CHAPTER 5

CONCLUSION

This work used gene replacement technique for the disruption of *pcpD* gene in the genome of *Sphingomonas chlorophenolica* ATCC 39723. The targeting vector was constructed by cloning of 0.8 kb of *pcpD* gene fragment into pUC 19, then, the clone gene was interrupted by insertion with 1 kb of kanamycin resistance gene.

Targeting vector was transformed into *S. chlorophenolica* and kanamycin resistance colonies were detected. From Southern blot analysis with DNA extracted from 27 kanamycin resistance clone show that all were *S. chlorophenolica* but did not have kanamycin resistance gene inserted into the genome. Therefore all of them were mutant strains of *S. chlorophenolica* with resistance to kanamycin. Extraction of plasmid DNA in mutant strains did not find the targeting vector. Therefore mutant strains resistant to kanamycin were not mediated by kanamycin resistance gene from targeting vector. In the future study, I recommend the increase in the homologous DNA length in targeting vector and disruption of the *pcpD* open reading frame in targeting vector with kanamycin resistance gene from transposonTn 5.