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開發具蛇種專一性的檢驗試劑來快速檢辨台灣蛇毒種類

研 究 報 告

執行機構：中央研究院

計畫主持人：陳水田

研究人員：林力雯、董志杰

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中文關鍵字：蛇毒蛋白、二維凝膠電泳、蛋白質表現圖譜、蛋白質體學、蛋白晶片

中文摘要

在這一年執行計畫期間，已陸續完成台灣特有蛇毒二維凝膠電泳的蛋白質表現圖譜及蛋白質鑑定。在二維凝膠電泳圖譜方面，六種台灣常見蛇毒以二維凝膠電泳分離染色後，已建立各種不同條件的蛇毒的蛋白質圖譜，且經影像分析比對將特異性的蛋白質點進行蛋白質鑑定分析，也使用多維的液相層析及蛋白質晶片方法進行樣品的初步篩選。用液相管柱層析法從天然蛇毒中分離特有蛇毒蛋白，作為抗原以製造出具專一性抗體。建立固相生太合成法以快速合成足夠量的特有蛇毒蛋白技術。在蛇毒蛋白質鑑定方面，以 MALDI-TOF 的方法將更多的蛋白質鑑定出，此外亦以 MSMS 的方法找出尚未鑑定出的蛇毒蛋白。研究結果找出蛇種特有蛋白標的物後，配合以 protein chip 的初步成果，嘗試發展出具蛇種專一性的檢驗試劑來鑑別台灣毒蛇的種類。

Keyword: Snake venom protein、two dimensional gel electrophoresis、protein expression map、 Proteomics、protein chip

英文摘要

In the second year of the project, the protein expression map of Taiwan snake venoms were established by two dimensional gel electrophoresis analysis. There are some snake-specific proteins discovered in certain snake venoms by gel image analysis from six species snake venoms. We try to use multi-dimensional liquid chromatography and Lab-on-a-Chip analysis of snake venoms to screen specific protein. Using the liquid chromatography to isolate specific protein as antigen which made the specific snake venoms antibody. The rapid peptide synthetic method made more specific snake proteins. Protein stops were extracted and identified by MALDI-TOF and database searching to determine the protein identification. Now we identified more proteins by narrow pH 2D gel. And we tried to identify unknown and non-identified proteins by tandem mass spectrometry. Combination of proteomics and protein chip technologies, rapid detection kit for Taiwan snake venoms will be set up.

（1）前言

我國將從民國九十年起全面實施週休二日，未來國人將有更多的機會到野外或山林間從事休閒活動，因此被毒蛇咬到的機會也隨之大幅增加。毒蛇咬傷中毒事件，一旦被毒蛇咬傷通常解救方法均為以抗體血清中和，目前國內預防醫學研究所所製作的抗蛇毒血清有抗百步蛇毒血清、抗雨傘節及飯匙倩蛇毒血清(抗神經性)及抗龜殼花及赤尾鮶蛇毒血清(抗出血性)，在醫院就醫主要就是針對被咬傷毒蛇種類給予抗蛇毒血清，但目前國內缺乏可鑑別病患被何種毒蛇咬傷之檢驗試劑，進而施以最正確、最專一之抗毒蛇血清來進行施救，因以混合之抗血清來中和，雖然有其效果，但反應時間長且可能有過敏情形發生（Thachil et.al. 1992；Jena et.al. 1993），因此研發一種試劑可於現場快速檢測何種毒蛇咬傷之檢驗試劑有其必要性。以利用正確的蛇毒血清來加以治療。

由於蛇毒抗原相當複雜，需找到專一性強之免疫原不易，1977 年 ELISA 首次被用來檢測被毒蛇咬傷之抗原檢測用(Theakston et.al 1977)，其抗體取得來源為多株抗體。後來也有用 ELISA 來檢測不同含毒動物之毒液抗原，故免疫原理之檢測試劑應可用來鑑別不同蛇毒（Coulter et.al. 1980, Theakston et.al, 1981, Labrousse et.al. 1988, Barral-Neto et.al. 1990, Audebert et.al. 1992, Chavez-Olortegui et.al., 1993, 1994））。蛇毒種類非常多，且不同毒蛇具有相同或類似之蛇毒種類，故找到一個某種毒蛇特有之蛇毒蛋白並不易，將可利用 2-D (two dimensional gel electrophoresis) proteomics 之技術，鑑別出不同蛇毒之特定抗原，並進行胺基酸序列分析，找到專一性強之抗原後，進行動物免疫、融合瘤製備及產生專一性、敏感性俱佳之單株抗體。單株抗體之製備方法為 N. Jern, G. Kohler 及 C. milstein 等人於 1975 年

即發表(Kohler, 1975)，單株抗體可被應用於不同領域，包括檢驗試劑之開發、醫學治療、生技產品之純化、農業及畜牧業及基礎研究，文獻中也發現有蛇毒之單株抗體及酵素連結免疫檢驗試劑(ELISA) (Hale, 1999；Selvanayagam, 1999；Yang, 1999；Amuy, 1997；Chavez-Olortegui, 1997)目前發展出之毒蛇咬傷檢驗試劑有酵素連結檢驗試劑及凝集檢測試劑，其原理均為利用抗原抗體之專一結合性之免疫原理，所檢測之對象為血液，所需檢測之檢測濃度為 ng/ml，反應時間均為 2 小時以上，近年來快速層析檢驗試劑之進展相當迅速，快速層析檢驗試劑之靈敏度亦可達到 ng/ml，且反應時間可於 5 分鐘之內即知結果，非常適於應用於現場篩檢，故有其研發之意義。

另一方面，為能提昇本土蛇毒抗血清品質，減少抗血清的過敏性副作用及增強其療效，有關本土蛇毒之成份特性研究也相當重要，以新的生化技術來分析及純化各種蛇毒的成分特性，尋找出毒素的主要成份，用於馬匹免疫取血，因而開發出品質更好的抗血清產品又是另外一個重要的研究課題。

本計劃從多方面研究不同蛇毒抗原之鑑別、分析、純化及合成，首先是以新的生化分析技術 2-D proteomics 首此針對台灣六大毒蛇(眼鏡蛇、雨傘節、百步蛇、龜殼花、赤尾鮶及鎖鏈蛇)為研究對象，經由各蛇毒蛋白質分佈圖譜的差異，尋找出各毒蛇之特有蛋白質分子作為抗源，以製造出具能分辨毒蛇種類之專一性抗體。利用協同計畫主持人已有多年製備單株抗體及開發 ELISA 檢驗試劑經驗，應可順利執行並完成本計畫。以開發出具蛇種專一性的檢驗試劑來快速診斷出病患被何種毒蛇咬傷，以利用正確的蛇毒血清來加以治療。第二是經由 proteomics 分析台灣六大毒蛇之結果，各種蛇毒的成分特性亦將會有更深入了解，對未來開發出品質更好的抗血清產品將有所助益。

（2）材料與方法

IPGphor, immobline drystrips, carrier ampholytes, ammonium persulphate and TEMED were from Amersham Pharmacia Biotecth（Uppsala, Sweden）. Second dimensional gels were cased and run in a Bio-Rad Multi-Cell casting chamber and Protean II xi Cell. Acrylamide was from AMRESCO. Urea and CHEAPS were from J.T. Baker. Thaiourea was from Aldrich. Iodoacetamide and glutaraldehyde were from Fluka. Sodium acetate , formaldehyde, acetic acid and citric acid were from Merck. DTE was from AppliChem. Silver nitrate was from Mallinckrodt. Methanol was from BDH. Deionized water preparing with a tandem Milli-Q system(Millipore)was used for all buffers.Ten kinds of snake venoms had been analyzed including four snake vemons from Taiwan (***Naja naja atra , Deinagkistrodon acutus, Bungarus multicintus***, ***Vipera russelli formosensis***, ***Trimeresurus mucrosquamatus*** and ***Trimeresurus stejnegeri*****).** The crude milking snake venoms are lyophilyzed immediately and stored at -20 oC

(一)利用 proteomics 新方法直接分別鑑定並比較各種蛇毒之專一性蛋白，並且加以分門別類，尋找出專一性蛇毒蛋白作為專一性抗源，以利製造出具專一性蛇毒抗體。

1.收集台灣六大毒蛇(眼鏡蛇、雨傘節、百步蛇、龜殼花、赤尾鮐及鎖鏈蛇)之蛇毒蛇毒將從預防醫學研究所或是從民間毒蛇養殖場收集而來，量完體機後經由冷凍乾燥，收集固體並稱重並計錄後保存於 -20^{o}C 下。

2.取 0.1 毫克固體蛇毒溶解於 0.35 毫升的覆水溶液，6000 轉 30 分鐘離心後取上清液加於 18 公分 IPG(immobilized pH gradient)等電位聚焦凝膠條(pH 3-10)，於 20^{o}C 下高電壓下(8000 V)跑 100 KVh，經由

平衡溶液作用後，跑第二維 SDS-PAEG(10%-20% acrylamide)。 跑完後利用銀染(silver stain)使蛋白質顯影。經由掃瞄器將影像輸入電腦，再以影像處理軟體 Image Master 來比較各種蛇毒蛋白分佈有差異的點。

3.取 1 毫克固體蛇毒溶解於 0.35 毫升的覆水溶液，6000 轉 30 分鐘離心後取上清液加於 18 公分 IPG 等電位聚焦凝膠條(pH 3-10)，於 20^{o}C 下高電壓下(8000 V)跑 100 KVh，經由平衡溶液作用後，跑第二維 SDS-PAEG(10%-20% acrylamide)。跑完後利用 Coomassie 染色使蛋白質顯影。經由掃瞄器將影像輸入電腦，再以影像處理軟體 Image Master 來比各種蛇毒蛋白分佈有差異的點。將蛋白質點由凝膠挖出並以蛋白水解胸處理後(in gel digestion)，再利用質譜儀分析蛋白質碎片，經由比對序列資料庫以鑑定出是何種蛋白質。

4.取 1 毫克固體蛇毒溶解於 0.35 毫升的覆水溶液，6000 轉 30 分鐘離心後取上清液加於 18 公分 IPG 等電位聚焦凝膠條(pH 3-10、4-7、6-11)，於 20^{o}C 下高電壓下(8000 V)跑 100 KVh，經由平衡溶液作用後，跑第二維 SDS-PAEG(10%-20% acrylamide)。跑完後利用 Coomassie 染色使蛋白質顯影。經由掃瞄器將影像輸入電腦，再以影像處理軟體 Image Master 來比各種蛇毒蛋白分佈有差異的點。

5.再次以上述步驟跑第二維 SDS-PAEG 後，利用 electroblotting 將蛋白質轉印到 PVDF 膜上，以 amido black 染色產生蛋白質點，挖下蛇毒蛋白分佈有差異的點進行氨基酸定序，經由比對序列資料庫以鑑定出是何種蛋白質。

6.將比對找出之蛋白質進行文獻查詢以再次確定蛋白質是否為該蛇毒特有之蛋白質。

7. Protein digestion and MALDI-TOF MS analysis

Protein spots were excised，destained with 50％ Acetinitril（ACN）in 25 nM ammonium bicarbonate buffer pH8.0 and dried in a speed vacuum. The dried gel pieces were swollen in 10 μl of 25 mm ammonium bicarbonate containing 0.1 μg trypsin（Sigma）.The gel pieces were then crashed with siliconized blue stick and left at 37℃ for at least 16 h. The gels were extracted with 50％ CAN,5％TFA and dried in speed vacuum. The pellets were dissolved with 0.1％TFA and the suspended solutions were purified with Zip-Tip（Millipore,US）.The purified solutions were concentrated into a volume of 10 μl. 1.5μl of the sample were applied onto spot matrix（0.1μl）. The matrix was consisted of α-cyano-4-hydroxycinnamic acid（Sigma）dissolved in 60 ％CAN containing 0.1％TFA. The digested mixtures were analyzed by matrix assisted laser desorption ionization time of flight（MALDI-TOF）MS （Micromass,UK）. The monoscopic peptide masses were matched with the theoretical peptide masses using Protein Prospector Software. Mass tolerance windows under 50 ppm were allowed.

8. HPLC analysis of snake venoms

Analysis of whole venoms was performed using a reverse-phase HPLC column (4.6 × 250 mm, Nucleosil 7C18, USA). Each snake venoms was injected into HPLC column with the final concentration of 1 mg/ml and a gradient of aqueous solvent system using from 0 to 100 %B for 60 min at the flow rate of 1 ml/min (A = 0.1% TFA, 5% ACN; B = 0.1% TFA, 95% ACN). The absorption was monitored at 280 nm.

9. Lab-on-a-Chip analysis of snake venom

Assay reagents such as 4-20% gradient polyacrylamide gel-dye mix, denaturing solution, lower marker mix, upper marker and destaining

solution were used according to the Agilent 2100 bioanalyzer assay kit. Whole snake venoms were dissolved in 0.1 M phosphate buffer pH 6.8 to a final concentration of 3.5 mg/ml. Each sample solutions (4 μl) was combined with 2 μl of denaturing solution containing 2-mercaptoethanol and upper marker in sample buffer. The mixtures were mixed and heated at 95°C for 5 min. Lower marker (84 μl) was added to each sample. Loading of samples and running conditions were performed according to Protein 200 Assay Protocol of Agilent 2100 bioanalyzer (Agilent Technologies, Waldbronn, Germany).

(二)利用 A 蛇毒抗體親和層析法將 B 蛇毒中與 A 蛇毒抗體產生雜交反應(cross reaction)之蛋白移除，再以 proteomics 新方法鑑定殘留之 B 蛇毒以找出 B 蛇毒特有之專一性抗原。

1.首先製備蛇毒血清抗體，由預防醫學研究所獲得或是以蛇毒為抗原來製被蛇毒抗體。

2.將各種蛇毒抗體與 CNBr 活化過的凝膠進行反應，將各種蛇毒抗體分別固定於凝膠上。

3.再將固定有 A 蛇毒抗體之凝膠填充於管柱上，準備進行抗體親和層析分離。

4.將 B 蛇毒溶液流入 A 蛇毒抗體親和層析管柱，B 蛇毒蛋白質會 A 蛇毒抗體產雜交反應的蛋白質將被吸附，而未被吸附部份則是 B 毒蛇與 A 蛇毒間有所差異的蛋白質。

5.將上述 B 毒蛇與 A 蛇毒間有所差異的蛋白質，再以 proteomics 方法來鑑定出是何種蛋白質。

6.將比對找出之蛋白質進行文獻查詢以再次確定蛋白質是否為該蛇毒特有之蛋白質。

(三)利用 A 蛇毒抗原親和層析法純化抗 B 蛇毒多株抗體，排除會與 A

蛇毒抗原產生雜交反應之 B 蛇毒抗體，因而取得具 B 蛇毒種類專一性之多株抗體，以發展為檢驗試劑。

1.首先製備各種蛇毒，由預防醫學研究所獲得或是從民間毒蛇養殖場收集而來，量完體機後經由冷凍乾燥，收集固體並稱重並計錄後保存於-20^{o}C 下。

2.將各種蛇毒與 CNBr 活化過的凝膠進行反應，將各種蛇毒分別固定於凝膠上。

3.再將固定有 A 蛇毒之凝膠填充於管柱上，準備進行 A 蛇毒親和層析分離。

4.將 B 蛇毒抗體溶液流入 A 蛇毒親和層析管柱，B 蛇毒抗體會 A 蛇毒產生雜交反應的抗體將被吸附，而未被吸附部份則是僅會變認 B 毒蛇之抗體，而不會變認 A 蛇毒之抗體。

5.上述之能變認 B 毒蛇之特有抗體以相同之方法將會與其他蛇毒產生雜交反應的抗體完全去除，剩餘的抗體就是 B 蛇毒種類專一性之多株抗體，可以以此嘗試發展為檢驗 B 蛇毒之試劑。

（3）結果：

1.2-D PAGE analysis of snake venoms

Six samples of Taiwan snake venoms in familes of Elapidae and Viperidae were separated on 2D gels using non-linear gradient pH 3-10 last year（圖 1）.In this year we using narrow pH range 2D gels to study non-abundant snake venoms protein spot and detect more spots. From wide pH range two-dimensional electrophoresis (pH 3-10), snake venoms appeared to have

abundant at some area on the gels. More than one proteins are overlapped at the same area. Narrow range two dimensional electrophoresis (pH 4-7 and 6-11) were used in order to separated the proteins of closely in molecular weight and pI. These results（圖 2、3） showed we can use different kinds of pH gradient to separate more and more spots from same area on gel and need to subprepare samples. From two-dimensional electrophoresis , pattern of proteins on 2D gel of snake venoms were compared using computer software (Imagemaster) for image comparison. Snakes that have similarity in 2D image or in related species are compared such as *Tr. mucrosquamatus* and *Tr. Stejnegeri* , two gel spots matched 18.44 % (圖 4), other results silme.

We set up multi-dimensioned chromatographic methods to subprepare snake venoms and compare differ from samples or methods（圖 5）. RP-HPLC separation of *N. naja atra*, *N. naja kaoutia*, *B. multicintus*, *B. fasciatus*, *O. Hannah*, *V. usselli formoseis*, *V. russelli siamensis*, *Tr. mucrosquamatus*, *Tr. stejnegeri* and *D. acutus* venoms（圖 6）. The samples were analyzed on their ultima time. Gel-like image of snake venoms resulted from the Agilent 2100 bioanalyzer. Gel-style

display of 10 separations plus a ladder on the microchip（圖 7）.

2.MALDI-TOF MS analysis and protein identification

In the studied proteome of snake venoms, some of protein spots on 2D gel (Sypro Ruby stained) are cut and trypsin digested. In gel digested samples are subjected for MALDI-TOF mass spectrometry. Peptide mass finger prints obtained MALDI-TOF are used for data base search for protein identification from ProteinProspector(MS-fit). 2-DE image（圖 8-11）of snake venoms indicated spot position number of proteins from in gel digestion and data base search results (Table 1-4).

（4）討論：

本計畫執行一年來已利用蛋白質體學技術及多維層析等技術去篩選及分離台灣蛇毒特異性的蛋白質，並已建立固相生太合成及特異性抗體製備的系統，整體來說雖有許多問題及困難，但都不斷被克服，此外尚有其它實驗還在進行及期刊發表中來不及備載。累積的研究經驗及成果，後續計畫年度將可有效率的完成本計畫以獲的具體成果。

（5）結論：

本年度執行計畫期間，本研究室陸續完成台灣蛇毒蛋白質特異性圖譜及其蛋白質鑑定、建立固相生太合成及特異性抗體製備的系統。在二維凝膠泳分析方面，以縮短酸鹼值的範圍以期可分析更多的蛇毒蛋白質，此外亦使用多維液相層析及蛋白質晶片測定之。而在蛋白質體學分析上，將鑑定出的蛋白質的資訊收集，藉結合前年研究的蛋白質體晶片之模擬系統建立分析的模式。一旦特異性蛇毒蛋白物確定後即可配合而發展具蛇種專一性的檢驗試劑。

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圖1

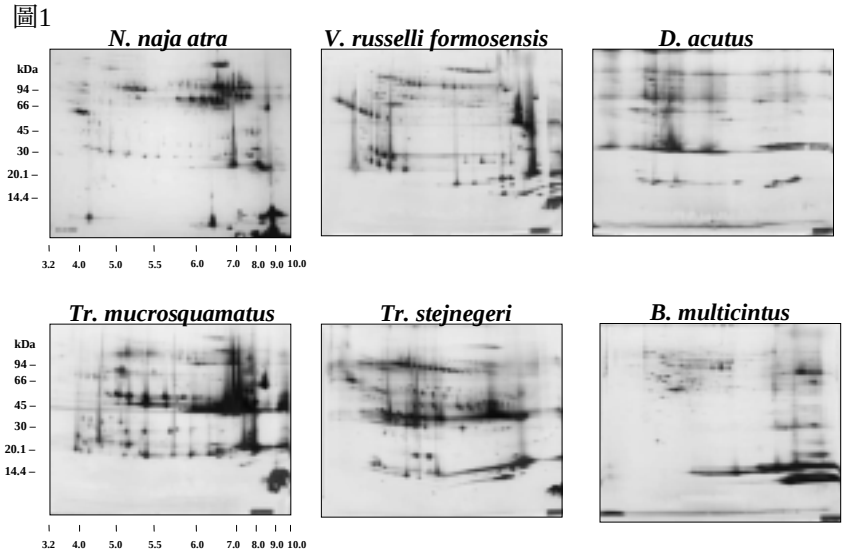

N. naja atra
V. russelli formosensis
D. acutus
kDa
94 –
66 –
45 –
30 –
20.1 –
14.4 –
3.2 4.0 5.0 5.5 6.0 7.0 8.0 9.0 10.0
Tr. mucrosquamatus
Tr. stejnegeri
B. multicintus
kDa
94 –
66 –
45 –
30 –
20.1 –
14.4 –
3.2 4.0 5.0 5.5 6.0 7.0 8.0 9.0 10.0

圖2

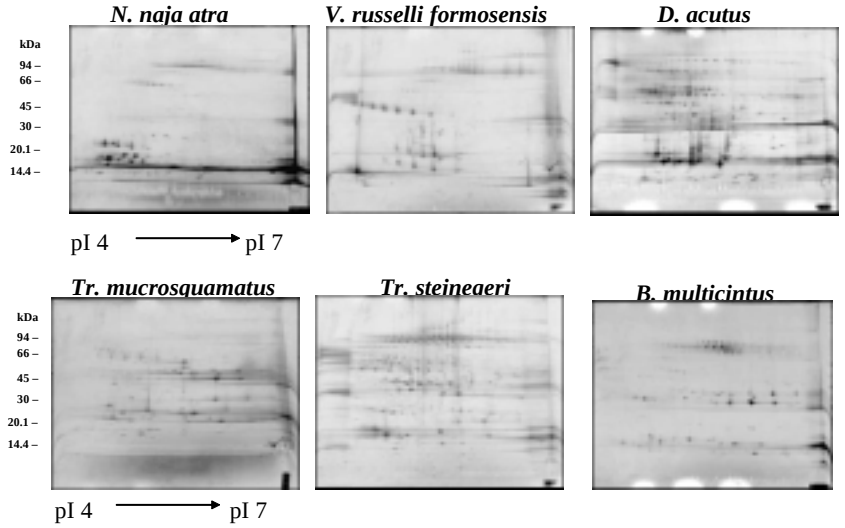
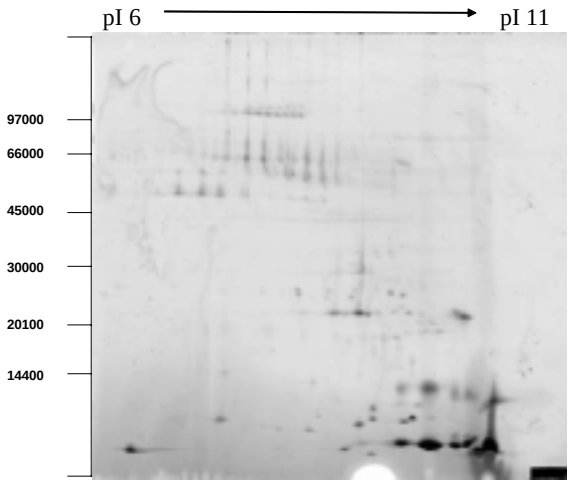

N. naja atra
V. russelli formosensis
D. acutus
kDa
94 –
66 –
45 –
30 –
20.1 –
14.4 –
pI 4
pI 7
Tr. mucrosquamatus
Tr. steinegeri
B. multicintus
kDa
94 –
66 –
45 –
30 –
20.1 –
14.4 –
pI 4
pI 7

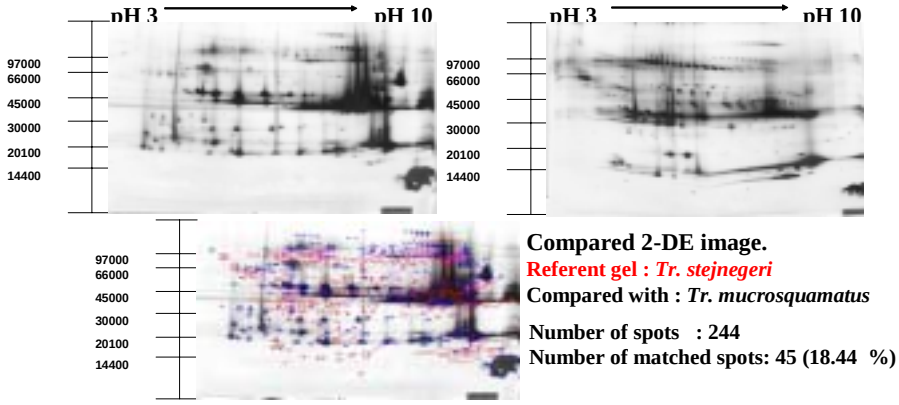
圖3



Sample; *N. naja kaouthia*

圖4

Trimeresurus mucrosquamatus ***Trimeresurus stejnegeri***



Compared 2-DE image.
Referent gel : *Tr. stejnegeri*
Compared with : *Tr. mucrosquamatus*
Number of spots : 244
Number of matched spots: 45 (18.44 %)

圖 5

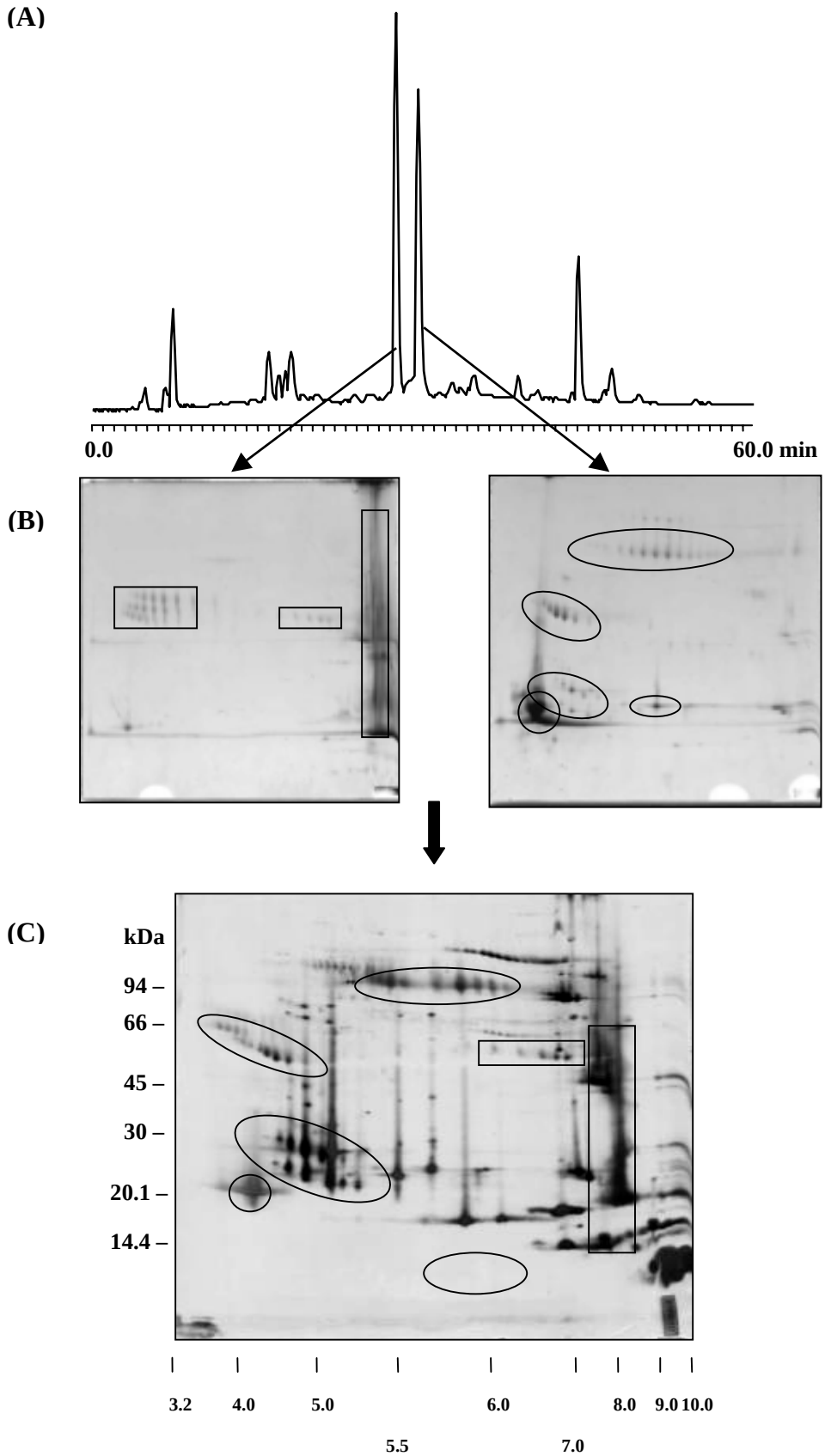


圖 6

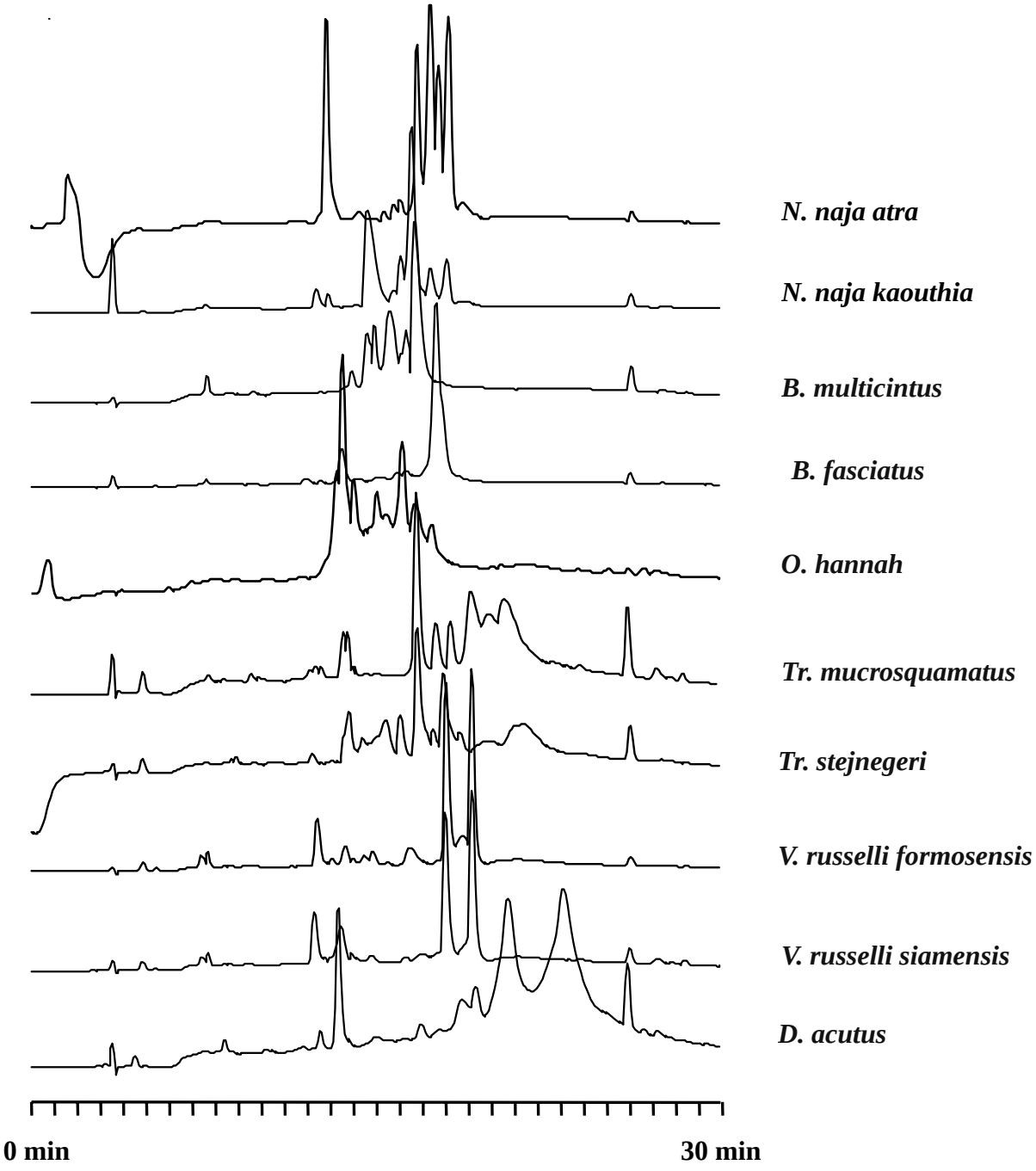
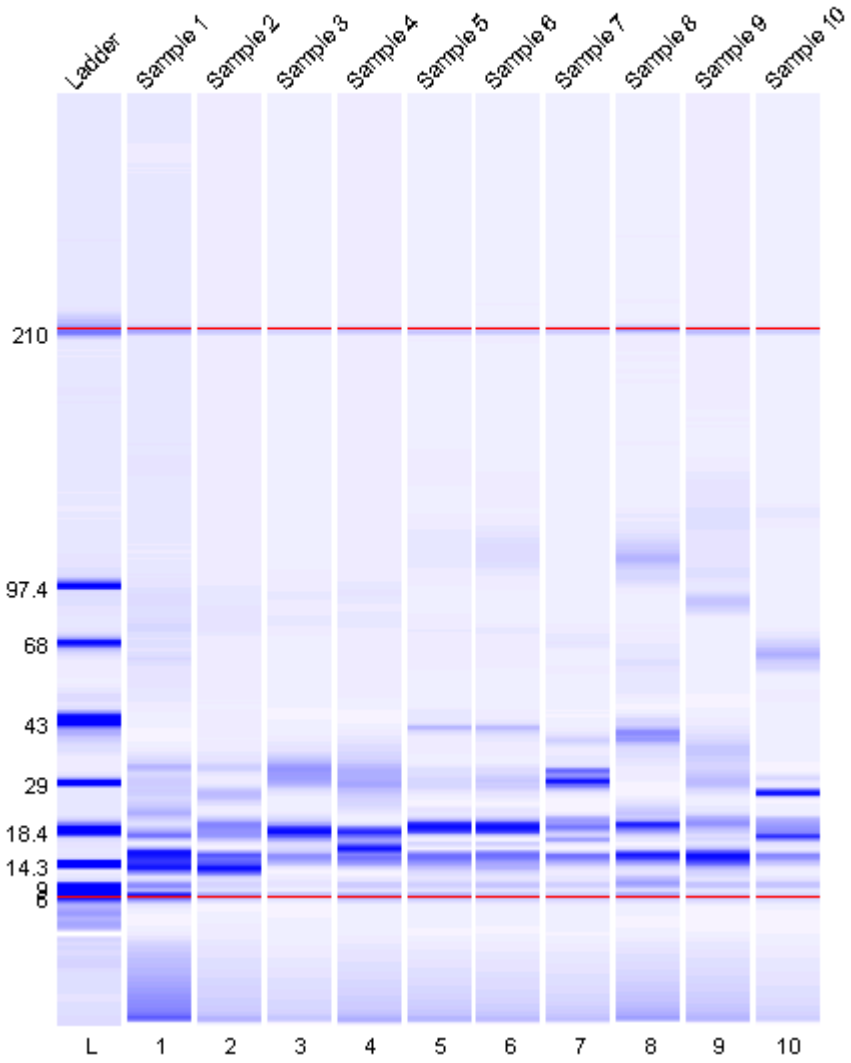

N. naja atra
N. naja kaouthia
B. multicintus
B. fasciatus
O. hannah
Tr. mucrosquamatus
Tr. stejnegeri
V. russelli formosensis
V. russelli siamensis
D. acutus
0 min
30 min

圖 7


Ladder
Sample 1
Sample 2
Sample 3
Sample 4
Sample 5
Sample 6
Sample 7
Sample 8
Sample 9
Sample 10
210
97.4
68
43
29
18.4
14.3
8
L
1
2
3
4
5
6
7
8
9
10

V. russelli formosensis

圖8

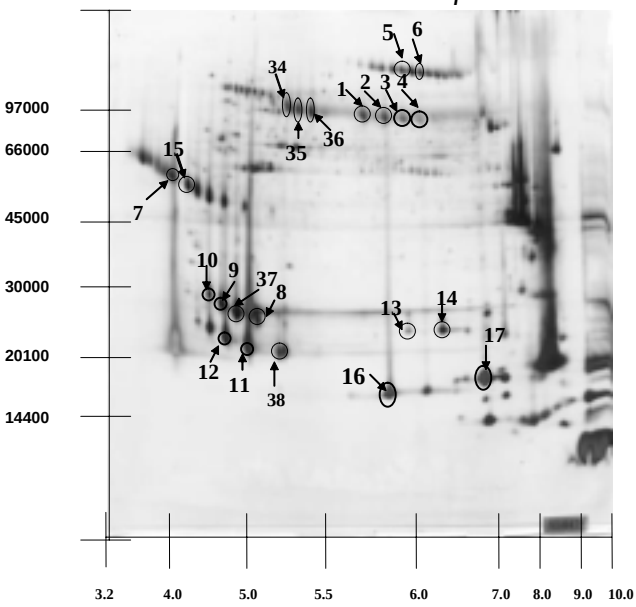


Table 1. Protein identification from data base search results (in similar to) of *V. russelli formosensis* venom .

Spot no.	MOWSE Score	Mass Matched	% Cov.	Prot. MW (Da)/pI	Accession	Species	Prot. name
1.	8.276e+005	9	20.0	96566/4.6	2499896	LACLA	ALANINE AMINOPEPTIDASE
2.	2.712e+007	15	25.0	98653/9.3	15604609	UNREADABLE	DNA POLYMERASE I (polA)
3.	6.116e+006	10	20.0	99543/8.8	16156304	UNREADABLE	disintegrin and metalloprotease domain 12 , isoform 1 preproprotein
4.	5.956e+006	14	25.0	94618/5.4	15612252 M	UNREADABLE	NADH oxidoreductase I
5.	4.841e+006	17	15.0	164669/8.7	7657269 M	UNREADABLE	K1AA0979 protein
6.	5.512e+006	11	12.0	182652/8.0	13626125	HUMAN	A disintegrin and metalloproteinase with thrombospondin motifs 9
7.	2.938e+005	11	25.0	60099/6.5	7548332	CANDIDA TROPICALIS	cytochrome P-450
8.	2.951e+004	6	55.0	17890/8.7	18538471 M	HUMAN IMMUNODEFICIENCY VIRUS TYPE 1	protease

圖9

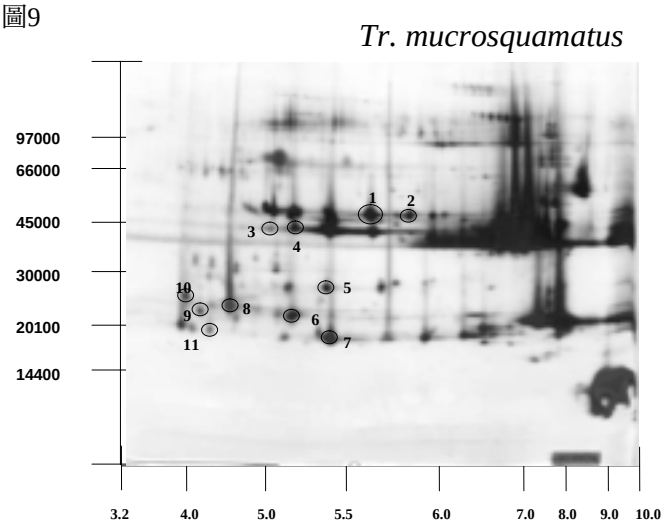


Table 2. Protein identification from data base search results (in similar to) of *Tr. mucrosquamatus* venom .

Spot no.	MOWSE Score	Mass Matched	% Cov.	Prot. MW (Da)/pI	Accession	Species	Prot. name
1.	4.259e+004	6	16.0	69249/ 7.9	5031623M	UNREADABLE	RAS guanyl releasing protein 2
2.	1571	4	11.0	74509/ 8.6	15893866M	UNREADABLE	Endo-arabinase related enzyme (family 43 glycosyl hydrolase domain and ricin B-like domain)
3.	2404	6	28.0	44747/10.1	21753225	Homo sapiens	unnamed protein product
4.	1925	4	16.0	53964/7.6	10176992	ARABIDOPSIS THALIANA	Similar to ATPase
5.	50.2	5	35.0	22457/6.2	6694971	CAPSICUM ANNUUM	putative proteinase inhibitor II
6.	845	4	22.0	20849/9.4	15611115 M	UNREADABLE	putative TYPE II DNA MODIFICATION ENZYME
7.	74.0	4	21.0	15870/8.7	9627075 M	UNREADABLE	transforming protein
8.	3127	4	12.0	75394/ 6.2	P40345	YEAST	HYPOTHETICAL 75.4 KDA PROTEIN IN AUT1-CSE2 INTERGENIC REGION
9.	2.00	6	31.0	25001/8.4	631967 M	ENCHYTRAEUS BUCHHOLZI	cysteine-rich protein CRP1
10.	8033	4	33.0	21116/9.5	22299381 M	UNREADABLE	probable peptidase
11.	5156	5	26.0	22576/5.3	17222586	MESOSTIGMA VIRIDE	NADH dehydrogenase subunit 9

圖10

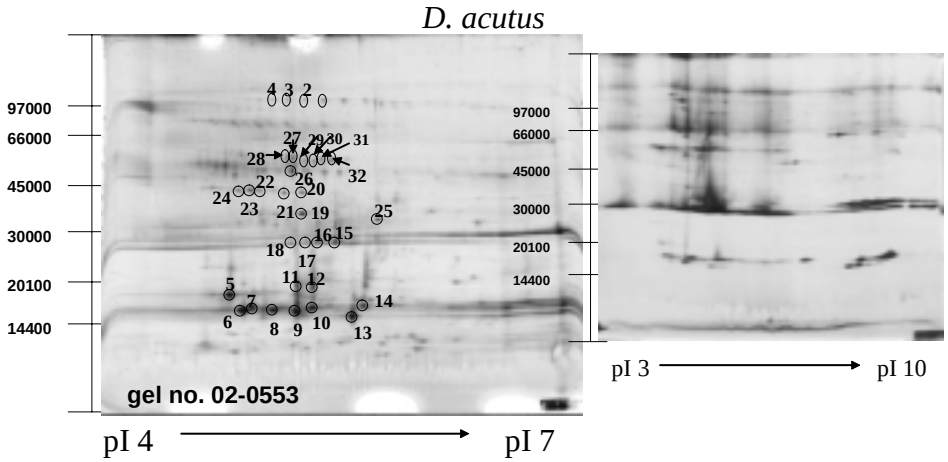


Table 3. Protein identification from data base search results (in similar to) of *D. acutus* venom .

Spot no.	MOWSE Score	Mass Matched	% Cov.	Prot. MW (Da)/pI	Accession	Species	Prot. name
2	1.67e+004	6	15.0	81708.4 / 6.30	Q05608	YERPS|7C09BDD4F9C9CBDF	Protein kinase ypkA precursor
4	8.73e+003	5	11.0	54563.5 / 6.04	Q93873	CAEEL|ACF06535338418B1	Probable NADH-ubiquinone oxidoreductase 49 kDa subunit
6	6153	4	57.0	8711/8.0	3088335	HOMO SAPIENS	ribosomal protein S2
8	594	4	40.0	14970/8.7	17482433	UNREADABLE	similar to ribosomal protein L10
10	157	4	20.0	19746/4.9	21359386	UNCULTURED PIG FAECES BACTERIUM	60 kDa chaperonin
12	2.02e+003	4	30.0	18197/5.6	P28574	MOUSE	MAX protein (MYN protein) (MYC-binding novel HLH/LZ protein)
13	839	4	40.0	15267/5.9	7446274 M	HUMAN	homeotic protein Hox 2.2
14	8135	4	40.0	18033/4.6	21693373	ENTEROCOCCUS FAECALIS	EF0119
15	3508	5	23.0	23701/5.8	20892533	UNREADABLE	hypothetical protein XP_156144
16	9412	5	14.0	68265/9.4	P55199	HUMAN	RNA polymerase II elongation factor ELL
17	1259	5	31.0	20269/6.0	11499701 M	UNREADABLE	inosine monophosphate dehydrogenase
18	6356	6	11.0	82468/5.9	Q99965	HUMAN	A disintegrin and metalloproteinase domain 2

圖11

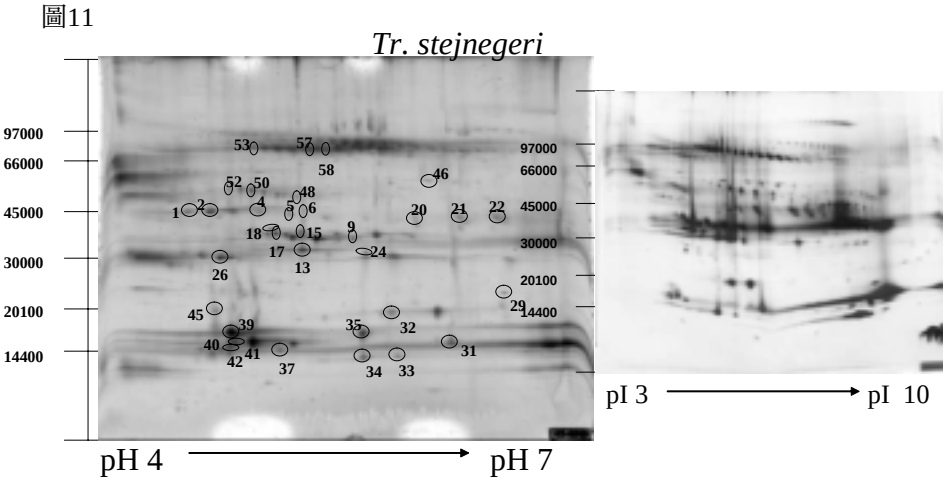


Table 4. Protein identification from data base search results (in similar to) of *Tr. stejnegeri* venom .

Spot no.	MOWSE Score	Mass Matched	% Cov.	Prot. MW (Da)/pI	Accession	Species	Prot. name
1	271	4	22.0	37572/6.6	461978 M	LYCES	(1->3)-BETA-GLUCAN ENDOHYDROLASE A
2	2478	5	19.0	47440/9.5	21626843 M	DROSOPHILA MELANOGASTER	CG30438-PA
4	4941	6	15.0	61889 /5.7	P50142	AJECA	Heat shock protein 60, mitochondrial precursor
5	2084	5	17.0	61889 /5.7	P50142	AJECA	Heat shock protein 60, mitochondrial precursor
6	7995	5	15.0	69838 /9.5	P57660	BUCAI	Chaperone protein hscA homolog
9	293	4	22.0	42531/5.6	21672267	AEROMONAS HYDROPHILA	dTDP-D-glucose-4,6-dehydratase
13	798	4	29.0	32670/6.6	17987017 M	UNREADABLE	SERINE ACETYLTRANSFERASE
15.	222	4	21.0	31850/5.0	21400637	UNREADABLE	abhydrolase
17.	567	6	14.0	37561/5.7	7673983 M	CANAL	3,4-dihydroxy-5-hexaprenylbenzoate methyltransferase
18	761	5	20.0	35889 /5.5	P13214	BOVIN	Annexin A4
20	3624	5	21.0	51996 /5.2	P48676	XENLA	Peripherin (Neuronal intermediate filament IF3)
21	4390	5	19.0	52012 /5.4	Q9CNL6	PASMU	Replicative DNA helicase
22	2767	5	20.0	40263/9.2	13736490	HUMAN IMMUNODEFICIENCY VIRUS TYPE 1	pol polyprotein
24	1155	6	29.0	29913/9.1	22788861 M	UNREADABLE	cAMP-dependent protein kinase catalytic chain 1