

CHAPTER V

CONCLUSIONS

1. The *alr* gene knockout mutant and *dadX* gene knockout mutant of *E. coli* BL21(DE3) were constructed by single insertion of group II intron.
2. The *dadX* and *alr* genes knockout mutant of *E. coli* BL21(DE3) had the intron insertion into both of *dadX* and *alr* genes.
3. The alanine racemase specific activity of *alr* gene knockout mutant and *dadX* gene knockout mutant were 1.88 and 5.47 unit/mg protein which were 3.3 and 1.1 times, lower than that of wild type respectively. In *dadX* and *alr* genes knockout mutant, alanine racemase activity could not be detected due to the mutant showed very slow growth rate.
4. The intron insertion in *alr* gene knockout mutant and *dadX* gene knockout mutant were stable upon the subculture at least 20 times. For *dadX* and *alr* genes knockout mutant, stability could not be performed since it did not survive on agar medium.