# Improvement of hydrogen yield of ethanol-producing Escherichia coli recombinants in acidic conditions


## ABSTRACT

Background: An effective single culture with high glycerol consumption and hydrogen and ethanol coproduction yield is still in demand. A locally isolated glycerol-consuming Escherichia coli SS1 was found to produce lower hydrogen levels under optimized ethanol production conditions. Molecular approach was proposed to improve the hydrogen yield of E. coli SS1 while maintaining the ethanol yield, particularly in acidic conditions. Therefore, the effect of an additional copy of the native hydrogenase gene hycE and recombinant clostridial hydrogenase gene hydA on hydrogen production by E. coli SS1 at low pH was investigated. Results: Recombinant E. coli with an additional copy of hycE or clostridial hydAwas used for fermentation using 10 g/L (108.7 mmol/L) of glycerol with an initial pH of 5.8. The recombinant E. coli with hycE and recombinant E. coli with hydA showed 41% and 20% higher hydrogen yield than wild-type SS1 (0.46 ± 0.01 mol/mol glycerol), respectively. The ethanol yield of recombinant E. coli with hycE (0.50 ± 0.02 mol/mol glycerol) was approximately 30% lower than that of wild-type SS1, whereas the ethanol yield of recombinant E. coli with hydA (0.68 ± 0.09 mol/mol glycerol) was comparable to that of wild-type SS1. Conclusions: Insertion of either hycE or hydA can improve the hydrogen yield with an initial pH of 5.8. The recombinant E. coli with hydA could retain ethanol yield despite high hydrogen production, suggesting that clostridial hydAhas an advantage over the hycE gene in hydrogen and ethanol coproduction under acidic conditions. This study could serve as a useful guidance for the future development of an effective strain coproducing hydrogen and ethanol.



**Keyword:** Clostridium; Ethanol production; Fermentation; Glycerol; hycE; hydA; Hydrogenase gene; Hydrogenase; Low pH; Microbial biotechnology; Recombinant clostridial hydrogenase