

A thermophilic cell-free cascade enzymatic reaction for acetoin synthesis from pyruvate

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Table

Strains, plasmids and primers	Properties and sequences	Sources
Strains		
<i>Caldicellulosiruptor owensensis</i> OL	DSM 13100, wild type	DSMZ
<i>Bacillus subtilis</i> IPE5-4	Wild type	Lab stock
<i>Escherichia coli</i> Top10	Cloning host	TianGen
<i>E. coli</i> BL21(DE3)	Expression host	Novagen
Plasmids		
pET28b	Overexpression vector; Kan ^r	Novagen
pET28b-coAHASL1	pET28b with <i>coilyB</i> gene of <i>C. owensensis</i>	This study
pET28b-coAHASS	pET28b with <i>coilyN</i> gene of <i>C. owensensis</i>	This study
pET28b-coAHASL2	pET28b with <i>coilyI</i> gene of <i>C. owensensis</i>	This study
pET28b-bsALDC	pET28b with <i>bsalsD</i> gene of <i>B. subtilis</i> IPE5-4	This study
Primers (5'-3')		
<i>coilyB</i> -F	GACGACGACAAGatgaaggtgaagatgacggtagcac	This study
<i>coilyB</i> -R	GAGGAGAAGCCCGGttaaggtatagttggctcatcag	This study
<i>coilyN</i> -F	GACGACGACAAGatgaaatatacactttcagtttgggtg	This study
<i>coilyN</i> -R	GAGGAGAAGCCCGGttactcatcctcctcagacttag	This study
<i>coilyI</i> -F	GACGACGACAAGatgaaattaacaggagctgaaattataatc	This study
<i>coilyI</i> -R	GAGGAGAAGCCCGGttaatcaataatctcatcaattggcg	This study
<i>bsalsD</i> -F	GACGACGACAAGatgaaacgmgaagcaayattcaagtgct	This study
<i>bsalsD</i> -R	GAGGAGAAGCCCGGttatcmgggcttccttcrgttgttc	This study

Table S1. Strains, plasmids, and primers used in this study.

Figure Legends

Figure S1. Genomic organization and phylogenetic tree analysis of ALS and

ALDC. (A) Genomic organization of the various *ilv* genes encoding ALS in *C. owensensis*. (B) Phylogenetic tree of *C. owensensis* ALS. (C) Phylogenetic tree of *B. subtilis* IPE5-4 ALDC. Abbreviations: AHASL, catalytic subunit of acetohydroxyacid synthase; AHASS, regulatory subunit of acetohydroxyacid synthase; KARI, ketol-acid reductoisomerase (EC 1.1.1.86); IPMS, 2-isopropylmalate synthase (EC 2.3.3.13); DHAD, dihydroxyaciddehydratase (EC 4.2.1.9). The trees were constructed using MEGA 5.05 with 1000 bootstrap values, and GenBank accession numbers of each protein were listed after scientific names.

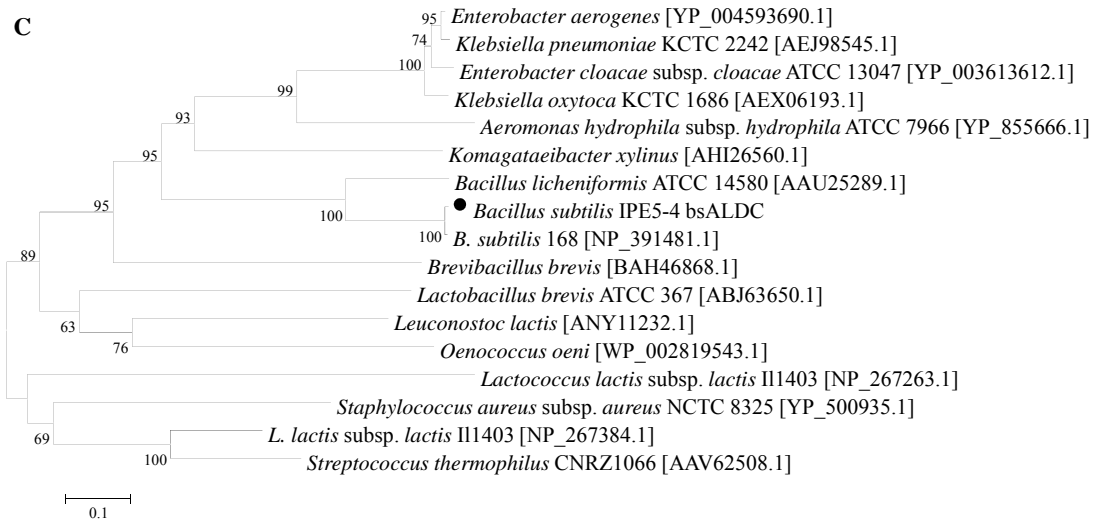
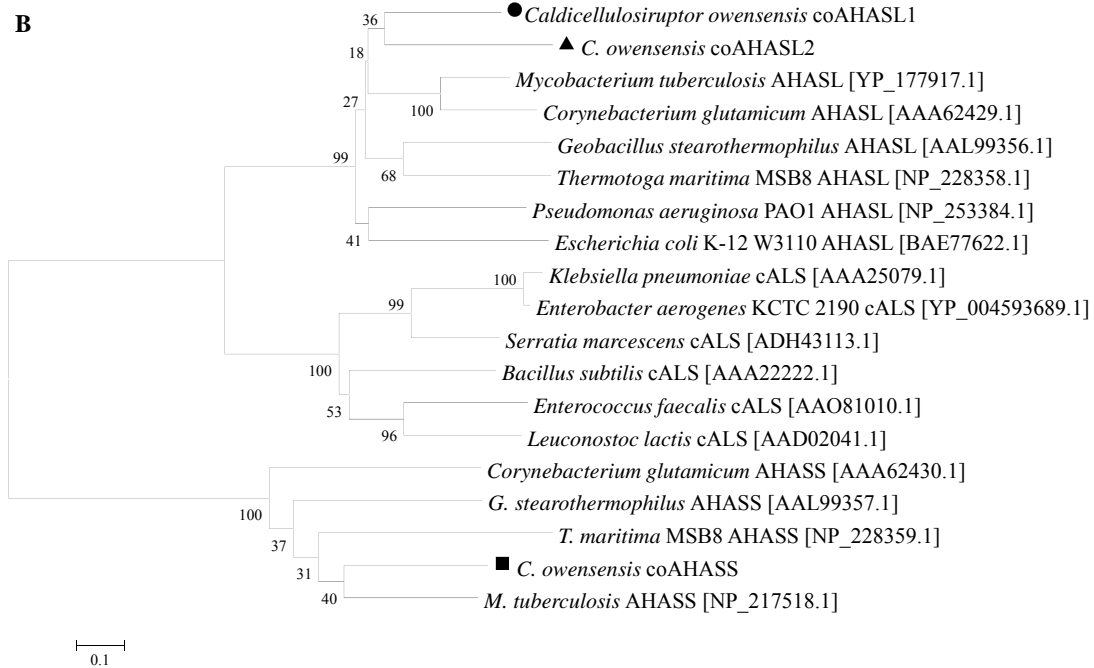
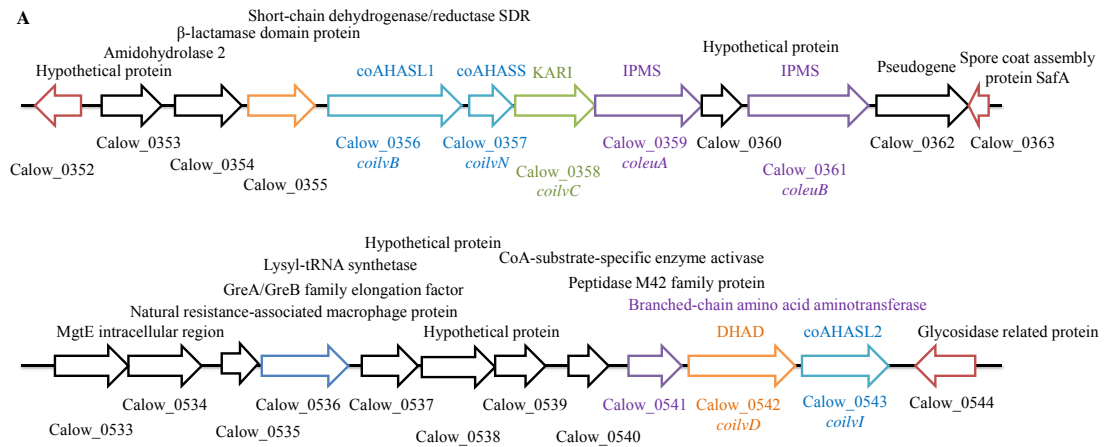


Figure S1