Isolation of [FeFe]-hydrogenase from *Clostridium tyrobutyricum* and the construction of recombinant plasmid

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A 4.3-kbp DNA region of *Clostridium tyrobutyricum* ATCC 25755 containing the putative hydrogenase gene (hydA) was cloned and sequenced. The 1,596-bp ORF (bp 1,139 to 2,734) as a putative hydA encodes 531 amino acids with a predicted molecular mass of 59,028-Da and it presents 82% and 75% identity with the [FeFe]-hydrogenase genes of *Clostridium kluyveri* DSM 555 and *C. acetobutylicum* ATCC 824, respectively. Transcription promoters and termination sequences of the hydA gene clusters were analyzed by using web-based programs. A putative ribosome binding site (AGGAGT) and -35 and -10 promoter regions (TTGATA and TACTATTAT) are located upstream of the hydA ATG start codon. The position of a putative rho-independent transcription terminator is downstream of the termination codon (TAA) of the hydA gene. A 11-bp inverted-repeat sequence is capable of forming a stable stem-loop structure. The recombinant plasmid containing [FeFe]-hydrogenase gene was successfully constructed for further research to improve hydrogen productivity.