CHAPTER 1

INTRODUCTION

1.1 Background

Microorganisms had been long recognised for their ability to utilise organic compounds as both carbon and energy sources by enzymes acting in an organised and sequential manner. Microbial remediation utilises microbial population (predominantly bacteria and fungi) as biological or biochemical catalyst, to degrade or transform hazardous or xenobiotic compounds to harmless/ less toxic compounds. Microbial bioremediation can also be defined as natural or managed biological degradation of environmental pollution and it is normally being carried out by the indigenous microorganisms (Scragg, 1999).

Anthropogenic activity is a major contribution to the release of xenobiotic compounds which lead to the plethora of Polyaromatic hydrocarbons (PAHs) and Persistent organic pollutants (POPs). The international market for remediation products and services is vast since the strict environmental regulations and legislation and the increasing public awareness for a better environment have driven the growth of the remediation sector. Physico-chemical treatment is usually costly and often the contaminant is not fully degraded. Alternatively, microorganisms can be used to degrade these xenobiotic compounds which have evolved as a cost-effective *in-situ* strategy for the remediation of contaminated sites.

Although microorganisms play a major role in the breakdown and mineralisation of man-made organic compounds, the kinetics of the biodegradation process may be much slower than desired

from public health or environmental considerations. The slow biodegradation of xenobiotic compounds in the natural environment may be caused by unfavourable physico-chemical conditions, availability of the nutrient, predation or the low enzymatic activity due to its gene expression. Bioremediation technology is focusing on enhancing this natural process either by altering the condition to the favourable state that organisms are able to degrade and mineralise the compound efficiently or by introduction of specifically engineered organism to clean up xenobiotic compounds particularly problematic compounds such as PAHs and POPs (Kumar *et al*, 2010)

Many *Burkholderia* species have been isolated for their potent capacities to biodegrade anthropogenic organochemical pollutants (O'Sullivan and Mahenthiralingam, 2005). The biodegradation capabilities may be due to their high genome plasticity through their large and complex genome sequence spread over two to four replicons as well as a large number of sequence insertions (Coenye and Vandamme, 2003; Lessie *et al*. 1996). In addition, the wide substrate diversity of *Burkholderi*a sp. makes them attractive bioremediation agents. Members in the *Burkholderia* genus have been shown in the laboratory setting but as well as in field trials to be capable of degrading many xenobiotic compounds including polychlorinated biphenyls (PCBs) (Bopp, 1986: Mukherjee-Dhar *et al.* 1997; Goris *et al*. 2004) , trichloroethylene (TCE) (Nelson *et al*. 1986; Zhang *et al*. 2000), organopesticides (2,4-D and 2,4,5-T) (Greer *et al*. 1990; Bhat *et al.* 1994; Rice *et al*. 2004), polycyclic aromatic hydrocarbons (PAHs) (Mueller *et al.* 1997; Balashova *et al.* 1998; Kim *et al*. 2003; Vacca *et al.* 2005) and munitions (RDX, TNT and DNTs) (Lee and Broadman, 2004). *Burkholderia xenovorans* LB400 has been the focus of many studies over the past two decades due to its exceptional ability to transform PCBs even though

this ability is not limited to PCB-degrading only. Bedard *et al*. (1986) reported that *B. xenovorans* LB400 can oxidize more than 20 congeners including some with 4, 5 and 6 chlorine substitutions on the biphenyl rings.

The ability of bacteria to adapt to various stress conditions including nutrient limitation by triggering a general and transcriptionally coordinated stress response has been known for many years. These bacteria possessed unique metabolism to deal with nutrient-related stress such as utilisation of xenobiotic compounds. This unique metabolism is activated when RNAP forms holoenzyme complex with sigma factor and initiate the transcription of the specific genes. Sigma (σ) subunits of RNA polymerase (RNAP) are important as determinants of differential gene expression since they are responsible for specific binding to DNA (Helmann and Chamberlin, 1988; Wosten, 1998). The σ subunit contributes to core RNAP ability to specifically recognise and initiate transcription from promoters. The complex formed by σ factor and polymerase core subunits constitutes the bacterial holoenzyme. Several alternative σ factors including σ^{54} have been observed to enable the cells to transcribe specific sets of genes in response to environmental stimuli. Alternative sigma factor-54 (also known as RpoN) has been demonstrated mediating several xenobiotic degradation pathways in bacteria as shown in toluene and m-xylene degradation pathway by the TOL plasmid of pWW0 *P. putida* mt-2 (Cases and de Lorenzo, 2005) and (methyl) phenol degradation encoded by plasmid pV1150 of *P. putida* CF600 (Powlowski and Shigler, 1994) and in transcription of biphenyl dioxygenase (encoded by *bphA* gene) that is responsible for the initial step in biphenyl degradation. Dibenzofuran, which has similar basic structure with biphenyl and PCBs are able to be degraded using biphenyl

dioxygenase via biphenyl degradation pathways by *Burkholderia xenovorans* LB400 (L'Abbee *et al*. 2005; Seeger *et al.* 2001).

1.2 Significance of study

Alternative Sigma Factor-54 (RpoN) was extensively studied especially in *Escherichia coli*, for example in nitrogen assimilation (Reitzer and Schneider, 2001). In *Burkholderia* species, it was shown that RpoN involved in pathogenicity characteristics such as pilus and biofilm formation. Studies show that RpoN is also involved in degradation of aromatic compounds such as toluene and xylene. Limited studies have been conducted on direct effects of RpoN on degradation of dioxin-like compounds via biphenyl degradation pathway in *Burkholderia* species especially in *Burkholderia xenovorans* LB400, well known PCB degraders. This study aimed primarily to address the relationship between the role of RpoN in selected *Burkholderia* species and degradation of dioxin-like compounds.

1.3 Objectives of the study

The main objective of this study is to determine the effects of inactivation of alternative Sigma Factor-54 (RpoN) which responsible in initiation of transcription process towards the ability of two selected *Burkholderia* species to degrade dibenzofuran. Specific objectives of this study are:-

1. To develop a single gene knockout system for *rpoN* genes (encoding RpoN) in *Burkholderia* species.

2. To determine the ability of wildtype strains and their single knockout mutants to degrade dioxins-like compounds such as dibenzofuran.
3. To determine the expression of biphenyl dioxygenase (encoded by *bph* genes) that is also involved in dibenzofuran degradation in both wild type and single gene knockout mutants.
4. To evaluate the bioremediation potential of the single gene knockout mutants using simple phytotoxicity test.