Overexpression of Thermophilic α -amylase in *Bacillus Licheniformis* using a High Efficiency Chromosomal Integration and Amplification Strategy

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Research

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Abstract

Highly efficient preparation of industrially important enzymes depends on development of the genetically stable and high-yield microbial cell factories, which is often a challengeable laboratory hard work. In aims to simplify strain development with high efficiency for enzyme overproduction, a new strategy based on chromosomal integration and amplification in *Bacillus* sp . was developed. A pair of plasmids, an integrated expression plasmid pUB'-Ex1 and a thermosensitive replicable plasmid pUB-MazF, were constructed. pUB'-Ex1 conditionally self-replicated in *Bacillus* sp . when the RepF in pUB-MazF expressed. pUB-MazF thermosensitively self-replicated in *Bacillus* sp . , which was easily cured from the host by inducing MazF expression with IPTG. *Bacillus licheniformis* BL-UBM that integrated with pUB-MazF was then transformed with pUB'-amyS derived from pUB'-Ex1 by in-frame cloning of amyS encoding a thermophilic α -amylase from *Geobacillus stearothermophilus* ATCC 31195. The transformant of *B. licheniformis* BL-UBM with pUB'-amyS was cultivated at 42 o C with the existence of 1 mmol/l IPTG and 500 μ g/ml kanamycin and the recombinants with high α -amylase activities were selected. All tested recombinants were extremely high genetic stability. One of which, recombinant BLiS-002, carried five copies of amyS and produced the highest yield of α -amylase. It could yield 50,753 U/ml of α -amylase in a 50-l bioreactor. The strategy developed in this study is of application potential for convenient and quick strain development for industrially important enzyme overexpression.

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the latest manuscript can be downloaded and [accessed as a PDF](#).

Figures

Figure 1

The physical maps of pUB-MazF (a) and pUB'-EX1 (b). pUB-MazF and pUB'-EX1 are E. coli-Bacillus sp. shuttle vectors. pUB-MazF had the functional repF which encodes a thermosensitive replicase for its automatic replication in Bacillus sp. host cells under the incubation temperature no more than 35°C. pUB-MazF also contained a complete mazF encoded an endoribonuclease under the control of a lactose-inducing promoter Pspac. The repF in pUB'-EX1 had been disrupted and its replication could only be restored while pUB-MazF existed and its repF functionally expressed. A 0.5 kb downstream fragment of B. licheniformis amyL (3'-amyL') was inserted in pUB'-EX1 to make it site-directed integration and amplification in B. licheniformis chromosome.

Figure 2

The confirmation of the replicon properties of pUB-MazF and pUB'-EX1 in B. subtilis and B. licheniformis. All strains were cultivated in LB with no antibiotic added under corresponding conditions for 48 h, the cultures were diluted and spread on LB plate complemented kanamycin and/or tetracycline and cultivated for 48 h. a pUB-MazF in B. subtilis WB600 or B. licheniformis BL-109. bThe property of pUB'-EX1 was confirmed in B. subtilis WB600 or B. licheniformis BL-109 harboring free pUB-MazF. cThe concentration of IPTG was 1 mmol/l.

Figure 3

The flowchart of α -amylase-overexpressing strain development based on pUB-MazF and pUB'-EX1. pUB-MazF was transformed into *B. licheniformis* BL109 and then forced to integrate into the chromosome under the selection pressure of 42°C and 2 µg/ml tetracycline, yielded a *B. licheniformis* recombinant BL-UBM. pUB'-amyS derived from pUB'-EX1 by cloning *amyS* encoding the mature thermophilic α -amylase from *G. stearothermophilus* ATCC 31195 was transferred into strain BL-UBM at 35°C on starch plates

containing 2 µg/ml tetracycline and 20 µg/ml kanamycin and obtained transformant BL-amyS. Recombinants BLiS-001~BLiS-010 were selected by incubating strain BL-amyS in LB medium supplemented 500 µg/ml kanamycin and 1 mmol/l IPTG at 42°C for 48 h and subsequently spread onto starch plates for another 24 h.

Figure 4

Genetic stability, copy number of amyS and fermentation performance of the recombinant *B. licheniformis*. a: The yields of α-amylase after 15 generations of ten recombinant strains with different copy number of amyS, open column: first generation, solid column: 15th generation; b: The time-course of AmyS yields during fermentation in a 50-l bioreactor, embedded box: SDS-PAGE profile of the fermentation broth while the enzyme activity maximum reached.