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CHARACTERIZATION OF ALLERGENS FROM DUST MITE (*TYROPHAGUS PUTRESCENTIAE*)

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CHARACTERIZATION OF ALLERGENS FROM DUST MITE
(TYROPHAGUS PUTRESCENTIAE)

By

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Hypersensitivity to dust mite allergens is one of the most common allergic reactions in the world with estimated 10% of the general population and 90% of individuals suffering from allergic asthma are sensitive to dust mites. *Tyrophagus putrescentiae* (TP) represents one of the common storage mites which has a worldwide distribution with particularly highly prevalence in tropical and subtropical regions and its explicit allergenic importance in causing mite sensitization has been well documented.

In an attempt to evaluate the allergenicity of *T. putrescentiae*, few immunological tests have been performed on *T. putrescentiae* crude extracts by using sera from allergic subjects. Dot blot screening revealed that 49.7% of 141 patient sera showed the presence of specific IgE towards TP mite components. There were at least 15 IgE binding components present in TP with molecular weights ranging from 10 to 150 kD with 15 and 77 kD appearing to be major allergens observed after immunoblotting. At the same time, the cross-reactivity studies were carried out in an effort to establish the antigenic relationship between *T. putrescentiae* and eight other mite species which is important for accurate allergy diagnosis as well as effective

immunotherapy for allergic patients. Although most of the mites' allergens share some degree of allergenic cross-reactivity or epitopes with *T. putrescentiae,* those mites somehow also contain unique allergens or epitopes with relatively low cross-reactivity with *T. putrescentiae* allergens. Also, cross-reactivity between *T. putrescentiae* and other mite allergens in this study was likely to be the result from multiple sensitizations of allergic subjects to coexisting mite species particularly the principal mite species (*Blomia* and *Dermatophagoides spp.*) in the studied environment.

Expressed sequence tags (ESTs) have led to rapid discovery of genes and has accelerated research by providing genetic materials for further investigation. This project has utilized the EST approach and resulted in the successful construction of a *T. putrescentiae* cDNA library with a titer of 1.54 x 10^7 pfu/mL. Putative mite allergens of group 2 and group 5 appeared to be the most highly abundant transcripts. EST catalogue generated from 2,305 *T. putrescentiae* ESTs clones revealed that 35% of the clones showed no significant homology to known genes in the GenBank database followed by 14% of the cDNA transcripts involved in the metabolism of the mite. It is interesting to note that, 10% of the transcripts showed significant homology to 15 groups of mite allergens (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 14, 15 and Mag 29) as well as 22 different panallergens. Hence, the ESTs approach has been demonstrated to be an excellent way of identifying new genes and successfully allowing the drafting of a gene expression profile in dust mite, *T. putrescentiae*.

In this project, *T. putrescentiae* ESTs full-length genes which were putatively identified as encoding group 5 (designated as TP 14 and TP 446) and 8 (TP 876)

allergens have been isolated from the cDNA library and their proteins have been successfully expressed in a bacterial system. In addition, full-length sequences of another isoallergen of putative group 8 (TP 215) mite allergen as well as panallergen homologues, thaumatin-like protein and aldehyde dehydrogenase were successfully obtained. However, only partial sequences of putative group 14 (M-177) mite allergen was able to be obtained. On the other hand, IgE binding profile of ten recombinant allergens from *T. putrescentiae* using 100 sera from atopic sera revealed that rTyr p 10 and rTyr p 2 have been recognized as *T. putrescentiae* major allergens by displaying high IgE binding reactivity of 80% and 60% respectively. We believed that with the efforts of isolating, characterizing and expressing *T. putrescentiae* putative allergens in this study could then facilitate the design of new immunotherapy agents for treatment of mite allergy in the future.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENCIRIAN ALERGEN DARIPADA KUTU HABUK *(TYROPHAGUS PUTRESCENTIAE)*

Oleh

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Hipersensitviti kepada kutu habuk merupakan salah satu reaksi alergi yang paling biasa berlaku di dunia dengan anggaran sebanyak 10 peratus daripada populasi secara am dan 90% daripada individu yang menderita akibat alergi asthma adalah sensitif kepada kutu habuk. *Tyrophagus putrescentiae* merupakan salah satu daripada kutu storan yang paling biasa di mana ia tersebar di merata dunia terutamanya di kawasan tropika and subtropika. Kepentingannya dalam menyebabkan sensitasi kutu habuk juga telah banyak dijalankan.

Dalam usaha untuk mengkaji kealergian terhadap *Tyrophagus putrescentiae,* beberapa ujian immunologi telah dijalankan ke atas ekstrak kasar *T. putrescentiae* dengan menggunakan serum daripada subjek alergik. Penyaringan blot bintik mendapati bahawa 49.7% daripada 141 serum pesakit menunjukkan kehadiran IgE spesifik terhadap komponen kutu TP. Terdapat sekurang-kurangnya terdapat 15 komponen TP yang bersifat pengikat IgE. Manakala, komponen TP yang berjulat dari 10 hingga 100 kD dengan 15 and 75 kD muncul sebagai alergen utama selepas kajian immunoblot dijalankan. Pada masa yang sama, kajian reaktiviti bersilang telah

dijalankan dalam usaha mengaitkan hubungan antigenik di antara *T. putrescentiae* dan lapan specis kutu-kutu yang lain. Kajian reaktiviti bersilang ini memainkan peranan yang penting dalam mendiagnosi alegi yang tepat juga untuk terapi immuno yang berkesan bagi pesakit alergik. Walaupun kebanyakan alergen daripada kutu berkongsi sebahagian daripada reaktiviti bersilang alergik atau epitop dengan *T. putrescentiae*, kutu-kutu tersebut juga menpunyai alergen tersendiri atau epitop yang mempunyai reaktiviti bersilang relatif yang rendah dengan *T. putrescentiae* alergen. Di samping itu, reaktiviti bersilang di antara alergen *T. putrescentiae* dan lapan kutu -kutu lain dalam kajian ini mungkin disebabkan daripada sensitasi berganda di kalangan subjek alergik terhadap specis kutu yang hidup bersama terutamanya specis kutu induk (specis *Blomia* dan *Dermatophagoides*) dalam kawasan kajian.

Tag jujukan ekspresi (TJE) telah menerajui penemuan gen-gen dengan pantas dan telah mendorong penyelidikan dengan membekalkan informasi genetik kepada siasatan selanjutnya. Projek ini telah menggunakan pendekatan TJE dan berjaya membina perpustakaan cDNA *T. putrescentiae* dengan titer 1.54×10^7 pfu/mL. Alergen kutu putatif daripada kelas 2 and 5 didapati muncul sebagai transkrip yang terbanyak. Lanjutan daripada itu, katalog TJE yang dijana daripada 2,305 klon TJE memberi penganggaran bahawa terdapat 35% daripada klon menunjukkan tiada pengertian kepada gen-gen yang tersedia ada di dalam pengkalan data GenBank. Selain itu, terdapat 14% daripada transkrip cDNA ini terlibat dalam proses metabolisma kutu. Daripada kajian ini, kami berjaya menemui 10% daripada transkrip yang menunjukkan homologi yang bererti terhadap 15 kelas alergen kutu (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 14, 15 and Mag 29) dan juga 22 panalergen yang berlainan. Oleh sebab itu, pendekatan ESTs tersebut telah terbukti sebagai satu cara

yang unggul dalam mengenalpastikan gen-gen yang baru dan melakar profil ekspresi gen dalam kutu habuk, *T. putrescentiae*.

Dalam projek ini, gen-gen yang berjujukan lengkap daripada *T. putrescentiae* yang dikenalpasti sebagai putatif kelas 5 (dinamakan sebagai TP 14 dan TP 446) dan 8 (TP 876) alergen telah berjaya diasingkan daripada perpustakaan cDNA dan protin masing-masing juga berjaya diekspreskan dalam sistem bakteria dengan baik. Lantaran itu, jujukan lengkap daripada satu lagi isoalergen yang merupakan putatif alergen kepada kutu kelas 8 (TP 215) dan panalergen yang menyerupai protin thaumatin dan aldehyde dehydrogenase juga diperolehi dengan berjayanya. Walaubagaimanapun, hanya sebahagian daripada jujukan lengkap alergen kutu kelas 14 diperolehi. Di samping itu, profil IgE penyikatan yang merangkumi sepuluh alergen rekombinan dengan menggunakan 100 serum daripada pesakit atopik menyatakan bahawa rTyr p 10 dan rTyr p 2 telah dikenalpasti sebagai alergen utama *T. putrescentiae* dengan mempamerkan reaktiviti IgE penyikatan yang tinggi dengan kadar sebanyak 80% dan 60% masing-masing. Kami yakin bahawa usaha pengasingan, pencirian dan pengekspresan alergen putatif *T. putrescentiae* dalam kajian ini akan memudahkan perekaan agen immunoterapi yang baru untuk alergi kutu-kutu pada masa yang akan datang.

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LIST OF ABBREVIATIONS

Symbol	Description
°C	degree Centigrade
AG	*Austroglycyphagus geniculatus*
AS	*Acarus siro*
BCIP	5-bromo-4-chloro-3-indolyl-phosphate
bp	base pair
BSA	bovine serum albumin
BT	*Blomia tropicalis*
CIE	cross-immunoelectrophoresis
CRIE	cross-radioimmunoelectrophoresis
dCTP	2'-deoxy-cytidine-5'-triphosphate
DF	*Dermatophagoides farinae*
dGTP	2'-deoxy-guanosine-5'-triphosphate
DMSO	dimethylsulphonyl oxide
DNA	deoxyribonucleic acid
dNTP	2'-deoxy-adenosine-5'-triphosphate
DP	*Dermatophagoides pteronyssinus*
DTT	dithiothreitol
dTTP	2'-deoxy-thymidine-5'-triphosphate
EDTA	ethylenediaminetetraacetic acid
EST	expressed sequence tag

EtBr	ethidium bromide
FAST	flouroallergosorbant test
GD	*Glycaphagus domesticus*
GSP	gene specific primer
His	histidine
HRP	Horseradish Peroxidase
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IPTG	isopropyl-β-thiogalactopyranoside
kD	kiloDalton
LB	Luria Bertani
LD	*Lepidoglyphus destructor*
MMLV	Moloney murine leukemia virus reverse transcriptase
mRNA	messenger RNA
NBT	nitroblue tetrazolium
NGSP	nested gene specific primer
OD	optical density
PBS	phosphate-buffered saline
PBST	phosphate-buffered saline Tween 20
PCR	Polymerase chain reaction
pfu	plaque forming unit
pI	isoelectric point

Poly-(A+)-RNA	polyadenylated RNA
RAST	Radioallerghosorbent test
RNA	ribonucleic acid
rpm	rotation per minute
RT	reverse transcriptase
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SM	*Suidasia medanesis*
SPT	skin prick test
TAE	Tris acetate EDTA
TBS	Tris-buffered saline
TP	*Tyrophagus putrescentiae*
Tris	Tris (hydoxymethyl)- aminomenthane
U	unit
V	volt
v/v	volume per volume
w/v	weight per volume
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside
α	alpha
β	beta
λ	lambda

CHAPTER 1

INTRODUCTION

There are many triggers of allergic responses like pollen, mold, biting insects, dust mites, cockroach, food, latex and certain drugs. Dust mite is a specific example of an allergy that results in asthma, rhinitis and atopic disease. Mites are normal inhabitants in our environment and play an important role in the biological recycling process by breaking down waste products of organic materials. Basically, mites can be broadly divided into two categories: the pyroglyphid mites, referred to as house dust mites, and the non-pyroglyphid mites, referred to as storage mites (eg. *Tyrophagus putrescentiae*). Mites feed on a variety of protein-rich substances, house dust mites primarily on shed human skin scales, while storage mites feed on plants and microorganisms. Mite allergens are present in mite bodies, secreta and excreta. Textile furnishings (e.g., carpets, mattresses, sofas, and curtains) are major reservoirs for mite allergens.

Since the early 1920s, mites were recognized as a possible source of allergens in house dust that causes asthmatic reactions. Reports from the surrounding Asia Pacific region such as Thailand (Malainual et al., 1995) and Indonesia (Woolcock et al., 1984) and Taiwan (Chang and Hsieh, 1989), indicated that the *Dermatophagoides spp.* is the most prevalent and predominant mites that are recognized as an important etiologic factor in allergic respiratory diseases. However, there were other studies conducted elsewhere in tropical and subtropical countries

(Hurtado and Parini, 1987; Fernandez-Caldas et al., 1993) such as Malaysia (Ho, 1986), Singapore (Zhang et al., 1997; Chew et al., 1999), have demonstrated that a high prevalence of non-pyroglyphid storage mite, *Blomia tropicalis.* In addition to that, studies in different parts of Europe (Cuthbert et al., 1979; van Hage-Hamsten et al., 1985; Terho et al., 1982; Iversen et al., 1990; Franz et al., 1997) as well as in United States (Marx et al., 1993; Campbell et al., 1989) have shown that storage mites can cause occupational allergy (Revsbesh and Andersen, 1987; Revsbech and Dueholm, 1990) among farm workers. However, since these mites are also found in homes, especially in regions with damp housing conditions (Spieksma and Spieksma-Boezeman, 1967), it is increasingly recognized that urban populations are also at risk of developing allergy to storage mites. Hence, those studies have indicated that storage mite allergens as a source of important environmental allergens and storage mite allergy as a worldwide problem.

Tyrophagus putrescentiae is one of the common storage mites that can be found worldwide, particularly in tropical and subtropical countries. However, to date, only a few studies have been performed on *T. putrescentiae* allergens and only Group 2 allergens have been well characterized. In conjunction with this, allergens from the major house dust mites, *Dermatophagoides spp.* and *Blomia spp*. have also been extensively studied. Thus, as a complement to the effort of designing a proper diagnosis for allergic diseases and immunotherapy, further characterization of the genome and proteome of *T. putrescentiae* particularly its allergenic proteins needs to be carried out.