CHAPTER VI

CONCLUSION

Organic solvent-tolerant bacteria can be isolated from soil using enrichment technique with toluene and cyclohexane vapor. Thirty-six bacterial isolates that grew on toluene or cyclohexane as a sole carbon and energy source were isolated. According to the primary test of the isolates for organic solvent utilization and tolerance, cells could be divided into 5 groups mainly according to their tolerance towards organic solvents with different log P_{ow} values : Group I bacteria tolerate to organic solvent with log P_{ow} 0.7-5.6 or exhibited tolerance to a broad rang of log P_{ow} value; Group II bacteria tolerate to organic solvent with log $P_{ow} > 3.0$; Group III bacteria tolerate to organic solvent with log P_{ow} 2.0-3.0; Group IV bacteria tolerate to organic solvent with log $P_{ow} < 2.0$; and Group V bacteria low exhibited tolerance to organic solvent. Within these five groups of organic solvent-tolerant bacteria, twelve bacteria isolates having comparatively good growth in the presence of organic solvent from each group were chosen for the secondary test. According to secondary test the isolates for organic solvent utilization and tolerance using various types of liquid medium supplemented with organic solvents, those four strains could be selected because growth of these bacterial strains were greater than another bacterial strain.

D. geothemalis T27 exhibited tolerance to a broad rage of organic solvent. Cells decreased in size increase to a direct contact to ethyl acetate. Fatty acid composition was no significantly changed when exposed to ethyl acetate. *D. geothermalis* T27 could degrade 88% and 70% of 2.838 mM and 0.709 mM ethyl acetate respectively in MSB liquid medium within 24 h. Inducible intracellular esterase was detected as a consequence of ethyl acetate exposure. Supplement Ca^{2+} was beneficial to cell growth and ethyl acetate tolerance.

B. cereus strain 4/1 exhibited tolerance to broad range log P_{ow} value of organic solvent. Cells did not change in size with exposure to chloroform. Fatty acid composition was not significantly changed. Addition of Ca^{2+} at all three concentrations tested improved cell tolerance to touene.

B. subtilis strain 45 exhibited tolerance to log P_{ow} value ≥ 3 of organic solvent. Cells did not change in size with a direct contact to *n*-decane. Fatty acid composition was not significantly changed when exposed to *n*-decane. Supplement Ca^{2+}, Mg^{2+} and Na^{+} improved *n*-heptane tolerance , but not cyclohexane and ethybenzene tolerance.

Brevibacillus agri strain 13 could withstand solvent toxicity with log $P_{ow} \leq 2$. Cells could not change in size with exposure to ethyl acetate. There was a change in fatty acid composition I that C16:1 was increased by 2.65 times when exposed to ethyl acetate. Supplement with Ca^{2+}, Mg^{2} and Na^{+} improved cell growth of *Brevibacillus* agri *s* strain 13.

Organic solvent are known to be extremely toxic to cells. They dissolve and accumulate in the bacterial cell membrane, resulting in changes in structural and functional integrity and cause cell lysis. Organic solvent-tolerant bacteria are a newly discovered group of microorganisms, which enable them to thrive in solvent-saturated environment. These bacteria are significant due to their immense potential in nonaqueous bio-catalysis, industrial processes involving biphasic organic aqueous fermentation systems, effluent treatment and bioremediation in hydrocarbon-saturated environments.