

Bioscience, Biotechnology, and Biochemistry

Plasmid copy number mutation in *repA* gene encoding RepA replication initiation of cryptic plasmid pHM1519 in *Corynebacterium glutamicum*

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Supplementary materials

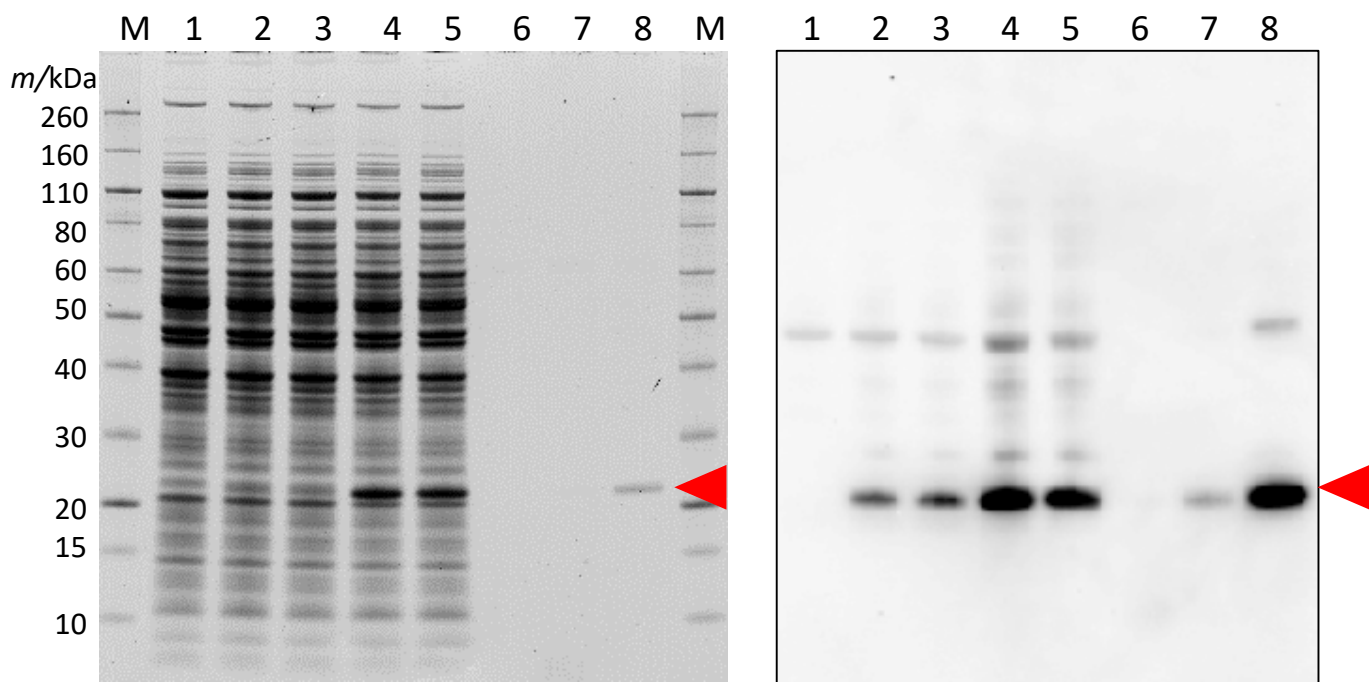


Fig. S1. SDS-PAGE analysis (A) and immunoblotting (B) of chloramphenicol acetyl transferase (CATase) produced from plasmids. For immunoblotting, rabbit anti-CATase antiserum was used. Cell-free extract of *Corynebacterium glutamicum* 2256L and purified CATase (16 mg/mL; Sigma-Aldrich) were employed as standards. Lanes: M, protein standard maker; 1, *C. glutamicum* 2256L (control); 2 and 3, strain 2256L harboring pPC4; 4 and 5, 2256L harboring pPC4H1; 6, 7 and 8, samples (4 μ L/lane) of 10⁻³, 10⁻², and 10⁻¹ dilutions of CATase, respectively. The position of CATase is indicated with a red arrow.

WT (Gly)	Conf: 9999999998689985422347889998764 Pred: HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH AA: RAQREKLAKSSQRQARKAKGNRLTIAGWFMT 410 420 430 440
Glu	Conf: 9999999998799997655556879999875 Pred: HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH AA: RAQREKLAKSSQRQARKAKENRLTIAGWFMT 410 420 430 440
Ala	Conf: 9999999999789998755559999999876 Pred: HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH AA: RAQREKLAKSSQRQARKAKANRLTIAGWFMT 410 420 430 440
Lys	Conf: 9999999998799998754445779999875 Pred: HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH AA: RAQREKLAKSSQRQARKAKKNRLTIAGWFMT 410 420 430 440
Asp	Conf: 9999999998799986532334779999875 Pred: HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH AA: RAQREKLAKSSQRQARKAKDNRLTIAGWFMT 410 420 430 440
Pro	Conf: 9999999998789873113036889999976 Pred: HHHHHHHHHHHHHHHHHHCCHHHHHHHHHHH AA: RAQREKLAKSSQRQARKAKPNRLTIAGWFMT 410 420 430 440
Gln	Conf: 9999999999799998654458999999976 Pred: HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH AA: RAQREKLAKSSQRQARKAKQNRRLTIAGWFMT 410 420 430 440

Fig. S2. Predicted secondary structure of the C-terminal region (from amino acids 410 to 440) of RepA and its mutants. The secondary structure was predicted using PSIPRED. The amino acid numbers of RepA are indicated below the sequence (AA). “H” stands for helix and “C” for coil. “Conf” represents the confidence of each prediction. The Gly (G) residue at position 429 of wild-type RepA and the confidence value for the prediction of its structure are indicated in blue. Each substituted residue and its confidence value is in red. A coil structure (highlighted in yellow) was only predicted on replacement of Gly429 to Pro.