# ENGINEERING OF *KLEBSIELLA OXYTOCA* TO PRODUCE D-LACTATE

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**Introduction.** Lactic acid is one of many chemicals with many potential applications in the food, pharmaceutical, polymer and chemical industries. Even though lactic acid bacteria produce lactic acid with high titers and productivities, the strains exhibit low growth rates in synthetic media without rich or complex nutrients. Lactic acid bacteria have complex nutritional requirements that increase costs associated with production, purification, and waste disposal. The inexpensive production of sugars from cellulosic substrates is also an essential step for the fermentative production of DL-lactate. *K. oxytoca* has the ability to utilize a broader range of carbon substrates including cellobiose and cellotriose derived from cellulosic substrates. The strain exhibits fast growth under anaerobic conditions, and have no special nutritional requirements. Therefore, it is an interesting to develop this microorganism as a new platform of biocatalyst for D-lactate production.

**Methods.** The metabolic pathway of wild type *K. oxytoca* M5a1 was altered by the elimination of the chromosomal genes, *adhE* (alcohol dehydrogenase E) and *pta-ackA* (phosphotransacetylase-acetate kinase A). The mutant strain, KMS004, was tested for D-lactate production in AM1 mineral salts medium containing sugarcane molasses and maltodextrin derived from cassava starch during simple, pH-controlled batch fermentations.

**Results.** *Klebsiella oxytoca* strain was constructed to produce optical pure D-lactate by pH-controlled batch fermentation in mineral salts medium. The alcohol dehydrogenase gene, *adhE,* and the phospho-transacetylase/acetate kinase A genes, *pta-ackA,* were deleted from the wild type. KMS004 (Δ*adhE* Δ*pta-ackA*) exhibited D-lactate production as a primary pathway for the regeneration of $NAD^+$. The strain produced 11-13 g/L of D-lactate in medium containing 2% (w/v) glucose with yields of 0.64-0.71 g/g glucose used. In sugarcane molasses, KMS004 produced 22-24 g/L of D-lactate with yields of 0.80-0.87 g/g total sugars utilized. Sugar analyses during sucrose fermentation indicated that different sugars were fermented at different rates. This result suggested that the presence of glucose and fructose in sugarcane molasses affected sucrose utilization in KMS004. The rate of sucrose metabolism dramatically increased after the exhaustion of glucose and fructose. KMS004 also utilized maltodextrin and produced D-lactate at a concentration of 33-34 g/L with yields of 0.91-0.92 g/g maltodextrin utilized. These D-lactate yields are higher than those of *E. coli*. The delay in maltodextrin utilization observed might result from catabolite repression due to a presence of glucose in the maltodextrin used in this study. In *Klebsiella* spp., the maltose/maltodextrin-utilizing (*mal*) system is subjected to catabolite repression. Thus, maltodextrin consumption started after glucose was depleted.

**Conclusions.** *K. oxytoca* was metabolically engineered to produce D-lactate. KMS004 (Δ*adhE* Δ*pta-ackA*) strain produced impressive titers and yields of optically pure D-lactate in a low-cost medium containing sugarcane molasses and maltodextrin without complex nutrients under simple-batch fermentations. Other genes involved in NADH re-oxidation under anaerobic fermentation should be further deleted to direct more carbon flux towards D-lactate. KMS004 would be an alternative strain for the development of economic D-lactate production from renewable substrates.

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