured water. The Drainage and Irrigation Department of Malaysia (DID Malaysia) is routinely monitoring 24 water quality parameters of these two rivers, but CP is not one of the monitored parameters.

1.2 Research Objectives

What is the prevalence and density of CP in our rivers? What are the common CP toxinotypes? Will river discharge significantly affect the density and prevalence of CP in river? Will CP data from our tropical rivers be different from other data which are mostly related to temperate countries? With these research questions in mind, this study was carried out with three objectives :

1) To investigate and compare CP densities among Sungai Selangor, Sungai Bernam and Tengi Canal
2) To analyze the correlation between CP densities and river discharge
3) To examine the toxinotype of CP isolates

1.3 Research Rationales and Limitations

Episodic non-compliance of faecal indicator organisms in downstream area notably seaside and marine aquaculture farms were reportedly caused by rainfall that increases stormwater runoff (Wilkinson *et al*., 2006; Krogh and Robinson, 1996). Therefore, river discharge as a relative measurement of rainfall and runoff is hypothetically chosen to be the determining factor of CP densities in this study. Besides river discharge, sediment may also be important (Garrido-Pérez *et al*., 2007). However, this parameter is not studied because of research limitation.

This study voluntarily adopted the bio-security principle whereby no *Clostridium perfringens* (CP) reference strains would be acquired from overseas research institutions. With only one Type A CP positive control, which was the commercially available ATCC 13124 from Oxoid Ltd, this study faced challenges especially in toxinotyping CP strains other than Type A. Nevertheless, the toxinotyping is expected to work well since established primers for this purpose (Yoo *et al*., 1997) would be used and representative Polymerase Chain Reaction (PCR) products be sequenced.

1.4 Significance of Study

CP has yet to be used as faecal pollution indicator in aquatic bodies of Malaysia. There are also very few CP related data in the South East Asia region that reflect the pollution level. Meanwhile, CP toxinotype from river water isolates has not been reported thus far. Therefore, this study served to provide preliminary data on CP densities and toxinotype from local river water, which may later be used for sustainable water supply management especially in river pollution control and microbial risk assessment.