Cloning and Heterologous Expression of Acetyl Xylan Esterase from *Aspergillus ficuum*

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Abstract

Xylan, the major hemicellulose component of many plants, occurs naturally in a partially acetylated form and lignin, the most resistant component in plant cell wall degradation, is also attached to β-1,4-linked-D-xylose backbone through the ester linkage. Esterases are required to release the esterified substituent and acetyl esterases are important in the complete degradation of acetylated polysaccharides, like pectins and xylans. The gene(*Axe*) encoding acetyl xylan estarase(AXE) was isolated from genomic λ library from *Aspergillus ficuum*. Nucleotide sequencing of the *Axe* gene indicated that the gene was separated with two intervening sequences and the amino acid sequence comparison revealed that it was closely related to that from *A. awamori* with the 92 % identity. Heterologous expression of AXE was conducted by using YEp352 and *Saccharomyces cerevisae* 2805 as a vector and host expression system, respectively. The *Axe* gene was placed between GAL1 promoter and GAL7 terminator and then this recombinant vector was used to transform *S. cerevisiae* 2805 strain. Culture filtrate of the transformed yeast was assayed for the presence of AXE activity by spectrophotometry and, comparing with the host strain, four to five times of enzyme activity was detected in culture filtrate of transformed yeast.

서론

Hemicellulos의 생분해 산물인 xylan은 자연상태에서 부분적으로 acetyl화 되어있는 형태로 존재하며 lignin 등도 hemicellulose의 β-1,4-D-xylose backbone에 연결되어 있는 α-(1,5) glucuronyl 또는 α-(1,3)-L-arabinose과 ester 결합을 하고 있는데 이러한 잔기 결합구조는 식물 세포벽의 생분해성을 어렵게 하고 있다. Xylan의 완벽한 분해를 위해서는 endoxylanase 또는 β-xylosidase 등의 분해 효소와 함께 xylan backbone으로부터 *O*-acetyl, arabinose, ferulic, uronic acid 등의 비 xylose 성분들을 제거해주는 acetyl esterase등이 반드시 필요로 하고 있다.

Acetyl xylan esterase (AXE, EC3.1.1.6)는 xylan의 xylose 잔기로부터 *O*-acetyl group을 제거 할 수 있는 활성을 가지고 있으며 cellulose 또는 hemicellulose 분해에 관련하는 여러 종류의 곰팡이, 세균, 식물, 동물 등에서 그 기능이 보고된바 있다. 하지만 최근 들어서는 acetyl 함량이 많은 hardwood의 xylan에 대해 특이성(specificity)이 높은 곰팡이 유래의 AXE 특성에 관해 관심이 많이 집중되고 있으며 현재까지 분리 정제되어진 곰팡이 유래의 AXE는 *Trichoderma reesei* (1), *Aspergillus awamori* (2), *Aspergillus oryzae* S85 (3) 등이 있다. 이들 효소에 대한 산업적 유용성의 증가로 인한 필요성으로 본 연구에서는 *Aspergillus ficuum*에서의 AXE 유전자의 분리를 시도하였으며 이를 cloning 한 뒤 yeast에서 발현시켜 그 기능을 확인 하였다.

결과 및 고찰

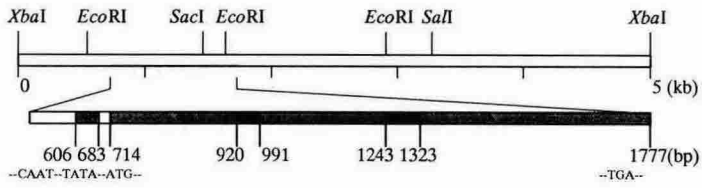

Fig.1. Partial restriction map of 5kb XbaI fragment and schematic drawing of *Axe* structure of *A. ficuum*. The DNA sequence with high similarity to the consensus sequence for TATA box is dotted (nt 606 to 683), the positions of intron (nt 920to 991and 1243 to 1323) are hatched, and the exons (nt 714 to 920, 991 to 1243, and 1323 to 1777) are shaded. Nucleotide sequence number starts from left *Xba*I position.

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A.ficuum :  MLSTHLLFLATTLLTSLLHPIAAHVAKRSGSLQQITDFGDNPTGVGMYIYVPNNLASNPG 60
            .::::::::. :::.::::::::  :  :::::::::.::::::::: ::::::::::::::::
A.awamori:  LLSTHLLFVITTLVTSLLHPIDGHAVKRSGSLQQVTDFGDNPTNVGMYIYVPNNLASNPG 61

A.ficuum :  IVVAIHYCTGTGPGYYSNSPYATLSEQYGFIVIYPSSPYSGGCWDVSSQATLTHNGGGNS 120
            ::::::::::::::::: .:::::::::::::::::::::::::::::::::::::::::::
A.awamori:  IVVAIHYCTGTGPGYYGDSPYATLSEQYGFIVIYPSSPYSGGCWDVSSQATLTHNGGGNS 121

A.ficuum :  NSIANMVTWTISEYGADSKKVYVTGSSSGAMMTNVMAATYPELFAAGTVYSGVSAGCFYS 180
            :::::::::::::.::::: ::.:::::::::::::::::::::::::: ::::::::::::::
A.awamori:  NSIANMVTWTISKYGADSSKVFVTGSSSGAMMTNVMAATYPELFAAATVYSGVSAGCFYS 181

A.ficuum :  DTNQVVGLNSTCAQGDVITTPEHWASIAEAMYPGYSGSRPKMQIYHGSVDTTLYPQNYYQ 240
            .:::::::::::::::::::::::::::::::::: ::::::::.::::::::.::::::::::.
A.awamori:  NTNQVVGLNSTCAQGDVITTPEHWASIAEAMYSGYSGSRPRMQIYHGSIDTTLYPQNYYE 241

A.ficuum :  TCKQWAGVFGYDYSAPEKTEANTPQTNYETTIWGDNLQGIFATGVGHTVPIHGDKDMEWF 300
            ::::::::::::::::::::::::::::::::::.:::::::::::::::::::::::::
A.awamori:  TCKQWAGVFGYDYSAPEKTEANTPQTNYETTIWGDSLQGIFATGVGHTVPIHGDKDMEWF 301

A.ficuum :  GFA 303
            :::
A.awamori:  GFA 304
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Fig. 2. Comparison of the deduced aminoacid sequences of acetylesterase of *A. ficuum* and *A. awamori*. The sequences were aligned to yield maximum homology with respect to identical(:) and functionally similar (.) amino acids.

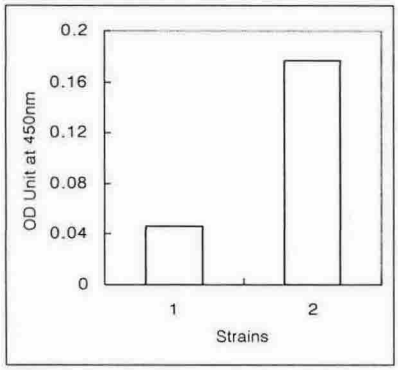

Fig. 3. Comparison of secreted AXE activity between 2805 yeast strain and its transformant. Heterologous expression of AXE was conducted by using YEp352 and *Saccharomyces cerevisae* 2805 as a vector and host expression system, respectively. All cultures were grown with galactose for induction at 30 °C, and total secreted protein was concentrated by ammonium sulfate. The enzyme activity was measured at 450 nm using *p*-nitophenyl acetate as a substrate.
1, Host strain; 2, Transformant.

요약

1. *A. ficuum*의 genomic library 검색을 통해 *Axe* 유전자를 포함하고 있는 5.0 kb의 *Xba*I DNA 절편을 cloning 했다. Cloning 된 절편의 부분 염기서열 결정 결과 약 1.4 kb의 AXE coding 부위를 확인했으며, cDNA cloning과 그 염기서열의 결정을 통해 AXE coding 부위 내에는 두 개의 intron 이 존재함이 확인되었다.

2. AXE coding 부위의 아미노산 잔기 서열 검색 결과 *A. awamori*의 AXE와 약 92 %의 상동성과 95%의 유사성이 있음이 확인 되었다.

3. 약 900 kb의 AXE의 cDNA를 yeast의 YEp352 vector의 GAL1 promoter의 전사 방향으로 cloning한 후 발현시킨 결과 형질전환체에서 acetyl esterase 활성을 확인 했으며, 활성도는 숙주균주에 비해 약 4-5배의 높은 OD unit로 나타내는 것을 확인 할 수 있었다.

참고문헌

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