CHAPTOR I

INTRODUCTION

1.1 Statement of problem

Shikimate is a key important metabolic intermediate in the shikimate pathway and is known as the direct precursor in the biosynthesis of aromatic amino acids (tyrosine, phenylalanine and tryptophan). This pathway is found only in microorganisms and plants. Therefore, shikimate is one of the important targets for the development of herbicides and anti-microbial compounds including several antibiotics. Recently, the importance of shikimate has been extensively established as it is a direct precursor in oseltamivir synthesis, the synthesis of compound against global pandemic flu infection, including avain flu.

Besides shikimate, dehydroshikimate has important roles in food industry. Dehydroshikimate is used as an antioxidant to extend food product shelf life and to improve food safety and quality. It inhibits a development of undesirable flavor, aroma, and color compounds triggered by peroxidation of lipids. Moreover, it has an evidence to suggest that many naturally derived antioxidants provides additional health benefits as antimutagenic, anticarcinogenic, or anti-inflammatory agents (Chang *et al.*, 2003).

In spite of the importance of the shikimate pathway to a variety of applications, little information has been accumulated about the shikimate pathway regarding interconversion of intermedietes. One reason can be that the metabolic location of dehydroshikimate and shikimate are remote from the initial substrate, glucose. 3-Deoxy-D-*arabino*-heptulosonate 7-phosphate, a key intermediate to

shikimate, is produced by combined two different metabolic pathways, phosphoenolpyruvate from the glycolysis and erythrose-4-phosphate from the pentosephosphate pathway. Therefore, it is complicate and difficult to control and modified genetically.

Recently, a novel route producing shikimate by oxidative fermentation of acetic acid bacteria, *Gluconobacter*, using quinate has been studied and developed. The quinate pathway is consisting of 3 steps: quinate→dehydroquinate→ dehydroshikimate→shikimate. This pathway is catalyzed by 3 important enzymes: quinate dehydrogenase (QDH), dehydroquinate dehydratase (DQD) and shikimate dehydrogenase (SKDH), respectively. In order to improve the oxidation fermentation to produce shikimate and its intermediates, understanding of the characteristics of these enzymes is essential. For large-scale production, large amount of these enzymes are needed. Therefore, molecular techniques will be used in order to express the proper enzymes in large amount.

In 2005, genome sequence of *G. oxydans* 621H was done (Prust *et al.*, 2005). To take advantage of the genome sequence database, *dqd* and *skdh* genes were cloned and overexpressed heterologously and homologously. Moreover, *glucose dehydrogenase* (*gdh*) gene was co-expressed with *skdh* gene to regenerate $NADP^+$. The suitable expression vector will be examined. The overexpression conditions will be investigated and optimized.

1.2 Objectives

1.2.1 Cloning of *dehydroquinate dehydratase* (*dqd*), *shikimate dehydrogenase* (*skdh*) and *glucose dehydrogenase* (*gdh*) genes from *G. oxydans* 621H.

1.2.2 Overexpression of *dqd*, *skdh* and *gdh* genes.

1.3 Scope of study

1.3.1 To clone *dqd*, *skdh* and *gdh* genes from *G. oxydans* 621H.

1.3.2 To overexpress *dqd*, *skdh* and *gdh* genes in *E. coli* BL21 (DE3).

1.3.3 To optimize the expression conditions of *dqd*, *skdh* and *gdh* genes in *E coli* BL21 (DE3).

1.3.4 To overexpress *skdh* genes in *G. oxydans* IFO3244.

1.4 Expected benefits

The *dqd*, *skdh* and *gdh* genes from G. *oxydans* 621H will be overexpressed in E. coli BL21 (DE3). This result will be useful information for industrial application in shikimate production.

1.5 Thesis organization

This thesis is comprised of six different chapters. First, Chapter I provides 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 and then these results are concluded.