CHAPTER IV

DISCUSSION

It had bee reported that quality and quantity of *A. mellifera* royal jelly could be effect by the different month of sampling (Sahinler and Kaftanoglu, 2001). In this study, royal jelly were produced in May, 2001 and May, 2002 as reported in the Table 3.1. The production of royal jelly was performed in the same period each year because the royal jelly production was influenced by several factors such as the weather, source of foods, the colony size, the disease, the pest and the other disturbances (Sahinler and Kaftanoglu, 2001; Schimidt, 1997; and Sahinler and Kaftanoglu, 1997). These influenced factors affected directly to the quality and quantity of royal jelly. Then, all periods of the collecting times were limited for the neighboring condition. May was the selected time for royal jelly production because most of qualified honeybee products were harvested in this month that showed the plentiful supply of foods, the suitable weather and the healthy of the honeybee. However, the different collecting time was not significant among the time of day (Jianke, Li. *et. al.*,1993). In hot weather like in Thailand, the collecting time is better in the morning because there are a long period of time to process the larvae grafting procedure that might increase the survival larvae and higher production of royal jelly. Studied on production of royal jelly in *A. cerana* by Kawinsaeksun (1993) showed that the quantity of produced royal jelly inverted to the age of the larvae and 3-day larvae gave the highest amount of royal jelly.

Suktawoncharoenpon (2001), Sihanunthavong (1999) and Songram (1997) had studied the distribution and population structure of honeybee *A. cerana* in Thailand using PCR-RFLP on mitochondrial DNA technique. They found that the honeybee *A. cerana* in Thailand was genetically divided into 3 populations: (1) Northern honeybee (north, northeast and central); (2) southern (south); and Samui Island (Samui island). These differences of populations were also considered in the royal jelly production. But at the borderline region of each population, the mix of population was found. Thus the individuals kept from each selected colony for royal jelly production were confirmed their populations by the same method as Sihanuntavong (1999) and Suktawoncharoenpon (2001).

Eventhough environment might effect the quantity and quality of the royal jelly production. In this study, the royal jelly from all colonies was not produced in the same location or same environment. They were produced in their own suitable environment for each population. The honeybees probable produced the highest royal jelly in their own places and the highest production was needed to compare with each other that might advantaged to apply for further beekeeping. The production of royal jelly can be performed only in northern and southern honeybees because they were fed in managed hive which easier to produce royal jelly. In Samui Island, the honeybee was fed in the native hive which was difficult to produce the royal jelly. Although the management of honeybee into managed hive had been tried, it was not successful. However, Samui island population was in the same phylogenic

lineages with those southern honeybees Therefore, the result might be the same as those in Southern honeybee.

Identification of honeybee population using PCR-RFLP of *Dra*I digested 3 mitochondrial regions showed that the individual composite haplotype of Samut Songkram and Banbkok province, Chumphon province and Samui Island were AAA, BBA and BCC, respectively. These results indicated that honeybee colonies at Samut Songkram, Bangkok and Chumphon were northern, northern and southern population, respectively

Under queenless condition, the worker honeybee tried to rear the new queen. The wax cups (made of *A. cerana* wax) were inserted between the middle of wax frames and incubated in the hive for honeybee recognition. The % acceptance indicated the probability of queen rearing. As the result, high % acceptance of both northern and southern honeybee was observed that showed the perfect harmony of worker in queen construction. The produced cups were also observed in this study. At the suitable number and size of acceptable cups (about 40 cups) (Kawinsaeksun, 1993), two factors probably effect to the number of the produced cups: (1) the healthy of the individual; and (2) the number of the survival larvae between the royal jelly production. The healthy honeybee could find the sufficient foods for secretion of royal jelly, and then abundant royal jelly was served in queen cell. While the royal jelly production method had been performed, the larvae had been transferred to the queen cell by spoon which might hurt the young larvae (0-1 day larvae) more than older larvae (2-3 day larvae). Then, the 2-3 day larvae were selected for

experiment. About 50 percents of produced cups and the amount of harvested royal jelly were observed. The royal jelly production of northern and the southern honeybees were not different (P = 0.05) in % acceptance, % produced cup and amount royal jelly per cup per day.

An aimed of the study was to quantify of the major compositions of royal jelly for basis data to advance the beekeeping in Thailand and to use as the data for further related studies. Then, the royal jelly harvested from the production experiment was analyzed. The determination of royal jelly composition followed by Manual for examination of royal jelly (National royal jelly fair trade conference) and method of Association of Official Analytical Chemists (A.O.A.C). Because of the composition of royal jelly was different and vary upon the environment, the royal jelly of *A. mellifera* produced in Thailand was also determined for comparing. Three brands of commercial royal jelly of *A. mellifera* were randomly selected for analysing in this study (A, B and C). The harvested royal jelly of *A. cerana* from each hive was pooled together because the only 2-3 g of royal jelly were produced in once that was not enough to examine all chemical components.

The moisture content, the royal jelly was evaporated until sample dried which observed by the constant weight. The *A. mellifera* royal jelly contained 64 - 67 %moisture contents, approximately, while the moisture in royal jelly of northern *A. cerana* was 48.8 % and southern *A. cerana* was 49.6 %. From the observation, the moisture content was directly related to the physical appearance of royal jelly. The royal jelly of *A. cerana* had higher viscosity than

A. mellifera. However, the difference of moisture within the same species, northern and southern, was not significant but the significant difference was found when compared between species, *A. mellfera* and *A. cerana* ($P > 0.05$). Referred to the composition standard moisture value, of royal jelly as food of the Ministry of Public Health, the percentages of moisture was determined in range of 62.5 to 68.5. In commercial samples (*A. mellifera*), the percent moisture was expected to be within the standard range of royal jelly. But in the harvested samples (*A. cerana*), both northern and southern, the percentages of moisture content was below the standard range.

In the opposite, the protein content of *A. cerana* royal jelly was higher than *A. mellifera* royal jelly. Both were in range of composition standard protein value (not less than 11%) of royal jelly announced by the Ministry of Public Health. Interestingly, the protein content of northern and southern *A. cerana* royal jelly in Thailand were very high (about 20 – 21 %) and many reports showed the enrich of essential amino acids was contained in protein content of royal jelly. Considered the difference of protein, the differences were significant both within species (northern: southern royal jelly protein) and between species (*A. mellifera*: *A. cerana*) ($P > 0.05$).

For the lipid and 10-HDA contents, crude lipid extracted from the harvested royal jelly (from *A. cerana*) was compared with commercial royal jelly (from *A. mellifera*). The quantity of *A. cerana* royal jelly lipid was lower than *A. mellifera*. Same as the crude lipid, the quantity of 10-HDA in royal jelly of *A. cerana* was lower than *A. mellifera*. The *A. mellifera* 10-HDA in

royal jelly was in range of standard value (more than 1.50 mg). The royal jelly of the northern and southern populations, which lowers than standard range, were about 0.9 and 1.3 mg, respectively. Compared northern and southern royal jelly lipid, some difference was found, significantly ($P > 0.05$). The 10-HDA, a component required in standard value of Ministry of Public Health (1990), had been quantified by gas chromatography. The amount of 10-HDA in *A. mellifera* samples were higher than 1.40% (standard value for royal jelly announced by the Ministry of Public Health. But, the amount of 10-HDA in *A. cerana* samples were below the composition standard value. From test of significant, there was no significant difference among the royal jelly within the same species ($P = 0.05$). But those of differences were found at very high level between *A. mellifera* and *A. cerana* royal jelly ($P > 0.05$).

The determination of Ash and Acidity showed the no significant difference among *A. carana* samples ($P = 0.05$). The ash of *A. mellifera* was in range of ash composition standard (less than 1.5%) for medicine. The ash of *A. cerana* was too high when compared with the standard range that related to the low percentage of moisture, directly.

The basis datas of chemical composition of Thai royal jelly showed some component difference including the variance of lipid component. The southern honeybee produced higher royal jelly lipid than northern honeybee, significantly. The southern honeybees were used for screening of expressed gene.

The royal jelly was the secretion of hypopharyngeal gland and mandibular gland. The hypopharyngeal gland function was to secrete protein components in royal jelly. The mandibular gland was known as the pheromone secretor in queen. Most pheromones were the unusual cholesterols, triglycerides and carboxylic acids (Young., 1977; William and Freure., 1959). Another function of mandibular gland related to production of some chemical substances contained in royal jelly (queen and larvae food). The gland components were compared with free fatty acid in royal jelly, the mandibular gland of young honeybee had the major fatty acid component as same as the component in royal jelly (Matsuyama, *et al.*, 1998). Thus, to study on expressed genes involved in lipid metabolism, the mandibular glands were used to be the source of mRNA. In the experiment, the mRNA could be extracted from 600 mandibular glands using oligo (dT) which attached mRNA at the end of 3'-poly (A) tail. The mRNA was changed to be cDNA by TimeSaver™ cDNA Synthesis kit (Amersham Pharmacia Biotech, England). Unexpectedly, the cDNA could not be clone to the *E. coli*. The procedures were checked and the results showed the reagents were good. A reason could be probably, the cDNA fragment was not ligated to the linker. Then, the recombinant clones were not found. As described above, the aim of cDNA cloning in this experiment was to screen the genes expressed in mandibular gland. Instead, Delta™ Differential display kit (Clonetech, USA.) was selected to construct the cDNA clone. Generally, the differential display kit was used to identify RNAs that were expressed in one RNA population but missing in another (Diachenko

et al., 1996). Referred to the procedure of manufacture, after the second strand cDNA was constructed using arbitrary primer and oligo (dT) primers, only the different bands of cDNA were eluted from acrylamide gel and reamplified, ligated to vector and transformed to host cell. In the experiment, the first strand cDNA was directly reamplified and electrophoresed in agarose gel. Then, the gel was stained with ethidium bromide and the cDNA was observed under UV light. Then, all observed cDNA were eluted, ligated and transformed to host cell. From the modified procedure, all PCR products amplified with random primers were cloned and the various cDNA clones was screened and sequencing.

The sequencing results, 23 independent genes matched by Blastn and 17 proteins deduced from nucleotide sequences by Blastx. The constitutive genes such as rRNA genes, aminotransferase gene and the cell division control gene were found. Moreover, the other genes or proteins were also found. The hypothetical proteins were proteins decided by theory, but the function and properties were not known, precisely. The dynein intermediate was the major minus end-directed microtubule motor in animal cell and associated with many cargoes in conjunction with the dynaction complex. HsdR restriction subunit was the gene for restriction modification enzyme. Nef attachable protein was the pseudogene, which like serine/threonine protein kinase. It offered clues on where and when certain gene. The p55 gene was the X-linked, which encodes for the palmitoylated erythrocyte membrane protein. Its function was not exactly known, but it was speculated that it could play a part in tumor

suppression. The putative-hydroxymate-type-ferrisiderophore receptor signal peptide was the outer membrane protein receptor of ferrichrome. The synaptojanin, which containing the nerve-terminal enriched inosital 5-phosphatase, was the protein function to the synaptic vesical endocytosis. SR-related CTD was the non-snRNP (spliceosomal small nuclear ribonucleoprotein particles) splicing factor enriched with the Ser-Arg (SR) family. Because of the most study on human nucleotide, many nucleotide sequences matched to the human genes and reported as the chromosome sequence. However, most of the sequences were unknown genes or proteins because they were not matched to any genes or proteins reported in the databases. Those genes might be characterized for the further studies.