

## **Engineering carbonic anhydrase for enhanced stability and useful in the production of biofuel**

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Carbonic anhydrase (CA) is an enzyme of great interest because of its potential industrial and clinical application. Recently, a new concept developed to capture CO<sub>2</sub> from the atmosphere for biofuel production, which involves the use of immobilized CA that catalyses the reversible hydration of CO<sub>2</sub> to HCO<sub>3</sub><sup>-</sup>. Due to enormous industrial and clinical application, engineered CA produced which is highly stable, which provides long-term stability. This engineered CA worked efficiently well at high temperature without losing their activity significantly. This captured CO<sub>2</sub> through engineered CA could be used as a potential carbon source to produce lipids for the generation of biofuel. We developed six engineered CAs and measured their activity, stability, and kinetics. The engineered CAs are, (i) CA with 6 N-terminal histidine (His), (ii) CA with 6 C-terminal His, (iii) CA with 6 N-terminal and 6 C-terminal His, (iv) CA with N-terminal Cellulose binding domain (CBD), (v) CA with C-terminal CBD and (vi) CA with N and C-terminal CBD along with wild-type CA. Cost-efficient production of stable enzyme system is critical for the development of the economically feasible CA-based application. All these variants and wild-type enzymes studied for their structure and confirmation by using UV-absorption, circular dichroism, and fluorescence spectroscopy. We found that variants showing good activity and relatively more stability at a higher temperature. These engineered CAs will be used in the production of biofuel.

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