Structural and functional analyses of bacterial organic acid exporters for the efficient fermentative production of organic acids

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Research aims

To date, organic acids, the raw materials used to produce many polymers, are mainly derived from fossil fuels. However, with the popularization of green biotechnology, many studies worldwide are seeking to produce organic acids through microbial fermentation. However, harvesting biologically produced organic acids is hindered by their strong polarity, which prevents them from crossing the cell membrane. Therefore, membrane transporters are necessary to mediate organic acid efflux. In this study, we investigated the organic acid export activity of bacterial membrane transporters, focusing on the following three subjects.

Subject 1: Expression and purification of aspartate:alanine exchanger (AAEx) transporters

Methods: In order to crystallize AAEx membrane transporters, we first needed to identify proteins having a good *in vitro* stability. To this end, we cloned the genes encoding bacterial homologs of AAEx transporters and constructed expression vectors. Next, we optimized the conditions for expressing and purifying these AAEx homologs.

Results: We have successfully expressed and purified AAEx transporters and are now optimizing the buffer conditions required to improve the purification step and crystallize AAEx transporters.

Subject 2: Establishment of *in vivo* methods for the functional analysis of AAEx transporters

Methods: To study the substrate transport mechanism of AAEx transporters, we selected a representative member of the AAEx transporter family, the aspartate:alanine antiporter (AspT) from *Tetragenococcus halophilus*[1]. To assess the export function of AspT, we exploited an *Escherichia coli* strain containing a deletion of the L-alanine exporter *ygaW* (MLA301Δ*ygaW*)[2]. This strain is unable to export L-alanine. Upon addition of L-Ala-L-Ala to the medium, the cells accumulate L-alanine, which inhibits cell growth. We hypothesized that the expression of a functional AspT in the MLA301Δ*ygaW E. coli* strain would restore

both the L-alanine export function and cell growth.

Results: First, we grew the *E. coli* MLA301Δ*ygaW* strain in a medium containing L-Ala-L-Ala to optimize the growth conditions, Isopropyl β-D-1-thiogalactopyranoside concentration, and growth time. In cells transformed with an empty vector, we observed growth inhibition. However, as anticipated, the expression of AspT restored L-Ala-L-Ala resistance and promoted cell growth (Fig. 1). We believe that this method will prove useful for analyzing the function of AAEx transporters.

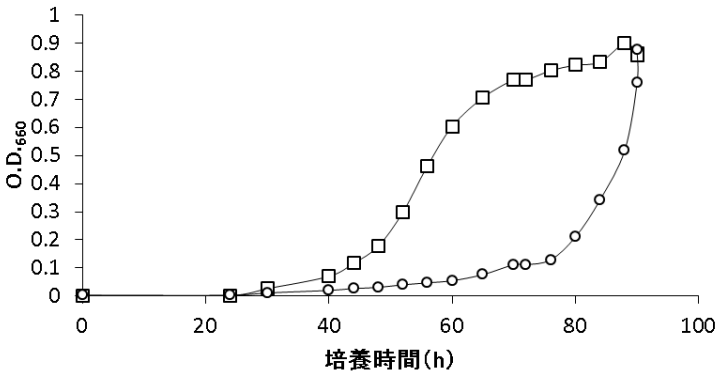

Fig. 1. AspT expression restores L-Ala-L-Ala resistance in L-alanine export-deficient cells
The L-Ala-L-Ala-sensitive MLA301Δ*ygaW E. coli* strain was transformed with either the pTrc_aspT expression vector (□) or the empty pTrc99A vector (○) vector. Cell growth was monitored using medium containing 0.5 mM L-Ala-L-Ala.

Subject 3: Establishment of a method to prepare stable proteoliposomes for *in vitro* functional analyses

Methods: The preparation of stable proteoliposomes is a prerequisite for *in vitro* functional analyses of transporters. Due to a lack of stability of the proteoliposomes, our previous method only allowed us to measure the transport activity for 1 h. We hypothesized that traces of detergents that were used to solubilize the transporters, might have remained in the preparation and destabilized the proteoliposomes. To eliminate these detergent traces, we employed Bio-Beads resin, which has a high affinity for detergents, in our liposome preparation protocol.

Results: Using Bio-Beads resin to optimize our proteoliposomes preparation method, we were able to prepare stable liposomes with a high transport activity. Moreover, the liposomes prepared using this method allowed us to measure the transport activity for more than 3 hours. We believe that this method will help us perform detailed functional analyses of AAEx transporters.

Conclusion

To facilitate the production of organic acids through microbial fermentation further, we aimed to solve the structure and investigate the function of AAEx transporters. We have made significant progress towards achieving these aims by successfully purifying AAEx transporters and establishing functional transport assays.

References

1) Sasahara, A., Nanatani, K., Enomoto, M., Kuwahara, S., and Abe, K. (2011) Substrate specificity of the aspartate:alanine antiporter (AspT) of *Tetragenococcus halophilus* in reconstituted liposomes. *J. Biol. Chem.* **286:** 29044-29052.
2) Hori, H., Yoneyama, H., Tobe, R., Ando, T., Isogai, E., and Katsumata, R. (2011) Inducible L-alanine exporter encoded by the novel gene *ygaW* (*alaE*) in *Escherichia coli*. *Appl. Environ. Microbiol.* **77:** 4027-4034.