Supplementary Figures

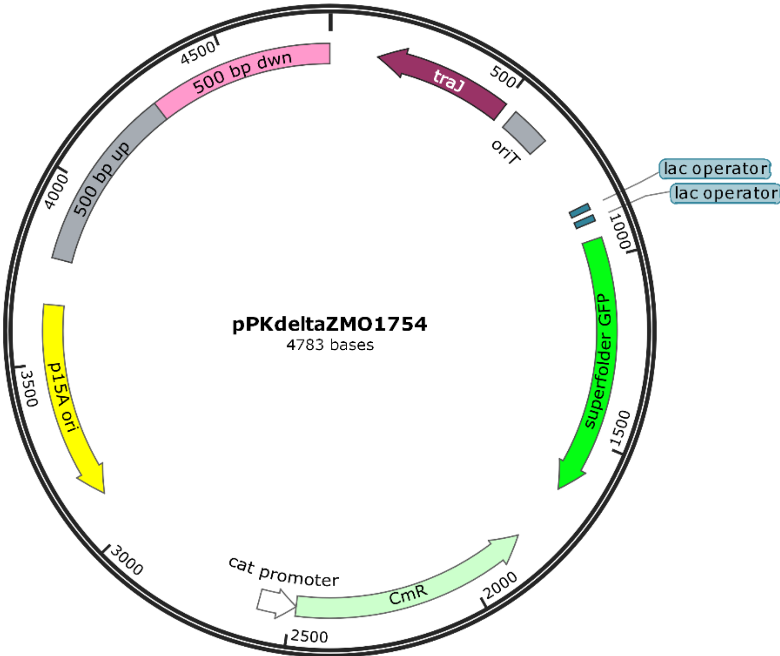


Figure S1. pPKΔZMO1754 map. 500 bp fragments directly upstream and downstream of ZMO1754 were amplified from ZM4 chromosome and Gibson assembly into pPK15534 digested with SpeI as described in Materials and Methods.

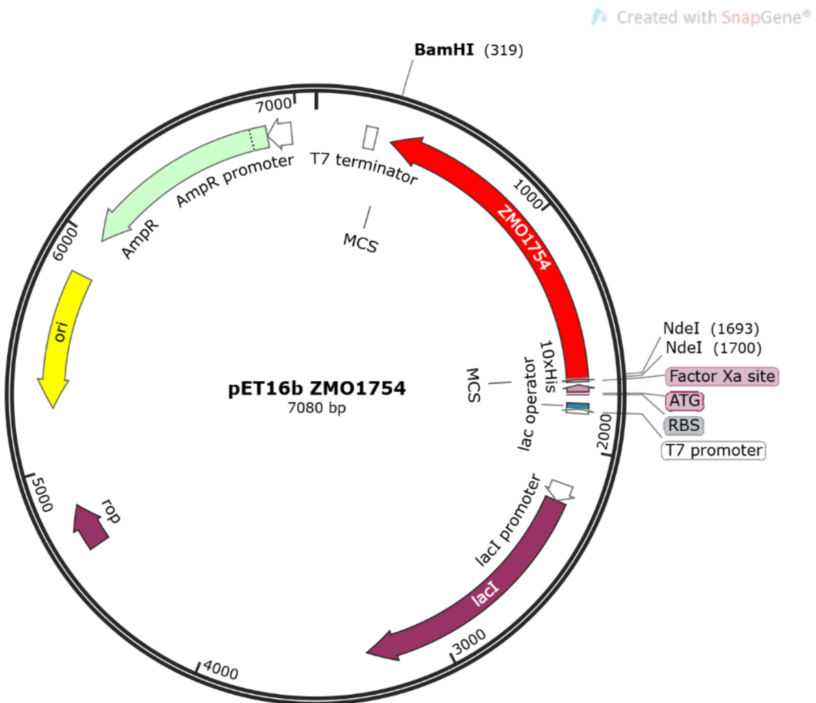


Figure S2. pET16b*aldB* map. The *aldB* (ZMO1754) gene was amplified from ZM4 and cloned to pET16b between NdeI and BamHI sites using Gibson Assembly as described in Materials and Methods. AldB protein was fused with 10 histidine residues at N-terminus and placed under T7 promoter of pET16b. Primers are listed in Table 2.

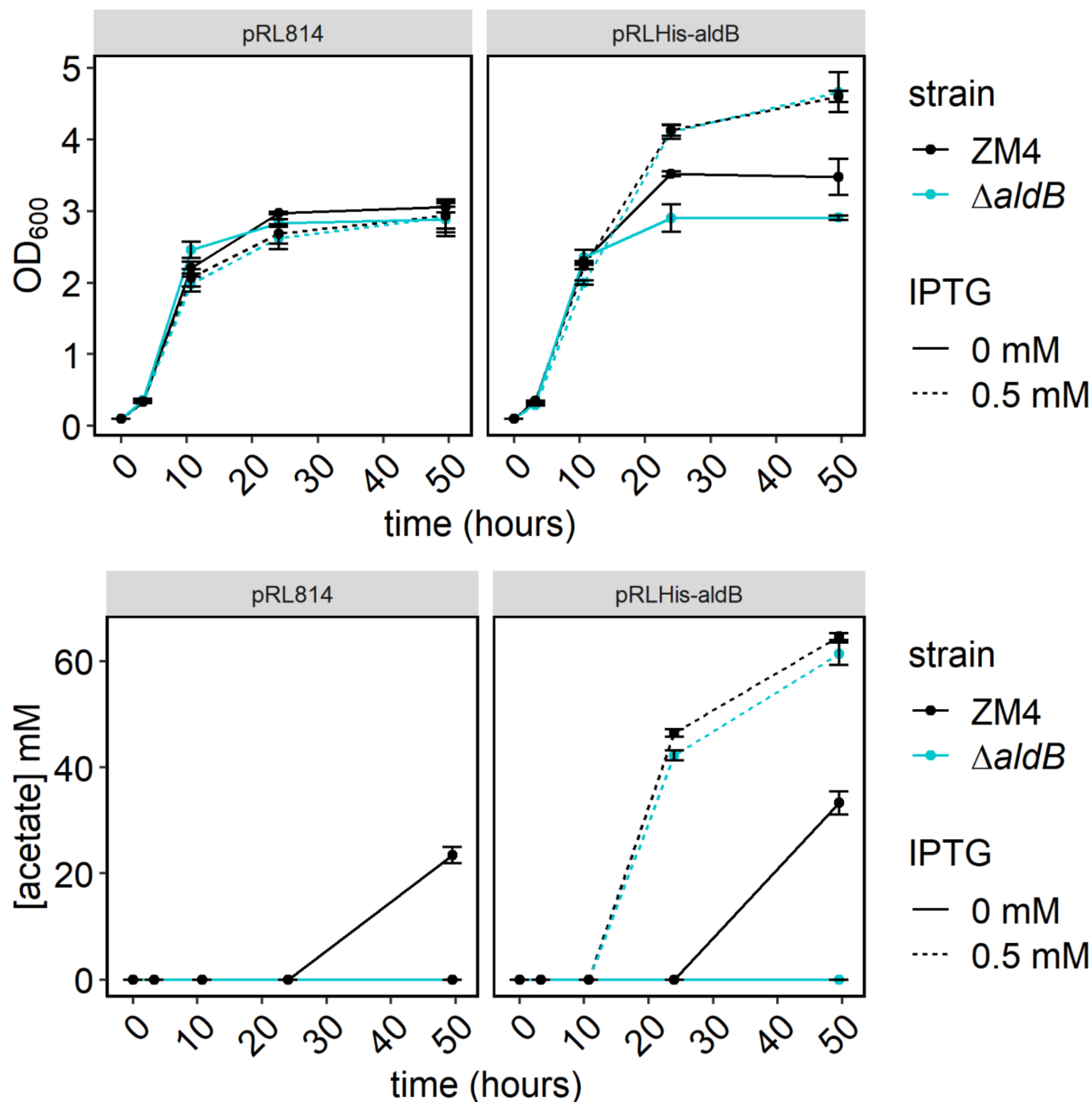


Figure S3. Growth and acetate production by *Z. mobilis* ZM4 WT (black) and Δ*aldB* (blue) bearing plasmid pRLHis-*aldB* with (dashed) and without (solid) IPTG induction. Strains bearing pRLHis-*aldB* or pRL814, were grown as in Figure 2 but media were supplemented with 100 g/ml of spectinomycin and IPTG was added to 0.5 mM, at time of dilution, when indicated. HPLC analysis was performed as in Figure 3 Points represent the average of three biological replicates and error bars represent standard error.

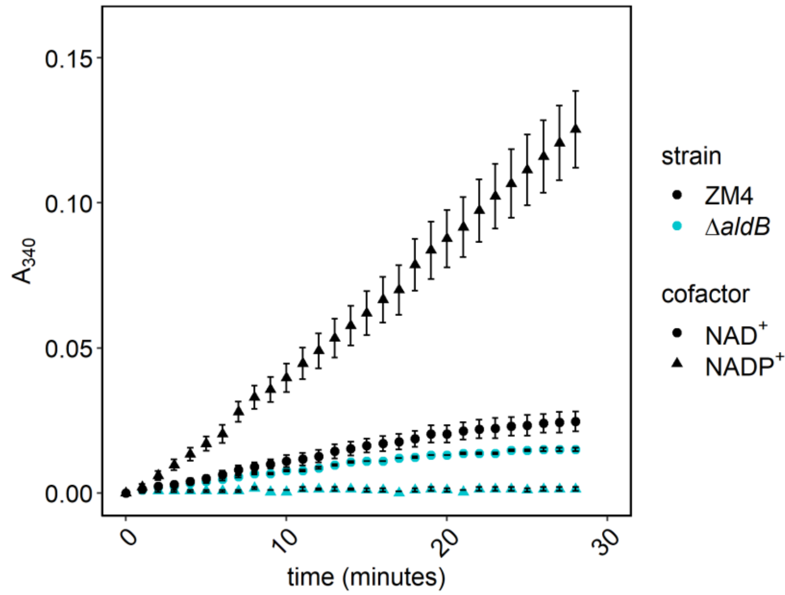


Figure S4. Acetaldehyde dehydrogenase activity in soluble protein fractions from ZM4 WT and Δ*aldB*. Soluble protein fraction (FI) was obtained as described in "Materials and Methods". Average, total protein concentration in FI was 5.7 +/- 0.5 mg/ml and 5.2 +/- 0.7 mg/ml for ZM4 and Δ*aldB*, respectively. Each enzymatic reaction contained: 0.1 M Tris HCl pH 8.0, 0.1 M KCl, 10 mM β-mercaptoethanol, 2 mM acetaldehyde and 0.67 mM $NAD^+$ or $NADP^+$ (protocol for yeast acetaldehyde dehydrogenase from Sigma-Aldrich). Reaction was started by adding 33 □l of FI and measured for 30 minutes at 25°C in 24-well microtiter plate. Absorbance at 340 nm in control without FI was subtracted from the reactions. Points represent the average of three independent experiments with three technical repeats and error bars represent standard error.

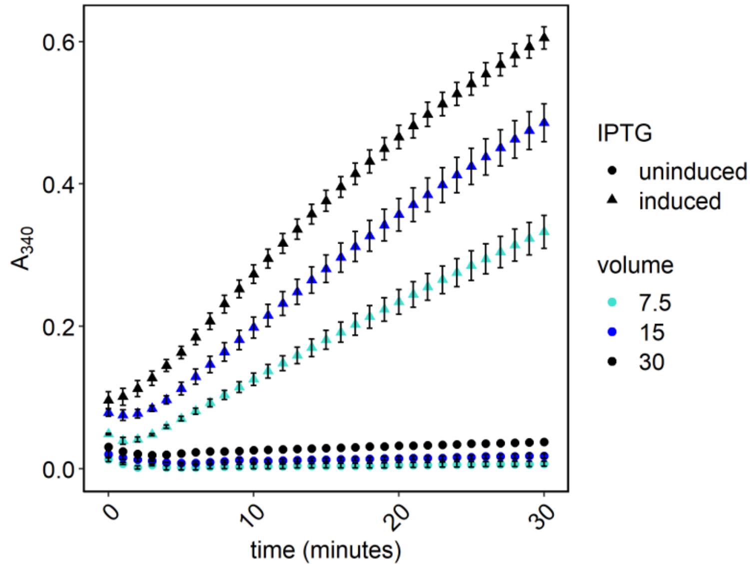


Figure S5. Acetaldehyde dehydrogenase activity in soluble protein fraction from *E. coli* BL21 carrying a plasmid expressing *aldB*. 50-mL cultures were grown in LB supplemented with ampicillin at 30ºC to $OD_{600}$ 0.4. At this point, IPTG was added to a final concentration of 0.1 mM, where indicated, and growth continued for 1.5 hours. FI was obtained as described in "Materials and Methods" for *Z. mobilis*. Average total protein concentration in three independent FI was 11±1.2 mg/ml and 8.4±1.1 mg/ml for uninduced and induced cultures, respectively. An enzymatic assay was performed as described in Figure S4, with NADP as a cofactor. The reaction was started by adding variable volumes of FI as indicated. Points represent the average of three independent FI and error bars represent standard error.

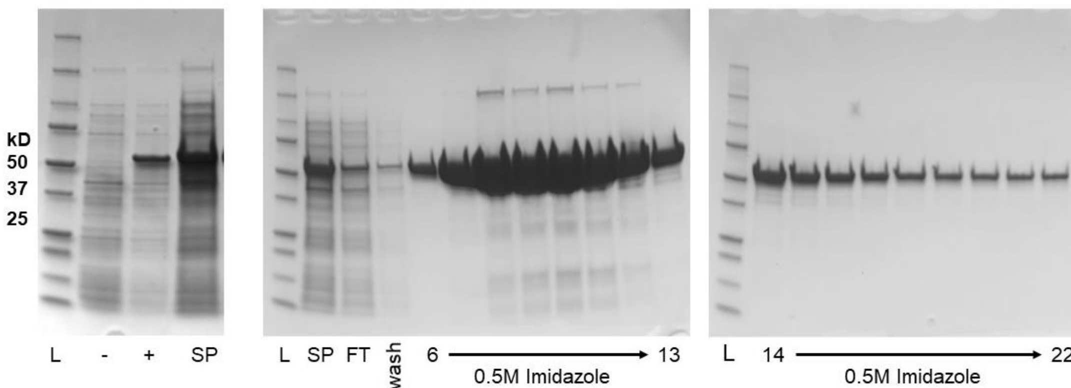


Figure S6. Ni-affinity purification of His-AldB. Protein was overexpressed in *E. coli* BL21(DE3) from pET16b*aldB* induced with IPTG as described in Materials and Methods. Soluble protein fraction was loaded on Ni-affinity column and the protein was eluted with IMAC C buffer containing 0.5 M imidazole. Protein on different stages of purification is visualized on Comassie stained SDS-PAGE as described in Materials and Methods. L, All Blue Protein Ladder; -/+ indicates induction with IPTG; SP, soluble protein fraction; FT, flow through; wash, IMAC B (20 mM imidazole buffer); 6-22, IMAC C fractions.

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CLUSTAL multiple sequence alignment by MUSCLE (3.8)
pomaceae        MAYESVNPATGETVKKYPDLSDAQVKEAIDRTFDVFQKDWGKRSIEDRSKILHKAAEIFR
francensis      MAYESVNPATGETVKKYPSFSDAQVKEAVDRAATVFKNDWSQRTIAERSKVLHKAADIFR
Z6              MAYESVNPATGEIVKKYPDFSDKQVKESVDRAATVFKNDWSQRTIAERSKVLHKAAEIFR
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B23394          EFFGPIAQIYAVKDEAEAIELANDSPYGLGGAVFAPDVEQGRKVAEQIETGMVAINKPLW
B4492           EFFGPIAQIYAVKDEAEAIELANDSPYGLGGAVFAPDVEQGRKVAEQIETGMVAINKPLW
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B1960           EFFGPIAQIYAVKDEAEAIELANDSPYGLGGAVFAPDVEQGRKVAEQIETGMVAINKPLW
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CU1             EFFGPIAQIYAVKDEAEAIELANDSPYGLGGAVFAPDVEQGRKVAEQIETGMVAINKPLW
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francensis      TAPELPFGGVKHSGYGRELSHFGIQEFINWKLIDGSAA-
Z6              TAPELPFGGVKHSGYGRELSHFGIQEFINWKLIDASAA-
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Figure S7. *Z. mobilis* AldB (ZMO1754) multiple sequence alignment. Alignment of AldB from different laboratory strains of *Zymomonas mobilis* was performed by MUSCLE. Origin and characteristics of different laboratory strains of *Z. mobilis* are described in (7).