Characterization of acetohydroxyacid synthase from the hyperthermophilic bacterium *Thermotoga maritima*

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Running title: Acetohydroxyacid synthase in *Thermotogales*
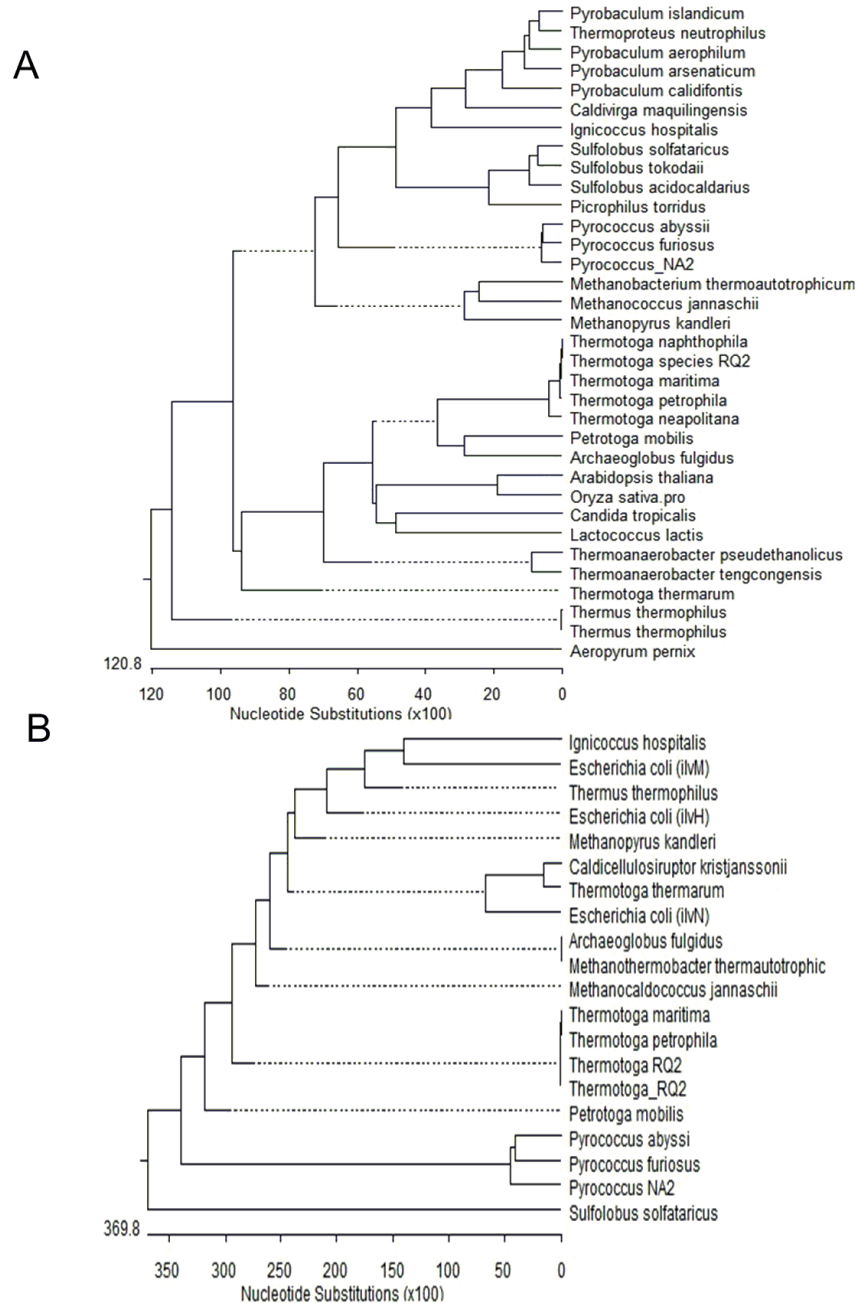
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Contents:

Supplementary Figure S1 Construction of expression plasmids

Plasmids constructs for **(A)** *ilvN*, the putative regulatory subunit (pETTm0549), **(B)** *ilvB*, the putative catalytic subunit (pETTm0549), and **(C)** *ilvB-ilvN*, the coupled catalytic and regulatory subunits (pETTm0548/9) of acetohydroxyacid synthase from *T. maritima*. For each clone, the coding sequences and their transcription direction is indicated with grey arrows; in all cases the amplified coding sequences were digested with NdeI–XhoI and inserted into the pET30a(+) vector; the plasmid pETTm0548/9 was constructed by insertion of the consecutive genes encoding both catalytic and regulatory subunits (*ilvB-ilvN*) in their natural order. The plasmid maps were generated using the SeqBuilder software (Lasergene, DNASTAR, Madison, USA).

Supplementary Figure S2 Phylogenic relatedness of AHAS subunits

The phylogenic relatedness was determined for **(A)** catlytic and **(B)** regultory subunits of AHAS. Genome sequences in the NCBI microbial genome database (http://www.ncbi.nlm.nih.gov/genomes/ MICROBES/microbial_taxtree .html) were used to retrieve the amino acid and nucleotide sequences of AHASs from various thermophilic and mesophilic organisms. The amino acid sequence homology and the phylogenetic tree were prepared based on the ClustalW analysis using MegAlign software (Lasergene, DNAStar, Madison, USA).