

BACILLUS SUBTILIS CELL FACTORY CONVERTING PHYTIC ACID INTO SCYLLO-INOSITOL, A THERAPEUTIC AGENT FOR ALZHEIMER'S DISEASE

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Key Words: *Bacillus subtilis*, inositol, bioconversion, phytic acid

Phytic acid, known as *myo*-inositol (MI) hexakisphosphate, is the principal storage form of phosphorus in many plant tissues, especially bran and seeds. Phytases comprise a group of phosphatases that can trim inorganic phosphates from phytic acid. *B. subtilis* laboratory standard strain 168 and its derivatives exhibit no phytase activity, whereas a natto starter secretes significant phytase actively.

The natto phytase gene was cloned into strain RIK1285, a protease-defective derivative of 168, to construct a random library of its N-terminal fusions with 173 different signal peptides identified in the 168 genome. The library was screened to assess the efficiency of phytase secretion based on clear zones around colonies on plates, which appeared when phytic acid was hydrolyzed.

The *pbp* signal peptide enhanced the secretion of the natto phytase most efficiently, i.e., twice that of the original signal peptide. The secreted phytase can be one of the enzymatic tools to liberate MI from phytic acid contained in agricultural wastes including rice bran.

As described above, MI is the most abundant inositol stereoisomer in nature and thus supplied cheap. On the other hand, *scyllo*-inositol (SI) is one of the inositol stereoisomers, rare in the nature, and expected as a promising disease-modifying therapeutic agent for Alzheimer's disease.

B. subtilis 168 has the ability to metabolize inositol stereoisomers, including MI and SI. Previously, we reported a *B. subtilis* cell factory with modified inositol metabolism that converts MI into SI in the culture medium. The strain was constructed by deleting all genes related to inositol metabolism and overexpressing key enzymes, *lolG* and *lolW*. By using this strain, 10 g/l of MI initially included in the medium was completely converted into SI within 48 h of cultivation in a rich medium containing 2% (w/v) Bacto soytone.

When the initial concentration of MI was increased to 50 g/l, conversion was limited to 15.1 g/l of SI. Therefore, overexpression systems of *lolT* and *PntAB*, the main transporter of MI in *B. subtilis* and the membrane-integral nicotinamide nucleotide transhydrogenase in *Escherichia coli* respectively, were additionally introduced into the *B. subtilis* cell factory, but the conversion efficiency hardly improved. We systematically determined the amount of Bacto soytone necessary for ultimate conversion, which was 4% (w/v). As a result, the conversion of SI reached to 27.6 g/l within 48 h of cultivation.

The *B. subtilis* cell factory was improved to yield a SI production rate of 27.6 g/l/48 h by simultaneous overexpression of *lolT* and *PntAB*, and by addition of 4% (w/v) Bacto soytone in the conversion medium. The concentration of SI was increased even in the stationary phase perhaps due to nutrients in the Bacto soytone that contribute to the conversion process. Thus, MI conversion to SI may be further optimized via identification and control of these unknown nutrients.

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