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Reprogramming *Escherichia coli* toward versatile and low-carbon footprint chemical synthesis

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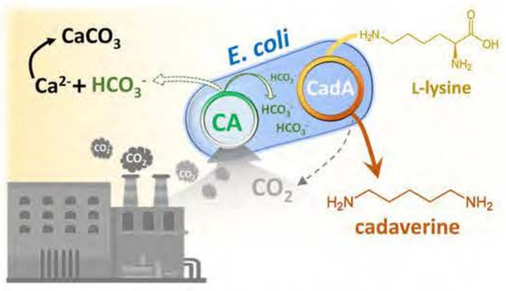

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The enzymatic capture and sequestration of CO_2 using carbonic anhydrase (CA) have been extensively studied as alternative solutions for CO_2 mitigation. Since CA is the main enzyme to catalyze the reversible hydration of CO_2 to bicarbonate. Among major evolutionary CA classes, human carbonic anhydrase (hCAII) is one of the α-CAs that exists in red blood cells and possesses high solubility and activity. Nonetheless, recombinant hCAII has poor thermostability and reduces biocatalyst efficiency. Research strategy: Dual promoters of heat shock and housekeeping proteins were employed to stimulate hCAII activity in recombinant *Escherichia coli* under heat environments.

Functional application: The effectiveness of recombinant hCAII as a biocatalyst is investigated for utilizing CO_2 release during cadaverine (DAP) synthesis *in vivo* and mineralizing CO_2-captured *in vitro*.

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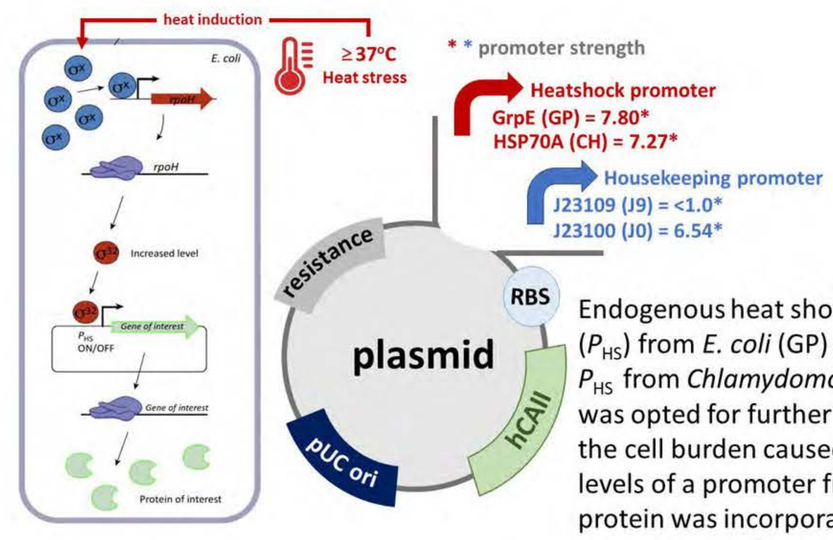
1.Genetic design of heat tolerance chassis

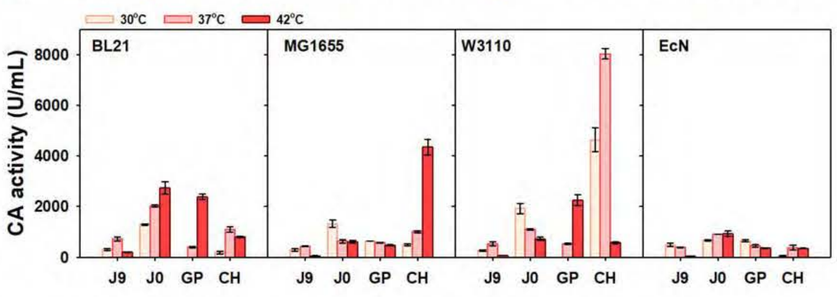
To suppress the production cost from the inducer usage (IPTG) and stimulate hCAII expression constitutively, a robust recombinant hCAII strain was genetically designed under heat induction.

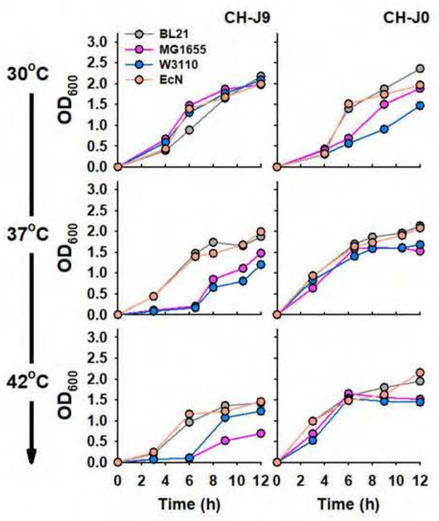


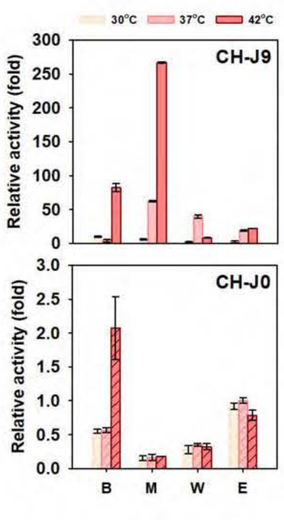
Endogenous heat shock promoter (P_{HS}) from *E. coli* (GP) and heterologous P_{HS} from *Chlamydomonas reinhardtii* (CH) was opted for further screening. Considering the cell burden caused by P_{HS}, two different levels of a promoter from *E. coli* housekeeping protein was incorporated as a dual-promoter system.

2.Evaluation of single and dual promoter efficiency in *E. coli* strains

The efficiency of a single promoter was functionally tested in four different *E. coli* strains at 30, 37, and 42°C. In short, CH promoter possessed higher heat sensitivity than GP promoter by having notable CA activity in MG1655 and W3110 strains.





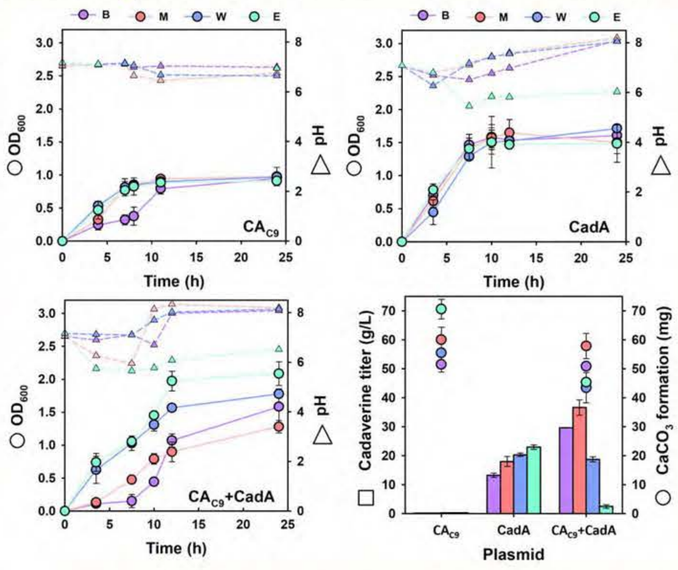


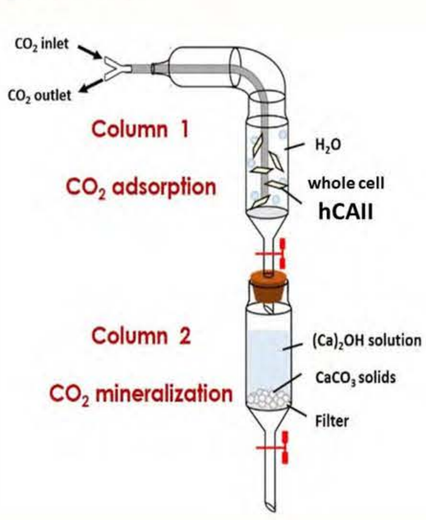
CH promoter was further coupled with J9 or J0, generating strong-weak or strong-strong promoter combinations, respectively.

Coupling J9 could not rescue the cell burden from CH promoter at higher temperatures and had retardation in the lag phase. Despite this, a combination of CH-J9 boosted CA activity of recombinant BL21 and MG1655 strains at 42°C by more than 50x, suggesting an effective and robust biocatalyst design.

3.Concurrent CO_2 utilization and cadaverine biosynthesis

B: BL21
M: MG1655
W: W3110
E: EcN

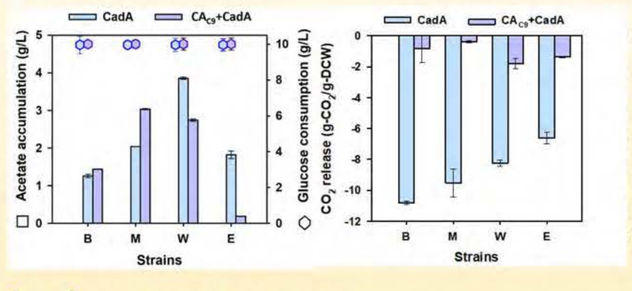




Of all *E. coli* strains, co-expression of CH-J9-hCAII (CA_{C9}) with lysine decarboxylase (CadA) in MG1655 strain showed a significant increment in cadaverine titer from 18.0 to 36.7 g/L. On the other hand, the remained cells of MG1655 harboring CA_{C9}+CadA from cadaverine (DAP) synthesis sustained for converting CO_2 through a two-column system and precipitating 57.9 mg-$CaCO_3$.

4.CO_2 sequestration of recombinant *E. coli* strains

$$CO_2\ release\ (gCO_2/gDCW) = ([CO_2]_{out} - [CO_2]_{in}) \times \frac{1}{DCW}$$
$$= \{([C_{biomass}] + [C_{metabolites}]_{out}) - [C_{metabolites}]_{in}\} \times \frac{1}{DCW}$$



Considering carbon input and carbon output, the CO_2 release of strains harboring CA_{C9} gene was strikingly lowered.
BL21 and MG1655 → DAP
W3110 and EcN → biomass

Take-home messages:

A novel of engineered dual-promoter systems to develop low-cost and highly active hCAII biocatalysts was explored for the first time. This genetic design successfully eliminated the inducer usage and augmented hCAII durability at high temperatures. Moreover, utilizing recombinant hCAII offered a win-win strategy for sequestering CO_2 release and improving cadaverine titer simultaneously during *in vivo* production, promoting a low carbon footprint in microbial cell factories.

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