การศึกษาสมบัติของ cDNA ที่เป็นรหัสของเลคตินในใบหม่อน

Characterization of partial cDNA clone encoding mulberry leaf lectin

จันทนา กาญจน์กมล[1], อมรรัตน์ พรหมบุญ[1], พรรณนภา ศักดิ์สูง[2] และ สุนันทา รัตนาโภ[1]

Chantana Kankamol[1], Amornrat Promboon[1], Panapa Saksoong[2] and Sunanta Ratanapo[1]

บทคัดย่อ

ได้แยกเลคตินจากใบหม่อนน้อยให้บริสุทธิ์และนำไปหากรดอะมิโนทางปลายเอ็น ลำดับกรดอะมิโนทางปลายเอ็นมีเปอร์เซ็นต์ความเหมือนกับเลคตินที่มีความจำเพาะกับน้ำตาลกาแลคโทสจาก *Morus nigra* และ

เลคตินจาก *Maclura pomifera* สูงถึง 85 และ 80 เปอร์เซ็นต์ตามลำดับ จากนั้นสังเคราะห์ชิ้น cDNA ของยีน

เลคตินด้วยปฏิกิริยา RT-PCR (Reverse transcription-polymerase chain reaction) **โดยใช้** degenerate primers **ที่สังเคราะห์มาจากลำดับกรดอะมิโนทางปลายเอ็นของ** MLL1 **และจากบริเวณอนุรักษ์ของยีนเลคตินในตระกูล** Moraceae **ชนิดอื่นที่มีความจำเพาะต่อน้ำตาลแตกต่างกัน พบว่าขนาดชิ้นดีเอ็นเอของยีนเลคติน** MLL1 **จากใบหม่อนน้อยที่ได้มีความยาว** 353 **คู่เบส มีเปอร์เซ็นต์ความเหมือนของลำดับนิวคลีโอไทด์เป็น** 93 81 **และ** 64 **เมื่อเปรียบเทียบกับ *Morus nigra* galactose-binding lectin jacalin และ *Morus nigra* mannose-binding lectin ตามลำดับ จากลำดับนิวคลีโอไทด์ของยีนเลคตินบางส่วนนี้ยังสามารถใช้ประโยชน์ในการหาลำดับนิวคลีโอไทด์ตลอดสายของเลคติน** MLL1 **ต่อไป**

ABSTRACT

Mulberry leaf lectin of Noi variety, MLL1 was purified and sequenced for N-terminal amino acids. The MLL1 sequence shows high homology of 85 and 80 % with *Morus nigra* galactose binding-lectin and *Maclura pomifera* agglutinin, respectively. The single PCR product of 353 bp was obtained by reverse transcription-polymerase chain reaction using degenerate primers designed based on the N-terminal amion acid sequence of MLL1 and the conserved amino acid sequences of Moraceae lectins of distinct carbohydrate binding specificity. The cDNA sequence of MLL1 was homologous to other lectin genes with high percentage of 93, 81 and 64 % with *Morus nigra* galactose binding-lectin, jacalin and *Morus nigra* mannose-binding lectin, respectively. The cDNA sequence of this partially cloned could be useful for further study of full length cDNA sequence of MLL1.

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1 **ภาควิชาชีวเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเกษตรศาสตร์**
Department of Biochemistry, Faculty of Science, Kasetsart University

2 **ภาควิชาพันธุศาสตร์ คณะวิทยาศาสตร์ มหาวิทยาลัยเกษตรศาสตร์**
Department of Genetic, Faculty of Science, Kasetsart University

INTRODUCTION

Many plants contain carbohydrate-binding proteins called lectins and agglutinins (Van Damme *et al*., 1998). Because of their binding specificity they have the capability to serve as recognition molecules within a cell, between cells, or between organisms (Chrispeels *et al*., 1991). An extensive study based on recent advances in the biochemistry, molecular cloning and structural analysis have subdivided plant lectins into seven families of structurally and evolutionarily related proteins (Van Damme *et al*., 1995 and Van Damme *et al*., 1998). The family of jacalin-related lectins has received a lot of attention because of their biological role in the plant defense. (Yang and Czapla, 1993 and Zhang *et al*., 2000). Jacalin is a lectin from *Artocarpus integrifolia* (jack fruit), a member of the Moraceae family (Sanjeev *et al*., 1992). Similar lectins were identified in other Moraceae plants such as osage orange (*Maclura pomifera*) (Young *et al*.,1989) and *Morus nigra* (Rabijns *et al*., 2001 and Yeasmin,,2001). Recently, Ratanapo *et al*. (1998) have discovered two isolectins from mulberry leaves (*Morus sp*. called Mon-Noi), MLL1 and MLL2 showing carbohydrate binding specificity to N-glycolylneuraminic acid. Each mulberry leaf lectin was a glycoprotein, having a native mol. wt of 51,000 and subunit mol. wt of 16,500. However, only the lectin MLL1 can induce the agglutination of a specific phytopathogenic bacteria, *Pseudomonas syringae pv* mori (Ratanapo, *et al*. 2001).

In this study, we reported the characterization of cDNA clone from young mulberry leaves, which encodes partial nucleotide sequence of MLL1. Analysis of the cDNA sequence reveals that the lectin MLL1 has sequence similarity to some Moraceae plant lectins.

MATERIALS AND METHODS

Plant material

Noi variety of mulberry, *Morus sp*. was collected from Nakonratchasima and Udonthani Sericultural Research Centers and cultivated at Faculty of Science, Kasetsart University.

N-terminal amino acid sequencing

The purified mulberry leaf lectin as described by Ratanapo *et al*. (1998) was determined for N-terminal amino acid sequence by Edman degradation method using 491 CLC Protein Sequencer (PE Applied Biosystems).

Isolation of Total RNA

Total RNA was extracted from young mulberry leaves, Noi variety, using TRIzol reagent as described in protocol of Gibco-BRL Life Technologies. A 0.1 g sample of lyophilized leaves was powdered in liquid nitrogen and mixed with 1 ml of TRIzol reagent. RNA was precipitated by mixing

with isopropyl alcohol and washed with 70% ethanol. Purity and quality of the RNA were checked by agarose gel electrophoresis and its concentration was estimated by measuring the A 260.

Cloning of MLL1 cDNA

Total RNA of young mulberry leaves was extracted and an Oligo(dT) primer used to synthesize the first strand cDNA by the Reverse transcription system kit from Promega as described by the manufactures' protocol. The following degenerate oligonucleotides were used to amplify the cDNA fragment of the MLL1 : GG(C/A)GT(G/C)GC (A/C)TT(T/C)GA(T/C)GA(T/C)GG (forward primer, MLF) and CCTTTGAATCC(A/G)(A/G)C(A/G)AT(C/T)(A/T)(A/T)GC (reverse primer, MLR). The forward primer was designed corresponding to N-terminal amino acid sequence of MLL1. The reverse primer was designed from the most conserved amino acid sequences of various Moraceae lectins. The PCR amplification was performed for 35 cycles : 1 min at 94°C, 1 min at 56°C and 1 min at 72°C, followed by a 10 min extension at 72°C added to the final cycle. The cDNA fragment was then purified from agarose gel using NucleoSpin Extract 2 in 1 kit (MACHEREY-NAGEL, Germany). The amplified cDNA product was inserted into the pGEM-T Easy vector (Promega) and cloned into *Escherichia coli* JM109 competent cells (Promega). Plasmid DNA was then extracted from the bacterial cells on a miniprep scale using the alkaline lysis method as described by Sambrook and Russell (2000) and sequenced by fiuorescence-base modification of the Sanger dideoxy chain termination method (Sanger *et al.,* 1997; Smith *et al*., 1987) using M13 universal and M13 reverse primers.

Sequence alignment

The advanced BLAST program (Altschul *et al*.1997) was used to search the nucleotide sequences database on the National Center for Biotechnology Information. The MLL1 cDNA sequence was aligned with other lectin sequences using CLUSTAL W @ ebi. ac.uk.

RESULTS AND DISCUSSION

The N-terminal amino acid sequence of the mulberry leaf lectin, MLL1 yielded the 20 amino acid residues ; GVAFDDGVYTGIRAINFEYK. Alignment of the sequence with those previously reported proteins including distinct carbohydrate-binding lectins of the Moraceae plant family and other non-related proteins is shown in fig.1. The MLL1 N-terminal sequence showd high homology of 85 and 80 % with *Morus nigra* galactose binding-lectin and *Maclura pomifera* agglutinin (MPA), respectively.

Oligonucleotides were designed corresponding to the N-terminal amino acid sequence of MLL1 and the conserved amino acid sequence of other Moraceae lectins using the codon degeneracy and the homologous sequence as shown in fig. 2. Amplification of MLL1-specific sequence was generated using reverse transcription polymerase chain reaction (RT-PCR). The total

RNA isolated from young mulberry leaves showed high quality, having uniform ultraviolet light absorption spectra with A 260/280 ratio between 1.6-1.9 and yielded 0.75 μg per mg of leaf fresh weight. The single PCR product obtained from the PCR amplification was about 350 bp in size which is the expected size. However, the cDNA sequence is a half of the size expected from the molecular weight of the lectin MLL1 (Mr = 51,000 dalton). The PCR product was inserted into the plasmid pGEM-T Easy vector and cloned into *Escherichia coli* JM109. The 353 bp cDNA clone was obtained by the Sanger's dideoxy method. The cloned PCR product was shown in fig. 3.

The nucleotide sequence of the MLL1 cDNA was aligned with three Moraceae lectins, *Morus nigra* galactose-binding lectin, jacalin and *Morus nigra* mannose-binding lectin, the percentage of homology was 93, 81 and 64 %, respectively (fig. 4). To elucidate the protein structure, carbohydrate-binding site and structure-function relationships of the lectin MLL1, the complete entire sequence of the lectin is necessary obtained. For further work, we plan to do 3′- and 5′-RACE using primers synthesized from the partial cDNA sequence of the lectin.

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1   GVAFDDGVYTGIRAINFEYK------- 20
2   GVAWDDGVYDGVR-------------- 13
3   GVAFDDGAYTGIREINFEYN------- 20
4   GVTFDDGAYTGIREINFEYN------- 20
5   GIMFDDGIYTGIRQIN----------- 16
6   GKAFDDGAFTGIREINLSINKETAIGD 27
7   GKAFDDGAFTGIREINLSINKETAIGD 27
8   GKAFDDGAFTGIREINLSYN------- 20
9   GNAWDDGSYTGIREINLSHG------- 20
10  ----DDGSYTGIRQIELSYK------- 16
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Figure 1 Alignment of the N-terminal amino acid sequences of MLL1 to other proteins

1 = N-glycolylneuraminic acid-binding lectin (MLL1): *Morus* sp. (Mon-Noi) ; 2 = putative myrosinase-binding protein: *Arabidopsis thalina* ; 3 = galactose-binding lectin: *Morus nigra* ; 4 = agglutinin alpha chain: *Maclura pomifera* ; 5 = unknown protein: *Arabidopsis thalina* ; 6 = jacalin: *Artocarpus heterophyllus* ; 7 = jacalin alpha chain: *Artocarpus champeden* ; 8 = jacalin: *Artocarpus integrifolia* ; 9 = mannose- binding lectin: *Morus nigra* ; 10 = mannose-binding lectin KM+: *Artocarpus integrifolia*

CLUSTAL W (1.82) multiple sequence alignment

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MLL         ------------------------------------------------------------
AAL09163.1  MASSSFLSLSFLVLLFSISSANTRKWSLSNGLDQKPISIIEAAIGVSEDLLNLNGMEAKN 60
P18674      ------------------------------------------------------------
AAA32678    MAYSSLFSLSVLALLFSISSADTRKWFLAKGINQNPIGIIEAAVGVSEDLLNLNGMEAKN 60
AAL10685    -----------------------------------------------------MAG-TSTN 7

                                      MLF
MLL         ------------------------GVAFDDGVYTGIRAINFEYK---------------- 20
AAL09163.1  NQQSGKSQTIVVGTWGAQVT-SSNGVAFDDGAYTGIREINFEYNNETAIGSIQVTYDVNG 119
P18674.     ------------------------GVTFDDGAYTGIREINFEYNSETAIGGLRVTYDLNG 36
AAA32678    NEQSGISQTVIVGPWGAKVSTSSNGKAFDDGAFTGIREINLSYNKETAIGDFQVVYDLNG 120
AAL10685    TQTTGTSQTVEVGLWG-----GPGGNAWDDGSYTGIREINLSHG--DAIGAFSVIYDLNG 60
                                  * ::*** :**** **:.:

MLL         ------------------------------------------------------------
AAL09163.1  TPFEAKKHASFIKGFTQVKISLDFPNEYIVEVSGYTGKLS----GYTVVRSLTFKTNKET 175
P18674      MPFVAEDHKSFITGFKPVKISLEFPSEYIVEVSGYVGKVE----GYTVIRSLTFKTNKQT 92
AAA32678    SPYVGQNHKSFITGFTPVKISLDFPSEYIMEVSGYTGNVS----GYVVVRSLTFKTNKKT 176
AAL10685    QPFTGPTHPGNEPSFKTVKITLDFPNEFLVSVSGYTGVLPRLATGKDVIRSLTFKTNKKT 120

                                      MLR
MLL         ----------------------------------------
AAL09163.1  YGPYGVTSGTHFKLPIQNGLIVGFKGSVGYWLDYIGFHLSL 216
P18674      YGPYGVTNGTPFSLPIENGLIVGFKGSIGYWLDYFSIYLSL 133
AAA32678    YGPYGITSGTPFNLPIENGLIVGFKGSIGYWLDYFSMYLSL 217
AAL10685    YGPYGKEEGTPFSLPIENGLIVGFKGRSGFVVDAIGVHLSL 161
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Figure 2 Two degenerate oligonucleotides derived from N-teminal amino acid residues of the mulberry leaf lectin, MLL1 (forward primer, MLF) and the most conserved sequences of some Moraceae lectins (reverse primer, MLR) ; *Morus nigra* galactose-binding lectin (AAL09163.1), *Maclura pomifera* agglutinin (P18674)jacalin (AAA32678) and *Morus nigra* mannose-binding lectin (AAL10685). The amino acid residues completely conserved in all the lectins are indicated (*). Gap (-) are introduced for maximum alignment.

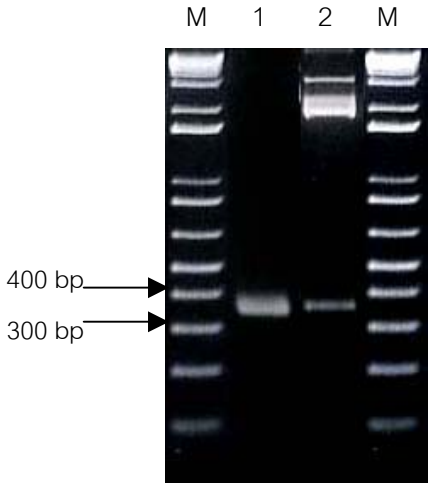

Figure 3 The cDNA clone of mulberry leaf lectin, MLL1

Lane M = 1 kb plus DNA ladder , Lane 1 = PCR amplification fragment of MLL1 cDNA

Lane 2 = insert pGEM-T Easy vector digested with *Eco*R 1

CLUSTAL W (1.82) multiple sequence alignment

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AY048576    ---AACATGGGGAGCCCAAGTGA---CTAGCTCTAATGGTGTGGCTTTTGATGACGGTGC 338
MLL         ------------------------------------GGAGTGGCATTTGACGACGGTTC 23
L03798.1    ---ACCATGGGGAGCCAAAGTAAGCACTAGCTCCAATGGTAAAGCTTTTGATGACGGTGC 296
AY048577Mo  TCAGACAGTAGAAGTGGGACTATGGGGAGGGCCTGGTGGTAATGCTTGGGACGATGGGTC 356
                                                **    ** *  ** ** **  *

AY048576    ATACACCGGAATAAGAGAAATCAATTTCGAATATAACAATGAAACTGCTATCGGGAGTAT 398
MLL         ATACACCGGAATAAGAGAAATCAATTTTGAATATAATAATGAAACTGCTATCGGGGGTAT 83
L03798.1    ATTCACCGGAATCAGAGAAATCAACCTTTCATATAATAAGGAGACCGCCATTGGGGACTT 356
AY048577    CTATACTGGAATTCGAGAAATCAATCTTTCTCATGGTGA------TGCCATTGGTGCCTT 410
             *  ** *****  **********  *     **    *       ** ** **     *

AY048576    TCAAGTGACCTACGATGTGAATGGTACGCCATTTGAAGCAAAAAAACATGCCAGCTTTAT 458
MLL           TCAAGTGACCTACGATGTGAATGGTACGCCATTTGAAGCAAAAAAACATGCCAGCTTTAT 143
L03798.1    CCAAGTTGTTTACGACTTGAATGGATCGCCATATGTAGGACAAAATCATAAAAGTTTTAT 416
AY048577    TAGTGTGATTTATGATTTGAATGGCCAGCCGTTTACAGGGCCCACACATCCAGGAAACGA 470
     **  ** ** ******* ***** **  * *** *

AY048576    TAAAGGCTTCACACAAGTGAAGATTTCCTTAGACTTTCCAAATGAGTATATCGTTGAAGT 518
MLL           TAAAGGCTTCACACCAGTGAAGATTTGCCTAGACTATCCAAGTGAGTATATAGTTGAAGT 203
L03798.1    AACAGGCTTCACACCAGTGAAGATTTCTCTAGACTTTCCAAGCGAGTATATAATGGAAGT 476
AY048577    ACCCTCTTTTAAAACGGTGAAGATTACACTGGATTTTCCAAACGAATTCTTAGTGAGCGT 530
      **** ********  ********** *** * *  **

AY048576    GAGCGGGTACACTGGTAAACT-----GAGCGGGTATACAGTGG-------TACGCTCTTT 566
MLL           GAGCGGGTACACTGGTAAAGT-----GAGCGGGTATATAGTAG---____TACGCTCTTT 251
L03798.1    GAGCGGGTACACTGGTAACGT-----GAGTGGGTATGTAGTAG-------TACGCTCTTT 524
AY048577    GAGTGGGTACACTGGTGTGCTTCCTCGACTGGCTACGGGGAAGGATGTTATACGCTCACT 590
            *** ************    *     **  ** **    *  *       *******  *

AY048576    AACATTCAAGACCAACAAAGAAACTTATGGACCATATGGAGTTACAAGCGGCACACATTT 626
MLL         AACATTCAAGACCAACAAAGAAACTTATGGACCATATGGAGTTACAAGCGGCACTTATTT 311
L03798.1    GACATTCAAGACTAATAAGAAAACCTATGGACCATATGGAGTTACAAGCGGCACACCTTT 584
AY048577    AACATTCAAAACCAACAAGAAAACCTATGGGCCATATGGAAAGGAAGAAGGCACACCTTT 650
             ******** ** ** **  **** ***** *********     *   *****   ***

AY048576    TAAGCTCCCAATCCAAAATGGCTTAATTGTTGGATTTAAAGGAAGTGTCGGCTATTGGCT 686
MLL         TAAGCTCCCAATCCAAAAGGGCTAAATTGCTGGATTCAAAGG------------------ 353
L03798.1    CAATCTCCCAATCGAAAATGGCTTAATTGTTGGATTCAAAGGAAGTATCGGCTACTGGTT 644
AY048577    CAGTCTCCCAATTGAAAATGGTTTAATTGTTGGATTCAAAGGACGAAGCGGCTTTGTTGT 710
    * ******** **** ** * ***** ****** *****
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Figure 4 Multiple alignment of the nucleotide sequences of MLL1 with *Morus nigra* galactose-binding lectin (AY 048576) jacalin (L03798.1) and *Morus nigra* mannose-binding lectin (AY048577). The nucleotides completely conserved in all the lectins are indicated (*). Gap (-) are introduced for maximum alignment.

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