

II. 귀리의 (1-3,1-4)-Beta-Glucanase cDNA Clone 의 분리와 확인

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II. Isolation And Confirmation Of An Oat (1-3,1-4)-Beta-Glucanase cDNA Clone

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실험목적

생장 발달중인 귀리 조직에서 활성을 나타내는 (1-3,1-4)-beta-glucanase 의 생리적 역할을 분자유전학적 방법으로 구명하기 위하여 (1-3,1-4)-beta-glucanase cDNA clone 을 분리하기 위함.

재료 및 방법

Gene bank에 등록되어 있는 식물체 beta-glucanase 효소들의 대부분에 잘 보존되어 있는 부위를 색출하여 동일 부위의 유전자 염기서열에 대한 30 bp oligonucleotide probe 를 합성하고, 이를 이용하여 귀리엽 cDNA library 를 screening 하여 secondary positive clones 을 선발하고, 이들의 염기서열을 결정, 분석한 후, pGEX-2T expression vector (Pharmacia Inc., NJ) 를 이용 cDNA insert 를 *Escherichia coli* 내에서 발현시켜 얻은 단백질의 특정 효소활성 여부를 조사 측정하였다.

실험결과 및 고찰

귀리엽 cDNA library 에서 선발된 clone 인 pOGL1 의 DNA 염기서열이 보리의 (1-3,1-4)-beta-glucanase 유전자의 염기서열과 90% 이상 동일하였고, pOGL1의 mature polypeptide coding sequence 에서 발현된 단백질이 (1-3,1-4)-beta-glucanase 활성을 나타내어 pOGL1이 (1-3,1-4)-beta-glucanase cDNA clone임을 확인하였다.

ACGAGAGAAAGAGTTTGAATCCCA ATG GCG AGC CAA GGT GTT GCC TCC ATG TTC GCT CTC GCA ITG CTC CTC 75
 H A S S G V A S H F A L A L L L
 GGA GCC TTC GCC TCC ATC CCA CAA AGC GTG GAG TCC ATC GGC GTT TGC TAC GGC ATG ACC GCC AAC AAC 144
 G A F A S I P Q S V E S I G V C Y G H S A N H
 CTG CCG GCG GCG AGC ACC GTG GTG GGC ATG TTC AAG TCC AAC GCG ATC AAC TCC ATG CCG CTG TAC CCG 213
 L P A N S T V V G H F K S N G I N S N R L Y A
 CCG GAC CAG GCG GCG CTT CAG GCC GTG GGA GCG AGC GCG GTG AAC GTG GTC GTC GCG GCG GCC AAC GAC 282
 P D Q A A L Q A V G S T G V N V V G A P H D
 GTC CTC TCC GCG CTC GCC GCT AGC CCT GCC GGC GCC TCC TGG GTG AGG AGC AAC ATC CAG GCG TAC 351
 V L S A L A A S P A A A A S W V R S H I D A Y
 CCG AAG GTC TGG TTC CCG TAC GTC TGC GTG GGC AAC CAG GTT GCC GGC GGC GCC ACC CAG AAC CTC CTC 420
 P K V S F R Y V C V G H E V A G G A T Q H L L
 CCG GCT ATG CAG AAC GTG CAG GCG GCG CTG GCG TCC GCG GCG GCG CAC ATC AAG GTG ACC ACG TCG 489
 P A M Q M V D G A L A S A G L G H I K V T T S
 GTG TCG CAG GCC ATC CTC GCG GTG TAC AGC CCG CCG TCG GCG TCC TTC ACG GCG GAG GCG GAC CCG 558
 V S Q A I L G V Y S P P S A G S F T G E A N A
 TTC ATG GCG GCC GTG GTG CAG TTC CTC GCG GCC ACC GCG AGC CCG CTC ATG GCG AAC ATC TAC CCG TAC 627
 F N D P V V D F L A R T G S P L H A H I Y P Y
 CTG GCC TGG GCC TAC AAC CCG AGC GCC ATG GAC ATG ACC TAC GCG CTC TTC ACC GCC TCC GCC ACC GTG 696
 L A W A Y N P S A M D H S Y A L F T A S G T V
 GTC CAG GAC GGC GCC TAC GCG TAC CAG AAC CTC TTC GAC ACC ACG GTG GAC GCC TTC TAC ACG GCG ATG 765
 V Q D G A Y G Y D N L F D T T V D A F Y T A M
 GGC AAG CAC GCG GCC GCG GCG GTG AAG CTG GTG GTC TCC GAG AGT GCG TCG GCG GCG GCG GCG GAG 834
 G E H G G A G V K L V V S E S C U P S A G G E
 GCT GCG ACC CCT ACC AAC GCC AGG ATC TAC AAC CAG TAC CTG ATC AAC CAC GTC GCG GCG GCC ACC CCG 903
 A A T P A M A R I Y N Q Y L I H N V G R G T P
 CCG CAC CCG GCG GCG ATC GAG ACC TAC GTG TTC GCG ATG TTC AAC GAG AAC CAG AAC GAC AAC GCG GTG 972
 R N P G G I E T Y V F A M F N E M Q K D N G V
 GAG CAG AAC TGG GCG CTC TTC TAC CCG AAC ATG CAG CAC GTC TAC CCG ATC AGC TTC TGA TCGAAGCAAGC 1043
 E Q N W G L F Y P N H Q H V Y P I S F
 ATCAGAGTACGTGGCTGGCTGGCTATACCGGTATGTCTCCATCCCGCGGTACATCGGATATAGCGGCTGTACCGGTACGTATGTGACAT 1134
 TATGTGTTTGTACAGTACAGGGGCTTGTATCCGACCGGTGAGACAGTATGCAGTACTCAGTCCGTACCGGTACCGGTACGTATGTGTA 1225
 CCGTATCCCTAGTATAGTATAGTAACTAGTATACGATTCCGATTGAAGGGAAGCAGATGTAACCGTCCCTTCTAGTGGAGAAG 1316
 CCTGATTCATAGGATGCTACACAGATGTGTACTATAGGTGTAGGTTACTATATGTGACAGGTTCAAGTTTCTGCAAAATTTTG 1407
 ATGAAATTTTGTTCGAAATAGAAAAAAAAAAAAAAAAAAAA 1408

Fig. The nucleotide sequence and deduced amino acid sequence of an oat (1-3,1-4)- β -glucanase cDNA clone, pOGL1. Possible polyadenylation signal sequences are underlined. Arrow indicates the putative NH₂-terminal residue of the mature enzyme. Standard one-letter amino acid codes are used.

Table. Nucleotide and amino acid sequence similarities (%) of pOGL1 with barley (1-3,1-4)- β -glucanase isoenzymes EI and EII

	Mature polypeptide		Signal peptide		3' UT ³
	NA ¹	AA ²	NA	AA	NA
EI	92	95	94	92	61
EII	89	90	91	92	53

1; nucleic acid, 2; amino acid, 3; untranslated sequence

Table (1-3,1-4)- β -glucanase activity of the GST-pOGL1 encoded β -glucanase fusion protein

Substrate	(1-3,1-4)- β -glucanase activity (μ g of Glc equiv./Reaction)
Barley β -glucan	40
Laminarin	<0.5
CM-cellulose	<0.5
Starch	<0.5