CHAPTER VI

CONCLUSIONS

6.1 *dqd* (GOX0437) gene

dqd is a gene encoding for dehydroquinate dehydratase (DQD) enzyme. The DQD is catalyzes dehydroquinate to dehydroshikimate. In this study, the *dqd* gene was cloned from *G. oxydans* 621H genome into pGEM-T Easy vector. The sequencing result shown that the insert size for *dqd* gene was 620 bps (83 bp upstream region, 483 bps coding region and 54 bps downstream region). Furthermore, the *dqd* gene was subcloned into pET-21a to express in *E. coli* BL21 (DE3). From the expression result, the DQD specific activity of *E. coli* BL21 (DE3)/pET-*dqd* when grown at 30°C was 4 fold (10.8 U/ mg) higher than that of 37°C.

6.2 *skdh* (GOX0859) gene

skdh is a gene encoding for shikimate dehydrogenase (SKDH) enzyme. From *G. oxydans* 621H genome database, two genes (GOX0859 and GOX1959) were annotated as SKDH. Therefore, two genes were cloned and overexpressed to check its biological activity. The *skdh* (GOX0859) gene was cloned from *G. oxydans* 621H genome into pGEM-T Easy vector. From the sequencing result, the insert size for *skdh* (GOX0859) gene was 971 bp (26 bp upstream region, 849 bps coding region and 19 bps downstream region). After that the *skdh* (GOX0859) open reading frame was subcloned into pET-21a. The SKDH (GOX0859) of *E. coli* BL21 (DE3)/pET-GOX0859 was very low (0.047 U/mg) which was similar to that of *E. coli* BL21 (DE3). Therefore, *skdh* (GOX0859) gene was subcloned into pCold I vector and co-

expressed with a chaperone vector, pG-KJE8 to improve SKDH activity. From, co-expression result, *skdh* (GOX0859) did not show SKDH activity. It is concluded that *skdh* (GOX0859) may not be the gene encoding for SKDH.

6.3 *skdh* (GOX1959) gene

skdh (GOX1959) is another one gene annotating as shikimate dehydrogenase. The *skdh* (GOX1959) gene was cloned from *G. oxydans* 621H genome into pGEM-T Easy vector. From the sequencing result, the insert size for *skdh* (GOX1959) gene was 971 bp (4 bp upstream region, 840 bps coding region and 19 bps downstream region). After that the *skdh* (GOX1959) open reading frame was subcloned into pET-21a. Gene expression of *E. coli* BL21 (DE3)/pET-GOX1959 showed significant SKDH activity (92.49 U/mg) with shikimic acid in a presence of $NADP^+$. Then, the expressed SKDH (GOX1959) was purified by Ni-NTA agarose column with 31.59% yield, 7.33 purification fold and specificity of 452.03 U/mg. The purified SKDH (GOX1959) was used to determine for its kinetic parameters. K_m and V_{max} for shikimate and $NADP^+$ were 170 μM, 169.5 μmole/min and 52.6 μM, 256.4 μmole/min, respectively. The molecular weight of SKDH (GOX1959) from molecular weight calibration curve by 12.5% SDS-PAGE was 30.9 kDa. Furthermore, the expression of *skdh* (GOX1959) was carried out using *G. oxydans* IFO3244 and pSG8 vector resulting in 10-fold increasing SKDH activity. IPTG did not effect to *skdh* (GOX1959) expression in *G. oxydans* IFO3244.

6.4 $NADP^+$ regeneration

$NADP^+$ regeneration was performed by coexpression of *gdh* (GOX2015) encoding glucose dehydrogenase and *skdh* (GOX1959) in pET-21a vector. The

optimum condition for *E. coli* BL21 (DE3)/pET-GOX1959-GOX2015 co-expression was obtained when grown in a 100-ml LB medium at 37°C with 0.2 mM IPTG induction. The SKDH and GDH activity was 2.8 fold increased (98.97 up to 279.24 U/mg) and 6.7 fold increased (0.15 up to 1.03 U/mg), respectively. To improve GDH activity, *gdh* gene was subcloned into pBluescript II SK (-) vetor and site-directed mutagenesis of Shine-Dalgano sequence was done. Moreover, pET-GOX1959 was co-expressed with pACGD vector harboring *gdh* gene from *Bacillus megaterium* in *E. coli* BL21 (DE3).However, the GDH activity was not observed.

6.5 Future work

- The *E. coli* BL21 (DE3)/pET-*dqd* will be coexpressed with chaperone vector to improve DQD activity.
- *skdh* (GOX1959) and *gdh* (GOX2015) coexpression will be improved.