802 *Bacillus brevis* による *Bacillus* sp. D-2 キトサナーゼの高分泌生産

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【目的】 我々は細菌由来キトサナーゼタンパク質の立体構造の解明を目的に、*Bacillus brevis* による *Bacillus* sp. D-2 キトサナーゼの高発現系を構築し、その高分泌生産物からのキトサナーゼの精製を試みた。

【方法及び結果】高分泌発現用ベクターとして pNY-301 を用い、*B.* sp. D-2 キトサナーゼ遺伝子を含むプラスミド pCG3-61 から、pCGΔN-41 と pCGΔC-372 を構築した。次いで、エレクトロポレーションによってこれらの構築プラスミドを *B. brevis* に導入した。次に、キトサナーゼ遺伝子 CGΔC-372 をもつ *B. brevis* によって生産されたキトサナーゼタンパク質の精製を試みた。その結果、一連のカラムクロマトグラフィーによる精製によって、培養液6リットルから 78mg のキトサナーゼが精製された。現在、キトサナーゼ遺伝子 CGΔN-41 をもつ *B. brevis* によって生産されたキトサナーゼの精製を試みている。

Efficent Production of a Chitosanase from *Bacillus* sp. D-2 by *Bacillus brevis* Mutant

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【Key word】 chitosanase, efficient production, *Bacillus brevis*, *Bacillus* sp. D-2

803 **Molecular cloning and high-level expression in *Escherichia coli* of fungal α-galactosidase from *Absidia corymbifera* IFO 8084**

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[Objective] Since raffinose became recognized as a low-calorie sweetener, and as contributory to the maintenance of human health by promoting the proliferation of intestinal *Bifidobacteria*, the demand for this galacto-heterooligosaccharide has increased. The objective of the study is to develop an effective method for synthesis of raffinose.

[Methods and Results] We found that α-galactosidase from *Absidia corymbifera* IFO 8084 was able to synthesize raffinose with the use of crude enzyme in batch process. Moreover, we found that the synthetic yield could be increased to some extent depending on the amounts of enzyme used. To analyze α-galactosidase from *Absidia corymbifera* IFO 8084 at molecular level and prepare a large amount of the enzyme more efficiently, a cDNA encoding α-galactosidase of *Absidia corymbifera* IFO 8084 was cloned and sequenced. An expression vector pET32Trx/galα was constructed by introducing the cDNA coding region into a thioredoxin fusion system, pET32-Ek/LIC. The resulting transformant pET32Trx/galα overproduced the active enzyme as a thioredoxin fused form in the host *E. coli*. By adopting an His-binding metal affinity chromatography, the recombinant α-galactosidase was purified to a homogeneity in a single step. The purified recombinant fusion α-galactosidase showed a very similar characteristics with the native one from *Absidia corymbifera* IFO 8084.

[Key Words] raffinose, α-galactosidase, *Absidia corymbifera*, cDNA cloning, heterologous expression