

Constitutive expression of CO-dehydrogenase and CO-Hydrogenase in *Citrobacter amalonaticus* Y19

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Citrobacter amalonaticus Y19 has a great potential as a biocatalyst for production of hydrogen (H₂) from oxidation of carbon monoxide (CO), via so-called the water gas shift reaction (WGSR). It has a high H₂ production activity and can grow heterotrophically on various sugars. However, transcription of the gene clusters for CO-dependent H₂ production, involving CO-dehydrogenase (CODH), CO-dependent hydrogenase (CO-*hyd*) and their maturation factors (CO-*hyp*), strictly required the presence of CO to activate the transcriptional regulatory protein CooA and glucose-deficient condition to avoid carbon catabolite repression (CCR). To express CO-dependent H₂ production activity constitutively while growing Y19 on glucose, the native CO-inducible promoters were replaced by strong and engineered constitutive promoters. All promoter-replaced strains (Y19-PRs) showed CO-dependent H₂ production activity while growing on glucose in CO-free condition. The best engineered strain Y19-PR1 showed a similar CO-dependent H₂ production activity as wild type Y19. Compared to wild type Y19, transcription of the CODH gene cluster in Y19-PR1 increased by 1.5-fold but that of the CO-*hyd* operon genes remained at a similar level.