CHAPTER III

RESULTS

3.1 Part I: Examination of royal jelly composition

3.1.1 Identification of the honeybee hive populations

The Northern, Southern and Samui Island honeybees hives were selected from Samut Songkram, Bangkok, Chumphon province and Samui Island, respectively. Genetic population of honeybee from various hives was investigated by PCR-RFLP of *Dra*I digested 3 mitochondrial DNA regions following Srihanuntavong (1999). The total DNA was extracted from thorax using proteinase K/ phenol-chloroform extraction method and high molecular weight DNA (larger than 23.1 kb). The concentration of extracted DNA was about 1.0 to 1.5 μg per individual as estimated by comparing its intensity of ethidium bromide-DNA complex with that of known amount of λ/*Hin*dIII marker in 0.7% agarose gel electrophoresis (Figure 3.1). About 50 ng of each total DNA sample was used as template for lrRNA gene, srRNA gene and intergenic COI-COII region PCR-amplification reactions. After electrophored in 1.2% agarose gel electrophoresis and ethidium bromide staining, PCR product sizes were determined under UV light using λ/*Hin*dIII and 100 bp DNA ladder as standard molecular size. The amplified products of lrRNA gene, srRNA gene and intergenic COI-COII region obtained were 755 (Figure 3.2), 410 (Figure 3.3) and 1710 (Figure 3.4) bp, respectively. The PCR products were restricted with *Dra*I restriction endonuclease (Figure 3.3 to 3.5).

The results showed that honeybee from Samut Songkram, Bangkok, Chumphon provinces and Samui island were northern and southern population bee since they had the composite haplotype of AAA, AAA, BBA and BCC, respectively (see Appendix A).

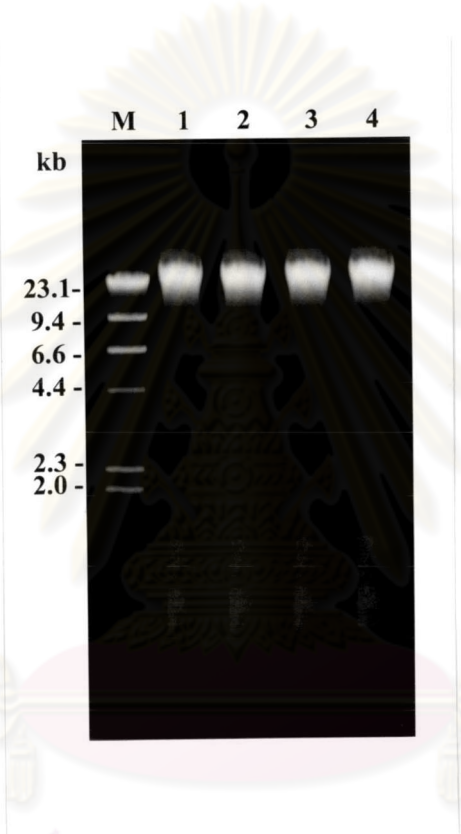

Figure 3.1 High molecular weight DNA extracted from thorax of *A. cerana* individuals

Lane M = λ/*Hin*dIII

Lane 1-4 = Total DNA from 4 individuals

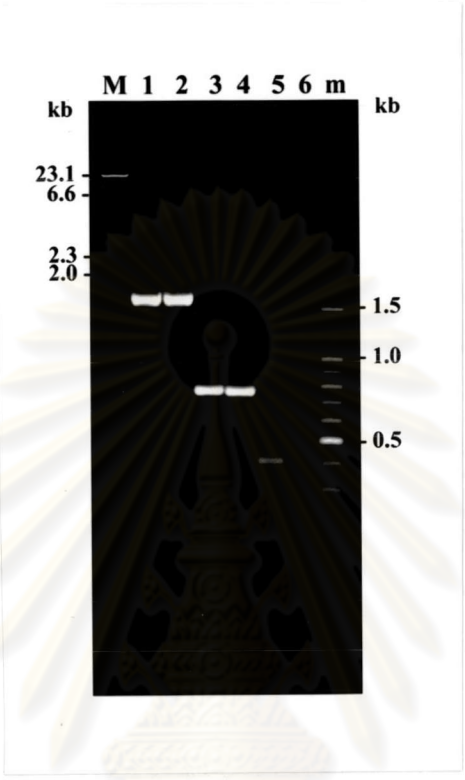

Figure 3.2 The amplified products of intergenic COI-COII region, lrRNA gene and srRNA of *A. cerana* individuals

Lane M and m = λ/*Hin*d III and 100 bp DNA ladder, respectively

Lane 1-2 = amplified products of inter COI-COII region

Lane 3-4 = amplified products of lrRNA gene

Lane 5-6 = amplified products of srRNA gene

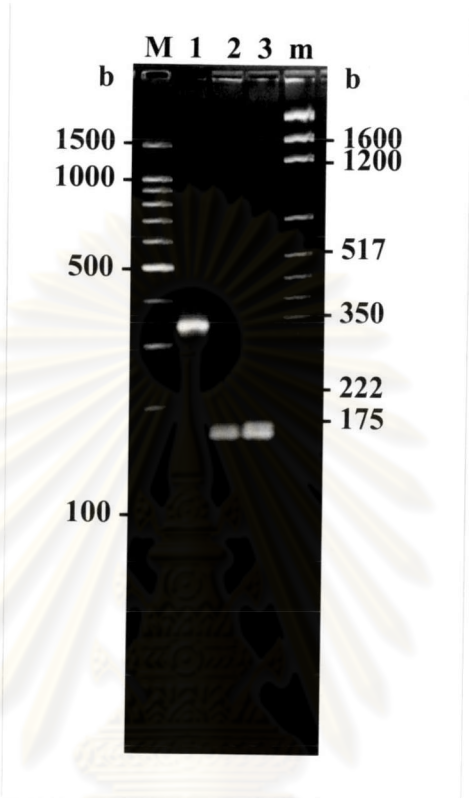

Figure 3.3 The amplified product of srRNA gene digested with *Dra*I restriction endonuclease of *A. cerana* individuals

Lane M and m = 100 bp DNA ladder and pGEM marker, respectively

Lane 1-3 = amplified products of srRNA gene of northern, southern and Samui island, respectively

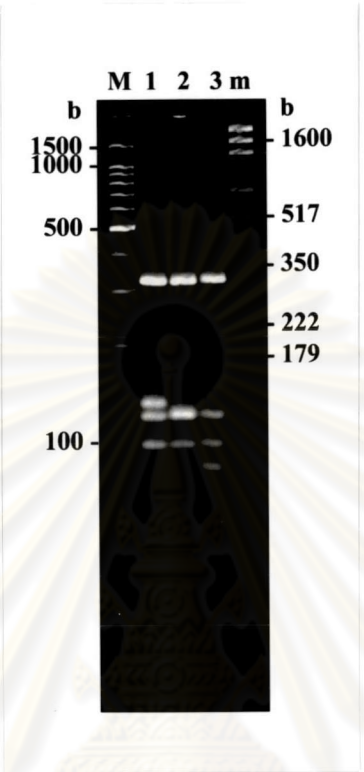

Figure 3.4 The amplified product of lrRNA gene digested with *Dra*I restriction endonuclease of *A. cerana* individuals

Lane M and m = 100 bp DNA ladder and pGEM marker, respectively

Lane 1-3 = amplified products of srRNA gene of northern, southern and Samui island, respectively

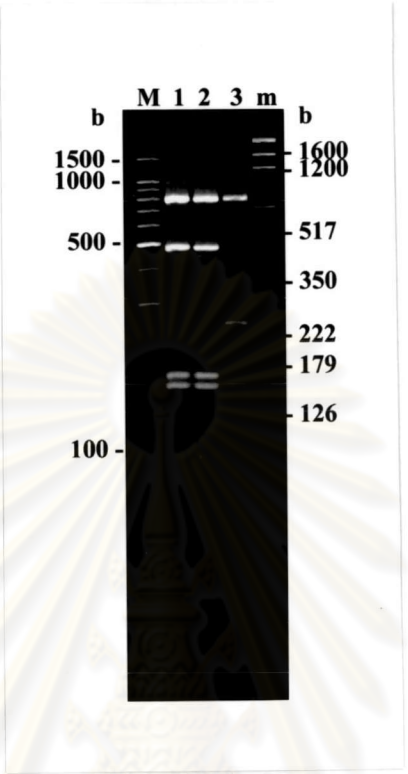

Figure 3.5 The amplified product of intergenic COI-COII region digested with *Dra*I restriction endonuclease of *A. cerana* individuals

Lane M and m = 100 bp DNA ladder and pGEM marker, respectively

Lane 1-3 = amplified products of intergenic COI-COII region of northern, southern and Samui island, respectively

3.1.2 Production of fresh royal jelly sample

Three commercial *A. mellifera* royal jelly were purchased or obtained from beekeepers and health-food stores in Thailand. Each commercial sample originated from Thailand and stored at -20 °C.

Harvested samples, *A. cerana* royal jelly were produced in Samut Songkram (hive number 1 and 2) and Bangkok (hive number 3), Chumphon (hive number 4 to 8) provinces and Samui Island in Thailand in May, 2001 and May, 2002 using modified method of Doolittle queen rearing (Doolittle, 1915). The result of royal jelly production from *A. cerana* in Samut Songkram, Bangkok and Chumphon provinces were shown in Table 3.1. Unfortunately, the production of royal jelly from 3 hives of *A. cerana* in Samui Island was not successful because they swarmed from the hives. In north and south of Thailand, the *A. cerana* had been commercially fed in managed hive while Samui Island honeybee had been fed in native hive. The frame in managed hive was separated from each other. Therefor each frame could be observed and managed easily. Unlike those for the native hive, the top of each honeybee frame was sticked to the ceiling of the hive and that made each frame could not be managed. Thus, in this study, only northern (North, North-east and Central of Thailand) and southern (South of Thailand) except Samui island honeybee hives were used for royal jelly production.

As the results shown in Table 3.1, under queenless condition, three hives of northern honeybee and five hives of southern honeybee were selected. The royal jelly was produced by the hive number 1 and 2 for three

times and hive number 3 to 8 for twice. The data showed in Table 3.1 were average values of production. After the grafting bar with cell cups (made of bee wax) was incubated in hive for 1 hour, the percentage of cup acceptance was calculated. Percentages of acceptance cup which was not destruction by worker bee of northern and southern production were 93.46± 6.59 and 100.0 %, respectively. Then, a very small amount of royal jelly and 1 day larvae were put into the incubated cell cup and transferred back to the hive. Two days incubation showed 50.16 ± 7.06 % and 52.51 ± 17.37 % of acceptable cell cups were served with royal jelly. The average amounts of royal jelly served in acceptable cups by northern and southern honeybee were 25.54 ± 5.26 and 23.63 ± 9.45 mg/cup/day.

Table 3.1 The production values of royal jelly produced by northern and southern honeybee

Sample	Hive no.	repeat	% Acceptance	% Produced cup	RJ per cup (mg/cup/day)
Northern	1	3	93.56	43.29	32.18
	2	3	86.83	57.4	25.23
	3	2	100	49.8	13.48
	Average		**93.46 ± 6.59**	**50.16 ± 7.06**	**23.63 ± 9.45**
Southern	1	2	96.43	68.52	33.11
	2	2	100	67.44	28.62
	3	2	93.33	32.14	21.12
	4	2	100	58.54	20.88
	5	2	95.12	35.9	23.98
	Average		**96.98 ± 2.97**	**52.51 ± 17.37**	**25.54 ± 5.26**

The difference between royal jelly production of northern and southern honeybees was statistical calculated using t-test. The analyzed datas shown in Table 3.2 indicated no significant difference between the production of royal jelly by these two population (P = 0.05).

Table 3.2 t-values of royal jelly production by northern and southern honeybees (*A. cerana*) in Thailand (two tail test)

	t critical	df	t Stat
% Acceptance	2.447	6	-1.066
% Produced cup	2.447	6	-0.217
Amount of royal jelly	2.447	6	-0.377

3.1.3 Chemical composition of royal jelly

A. mellifera and *A. cerana* royal jelly were used to determine the crude moisture, crude protein, acidity, ash, crude lipid and 10-hydroxy-δ-2-decenoic acid contents. Since *A. cerana* royal jelly obtained from each colony was not enough for all analysis (at least 8 grams were required). Therefore, royal jelly from the same population (northern and southern populations) was pooled and analyzed as average content values.

The results of the crude moisture determination for the *A. cerana* and *A. mellifera* royal jelly were repeated for five times and then as showed in Table 3.3. The moisture content of three brands of commercial *A. mellifera* royal jelly ranged from 61.9 to 68.2 % with mean 66.5, 64.3 and 64.7 %, respectively. The moisture content of northern and southern *A. cerana* royal jelly were less than *A. mellifera* with means 48.8 and 49.6, respectively. The datas were shown in Table 3.3.

Table 3.3 The royal jelly moisture content (%) of commercial honeybee (*A. mellifera*), northern and southern honeybees (*A. cerana*) in Thailand

	% Moisture content in *A. mellifera* royal jelly			**% Moisture content in *A. cerana* royal jelly**	
Repeat	**A**	**B**	**C**	**N**	**S**
1	67.9	67.0	65.8	52.9	49.7
2	65.3	62.7	64.9	53.5	49.6
3	66.5	63.4	64.6	45.2	49.7
4	68.2	61.9	63.8	43.5	49.7
5	64.6	66.4	64.3	48.7	49.6
Average ± SD	**66.5 ± 1.6**	**64.3 ± 2.3**	**64.7 ± 0.7**	**48.8 ± 4.4**	**49.6 ± 0.1**

The crude protein content of the royal jelly samples were determined by Kjeldahl method and were calculated by multiplying % crude nitrogen by the conversion factor of 6.25 (Haydak, 1943; Weaver and Kuiken, 1951; Lercker *et al.*, 1981). The crude protein content of *A. mellifera* royal jelly ranged from 15.7 to 16.5 %. The crude protein contents of northern and southern *A. cerana* honeybees were 21.1 and 22.6 %, respectively. The datas were shown in Table 3.4.

Table 3.4 Total crude protein content (%) of commercial honeybee (*A. mellifera*), northern and southern honeybees (*A. cerana*) in Thailand

	% Protein in *A. mellifera* royal jelly			**% Protein in *A. cerana* royal jelly**	
Repeat	**A**	**B**	**C**	**N**	**S**
1	15.6	16.4	24.2	19.4	22.4
2	16.5	20.8	24.6	18.5	24.4
3	20.3	18.3	11.6	20.6	21.9
4	12.3	24.5	10.0	21.4	22.0
5	21.7	15.1	15.5	18.9	21.6
6	14.0	14.8	15.4	21.5	22.9
7	13.9	10.3	11.8	21.8	21.7
8	15.8	12.0	12.2	18.6	23.5
Average ± SD	**16.3 ± 3.2**	**16.5 ± 4.6**	**15.7 ± 5.7**	**20.1 ± 1.4**	**22.6 ± 1.0**

The acidity was determined by titration with basis reagent (NaOH) and calculated in term of milliliter of NaOH per gram royal jelly. The royal jelly from each individual was titrated for four repeats. The acidity of *A. mellifera* royal jelly ranged from 34.8 to 42.6 ml of 1 N NaOH/ 100 g royal jelly. The acidity of *A. cerana* royal jelly ranged from 45.0 to 47.1 ml of 1 N NaOH/ 100 g royal jelly. The datas were shown in Table 3.5.

Table 3.5 The acidity (ml of 1 N NaOH/100 g royal jelly) of commercial royal jelly (*A. mellifera*), northern and southern honeybees (*A. cerana*) royal jelly in Thailand

	Acidity of *A. mellifera* royal jelly			**Acidity of *A. cerana* royal jelly**	
Repeat	**A**	**B**	**C**	**N**	**S**
1	35.5	40.1	41.1	52.8	45.8
2	36.5	45.3	37.3	41.8	44.6
3	33.4	43.2	40.3	45.7	46.5
4	33.6	41.8	38.4	48.2	42.9
Average ± SD	**34.8 ± 1.5**	**42.6 ± 2.2**	**39.3 ± 1.7**	**47.1 ± 4.6**	**45.0 ± 1.6**

Ash content of royal jelly was determined following A.O.A.C. method. Some difference between commercial and harvested royal jelly was found. Ash content of *A. mellifera* royal jelly samples ranged from 1.1 to 1.3 % whereas ash of *A. cerana* royal jelly ranged from 1.7 to 1.8 %. The data was shown in Table 3.6.

Table 3.6 Ash (%) of commercial royal jelly (from *A. mellifera*) and harvested royal jelly (from *A. cerana*)

	% Ash in *A. mellifera* royal jelly			**% Ash in *A. cerana* royal jelly**	
Repeat	**A**	**B**	**C**	**N**	**S**
1	1.4	1.4	1.0	1.8	1.8
2	1.2	1.6	0.9	1.6	1.9
3	1.1	1.2	1.2	2.1	1.4
4	1.4	1.1	1.2	1.9	1.6
Average ± SD	**1.3 ± 0.2**	**1.3 ± 0.2**	**1.1 ± 0.2**	**1.8 ± 0.2**	**1.7 ± 0.2**

Lipid contents of *A. mellifera* royal jelly and *A. cerana* royal jelly were determined as the total lipid using diethyl ether extraction in sohxlet apparatus. The lipid content of *A. mellifera* royal jelly ranged from 6.0 to 7.6 %. The lipid contents of northern and southern *A. cerana* royal jelly were 4.6 and 6.9 %, respectively (Table 3.7).

Table 3.7 The lipid content (%) of commercial honeybee (*A. mellifera*), northern and southern honeybees (*A. cerana*) royal jelly in Thailand

	% Lipid in *A. mellifera* royal jelly			**% Lipid in *A. cerana* royal jelly**	
Repeat	**A**	**B**	**C**	**N**	**S**
1	7.8	6.5	6.0	4.4	7.0
2	7.4	6.8	5.9	4.7	6.8
Average ± SD	**7.6 ± 0.3**	**6.7 ± 0.2**	**6.0 ± 0.1**	**4.6 ±0.2**	**6.9 ± 0.1**

The 10-HDA, major fatty acid component in royal jelly was quantified by gas chromatography using 5% Silicone SE-30 column. The condition was followed as described in 2.1.9 in Materials and Methods. The results were shown in Table 3.8

Table 3.8 Quantities of 10-HDA (%) contained in lipid fraction of commercial (from *A. mellifera*), northern and southern (from *A. cerana*) royal jelly

	% 10-HDA in *A. mellifera* royal jelly			% 10-HDA in *A. cerana* royal jelly	
Repeat	**A**	**B**	**C**	**N**	**S**
1	2.92	2.72	2.56	1.04	1.66
2	2.33	2.46	2.34	0.76	1
Average ± SD	**2.6 ± 0.4**	**2.6 ± 0.2**	**2.5 ± 0.2**	**0.9 ± 0.2**	**1.3 ± 0.5**

From the results above, all determination of chemical composition were concluded in Table 3.9. The significant difference of each chemical composition between northern and southern honeybees was calculated using T-test as shown in Table 3.10. There were no significant in the differences between the moisture, acidity, ash and 10-HDA (t Stat values < t Critical values). The protein and lipid components were different, significantly.

The significant differences of the royal jelly chemical composition between commercial and harvested royal jelly were also compared as same as above and shown in Table 3.11. The moisture, protein, acidity, ash and the 10-HDA were different, significantly whereas only total lipid components were not significant difference.

Table 3.9 Chemical composition of royal jelly of *A. mellifera* and *A. cerana* in Thailand

	A. mellifera			*A. cerana*	
	A	**B**	**C**	**North**	**South**
Moisture (%) (r =5)	66.5 ± 1.6	64.3 ± 2.3	64.7 ± 0.7	48.8 ± 4.4	49.6 ± 0.1
Protein (%) (r = 8)	16.3 ± 3.3	16.5 ± 4.6	15.7 ± 5.7	20.1 ± 1.4	22.6 ± 1.0
Lipid (%) (r = 2)	7.6 ± 0.3	6.7 ± 0.2	6.0 ± 0.1	4.6 ± 0.2	6.9 ± 0.1
10-HDA (%)(r =2)	2.6 ± 0.4	2.6 ± 0.2	2.5 ± 0.2	0.9 ± 0.2	1.3 ± 0.5
Ash (%) (r = 4)	1.3 ± 0.2	1.3 ± 0.2	1.1 ± 0.2	1.8 ± 0.2	1.7 ± 0.2
Acidity* (r = 4)	34.8 ± 1.5	42.6 ± 2.2	39.3 ± 1.7	47.1 ± 4.6	45.0 ± 1.6

* ml of NaOH/ 100 g royal jelly
r = replicates

Table 3.10 The differences between northern and southern honeybees royal jelly in chemical components (two tails test)

	df	**t (P =0.01)**	**t (P =0.05)**	**t Stat**
Moisture	8	3.355	2.306	-0.440
Protein	14	2.977	2.145	-4.088
Acidity	6	3.707	2.447	0.892
Ash	6	3.707	2.447	1.098
Lipid	2	9.925	4.303	13.035
10-HDA	2	9.925	4.303	-1.200

Table 3.11 Comparison of the differences between commercial (from *A. mellifera*) and harvested (from *A. cerana*) royal jelly (two tails test)

	df	t (P =0.01)	t (P =0.05)	t Stat
Moisture	23	2.807	2.069	16.551
Protein	38	2.704	2.024	-4.441
Acidity	18	2.878	2.101	-4.341
Ash	18	2.878	2.101	-5.744
Lipid	8	3.355	2.306	1.519
10-HDA	8	3.355	2.306	7.498

3.2 Part II: Cloning, screening and sequencing of cDNA of mandibular gland

The honeybee individuals from Chumphon province, Thailand, were shock-frozen in liquid nitrogen. Six hundreds of nurse bee mandibular glands were dissected from three hundreds honeybee individuals under microscope. Then, the mRNA of pooled samples was extracted using QuickPrep® Micro mRNA Purification Kit according to manufacturer's instructions (Amersham Phamacia Biotech, England). The mRNA concerntration was 137 µg/ml estimated by absorbance measurement at 260 nm (Table 3.12 and Figure 3.6). The cDNAs were first constructed by TimeSaver™ cDNA synthesis kit (Amersham Phamacia Biotech, England) following the manufacturer protocol.

Unfortunately, cDNA cloning was not succeeded. Afterwards, the second cDNA construction was performed by modifying Delta® Differential Display Kit (Clontech, USA.). The mRNA was treated with DNaseI. The first-strand

Table 3.12 Concentration and purity of mRNA from triplicate experiment

	A 260	**A260/A280**
Average	0.1714 ± 0.0003	1.8969 ± 0.1291
Total Concentration (μg/ml)	137*	

* 1 A260 = 40 μg/ml

cDNA was constructed in the reverse transcription reaction using oligo(dT) primer. The second-strand cDNA was then performed by PCR amplification using 18 combination of arbitrary and oligo(dT) primers. The obtained PCR products were reamplified by the same arbitary and oligo (dT) primers as the second-strand cDNA construction to increase amount of cDNA concentration (Figure 3.7). Then, the PCR products were loaded into 1.2% agarose gel and electrophoresed in 1X TBE buffer. After electrophoresis, the cDNAs larger than 300 bp were eluted from agarose gel using QIAquick® Gel Extraction kit (QIAGEN, Germany) and ligated to pGEM-T easy plasmid vector (Promega Co., USA). The ligated plasmids were then transformed to bacteria *E. coli*

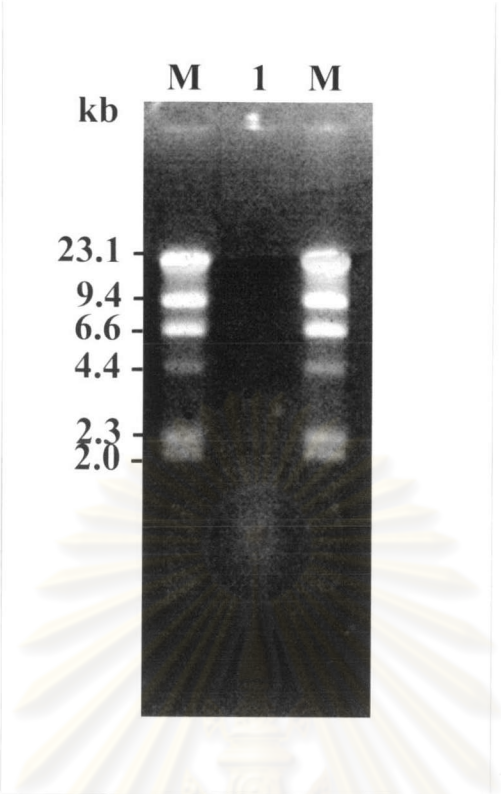

Figure 3.6 The mRNA extracted from the mandibular glands of *A. cerana*

Lane M λ/*Hin*d III

Lane 2 mRNA extracted from mandibular gland

JM 109 by electroporation. Blue/white colony screening was performed and total 7,829 white colonies were picked up. The recombinant plasmids of 3,250 colonies were randomly extracted. The size of cDNA clones were calculated by comparing with size-known DNAs on 0.8% agarose gel electrophoresis (Figure 3.8). The size of cDNA clones were ranging from 300 to 1000 base pairs. Before the sequencing, the clones were rechecked (Figure 3.9) using T7 and SP6 primers, performing the sequencing reactions and sequencing following

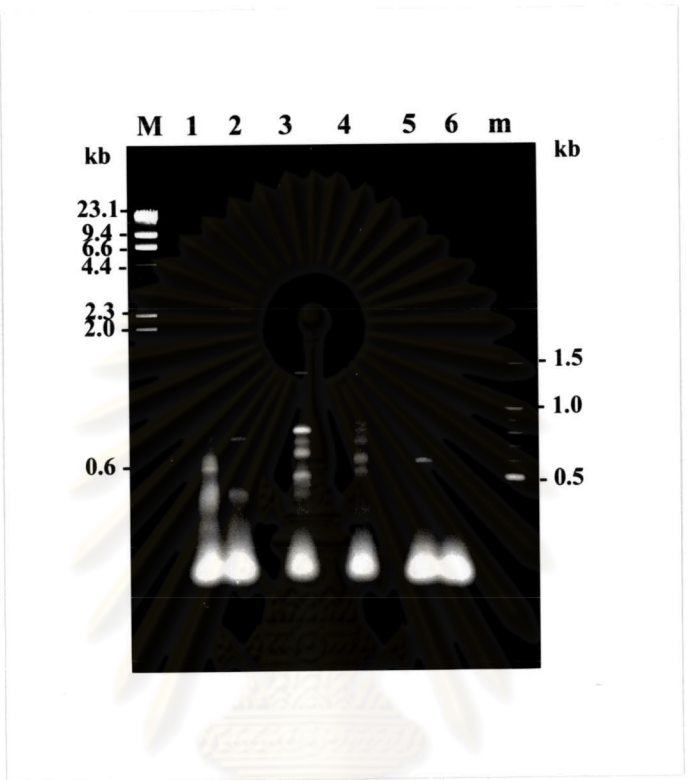

Figure 3.7 The cDNA-reamplified products by arbitrary and oligo (dT) primers

Lane M and m λ/*Hin*d III and 100 bp DNA ladder

Lane 1-6 reamplified products from various primers

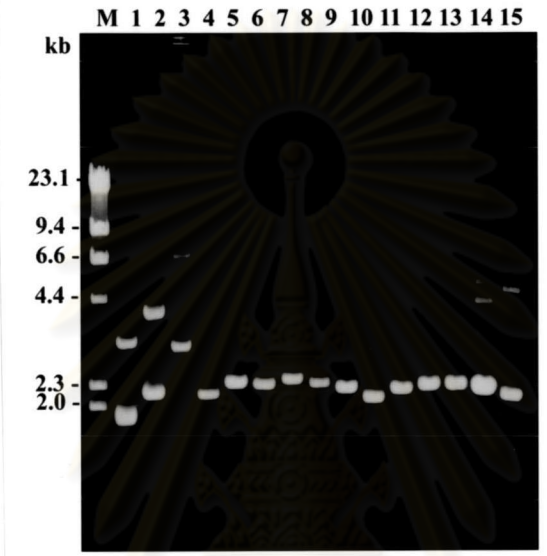

Figure 3.8 Recombinant plasmid extracted from cDNA clones on 0.8% agarose gel

Lane M λ/*Hin*d III

Lane 1-15 recombinant plasmids

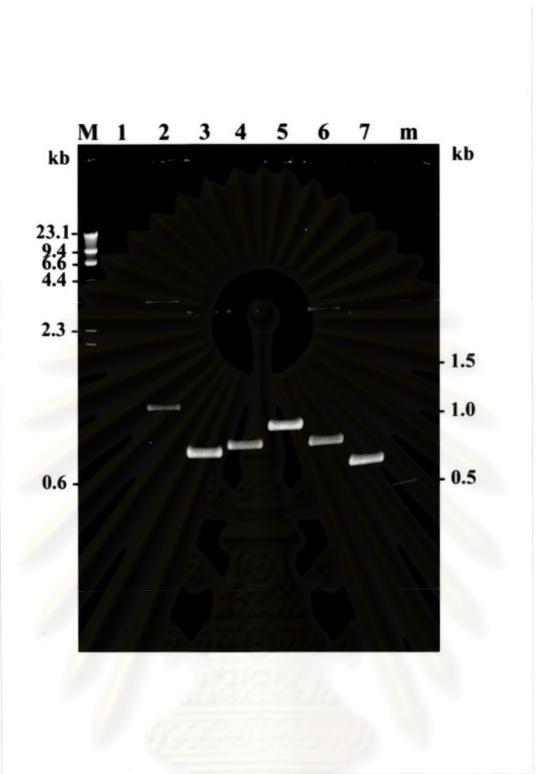

Figure 3.9 PCR of fragments inserted in recombinant plasmid using T7 and SP6 primers

Lane M and m λ/*Hin*d III and 100 bp DNA ladder

Lane 1-7 amplified products of inserts from clones

the protocol of manufacture. Two types of automated sequencer: ABIprism DNA sequencer models 377; and ALFexpress DNA sequencer, were used. The nucleotide sequences were analyzed by homology searching against GenBank DNA and protein databases using Blastn and Blastx programs through the National Center for Biotechnology Information (NCBI). The authors, on request, will provide sequence alignments used for inferring homology with known genes. A total of 110 cDNA clones were sequenced and analyzed.

The results of Blastn analysis showed that the expressed cDNA fragments were derived from 23 independent genes including some constitutive genes (Table 3.13) and results of Blastx analysis showed the protein deduced from cDNA sequences. Seventeen deduced proteins were listed in Table 3.14.

The deduced proteins were divided by function of gene expression/ regulation and protein synthesis, metabolism, internal/external mobility, signaling and communication and the unidentified function as shown in Table 3.15. The known genes and unknown genes were searched for open reading frame (ORF). The 11 (20.7%) and 16 (28.1%) genes of known and unknown genes had complete open reading frames, respectively (Table 3.16). The known genes with complete ORF were listed as deduced proteins in Table 3.17.

Table 3.13 Genes expressed in mandibular gland (Blastn)

Gene	Closest species	Accession no.	R*	%EST	E-value
2'-deoxyribonucleotide metabolism control intermediate	*Rastonia solasnacuarum*	AL646072.1	4	3.63	e-122 to e-131
3BAC RP11-56104	*Homo Sapiens*	AC074043.12	2	1.81	3e-26 to 1e-80
Alpha-satellite Clone PYAM9-60	*Homo Sapiens*	HUM18ASA	3	2.72	2e-86 to 0.0
BAC clone CTD-2053 H7	*Homo Sapiens*	AC025223.6	2	1.81	0
BAC clone RP11-93M13	*Homo Sapiens*	AC110766.5	3	2.72	0
Cellular RNA induction of interferon responsive RNAs	*Homo Sapiens*	AF026945	4	3.63	e-04 to 2e-06
Chromosome 1	*Homo Sapiens*	AC103592.2	1	0.90	0
Chromosome 16 clone RP11-96K14	*Homo Sapiens*	AC133569.4	7	6.36	0
Chromosome 5 clone RP-11-458K15	*Homo Sapiens*	AC120120.2	1	0.90	e-84
Chromosome X clone bwxD501	*Homo Sapiens*	AC004677	2	1.81	0
Chromosome X P21.1-21.3 from clone RP13-202 B6	*Homo Sapiens*	AL591625.8	2	1.81	0
Chromosome X seq. from clone RPt1-262B12	*Homo Sapiens*	AL591202.8	2	1.81	e-133
Epithelium cancer oncogene	*Homo Sapiens*	AB020875.1	1	0.90	2e-52
Genomic Chromosome 21	*Homo Sapiens*	AP00278.1	1	0.90	e-174
Genomic Chromosome 9	*Homo Sapiens*	AP006254.1	5	4.54	3e-38 to e-131
GM/1000 chromosome	*Rastonia solasnacuarum*	AL646063.1	2	0.90	5e-27
Homo Sapiens 12 BAC RP11-657 P13	*Homo Sapiens*	AC119042.9	3	2.72	5e-77 to 3e-84
Micrococcus luteus 23S rRNA gene	*Micrococcus luteus*	MLRN23S	1	0.90	e-102
Microsatellite DNA clone pEc CIR247	*Entandrophragma cylidricum*	ECY420893	2	1.81	5e-53 to 4e-75
PAC clone RP5-1039L24	*Homo Sapiens*	AC005283	3	2.72	0
putative cell division control	*Dendrobium grex Madame Thong-IN*	AF107586	4	3.63	4e-05 to 3e-05
srRNA gene clone Fn5-2	Uncultured soil fungus	AF515414.1	1	0.90	3e-07
Unknown	-	-	54	49.09	> 1e-04

* = Redundancy, $e = 10^x$

Table 3.14 Protein expressed in mandibular gland (Blastx)

Gene	Closest species	E-value	R*	%EST
Conserved hypothetical protein	*Corynebacterium efficiens* YS-314	2E-24	1	0.90
Dynein intermediate, ciliary	*Anthocidaris crassispina*	1e-33 to1e-32	6	5.45
HsdR restriction subunit	*Campylobacter jejuni*	5e-15 to 6e-15	3	2.72
hypothetical protein	-	7e-29 to 3e-5	11	10.00
LDO 2379p (cDNA)	*Drosophila melanogaseter*	7e-9 to 2e-8	6	5.45
nef attachable protein	*Aster yellow phytoplasma*	1e-11 to 2e-10	4	3.64
nef attachable protein	*Homo Sapiens*	5e-19 to 8e-16	7	6.36
p55	*Theileria orientalis*	2e-05	1	0.90
Probable lipoprotein transmenbrane	*Rastonia solanacearum*	3E-29	1	0.90
Probable pyridoxal phosphate aminotransferase protein	*Rastonia solanacearum*	2e-12	1	0.90
Probably transposase-human transposon	*Homo Sapiens*	2e-08 to 3e-08	2	1.81
Putative-hydroxamate-type-ferrisiderophore receptor signal peptide	*Rastonia solanacearum*	5e-84 to1e-72	4	3.64
reverse transcriptase honolog-human transposon	*Homo Sapiens*	1e-33 to 2e-31	2	1.81
Sodium-Potassium transporting ATPase beta-chain	*Artemia sp.*	1e-07	2	1.81
SR-related CTD associated factor	*Danio rerio*	3e-7	1	0.90
synaptojanin	*Homo Sapiens*	8e-12 to	1	0.90
Unknown	-	> 1e-4	57	51.82

*= Redundancy, $e = 10^x$

Table 3.15 Functional categories of the deduced proteins

Functional Category	Proteins	Number in Category	%EST analyzed
Gene expression, Regulation and protein synthesis	Nef attachable protein	19	17.3
	Probably transposase-human transposon		
	Reverse transcriptase homolog-human transposon		
	HsdR restriction subunit		
	SR-related CTD associated factor		
Metabolism	Putative-hydroxamate-type-Ferrisiderophore receptor signal transposon	8	7.3
	Sodium-Potassium transporting ATPase beta-chain		
	Probable lipoprotein transmembrane		
	Probable pyridixal phosphate aminotransferase protein		
Internal/External mobility	Dynein intermediate, ciliary	6	5.5
Signaling & Communication	synaptojanin	1	0.9
Unidentified function	Hypothetical protein	19	17.3
	LDO protein		
	p55 protein		
Unknown gene	-	57	51.8

Table 3.16 The number of clones with complete ORF and incomplete ORF

	Known gene	**Unknown gene**
Total clones	53	57
Number of clones with Complete ORF	11 (20.8%)	16 (28.1%)
Number of clones with Incomplete ORF	42 (79.2%)	41 (71.9%)

Table 3.17 The gene with complete ORF

Genes	**Number of clones**
Probable lipoprotein transmembrane	1
Dynein intermediate	3
Hypothetical protein	3
Putative-hydroxamate-type-ferrisiderophore receptor signal peptide	2
HsdR restriction subunit	2
Total clones	11