CHAPTER I

INTRODUCTION

1.1 Statement of problem

Organic solvents are a group of chemicals of varying volatility and lipophilicity. They are widely used as constituents of paints, varnishes, dyes, inks, adhesives and aerosol spray products, and are also commonly used as fuel additives. The prevalence of organic solvents in the environments means that exposure to solvents is generally unavoidable and humans are generally exposed for prolonged periods and often in combination with other compounds.

Environments having high concentrations (10 to 50%, v/v) of organic solvents are considered extreme and bacteria that are able to tolerate such environments have recently been recognized as a subgroup of the extremophiles (Aono,1998). Lipophilic hydrocarbons are harmful to bacteria because they accumulate in membrane lipid bilayers, thus affecting the structural and functional properties of the membranes (Sikkema *et al.*,1995), and cytoplasmic membranes are the primary site of cellular damage by both organic solvents (Sikkema *et al.*,1995).

The first report of an organic-solvent-tolerant bacterium was by Inoue and Horikoshi in 1989 (Inoue and Horikoshi,1989). They discovered a strain of *Pseudomonas putida* IH-2000 which could actively grow and multiply in the presence of 50% (v/v) toluene. This finding came as a surprise because it had been known for a long time that toluene and other solvents such as benzene or *n*-octanol were very toxic to microorganisms. This surprising observation was confirmed by others (Cruden *et*

al.,1992; Ramos *et al.*,1995) and the search to uncover the mechanisms behind this extraordinary characteristic began.

Reported of organic-solvent-tolerant gram-negative bacteria are *Pseudomonas* strains, for example *P. putida*, *P. aeruginosa*, *P. fluorescens*, and etc. white *P. putida* IH-2000. (Inoue and Horikoshi,1989), and *P. putida* DOT-T1E. (Ramos *et al.*,1995) and gram-positive bacteria like *Bacillus, Rhodococcus* and *Arthrobacter* tolerant to benzene have been reported (Abe *et al.*,1995; Kato *et al.*,1996; Paje *et al.*,1997).

The solvent-tolerant bacteria can be used in environmental biotechnology as well as in biotechnological production processes in two-liquid water–solvent systems. There is considerable interest in the development of technology for the production of value-added chemicals from hydrophobic substrates such as petroleum hydrocarbons by using biocatalysts. Enantiospecific oxidation and reduction of aromatic and aliphatic hydrocarbons have especially attracted much interest, because many important chemicals can be produced by these reactions. Since cofactors and their regeneration are required for oxidation and reduction reactions, whole cells are favored as biocatalysts. Therefore, solvent-tolerant bacteria as hosts and genes encoding enzymes that catalyze reactions of interest are necessary for the development of bioconversion processed for hydrophobic substrates.

Moreover, solvent-tolerant bacteria can be used in the environmental bioremediation of toxic wastes could be enhanced to withstand higher concentrations of the pollutants. Since most natural contaminated sites are saturated with solvents such as benzene, toluene, etc., organic solvent tolerant bacteria with the requisite catabolic potential can be of vital importance in cleanup operations. For instance, a *Rhodococcus* sp. strain 33 isolated from a contaminated site in Sydney can degrade benzene at concentrations of 200 ppm and tolerate high concentrations of benzene, which are

necessary characteristics for a culture if it has to be used in cleaning up marine oil spills *(Paje et al.,1997)*. Abe *et al.* isolated an organic solvent tolerant bacterium from deep sea sediment samples after treatment with 50% v/v benzene. This strain, *Bacillus* DS-1906, showed polyaromatic hydrocarbon degrading ability in the presence of organic solvent. It degraded 48% of naphthalene solubilized in *n*-hexane and the amount degraded was more in the presence of solvent (Abe *et al.*,1995).

Above mentioned, Bacterial cells with enhanced solvent tolerance would be extremely useful in the fields of applied microbiology and biotechnology. Bacteria used in the environmental bioremediation of toxic wastes could be enhanced to withstand higher concentrations of the pollutants. In the industrial production of fine chemicals, organic solvent tolerant bacteria would be better able to withstand extraction of the chemical end-product with a second phase of organic solvent. The further characterization of these specialized organic solvent-tolerant bacteria will provide us with the knowledge required for their use in biotechnological applications.

1.2 Objectives and expected results

There are three objectives of this research:

1.2.1 To screen, isolate and identify of organic solvent-tolerant bacteria

1.2.2 To characteriza of organic-solvent tolerant bacteria

1.2.3 To determination of factors involving organic-solvent tolerance

The organic-solvent tolerant bacterial isolated fromthis work may have potential for bioremediation and biotechnology applications.

1.3 Hypothesis

Bacteria screened and isolated from various soil in Thailand which has been exposed to hydrocarbon and organic solvent wastes might have the ability to tolerate organic solvent.

1.4 Scope of study

1.4.1 Screening and isolation of organic solvent tolerant bacteria from soil

1.4.2 Primary test of the isolates for organic solvent utilization and tolerance

1.4.3 Secondary test of the isolates for organic solvent utilization and tolerance

1.4.4 Identification of the organic solvent-tolerant bacteria

1.4.5 Characterization of the organic solvent-tolerant bacteria

1.4.5.1 Effect of types and concentrations of organic solvent on growth and tolerance

1.4.5.2 Effect of organic solvent on cell morphology

1.4.5.3 Determination of cell fatty acid composition

1.4.5.4 Organic solvent utilization using resting cell technique

1.4.5.5 Determination of enzymatic activity involving organic solvent utilization

1.4.6 Factors involving organic solvent tolerant of the bacteria

1.4.6.1 Effect of divalent ion on cell growth and organic solvent tolerant

1.4.6.2 Effect of nutrient on cell growth and organic solvent tolerant

1.5 Thesis Organization

This thesis is comprised of seven chapters. First, chapter I provide the introduction part of this research. Second, the theoretical background and literature review are described. Third, the research methodology is explained. After that, the results and discussions are demonstrated; then results are being concluded. Finally, the suggestion and future works are orderly described.