CHAPTER 1
INTRODUCTION

Pyrene, fluoranthene and phenanthrene are polycyclic aromatic hydrocarbons (PAHs). Structure of pyrene consists of four fused benzene rings by which fluoranthene possesses three aromatic rings being fused with another five membered ring, and phenanthrene contains three fused benzene rings (Sutherland *et al.*, 1995). These substances are formed during the combustion of fossil fuels, by-products of industrial processing as well as natural constituent of unaltered fossil fuel (Wilson and Jones, 1993). PAHs could contaminate environment by many ways for example: direct aerial fallout, leakage of industrial or sewage effluent, accidental discharges during transport, or the use and disposal of petroleum product (Cerniglia, 1992).

Environment contaminated with increasing amounts of PAHs is considered hazardous due to their potential toxic, carcinogenic and teratogenic properties. Many of such including pyrene, fluoranthene and phenanthrene are listed as priority pollutants by the U.S. Environmental Protection Agency (Patnaik, 1992).

Although PAHs may undergo photolysis, chemical degradation and volatilization, the major decomposition process is biodegradation (Cerniglia, 1992) which involves the use of microorganism to degrade and detoxify these hazardous organic compounds to harmless substances (Baker and Herson, 1994). The biodegradability of PAHs depends on their chemical structure and corresponding physicochemical properties (Weissenfels *et al.*, 1991). Two- and three-ring PAHs degradation has been extensively studied, whereas less is known about that of higher-molecular weight PAHs, the recalcitrant compounds such as pyrene and fluoranthene (Sepic *et al.*,1997).

Co-metabolism which is the oxidation of substances without utilization of the energy derived from the process to support microbial growth (Horvath, 1972) is a valuable alternative means to detoxify the recalcitrant compounds (Mueller *et al.*, 1989). Furthermore, in practice most sites are contaminated with a wide variety of PAHs such as it has been documented that phenanthrene, pyrene, fluoranthene and other 12 priority pollutant PAHs are found as major constituents of creosote production area and site with wood treatment activities (Wilson and Jones, 1993). Consequently, co-

metabolism might also be factor affecting the persistence of PAHs in the environment. Previous studies of PAH degradation by bacteria presented evidences that there are interaction between PAHs in the mixture that could influence the process of biodegradation. Examples of this are; *Pseudomonas putida* growing on naphthalene is found capable of co-metabolizing fluoranthene (Barnsley, 1975), *Alcaligenes denitrificans* WW1 could co-metabolize pyrene in the presence of fluoranthene (Weissenfels *et al.*, 1991). *Mycobacterium* sp. strain PYR-1 and RJGII-135 could co-metabolize pyrene and fluoranthene or pyrene alone when grew in mineral salts medium supplemented with peptone, yeast extract and soluble starch (Heitkamp *et al.*, 1988a; Grosser *et al.*, 1991). Phenanthrene is often used as a model for studying the metabolism and co-metabolism of carcinogenic PAHs. For example, Bouchez *et al.* (1995) demonstrated that unidentified bacterium *S Phe Na 1* could co-metabolize fluoranthene in the presence of phenanthrene. Moreover phenanthrene could stimulate microbial growth and degradation of dibenz(a,h)anthracene as well as benzo(a)pyrene when added to cultures containing these compounds (Juhasz *et al.*, 1997).

In Thailand, studies have been conducted and revealed that many PAHs were found to contaminate in Bangkok Metropolitan and its vicinity (Panther *et al.*, 1996) as well as the city of Chiang-Mai (Amagai *et al.*, 1999). According to the report on the uptake and accumulation of PAHs in food chain (Means *et al.*, 1980) as well as their toxicity described above, the finding of PAHs contamination in Thailand may cause serious health problems to Thai population. As a consequent, there is considerable interest in the study on biodegradation for the removal of these substances from environment.

Advances in the use of microorganism for biodegradation depend on understanding of the biochemistry and genetics of PAHs degrading pathways in microorganisms (Schneider *et al.*, 1996). Generally, the initial step in the aerobic microbial biodegradation of aromatic compounds is the introduction of two hydroxyl groups into the benzene ring, forming *cis*-dihydrodiols. This reaction is catalyzed by dioxygenase (Butler and Mason, 1997).

Recently, *Sphingomonas* sp. P2 was isolated from petroleum contaminated soil in Thailand for its ability to utilize phenanthrene as sole carbon and energy sources

(Supaka *et al.*, 1999). Preliminary study by Mr. Nuttapun Supaka (personal communication) suggested the possibility that this strain can co-metabolized pyrene and fluoranthene by using phenanthrene as growth substrate. It was interesting to verify co-metabolic products obtained from the degradation since there had not been found in the previous investigation of this bacterium.

The purpose of this study was to identify metabolites of pyrene and fluoranthene degradation via co-metabolism with phenanthrene by *Sphingomonas* sp. P2. Since the enzyme produced by this phenanthrene degrader could widely hydrolyze many other priority PAH pollutants, the dioxygenase genes involved in phenanthrene degradation was preliminarily studied as the second objective.

To identify metabolites from catabolism of such compounds, large-scale cultivation using two 30-liter fermenters were carried out. Solvent extraction as well as purification process namely, silica gel open column, thin-layer and high performance liquid chromatography were used to isolate and purify the metabolites. In order to elucidate their structures, gas chromatography-mass spectral, proton and carbon nuclear magnetic resonance spectral analyses were employed for the purpose.

Additional experiments of polymerase chain reaction and shot gun cloning experiment were conducted with the aim of understanding more in reference to the dioxygenase genes.