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Directed evolution of carbonic anhydrase to improve microbial induced calcium carbonate precipitation (MICP) efficiency

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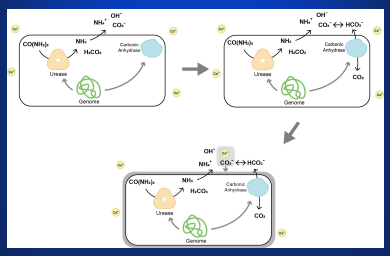
Background

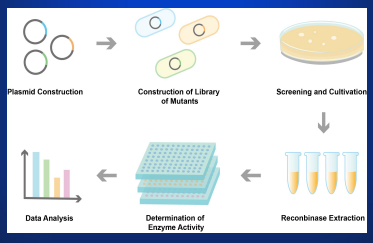
- Microbial induced calcium carbonate precipitation (MICP) is a natural phenomenon that microorganisms form calcium carbonate deposits in their surrounding microenvironment through metabolism. Most microorganisms in nature can generate cementation substances through their own metabolic activities, causing cementation between particles, thus improving the strength of soil and reducing its water permeability. Microbial cement has the characteristics of environmental protection, economy and efficiency, and has become a research hotspot in the fields of biology, civil engineering and environment.
- There are two key enzymes for biomineralization, namely urease and carbonic anhydrase. Urease hydrolyzes urea to increase cell pH and carbonate concentration, forming alkaline conditions for calcium carbonate deposition.
- MICP research has been focused on screening strains with higher urease activity, in order to quickly produce CO_3^{2-} by accelerating the decomposition of urea, but the activity of carbonic anhydrase of the strain has been ignored.



Our Project

According to previous preparation, we express carbonic anhydrase in Escherichia coli and modify it by error-prone PCR, which is a widely used directed evolution technique. We obtain several mutants and measure their enzyme activity successfully.

The structure of these mutants was predicted and analysed by means of bioinformatics. And we constructed phylogenetic lgenic trees of different species including target species of carbonic anhydrase.



Design & Results

Bioinformatics analysis of mASCA

- The gene sequence analysis(*Fig.1*) and 3D structure prediction(*Fig.2*) of mASCA was conducted with Phyer2 protein structure server. After finding the populations with high sequence similarity using Blastp server in NCBI, the sequences were aligned and the tree was built using the neighbor joining method in software Mega(*Fig.3*).

Fig. 1

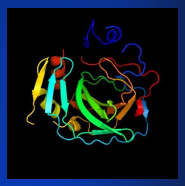


Fig. 2

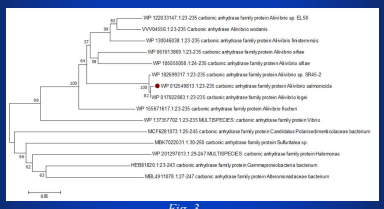


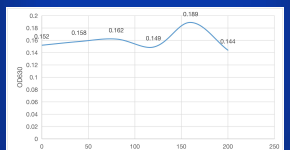
Fig. 3

Evolutionary relationships of carbonic anhydrase of Aliivibrio salmonicida and other relevant species.

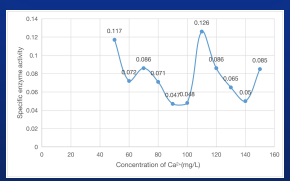
- The carbonic anhydrase of the target species *Aliivibrio salmonicida* has the closest evolutionary relationship with the protein corresponding to *Aliivibrio fischeri*, and the furthest evolutionary relationship with *Gammaproteobacterial bacterium*. It is in a relatively late evolutionary position, and the gene separation is late from other proteins.

Analysis of physical and chemical properties of mASCA

- We mainly analyzed the effect of Ca^{2+} concentration on mASCA expression and found the optimum Ca^{2+} concentration. In conclusion, 110mg/L Ca^{2+} concentration should be selected as the value of the subsequent evolution and expression process as the most suitable choice.



Effect of Ca^{2+} concentration on bacterial growth

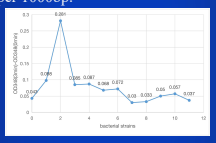


Effect of Ca^{2+} concentration on specific enzyme activity of CA

Design & Results

Analysis of error-prone PCR results

- We used the optimum condition of error-prone PCR with the number of cycles is 35 and the expected mutation value is 6 per 1000bp.



Values of the enzyme expressed by the mutant bacterial strains and by the original bacterial strain.

- Then we constructed the plasmids that contained error-prone PCR products and successfully transformed into *E. coli* BL21. At last, We determined the activity of recombinant enzymes obtained from 10 mutant bacterial strains and the original bacterial strain. No.0 is the original bacterial strain while the No.1 to No.11 are the mutant bacterial strains.

Discussion

- In this experiment, the carbonic anhydrase gene was successfully expressed and mutated. After mutating, the enzyme activity of Ep2 ncreased greatly, indicating that this method was effective in the evolution of carbonic anhydrase.
- At present, many researchers have used genetic engineering methods to heterologous express urease from different species, and previous studies have shown that urease is also feasible. Not only that, we aslo mutated the carbonic anhydrase gene in *Escherichia coli* based on the error-prone PCR technology, and screened the mutant strains from the established carbonic anhydrase mutation library.
- Urease and carbonic anhydrase have synergistic effects in MICP, and both of them can produce mutations under error-prone PCR, which has a certain possibility of co-evolution.
- In the future, we will sequence the mutants in order to identify the mutation sites and introduce mutated carbonic anhydrase gene and non-mutated carbonic anhydrase gene into *Escherichia coli*, and even use EvolvR system to conduct multi-window directed evolution of the urease gene, so that the evolved urease and carbonic anhydrase can cooperate in MICP. It may improve the relatively low urease activity in *E. coli* and improve the efficiency of bacterial mineralization, which is more efficient and suitable for laboratory research or ecological engineering, civil engineering and other fields.

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Reference:

[1] WANG L, WANG XX, LI F, CUI MJ, YANG XX, YANG M, YAN YJ. Advances of enzymes related to microbial cement. Chin J. Biotech, 2022, 38(2): 506-517. DOI: https://doi.org/10.13345/j.cjb.210127.
[2] ACHAL V, PAN X.Characterization of Urease and Carbonic Anhydrase Producing Bacteria and Their Role in Calcite Precipitation[J/OL]. Current Microbiology, 2011, 62(3):894-902. DOI: https://doi.org/10.1007/s00284-010-9801-4.
[3]JUN S Y, KIM S H, KANTH BK, et al. Expression and characterization of a codon-optimized alkaline-stablecarbonic anhydrase from *Aliivibrio salmonicida* for CO_2 sequestration applications[J/OL].Bioprocess and Biosystems Engineering, 2017, 40(3): 413-421. DOI: https://doi.org/10.1007/s00449-016-1709-3.
[4] WU H, TIAN X, DONG Z, et al. Engineering of *Bacillus amyloliquefaciens* α-amylase with improved calcium independence and catalytic efficiency by error-prone PCR[J]. Starch-Stärke，2018，70（3−4）：1700175. DOI: https://doi.org/10.1002/star.201700175
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