CHAPTER 5
CONCLUSION

In this study, the *K. oxytoca* KMS006 was developed as a novel potential microbial platform for industrial-scale succinic acid production. The combining metabolic engineering and metabolic evolution strategy was successfully employed for improving the succinate production in both *K. oxytoca* KC004 (Δ*adhE*Δ*pta-ackA*Δ*ldhA* Δ*budAB* Δ*pflB*) in mineral salts medium. The evolved *K. oxytoca* KC004-TF160 significantly enhanced the succinate concentration, yield, and productivity up to 84 g/L, 0.84 g/g, and 0.87 g/L/h, respectively, during anaerobic conditions. No byproducts including ethanol, lactate, formate and 2,3-butanediol were detected by the *K. oxytoca* KC004-TF160. Only acetate at 14 g/L was detected. Additionally, *K. oxytoca* KC004-TF160 could produce succinate with the yield in the range of 0.41, 0.67, 0.80, 0.81, and 0.82 g/g from lactose, xylose, fructose, sucrose, and maltose respectively. Furthermore, *K. oxytoca* KC004-TF160 has the ability to produce succinate with the yield of 0.87 g/g from non-pretreated sugarcane molasse without the addition of any nutrients or plasmids. While the further reducing acetate formation of *K. oxytoca* KC004-TF60 resulted in *K. oxytoca* KP001-TF60 (Δ*adhE*Δ*pta-ackA*Δ*ldhA*Δ*budAB*Δ*pflB* Δ*tdcD*Δ*pmd*), which improved succinate yield by approximately 4.76%. The *K. oxytoca* KP001-TF60 significantly enhanced the succinate yield up to 0.88 g/g of glucose, whereas acetate levels of less than 1 g/L were detected in batch fermentation. Unfortunately, this strain could only consume 60 g/L of glucose, which is a limitation in industrial-scale production. Unlike *K. oxytoca* KMS006, the increased activity of the *pck* gene was responsible for the increased succinate yield and ATP production, whereas the activities of the *pdh*, *tdcE*, *tdcD* genes were responsible for acetyl-CoA and acetate formation which is the primary mechanism for energy sources and redox balance. Increased *pck* activity may cause nucleotide variations in the genes *cyaA*, *ptsG*, *agaC*, and *CsrB* genes. While these variations may affect glucose metabolism when deficient acetate mechanism, resulting in decreased glucose consumption and yeast extract requirement for *K. oxytoca* KP001-TF60. Even though, *K. oxytoca* KC004-TF160

and *K. oxytoca* KP001-TF60 were able to efficiently produce succinate equivalent those of previously native producer and developed *E. coli* strains. Therefore, the newly developed *K. oxytoca* KC004-TF160 strain may serve as one of the potential microbial platforms for the commercial production of succinate, while *K. oxytoca* KP001-TF60 may further improve for succinate producers in the future.