1.1 **Background of the study**

Microbial pollution of water is a growing environmental health and public health crisis worldwide. Fecal contamination from humans and animals is believed to be a major cause for increased microbiological and nutrient loads in coastal and inland waterways (Lipp *et al*., 2001). Microbial contamination in waterways can originate from various sources such as wastewater treatment outfalls, municipal waste and discharge from large-animal feeding operations. Indicator microorganisms are used to predict the presence of the potential risk associated with pathogenic microbes and total coliforms have been used extensively for many years as indicators for determining the sanitary quality of surface waters (Scott *et al*., 2002).

Escherichia coli is a gram negative bacterium that is well recognized as a main commensal inhabitant of mammals' gastrointestinal tract. It is a major facultative anaerobe and harmless saprophyte. However Larulle (1889) was the first to suggest the possible role of *E. coli* as a pathogenic organism. The organism typically colonizes the infant gastrointestinal tract within hours of life and thereafter, both *E. coli* and the host derive mutual benefit (Drasar and Hill, 1974). In the debilitated or immunosupressed host, even normal non pathogenic strains of *E. coli* can cause infection.

Diarrhea caused by *E. coli* is one of the main diseases associated with water supply and sanitation. Six major pathogenic *E. coli* strains are enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E.*

coli (EaggEC), enteroinvasive *E .coli* (EIEC) and diffusely adherent *E. coli* (DAEC) (Smith & Fratamico, 2005). Pathogenic forms of *E. coli* associated with human and animal diseases are diverse. Certain pathogenic strains cause enteric diseases ranging in symptoms from cholera-like diarrhea to severe dysentery (Liu *et al*., 2007), while other *E. coli* strains may colonize the urinary tract, resulting in cystitis or septicemia (Donnenberg *et al*., 2001). In the United States alone approximately 4,000 confirmed *E. coli* O157:H7 cases are reported each year (Lindsay David, 2000). *E. coli* O157:H7 outbreaks associated with both drinking and recreational water raise concerns about waterborne illness outbreak.

In Malaysia, the active promotion of environmental sanitation has improved the health of the population, with coverage reaching almost 100% for all states with the exception of Kelantan and Sabah (Safurah *et al*., 2007). In the present research, waterways around Bachok, Kelantan were studied. Bachok is a territory and town in Kelantan. It is a rural area approximately 20 kilometers south east of Kota Bahru city (Fig. 1.1a). It borders Pasir Puteh to the south and Kota Bharu to the west. The focus of Bachok's economy is agriculture, primarily tobacco plantations. The population of Bachok is about 116,128 in 2005. The annual report of Ministry of Health Malaysia (1998) reported that Kelantan has more cases of food and waterborne disease than any other states in Malaysia (Ministry of Health, 1998).

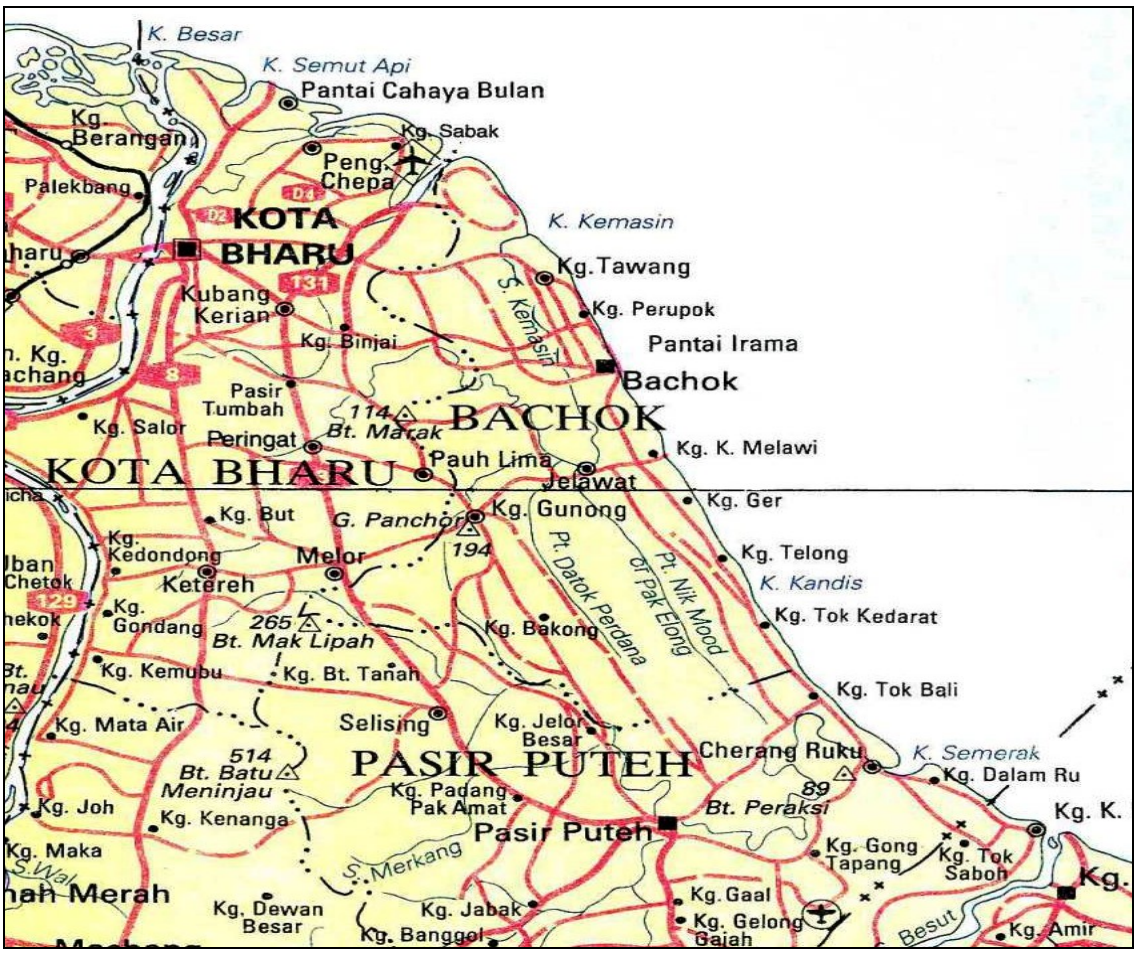

Fig. 1.1a Sampling sites around Bachok, Kelantan.

Earlier detection and differentiation of diarrheagenic *E. coli* are usually based on a combination of biochemical tests, serotyping and phenotypic assays based on virulence characteristics and molecular detection methods. However recent studies had simplified and accelerated differential diagnosis where novel multiplex PCR (mPCR) for the simultaneous detection and differentiation of the major categories of intestinal pathogenic *E. coli* strains were designed and evaluated (Kong *et al*., 2001). This was achieved by combining few specific primer pairs in a single reaction mixture. Muller and coworkers (2007) have even identified several strains that expressed unusual virulence factor profiles apparently representing intermediate pathotypes using mPCR assay. This

not only serves as a further example of the plasticity of the *E. coli* genome but also emphasizes the need for the differential identification of specific pathotypes in order to facilitate appropriate countermeasures (Muller *et al*., 2007).

The mPCR assay for the detection of pathogenic *E. coli* has been applied in Malaysia and other Asian countries. For an example, Alhaj *et al*. (2007) have developed a novel single mPCR for detection of EPEC and Shiga toxin producing *E. coli* (STEC). While, Kong and coworkers (1999) from Hong Kong have developed a versatile mPCR method that is capable of simultaneous detection of seven different virulence genes found in various combination in ETEC, EHEC and EPEC strains of pathogenic *E. coli*.

For the study of infectious disease outbreaks, DNA based techniques have been applied intensively. An advantage of these genotypic methods is that they do not depend on the expression of specific gene products for detection of the source of outbreak. PCR based methods permit sensitive detection of bacterial organisms in diseased samples because cultivation of organisms prior to typing is not required. Several typing methods can identify differences in genetic composition of microbial population. These techniques involve bacterial restriction endonuclease analysis, pulsed field gel electrophoresis (PFGE) and repetitive sequence based polymerase chain reaction (rep-PCR). Repetitive sequences are present in the genome of all the organisms. Genomic fingerprinting with these repetitive sequences based probes has been used to distinguish unrelated organisms. This is because individual bacterial strains vary with respect to the distances between the repetitive sequences. Repetitive sequence based genomic fingerprinting makes use of DNA primers complementary to naturally occurring, highly conserved,

repetitive DNA sequences present in multiple copies in genomes of most gram-negative and several gram-positive bacteria (Lupski and Weinstock, 1992).

Rep-PCR is a potential subtyping tool for identification of the source of environmental *E. coli* population owing to its success in classifying the host source, reproducibility, cost effectiveness and easy operational procedures (Mohapatra *et al*., 2007). Therefore, this method has been used in this study for genomic differentiation of the isolates.

1.2 Objectives of the study

Thus, the objectives of the study were:

1) To isolate and confirm the presence of *E. coli* in selected aquatic environments in Bachok, Kelantan by applying both phenotypic and genotypic methods.
2) To determine the prevalence of their virulence genes such as Heat-stable toxin 1 (ST1), Heat-labile toxin 2 (LT2), Verotoxin 1 (VT1), Verotoxin 2 (VT2) and Attachment and effacement (*eaeA*).
3) To analyze the genomic diversity among the isolates based on REP-PCR fingerprinting profiles
4) To compare the diversity of the environmental *E. coli* isolates with clinical and food isolates.

1.3 Scope of the study

The study was carried out for one semester (May to December 2008) as part of the partial fulfillment for the Master of Biotechnology (M.Biotech) program.