

โปรตีนโอมิกส์จากเมล็ดมะขามเทศ *Pithecellobium dulce*



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PROTEOMICS OF MANILA TAMARIND SEEDS *Pithecellobium dulce*

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(PROTEOMICS OF MANILA TAMARIND SEEDS *Pithecellobium dulce*)

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มะขามเทศ (*Pithecellobium dulce*) จัดเป็นพืชในวงศ์เดียวกับพืชตระกูลถั่วซึ่งเป็นไม้ผลพื้นเมืองอีกชนิดหนึ่งที่มีคุณค่าทางโภชนาการ โดยเป็นแหล่งโปรตีนที่สำคัญ ในการศึกษาครั้งนี้มีวัตถุประสงค์เพื่อระบุชนิดโปรตีนที่เป็นองค์ประกอบในเมล็ดมะขามเทศ ด้วยวิธีทางโปรตีโอมิกส์ โดยใช้เทคนิคทางเจลอิเล็กโตรโฟเรซิสแบบ 2 มิติ และหาลำดับกรดอะมิโนของโปรตีนด้วยเทคนิคทางแมสสเปคโตรเมทรี จากแผ่นเจลพบว่าสามารถแยกจุดโปรตีนได้ทั้งหมด 317 จุด โดยโปรตีนจากเมล็ดมะขามเทศส่วนใหญ่ที่มีความเข้มและปริมาณโปรตีนมาก จะแสดงจุดอยู่บริเวณที่เป็นกรดเล็กน้อย และอยู่ในช่วงมวลโมเลกุล 55-97 กิโลดาลตัน บนแผ่นเจลอิเล็กโตรโฟเรซิสแบบ 2 มิติ ซึ่งจุดของโปรตีนจำนวน 96 จุด ได้ถูกนำมาวิเคราะห์ด้วยเทคนิคนาโนลิควิดโครมาโทกราฟีแทนแควมแมสสเปคโตรเมทรี (LC/MS/MS) พบว่า สามารถระบุชนิดโปรตีนได้ 27 ชนิด และโปรตีนอีก 4 ชนิดจากกลุ่มของโปรตีนที่มีปริมาณโปรตีนมาก โดยได้จากการนำข้อมูลทางแมสสเปคโตรเมทรีของโปรตีนเหล่านี้ไปสืบค้นกับฐานข้อมูลโปรตีน MASCOT และเปรียบเทียบความเหมือน หรือ ความคล้ายคลึงของลำดับกรดอะมิโนบนสายโปรตีนในฐานข้อมูล (MS-BLAST) ตามลำดับ นอกจากนี้งานวิจัยนี้ยังสนใจที่จะศึกษาโปรตีนที่มีฤทธิ์ทางชีวภาพของโปรตีนจากเมล็ดมะขามเทศ พบว่าสามารถแยกโปรตีนบริสุทธิ์จากเมล็ดมะขามเทศที่มีฤทธิ์ยับยั้งการเจริญเติบโตของเชื้อรา *Macrophomina phaseolina* ซึ่งมีมวลโมเลกุลเท่ากับ 14.4 กิโลดาลตัน ได้จากการสกัดด้วยสารละลายทริสบัฟเฟอร์และตกตะกอนโปรตีนด้วยเกลือแอมโมเนียมซัลเฟตเข้มข้น แล้วจึงนำไปแยกโปรตีนให้บริสุทธิ์โดยใช้เทคนิคแอนไอออนเอ็กซ์เชนจ์ และเจลฟิลเทรชันโครมาโทกราฟี จากการวิเคราะห์ลำดับกรดอะมิโนของโปรตีนบริสุทธิ์ ด้วยเทคนิคแทนแควมแมสสเปคโตรเมทรี และสืบค้นกับฐานข้อมูลโปรตีน MASCOT พบว่าโปรตีนนี้มีลำดับกรดอะมิโนบางส่วนที่คล้ายคลึงกับโปรตีนพบว่า มีลำดับกรดอะมิโนบางส่วนคล้ายคลึงกับโปรตีนไลโซไซม์ (lysozyme) จากไข่ขาวของไข่ไก่

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NARUMON SAWASDIPUKSA : PROTEOMICS OF MANILA TAMARIND SEEDS *Pithecellobium dulce*. THESIS ADVISOR : ASSOC. PROF POLKIT SANGVANICH, Ph.D., 114 pp.

Pithecellobium dulce Benth. belongs to the Leguminosae family, which contains important components of human diets owing to their high protein content. The present study aimed to identify seed proteins from *Pithecellobium dulce* using a proteome approach by two-dimensional gel electrophoresis (2-DE) and tandem mass spectrometry. The 2-DE protein map revealed a total of 317 distinct protein spots, including a cluster proteins located in the acidic region with molecular masses of 55–97 kDa. Ninety-six of the most abundant protein spots were analysed using nano liquid chromatography/tandem mass spectrometry (nano-LC/MS/MS), from which 27 proteins and further four proteins from the highly abundant protein cluster were successfully identified through the query of acquired tandem mass spectral data used in Mascot and MS-driven BLAST homology searches, respectively. Moreover, the biological activity of *Pithecellobium dulce* seed proteins was identified in this research. An antifungal activity protein toward *Macrophomina phaseolina* with a molecular mass of 14.4 kDa was purified using a procedure that involved Tris buffer extraction, ammonium sulfate precipitation, anion exchange chromatography, and gel filtration chromatography. The result acquired from tandem mass spectrometry with Mascot database searching shows that this protein has partial amino acid sequences similar to chicken egg white lysozyme.

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CONTENTS

	Page
ABSTRACT (THAI).....	iv
ABSTRACT (ENGLISH).....	v
ACKNOWLEDGEMENTS.....	vi
CONTENTS.....	vii
LIST OF TABLES.....	xi
LIST OF FIGURES.....	xv
LIST OF ABBREVIATIONS.....	xvii
 CHAPTER	
I. INTRODUCTION.....	1
II. THEORETICAL AND LITERATURE REVIEWS.....	3
2.1 Leguminosae.....	3
2.2 <i>Pithecellobium dulce</i>	4
2.2.1 General Background.....	4
2.2.2 Morphology Description.....	5
2.2.3 Chemical Constituents.....	5
2.2.4 Uses and Applications.....	5
2.3 Protein Extraction and Precipitation.....	6
2.3.1 Protein Extraction.....	6
2.3.2 Protein Precipitation	6
2.4 Separations of Proteins.....	8
2.4.1 Ion Exchange Chromatography.....	10
2.4.2 Gel Filtration Chromatography.....	11
2.4.3 Gel Electrophoresis.....	14
2.4.3.1 Separations Based on Protein Molecular Weight.....	15
2.4.3.2 Separations Based on Protein Isoelectric Point.....	16
2.4.3.3 Two-Dimensional Electrophoresis.....	16
2.4.3.4 Protein Detection.....	17

CHAPTER	Page
2.5 Mass Spectrometry.....	18
2.5.1 Matrix Assisted Laser Desorption Ionization/ Time of flight Mass Spectrometry (MALDI/ToF MS).....	18
2.5.1.1 Matrix-Assisted Laser Desorption Ionization (MALDI)....	18
2.5.1.2 Time of Flight Mass Analyzer.....	20
2.5.2 Electrospray Ionization Mass Spectrometry.....	22
2.5.2.1 Electrospray Ionization (ESI)	22
2.5.2.2 Quadrupole Mass Filter.....	24
2.6 Tandem Mass Spectrometry.....	26
2.6.1 Collisionally Activated Dissociation (CAD).....	28
2.6.2 Electrospray Ionization Quadrupole/Time of Flight Mass Spectrometer (ESI-Q/TOF MS).....	29
2.6.3 Tandem Mass Spectrometry for Peptide Sequencing.....	30
2.7 Identification of Proteins.....	33
2.7.1 Protein Identification Using Peptide Mass Mapping.....	36
2.7.2 Protein Identification Using Tandem Mass Spectrometry.....	38
2.8 Literature Reviews.....	40
III. EXPERIMENTAL.....	43
3.1 Materials.....	43
3.1.1 Plant Material.....	43
3.1.2 Fungi.....	43
3.1.3 Chemicals.....	43
3.1.4 Apparatus and Instruments.....	44
3.2 Methods.....	45
3.2.1 Protein Profiling of <i>Pithecellobium dulce</i> Seeds Using 2-DE and MS/MS.....	45
3.2.1.1 Extraction and Precipitation.....	45
3.2.1.2 Protein Quantification.....	45
3.2.1.3 Two-Dimensional Gel Electrophoresis.	46

CHAPTER	Page
3.2.1.4 Gel Image Analysis.....	47
3.2.1.5 In-Gel Trypsin Digestion.....	47
3.2.1.6 Protein Identification Using Tandem Mass spectrometry..	47
3.2.1.6.1 LC-ESI-MS/MS.....	47
3.2.1.6.2 Data Analysis and Mascot Searching.....	48
3.2.1.6.3 <i>De novo</i> Sequencing and Sequence-Similarity Searches.....	48
3.2.2 Purification of Antifungal Protein from <i>Pithecellobium dulce</i> Seeds.....	49
3.2.2.1 Extraction and Precipitation.....	49
3.2.2.2 Biological Activity Test.....	49
3.2.2.2.1 Hemagglutination Activity.....	49
3.2.2.2.2 α -Glucosidase Inhibitory Activity.....	49
3.2.2.2.3 Antioxidant Activity.....	50
3.2.2.2.4 Antifungal Activity.....	50
3.2.2.3 Protein Purification by Column Chromatography.....	51
3.2.2.4 SDS-PAGE.....	52
3.2.2.5 Protein Identification.....	52
IV. RESULTS AND DISCUSSION.....	53
4.1 Protein Profiling of <i>Pithecellobium dulce</i> Seeds Using 2-DE and MS/MS.....	53
4.1.1 Protein Extraction and Precipitation.....	53
4.1.2 2-DE of Crude Protein from <i>Pithecellobium dulce</i> Seeds.....	53
4.1.3 Protein Identification Using Tandem Mass Spectrometry.....	55
4.1.3.1 Data Analysis and Mascot Searching.....	55
4.1.3.2 <i>De novo</i> Sequencing and Sequence-Similarity Searches...59	59
4.2 Purification of Antifungal Protein from <i>Pithecellobium dulce</i> Seeds.....	61

CHAPTER	Page
4.2.1 Extraction and Precipitation.....	61
4.2.2 Biological Activity Screening of Crude Protein.....	62
4.2.3 Purification of Antifungal Protein by Column Chromatography.....	62
4.2.4 Identification Antifungal Protein Using LC-ESI-MS/MS.....	67
V. CONCLUSION.....	69
REFERENCES.....	70
APPENDICES.....	83
APPENDIX A.....	84
APPENDIX B.....	86
APPENDIX C.....	98
APPENDIX D.....	111
VITA.....	114

LIST OF TABLES

Table	Page
2.1 Protein properties used during chromatographic purification.....	9
2.2 Functional groups used on ion exchangers.....	11
2.3 Supports for gel filtration chromatography.....	13
2.4 Protein fractionation range of some gels.....	13
2.5 Common MALDI matrices used in biological applications.....	20
2.6 The Masses of common 20 amino acids.....	32
2.7 Specific chemical and enzymatic cleavage of protein.....	37
2.8 Examples of the databases search programs and their internet addresses that can be used for protein identification.....	39
3.1 Preparation of single-percentage gel (12%) for SDS-PAGE of 2-DE.....	46
3.2 Composition of potato dextrose agar.....	51
3.3 Composition of the gel solutions for one 8×9 cm gel.....	52
4.1 Protein identified in <i>P. dulce</i> seeds by LC-ESI-MS/MS analysis.....	57
4.2 Protein Identification of 2D Gel Spots from <i>P. dulce</i> by MS-BLAST.....	60
4.3 Comparison of the amino acid sequences of query peptides from <i>P. dulce</i> and sequences from NCBI-Nr database.....	61
4.4 Purification of antifungal protein from <i>P. dulce</i> seeds (100 g).....	62
1A Solutions for 2D-PAGE.....	84
2A Coomassie gel stain and destain solutions.....	85
1B Peptide sequences acquired from MS/MS fragmentation of tryptic peptides from an identified protein spot 17 using Mascot search.....	86
2B Peptide sequences acquired from MS/MS fragmentation of tryptic peptides from an identified protein spot 19 using Mascot search.....	86
3B Peptide sequences acquired from MS/MS fragmentation of tryptic peptides from an identified protein spot 25 using Mascot search.....	87
4B Peptide sequences acquired from MS/MS fragmentation of tryptic peptides from an identified protein spot 29 using Mascot search.....	87

Table	Page
5B Peptide sequences acquired from MS/MS fragmentation of tryptic peptides from an identified protein spot 32 using Mascot search.....	88
6B Peptide sequences acquired from MS/MS fragmentation of tryptic peptides from an identified protein spot 33 using Mascot search.....	88
7B Peptide sequences acquired from MS/MS fragmentation of tryptic peptides from an identified protein spot 41 using Mascot search.....	88
8B Peptide sequences acquired from MS/MS fragmentation of tryptic peptides from an identified protein spot 48 using Mascot search.....	89
9B Peptide sequences acquired from MS/MS fragmentation of tryptic peptides from an identified protein spot 49 using Mascot search.....	89
10B Peptide sequences acquired from MS/MS fragmentation of tryptic peptides from an identified protein spot 54 using Mascot search.....	90
11B Peptide sequences acquired from MS/MS fragmentation of tryptic peptides from an identified protein spot 59 using Mascot search.....	90
12B Peptide sequences acquired from MS/MS fragmentation of tryptic peptides from an identified protein spot 66 using Mascot search.....	91
13B Peptide sequences acquired from MS/MS fragmentation of tryptic peptides from an identified protein spot 67 using Mascot search.....	91
14B Peptide sequences acquired from MS/MS fragmentation of tryptic peptides from an identified protein spot 69 using Mascot search.....	92
15B Peptide sequences acquired from MS/MS fragmentation of tryptic peptides from an identified protein spot 70 using Mascot search.....	92
16B Peptide sequences acquired from MS/MS fragmentation of tryptic peptides from an identified protein spot 71 using Mascot search.....	93
17B Peptide sequences acquired from MS/MS fragmentation of tryptic peptides from an identified protein spot 72 using Mascot search.....	93

Table	Page
18B Peptide sequences acquired from MS/MS fragmentation of tryptic peptides from an identified protein spot 74 using Mascot search.....	93
19B Peptide sequences acquired from MS/MS fragmentation of tryptic peptides from an identified protein spot 76 using Mascot search.....	94
20B Peptide sequences acquired from MS/MS fragmentation of tryptic peptides from an identified protein spot 77 using Mascot search.....	94
21B Peptide sequences acquired from MS/MS fragmentation of tryptic peptides from an identified protein spot 78 using Mascot search.....	95
22B Peptide sequences acquired from MS/MS fragmentation of tryptic peptides from an identified protein spot 80 using Mascot search.....	95
23B Peptide sequences acquired from MS/MS fragmentation of tryptic peptides from an identified protein spot 86 using Mascot search.....	95
24B Peptide sequences acquired from MS/MS fragmentation of tryptic peptides from an identified protein spot 87 using Mascot search.....	96
25B Peptide sequences acquired from MS/MS fragmentation of tryptic peptides from an identified protein spot 88 using Mascot search.....	96
26B Peptide sequences acquired from MS/MS fragmentation of tryptic peptides from an identified protein spot 93 using Mascot search.....	96
27B Peptide sequences acquired from MS/MS fragmentation of tryptic peptides from an identified protein spot 94 using Mascot search.....	97
1C Amino acid sequences of peptide precursor using BLAST search of protein spot 1.....	102
2C Amino acid sequences of peptide precursor using BLAST search of protein spot 8.....	105
3C Amino acid sequences of peptide precursor using BLAST search of protein spot 13.....	106

Table		Page
4C	Amino acid sequences of peptide precursor using BLAST search of protein spot 25.....	108
1D	Peptide sequences of tryptic peptides from the purified antifungal protein (G4) using Mascot search.....	113

LIST OF FIGURES

Figure	Page
2.1 Flower morphology of three sub-families belongs to Leguminosae.....	3
2.2 <i>Pithecellobium dulce</i> flowers, leave, pods, pulps and seeds.....	4
2.3 Protein dissolving in water when absent salt and increasing salt.....	7
2.4 Aggregation of proteins by interactions in an aqueous-organic solvent mixture.....	8
2.5 Ion exchange resins with functional group structure.....	11
2.6 The polymerization reaction of acrylamide and methylenebisacrylamide...	14
2.7 Mechanism of SDS to breaks up the interactions between proteins.....	15
2.8 Two-dimensional electrophoresis processes.....	17
2.9 Matrix Assisted Laser Desorption/Ionization (MALDI) source.....	19
2.10 MALDI ion formation.....	19
2.11 Linear time of flight mass spectrometer.....	21
2.12 Reflectron time of flight mass spectrometer.....	22
2.13 The electrospray ionization source.....	22
2.14 The electrospray ionization formation.....	23
2.15 The quadrupole instrument.....	25
2.16 The stability region for ion trajectories in the quadrupole mass filter.....	25
2.17 A schematic representation of the tandem mass spectrometry scan modes...	28
2.18 Schematic diagram of Electrospray ionization quadrupole/ Time of flight mass spectrometer.....	30
2.19 The peptide fragmentation.....	31
2.20 Immonium ion and internal fragment.....	32
2.21 Strategy for the identification of proteins based on mass spectrometry.....	36
4.1 2-DE protein profile of <i>Pithecellobium dulce</i>	54
4.2 Anion exchange chromatography using Resource Q column of 80% saturation ammonium sulfate precipitated from <i>P. dulce</i> seeds extract.....	63

Figure	Page
4.3 Inhibitory activity of the protein fractions from Resource Q column (Q1-Q4) toward <i>Macrophomina phaseolina</i>	63
4.4 SDS-PAGE (12% gel) of fraction Q1 from Resource Q column.....	64
4.5 Gel filtration chromatography using Superdex 200 column of Q1.....	64
4.6 Inhibitory activity of the purified antifungal protein (G4) toward <i>Macrophomina phaseolina</i>	65
4.7 SDS-PAGE (12% gel) of the fractions (G1-G4) from Superdex 200 column.....	65
4.8 Determination of the minimum concentration and effect of temperature on antifungal activity toward <i>Macrophomina phaseolina</i> of G4.....	66
1C The product ion spectrum of precursor ion at m/z of 837.4 (spot 1).....	98
2C The product ion spectrum of precursor ion at m/z of 797.03 (spot 8).....	98
3C The product ion spectrum of precursor ion at m/z of 797.02 (spot 8).....	99
4C The product ion spectrum of precursor ion at m/z of 631.8 (spot 13).....	99
5C The product ion spectrum of precursor ion at m/z of 897.0 (spot 25).....	100
6C The product ion spectrum of precursor ion at m/z of 672.1 (spot 25).....	100
7C The product ion spectrum of precursor ion at m/z of 678.9 (spot 25).....	101
8C The product ion spectrum of precursor ion at m/z of 647.0 (spot 25).....	101
1D Antifungal activity screening of crude protein from <i>P. dulce</i> seeds toward (I) <i>Macrophomina phaseolina</i> , (II) <i>Phymatotrichopsis omnivora</i> , and (III) <i>Fusarium avicenarium</i>	111
2D Mascot search result of the purified antifungal protein (G4).....	112

LIST OF ABBREVIATIONS

2-DE	Two-dimensional electrophoresis
µg	microgram
µl	microliter
AC	Affinity chromatography
ACN	Acetonitrile
APS	Ammonium persulfate
Bis	<i>N,N'</i> -methylenebisacrylamide
BSA	Bovine serum albumin
°C	degree Celsius
C	Crosslinking factor [%]
cal	Calculation
CCA	α-Cyano-4-hydroxycinnamic acid
CE	Capillary electrophoresis
CHAPS	3-(3-cholamidopropyl)dimethylammonio-1-propane sulfonate
CID	Collision-induced dissociation
cm	centimeters
CM	Carboxymethyl
Da	Dalton
DC	Direct current
DEAE	Diethylaminoethyl
DPPH	2,2-Diphenyl-1-picrylhydrazyl
DTT	Dithiothreitol
2-DE	Two-dimensional polyacrylamide gel electrophoresis
exp.	Experimental
EDTA	Ethylenediaminetetraacetic acid
e.g.	for example
ESI	Electrospray ionization
ESI-Q/TOF	Electrospray ionization Quadrupole time-of-flight
EtOH	Ethanol
Fmol	femtomole

g	gram
GE	gel electrophoresis
GF	Gel filtration
h	hour
HIC	Hydrophobic interaction chromatography
HPLC	High performance liquid chromatography
HU	Hemagglutinating unit
IAA	iodoacetamide
IDA	Information-dependent acquisition
IEF	Isoelectric focusing
IEX	Ion-exchange chromatography
IPG	Immobilized pH gradients
kDa	kilo Dalton
kVh	kilo volt-hour
kg	kilogram
LC-ESI-MS/MS	Liquid Chromatography/Tandem Mass Spectrometry
mA	milliampere
MALDI	Matrix Assisted Laser Desorption Ionization
mbar	millibar
mg	milligram
min	minute
ml	milliliter
MOWSE	Molecular weight search score
mm	millimeter
mM	millimolar
MS	Mass spectrometry
MS-BLAST	Mass spectrometry-driven BLAST
MS/MS	Tandem Mass spectrometry
MW	Molecular weight
m/z	mass per charge ratio

Native PAGE	Non-denaturing polyacrylamide gel electrophoresis
NCBI	National Center of Biotechnology Information
NL	Non-Linear
No	Number
nm	nanometer
nM	Nanomolar
OD	Optical density
pI	Isoelectric point
PMF	Peptide mass fingerprint
PNPG	<i>p</i> -nitrophenyl - α -D-glucopyranoside
ppm	part per million
Q	Quaternary ammonium
Q/TOF	Quadrupole Time of flight
RF	Radio frequency
rpm	Revolutions per minute
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
TCA	Trichloro acetic acid
TEMED	<i>N,N,N',N'</i> -tetramethylethylenediamine
TFA	Trifluoro acetic acid
theor.	Theoretical
TOF	Time of flight
Tris	Tris(hydroxymethyl)-aminoethane
U	Unit
UV	Ultraviolet spectroscopy
V	Volt
v/v	volume by volume