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BAHAGIAN PENYELIDIKAN & PEMBANGUNAN
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Laporan Akhir Projek Penyelidikan Jangka Pendek

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Nama Penyelidik-Penyelidik Lain (Jika berkaitan) : Dr Alexander Chong Shu Chien

2) Pusat Pengajian/Pusat/Unit : Sains Kajihayat

3) Tajuk Projek: Pencirian Protein Simpanan dalam Larva Semut isi rumah

For: *Sociobiology*

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Dietary Influence on Larval Storage Protein of the Pharaoh's ant, *Monomorium pharaonis* (L.) (Hymenoptera: Formicidae)

by

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ABSTRACT

This preliminary study reported the influence of dietary protein levels on larval storage proteins of the Pharaoh's ant, *Monomorium pharaonis*. Small *M. pharaonis* colonies of 6 queens and 150 – 200 workers (without presence of brood) were subjected either high, normal or limited protein diets and allowed to breed until they become established colonies. Larvae from each colony were sampled and divided according to their stages (L_1 – L_4). The larval homogenates were subjected to silver-stained SDS-PAGE. Results indicated that dietary protein levels affected the patterns of larval storage proteins in *M. pharaonis*. In addition, there appeared to be variation in protein storages among different larval stages. Larger larval stages (L_3 and L_4) were seen to have a higher diversity of proteins and higher absolute protein contents than those of L_1 and L_2. The possible implications of the findings on roles and responsibilities of the *M. pharaonis* larvae in a colony are discussed.

Key words: Storage protein, dietary influence, *Monomorium pharaonis*, larva

INTRODUCTION

The Pharaoh's ant is one of the world's most important tramp species. It possesses specific characters that enable them to spread through human activities and settle successfully far from their original habitat (Børgesen 1995). Tramp ants are species with small sterile workers that are usually monomorphic, widely distributed throughout the world by human activities, and often live in close association with humans. Besides that, tramp ants are also polygynous where queens are equally fertile and live unicolonial colonies. Sociotomy, or budding remains to be the main method of colony reproduction in replacement of nuptial flights (Passera 1994). However, very little information regarding diet and its effects on ant colonies is available.

In ants, a colony's dietary condition might actually have effects on the production of reproductives. Wheeler & Martinez (1995) found that patterns of resource consumption, storage and use could be an important aspect of caste specialization. A combination of egg enrichment and rich diet induces queen determination (Gösswald & Bier 1954a, 1954b; Wheeler & Martinez 1995). Food supply was also demonstrated to be an important proximate influence on sex investment where fed colonies of *Formica podzolica* were female biased and unfed colonies were male biased (Deslippe & Savolainen 1995).

The body weight of Pharaoh's ant's queen was also found to be significantly affected by the presence or absence of larvae (Børgesen & Jensen 1995). In *Leptothorax acevorum*, 93% of the queens' liquid nourishment was obtained from oral secretions from larvae (Bourke 1991). Wilson (1974) and Tschinkel (1988) also made similar observations in *Leptothorax curvispinosus* and *Solenopsis invicta* respectively.

Numerous studies have shown the importance of larva in regulating colony nutrient flow and distribution. (Wilson 1976; Børgesen 1989). In fire ants (*S. invicta*), larva plays an important role in distribution of food within the colony via different levels of interactions with its workers. (Cassill & Tschinkel 1999). Colony fecundity of Pharaoh ant (*Monomorium pharaonis* L.) was also reported to be dependent on transfer of nutrients from larvae to queens (Børgesen 2000). There seems to be a preference to feed on amino acids as compared to sucrose in fire ant larva (Cassill & Tschinkel 1999). Important storage proteins needed for colony development and metamorphosis have also been isolated and identified from larva of several ant species (Wheeler & Buck 1995). All these point to the importance of elucidating the role of ant larva in regulation of colony nutrient storage and transfer.

As a prerequisite to further understand nutrient dynamics in Pharaoh's ant, we report here a study on the effects of different levels of protein supplementation on storage protein patterns in the larvae stages under laboratory conditions.

MATERIALS AND METHODS

This project was conducted with Pharaoh's ants that have been cultured in the Urban Entomology Laboratory since 1995. Ants were separated from the original stock colony with 6 queens and 150-200 workers without presence of broods. Nine similar colonies were prepared in aluminium trays (40 x 24.5 x 8 cm) with fluon-coated inner sides.

Three colonies were subjected to a high-protein dietary treatment via feeding with proteinaceous food such as lobster cockroach (*Nauphoeta cinerea*), tuna fish, and egg yolk daily. Different types of protein foods were given alternately to avoid satiation. Another three colonies were given a limited-protein dietary treatment with proteinaceous food given only once a week. The remaining colonies were treated to a normal protein dietary regime where they were given proteinaceous food similar to those of the stock cultures, once every three days. All colonies were given 10% sucrose solution *ad libitum*.

These experimental colonies were allowed to proliferate before protein sampling. We also identified and distinguished four stages of larvae (L_1 to L_4) according to size differences, similar to the classification done by Edwards (1986).

Larvae (50-50ug) were separated and carefully placed into Eppendorf® tube, followed by homogenization in 10 µl of cold deionized distilled water, and subsequent addition of 50 µl of cold distilled water. Homogenate was centrifuged at 13,200 rpm, 4°C for 20 minutes. The resulting supernatant was transferred in a clean Eppendorf® tube and used for analysis. Protein concentration of supernatant was

carried out using Bradford method using the Model 680 Microplate Reader (BIORAD®) at 595nm absorbance.

Electrophoretic separation of muscle proteins was carried out using the denaturing SDS-PAGE method of Laemlii (1970). Briefly, supernatant was diluted in sample buffer (60mM Tris-HCl, pH 6.8; 25% glycerol, 10 % sodium dodecil sulphate, 14.4 mM 2-mercaptoethanol and 0.1 % bromophenol blue) at ratio of 5:1 (v/v). This mixture is then vortexed, followed by heating at 100^0C for 10 min and centrifugation at 12,000 rpm for 5 min. A total of 5.30 μg protein was loaded in 12.5% SDS PAGE gel followed and run at 200 V. Molecular weight markers from 10kDa to 250kDa (BIORAD®) were also loaded for molecular weight estimation of proteins. Gels were then stained with silver nitrate and scanned with Densitometer GS800 (BIORAD®), followed by documentation and band analysis with the Quantity One (BIORAD®) software.

RESULTS AND DISCUSSION

Bradford assay revealed differences in protein content among different larval stages from colonies receiving different dietary protein treatments. Results show that the L_3 stage larva samples showed the highest protein content in both limited and high protein treatments as compare to the other 3 stages. The magnitude of this difference was also higher under limited protein supply condition. There do not seem to be any significant differences in protein content between all larval stages when an intermediate supply of protein was given.

Electrophoretic profile (Figure 1-4) revealed differences in intensity of several bands, indicating changes in protein expression resulted by different dietary protein treatment in respective stages of larva. Since homogenized crushed larvae were used, our protein profile consists of mainly structural proteins and hemolymph. Among the

two, hemolymph protein content is more dynamic and readily influenced by factors such as developmental processes, temperature, water content and food quality (Mullins 1985; Consoli & Vinson 2002).

Differences in protein expressions resulting from different dietary protein treatments occur mainly in the molecular weight region of 20kDa - 75kDa for all larval stages. In L_2, L_3 and L_4 stages, expression of several proteins in this region was comparatively lower in colonies receiving limited dietary protein regime. This higher dietary proteins-higher expression trend is clearly shown in the L_4 larva stage, giving suggestion to the role of latter stages larva in handling protein-based food colony nutrient regulation. The role of larger larva in colonial nutrient distribution has been shown in this species, where queens select large larvae to feed from their stomodeal secretions (Børgesen 1989). The same study also showed that removal of these large larvae resulted in decreased egg production. During our experiments, we observed that the larger larvae were usually the first to feed from foragers returning with food particles before distributing the nutrients to the rest of the colony through trophallaxis. We postulate that larger larva helped in digestion and even enrichment of nutrients, which are essential to colonial queens. In fire ants, the foragers are responsible for regulating the flow of food from the environment into the nest while other adults and larvae regulate food distribution inside the nest (Cassill & Tschinkel 1999). More relevantly, numerous studies have shown the ability of larva to regulate colonial nutrient distribution using various factors such as larval size, hunger level and even food quality as regulators (Cassil & Tschinkel 1995, Cassill *et al* 1998).

A clear difference is seen in the L_1 stage, where highest density of protein was obtained with intermediate supply of protein (Figure 1). This was most probably due to the fact that proteinaceous food given in this study was mostly solid or semi-solid. L_1 were most probably fed with secondary protein after older larvae regurgitate it

back to more important members of the colony, i.e. the queens. An earlier experiment demonstrated that solid food primarily went to bigger larvae (L_3 and L_4) while liquid food was given to smaller larvae (L_1 and L_2). Cassill & Tschinkel (1999) also reported that fire ant larvae preferred soluble proteins (amino acids) as compare to the solid form. Figure 5 shows a photo of different stages of brood being fed with solid and liquid food respectively. Solid food was given in dyed tuna fish (blue) and liquid food was given in form of dyed sucrose (red).

Although our studies did not specifically identified the protein, the changes in expression of high molecular weight proteins in L_4 larval could be associated with the large molecular weights storage proteins which are important for metamorphosis in numerous species of insects (Levenbook 1985, Shipman *et al.* 1987) and beetles (DeKort & Koopmanschap 1994; DeKort & Koopmanschap 1987; Duhamel & Kunkel 1983; Jamroz *et al.* 1996). Holometabolous insects in particular gather large quantities of protein during larval period as storage proteins, which are normally used during metamorphosis (Wheeler & Buck, 1996). Metamorphosis in insects is a good example of a period when lack of food is coupled to a high demand for raw materials for building and remodeling tissues (Wheeler & Martinez, 1995). These proteins are accumulated in times of dietary surplus and are subsequently used during shortfalls of protein supply. The lower expression of these proteins when treated with limited protein regime in our study could be due to intensified utilization of these proteins to ensure continuous supply of proteins. Telang *et al.* (2002) also showed increased levels of storage proteins along with dietary protein levels.

Results from this study showed that larval protein profile in Pharaoh's ants varies with different levels of dietary protein. Different stages of larvae may also have difference roles and responsibility in a colony. We foresee that colony conditions may also affect these proteins. These include the presence and absence of queens. This

remains to be questionable and further experiments are needed to verify these hypotheses.

ACKNOWLEDGMENTS

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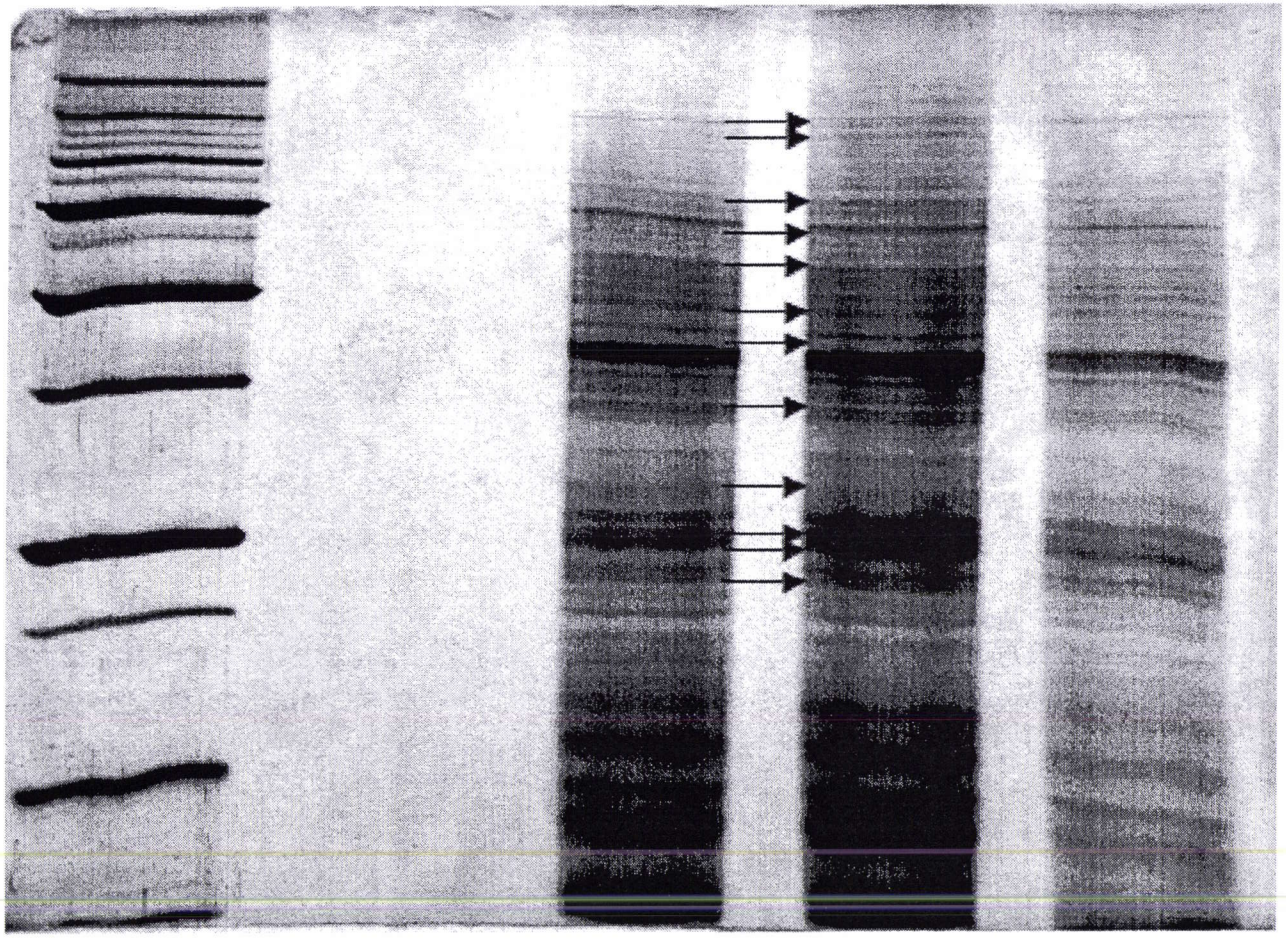

Figure 1: Protein profiles of larval stage 1 after subjected to dietary protein treatments (LP-low, NP-normal, HP-high). Arrows showed bands with changed protein expression as result of among the different dietary treatments.

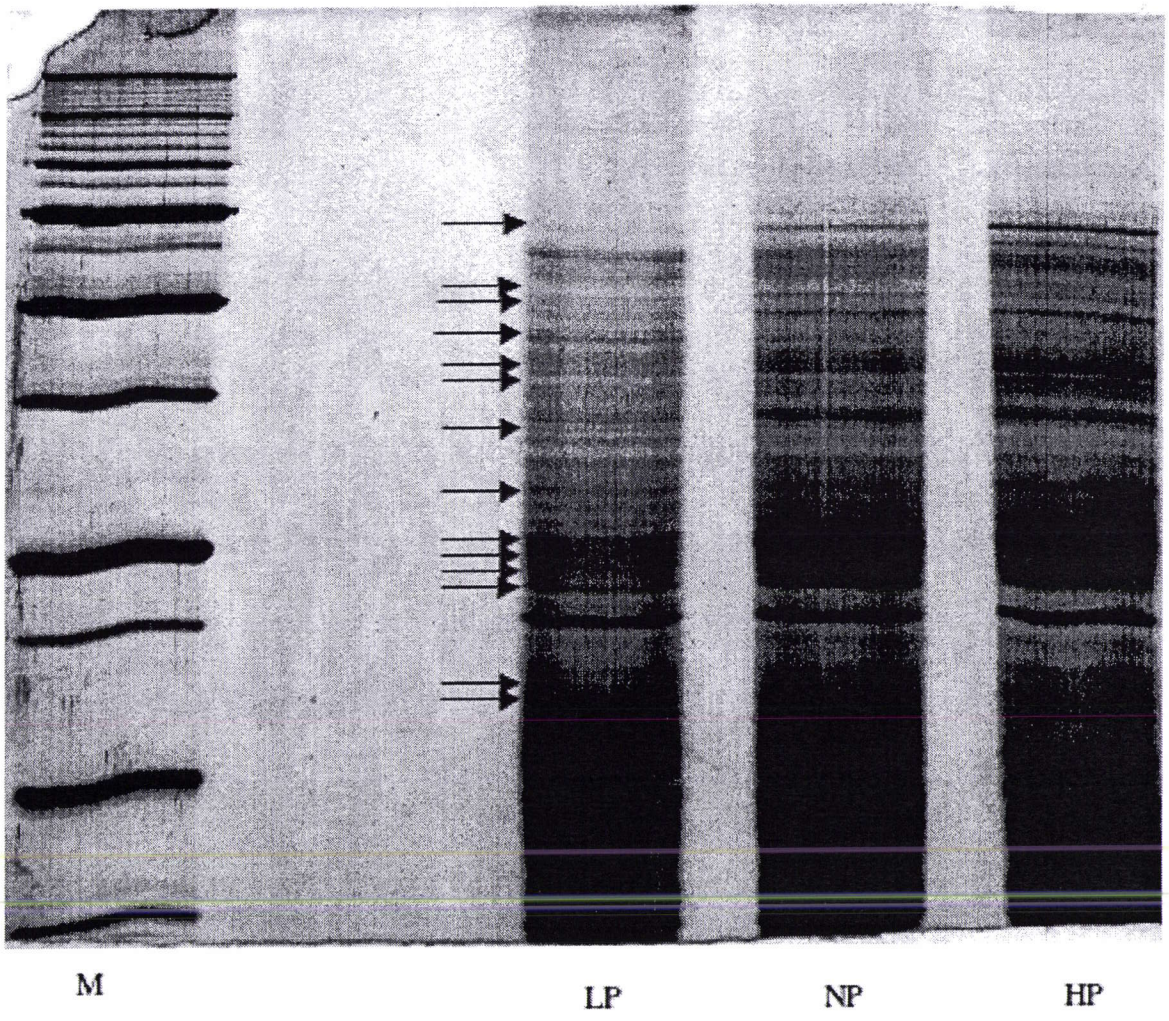

Figure 2: Protein profiles of larval stage 2 after subjected to dietary protein treatments (LP-low, NP-normal, HP-high). Arrows showed bands with changed protein expression as result of among the different dietary treatments.

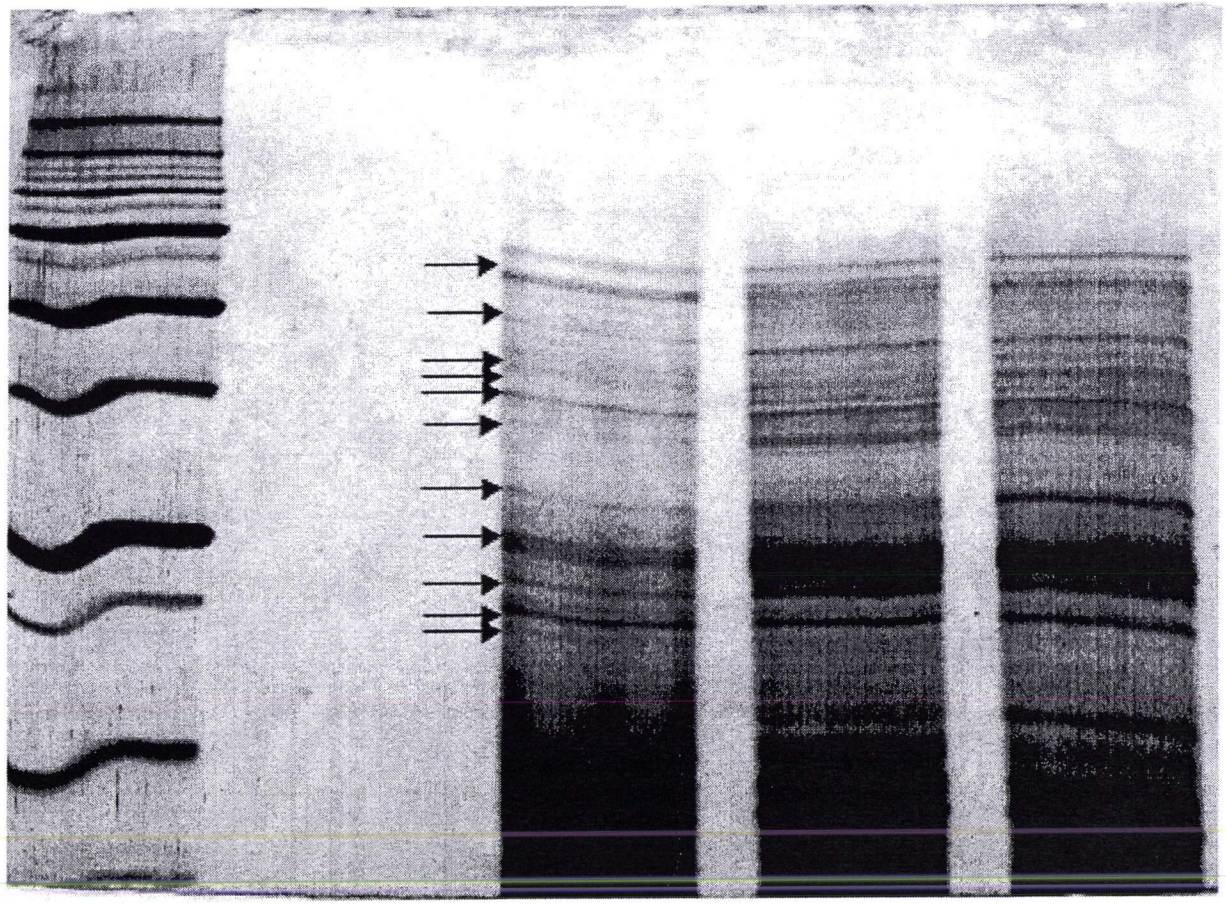

Figure 3: Protein profiles of larval stage 3 after subjected to dietary protein treatments (LP-low, NP-normal, HP-high). Arrows showed bands with changed protein expression as result of among the different dietary treatments.

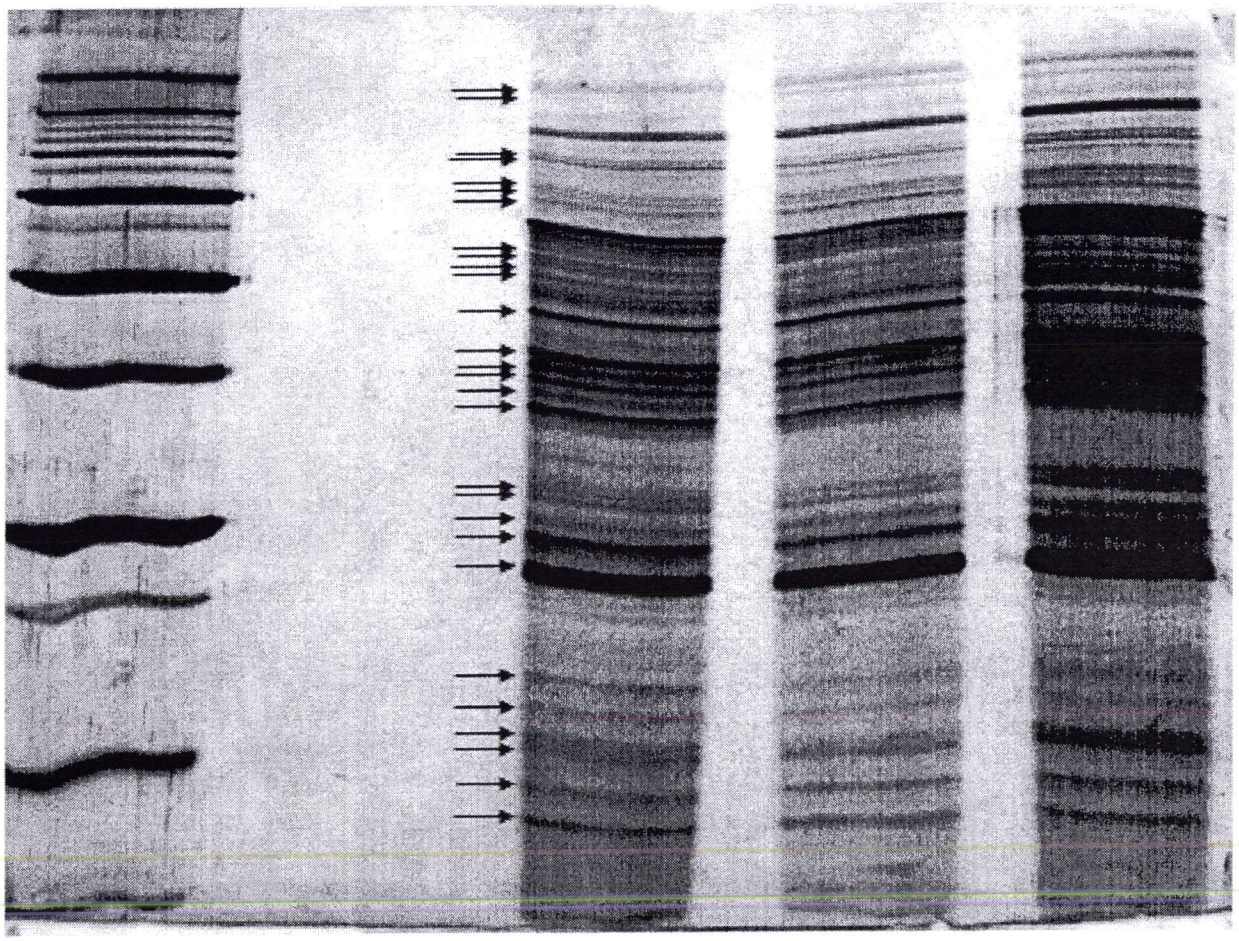

Figure 4: Protein profiles of larval stage 4 after subjected to dietary protein treatments (LP-low, NP-normal, HP-high). Arrows showed bands with changed protein expression as result of among the different dietary treatments.

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Pejabat Ketua Pustakawan

Ruj. Kami : FPP 2002/287
Tarikh : 13 Januari 2005

Profesor Poh Bo Long
Pusat Pengajian Sains Kimia

22579

Laporan Akhir Projek Penyelidikan Fundamental :
" Synthesis and Study of water – Soluble Supramolecules for Molecular Recognition of Carbohydrates"
(No. Akaun : 203/PKIMIA/670002)

Dengan segala hormatnya perkara di atas dirujuk.

Terlebih dahulu suka saya ucapkan terima kasih di atas satu salinan laporan akhir untuk projek penyelidikan fundamental ***"Sínthesis and study of water – soluble supramolecules for molecular recognition of Carbohydrates ".***

Seterusnya walaupun projek ini telah selesai, Jabatan Bendahari telah dinasihatkan untuk menangguhkan penutupan akaun projek kepada **28 FEBRUARI 2005**. Tempoh ini diberi untuk membolehkan penjelasan semua urusan tuntutan dan bayaran yang telah dikomitkan di dalam tempoh projek. Walaubagaimanapun, tuan dinasihatkan supaya tidak mengeluarkan borang-borang pesanan baru di dalam tempoh ini.

Sekian, terima kasih.

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Encik Kang Beng Chin
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Y. Bhg. Datin Masrah Hj. Abidin
Pemangku Ketua Pustakawan
Perpustakaan

] Disampaikan satu salinan
] laporan akhir projek untuk
] simpanan Perpustakaan.

Puan Zanita Zakaria
Penolong *Bendahari*
Unit Kumpulan Wang Penyelidikan
Jabatan Bendahari

] Diharap puan dapat menutup
] akaun projek tersebut mulai
] ***28 FEBUARI 2005***

/liza/laporan akhir

FINAL REPORT

A. PROJECT DESCRIPTION **Date of this report**: 26/8/04

1. **Account No: 304 670002** [ ][ ] [ ][ ][ ]-[ ] [ ][ ][ ]-[ ]

2. **Project Title: Synthesis and study of water-soluble supramolecules for molecular recognition of carbohydrates**

3. **Project Leader:** Poh Bo Long

4. **Co-researchers:** _Kang Beng Chin, Teem Chin Mean, Ainnie Rahayu bt Abdullah

5. **Starting date of project:** July 2002 [ ][ ] [ ][ ][ ][ ]
(Month)

6. **What was the duration of the project?** 24 [ ][ ] months

7. **This project was completed:** a x [ ] within the period originally proposed; or
b [ ] extended beyond the proposed period

8. **a. Original project objectives** *[As described in the application form]*

___To synthesize two water-soluble supramolecules and study their potential as carbohydrate receptors.

b. Objectives achieved *[State the extent to which the project objectives were achieved]*

_____Three water-soluble supramolecules have been synthesized. They were found to be able to act as carbohydrate receptors in water.

c. **Objectives not achieved** *[State the objectives that were not achieved and explain why]*

8. **How many man-months did the project involve?**

☐☐ Man-months

9. **What were the total project expenses?** RM 92,000.00

10. **Abstract of main findings:** *[Please describe in not more than 200 words in Bahasa Malaysia and English major/critical findings of the project. This abstract is for publication in USM's Annual Research Report. For additional space, please attach].*

Three water-soluble carbohydrate receptors have been synthesized. The first one is the chair conformer of cyclotetrachromotropylene, a cyclic tetramer (A). The other two are 1,1-bis(4,5-dihydroxy-2,7-disulfonato-3-naphthyl)ethane, tetrasodium salt (B) and 1,1-bis(4,5-dihydroxy-2,7-disulfonato-3-naphthyl)phenylmethane, tetrasodium salt (C). Compounds B and C exist as dimers, providing a cavity for encapsulation.

The three compounds complex with α-, β- and γ-cyclodextrins in an aqueous medium. The stability constant for A as host is α>β>γ_whereas for B and C as hosts the trend is β>γ>α.

d	received other sources of funding	x	
e	activated communication with other researchers, both local and international		
f	others (please specify)		

For each box 4, please give details *[such as source, amount, type/nature, masters or PhD, name of agency and/or students, countries, etc.].*

___a. training for eight final year chemistry students.
c. 2 PhD and 2 MSc students.
_____d. IRPA

C. BENEFITS OF THE PROJECT

14. Outputs of the Project and Potential Beneficiaries *[Please describe as specifically as possible the outputs achieved and provide an assessment of their potential and their significance to the advancement of knowledge in the relevant areas].*

Compound A has the potential in medical application because its boat conformer has anti-viral and anti-coagulation properties.
The ability of the non-cyclic B and C to complex with the cyclodextrins opens a search for non-cyclic compounds as receptors for carbohydrates.

15. Organisational Outcomes *[Please describe as specifically as possible the organisational benefits arising from the project and provide an assessment of their significance].*

11. **Please provide a maximum of 5 key words which describe your research project (these key words will be keyed into the university's research database).**

__supramolecules, carbohydrates, complexation, cyclodextrins

B. RESULTS AND OUTPUTS

12. **To the best of your knowledge, to which *core research activities* (i.e. *niche* research areas) at USM that your project may have contributed and/or strengthened? Please explain briefly.**

_____Our research on supramolecular chemistry is the only one in the country. We have earlier published many papers in international journals in this important area. We have also trained ten PhD/MSc students as well as many final year project students in this area.

13. **This project has** [*please 4appropriate box]*

	ITEMS	YES	NO
a	resulted in human resource training	x	
b	been patented		
c	trained post-graduate students	x	

d	received other sources of funding	x	
e	activated communication with other researchers, both local and international		
f	others (please specify)		

For each box 4, please give details *[such as source, amount, type/nature, masters or PhD, name of agency and/or students, countries, etc.].*

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15. Organisational Outcomes *[Please describe as specifically as possible the organisational benefits arising from the project and provide an assessment of their significance].*

16. **National Impact** *[Please describe as specifically as possible the organisational benefits arising from the project and provide an assessment of their significance].*

D REPORTS, PAPERS AND PUBLICATIONS

17. List of reports and conference/seminar papers written:

__1. Annie Rahayu bt Abdullah, 'Synthesis and complexation study of the conformational isomer of cyclotetrachromotropylene', MSc thesis, USM, 2004.

2. Teem Chin Mean, 'Sintesis dan kajian pengkompleksan suatu terbitan siklotetrakromotropilena', MSc thesis, USM, 2004.

3. Kang Beng Chin, 'Synthesis and complexation study of a self-assembled host, bis-(4,5-dihydroxy-2,7-disulfonato-3-naphthyl)ethane, tetrasodium salt', PhD thesis, USM, 2004.

4. B.L. Poh and C.L. Loh, paper presented at the 39th IUPAC Congress, 10-15 August 2003, Ottawa, Canada.
'Transport method for determining the stability constants of complexes with cyclodextrins (α-, β- and γ-) and starch as hosts, molecular iodine and triiodide anion as guests in water'.

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18. List of scientific publications *[including name(s) of co-author(s), date of publication, location and name of publisher. Please attach pre-print or re-print copies of the publications]*

_1. B.L. Poh and A.R. Abdullah, J. Inclu. Phenom., under review for publication.
'Isolation of the second conformer of cyclotetrahromotropylene and its complexation of alcohols and cyclodextrins'.

E. EQUIPMENT PURCHASED

19. Please list out the equipment purchased for the project

No	ITEMS	Price	Date of Purchase
1			
2			
3			
4			
5			

F. OTHER INFORMATION

20. Please provide other relevant information which you think would be useful to future research activities at USM, especially those that are related to the project which you have completed.

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21. Please provide reprint(s), galley proof(s), paper(s), or chapter(s) which should reflect the final results and findings of the research. All publications must acknowledge the grantee. A copy of all published article(s) or chapter(s) must be sent to the R&D Office.

SIGNATURE : _____