Specific bioproduction using thermophilic cells that produce mesophilic enzymes under temperate conditions

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Research objectives

Microorganisms can serve as hosts for the efficient production of beneficial metabolites where the productivity is often enhanced by metabolic engineering by enabling host organisms to activate specific pathways leading to the metabolites. The engineering generally involves disruption and/or forcible expression of genes relevant to the metabolic pathway. The process is challenging, requiring substantial effort and time, and occasionally results in involuntary phenotypes such as inefficient growth of host cells. Here, we designed an engineering approach for more facile bioproduction of specific metabolites. In this approach, mesophilic enzymes are overproduced in thermophiles at moderate temperatures. Since thermophilic enzymes in thermophiles are less active at moderate temperatures, the mesophilic enzymes could dominate in thermophiles to catalyze specific reactions under the conditions. In order to evaluate this approach, the present study was conducted to produce mesophilic enzymes for inositol conversion[1)] in a thermophile (*Geobacillus kaustophilus* HTA426) and to analyze whether the cells can specifically convert inositol at moderate temperatures.

Methods

The *cspX* promoter (P_{cspX}) was previously identified to express at moderate temperatures in *G. kaustophilus*. This promoter was cloned in the pGKE119 vector[2)] to construct pGKE120, which was then used to clone *iolG–iolX* and *iolG–iolW* genes from *Bacillus subtilis* at the P_{cspX} downstream. *Geobacillus kaustophilus* was transformed with the resultant plasmids and cultured at 60℃ in LB medium until the optical density at 600 nm was equal to 1. The cells were additionally incubated at 40℃ for 24 h and then permeabilized by the addition of surfactants and lysozyme to the culture. The culture was incubated at 30℃ for 6 h in the presence of *myo*-inositol (MI; 10 g/L) and NAD^+ (1 mM) and subjected to high performance liquid chromatography for the detection of *scyllo*-inositol (SI), that was converted from MI.

Results

IolG catalyzes NAD^+-dependent MI oxidation to produce *scyllo*-inosose, whereas IolX and IolW reduce *scyllo*-inosose using NADH and NADPH, respectively, as electron donors, to produce SI (Fig. 1A). *Geobacillus kaustophilus* that produced IolG and IolX converted 30% of MI (10 g/L) into SI, following incubation at 30℃ for 6 h. A prolonged incubation did not increase the conversion rates potentially because of the chemical equilibrium between MI and SI. The cells converted 25% of MI even when incubated at 10℃ for 6 h. The cells also converted a higher concentration (60 g/L) of MI at 30℃ for 24 h at a rate of 27%, which indicates that this system can produce 16 g/L SI. The reaction proceeded in the cell culture without washing cells. Cell permeabilization with a higher concentration of lysozyme decreased the conversion rates. A lower concentration (0.1 mM) of NAD^+ decreased the conversion rates but a higher concentration (2 mM) had no effect. *Geobacillus kaustophilus* that produced IolG and IolW also produced SI; however, the reaction required NADPH and the rate was lower compared to the cells that produced IolG and IolX. Unfortunately, the cells could not produce SI without permeabilization even when *iolT* was simultaneously expressed along with *iolG* and *iolX*. This was attributable to inefficient MI uptake at moderate temperatures.

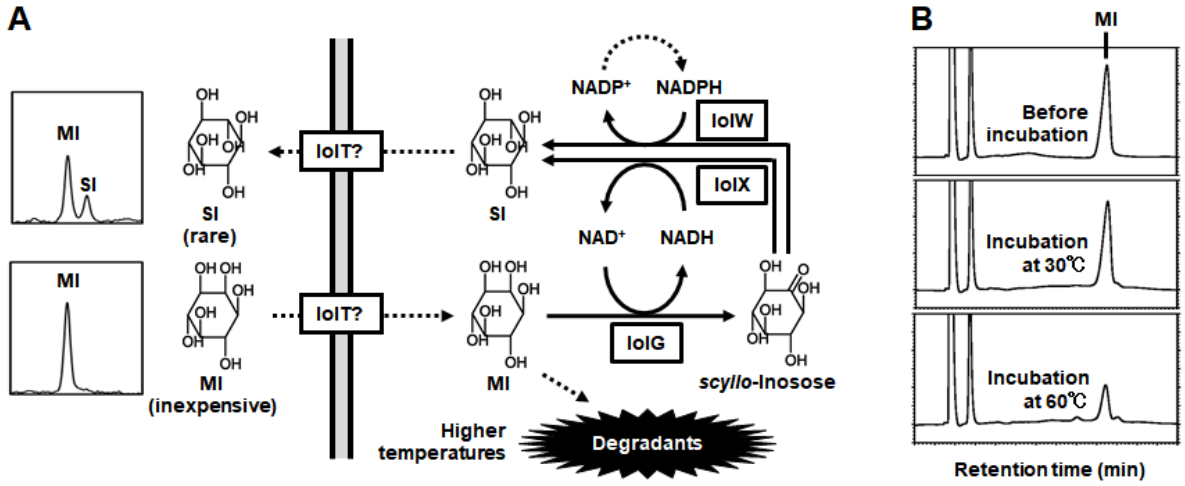

Fig. 1 Evaluation of an engineering approach that produces mesophilic enzymes in thermophiles at moderate temperatures. (A) Reactions catalyzed by Iol enzymes from *Bacillus subtilis*. IolG and IolX (or IolW) convert *myo*-inositol (MI) into *scyllo*-inositol (SI). IolT is potentially involved in MI uptake. *Geobacillus kaustophilus* can intrinsically catabolize MI. (B) MI degradation by *G. kaustophilus* is repressed at moderate temperatures. MI was incubated with the permeabilized cells for 24 h at respective temperatures.

To see whether MI is involuntarily degraded by intrinsic enzymes in *G. kaustophilus*,

cells carrying pGKE120 were cultured and incubated with MI following permeabilization. MI was notably decreased at 60℃ but not at 30℃ (Fig. 1B). This observation suggested that Iol enzymes catalytically dominated in thermophiles at moderate temperatures, supporting our concept. We also analyzed the *Geobacillus* spp. in which P_{cspX} functions. The gene encoding for a yellow fluorescence protein (Venus) was cloned in pGKE120 and introduced into the *Geobacillus* spp. The cells were cultured at 60℃ in LB medium until the optical density at 600 nm was equal to 1 and then at 40℃ for 24 h. Fluorescence analysis showed that Venus was produced more efficiently in *G. stearothermophilus* 10 than in *G. kaustophilus*, suggesting that *G. stearothermophilus* 10 is a promising host for our approach. In contrast, Venus was negligibly produced in *G. subterraneus* DSM 13552, *G. thermoglucosidasius* DSM 2542, and *G. thermoleovorans* DSM 5366; therefore, *Geobacillus* spp. in which P_{cspX} functions may be in minority.

Conclusion

Geobacillus kaustophilus that produced IolG and IolX were readily permeabilized and efficiently produced SI from MI. Since involuntary MI degradation was repressed at moderate temperatures, our approach could be effective for specific SI production. There are two similar approaches to prepare specific enzyme cocktails. One approach produces thermophilic enzymes in mesophiles and inactivates mesophilic enzymes by heat treatment. This approach requires reactions at elevated temperatures that cause vitamin degradation and cannot use mesophilic enzymes. The other produces mesophilic enzymes in psychrophiles and inactivates psychrophilic enzymes by incubation at moderate temperatures. Although this approach allows reactions at moderate temperatures, psychrophiles generally require a cooling system for cultivation and grow slowly. In contrast to these approaches, our approach does not require heat treatment and the reaction proceeds at moderate temperatures. The cells rapidly grow and can efficiently produce enzymes. Importantly, our approach could use viable cells for bioproduction when the substrates are incorporated into cells. This approach has the potential to serve as a new strategy for the facile and efficient production of diverse metabolites.

References

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2) Kurashiki, R., Mizuno, T., Murata, K., Ohshiro, T., and Suzuki, H. (2020) A plasmid vector that directs hyperproduction of recombinant proteins in the thermophiles *Geobacillus* species. *Extremophiles* **24**: 147.