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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

委 託 研 究 成 果 報 告

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中文關鍵詞：蛇毒蛋白、二維凝膠分析、蛋白質表現圖譜、蛋白質體學、蛋白晶片

中文摘要

在這一年執行計劃期間，已完成台灣特有蛇毒二維凝膠蛋白圖譜、蛋白質鑑定及蛇毒蛋白 chip 之模擬系統。在台灣蛇毒二維凝膠圖譜方面：台灣六種常見毒蛇之蛇毒蛋白以二維凝膠電泳分離染色後已建立出各蛇毒的蛋白質分佈圖譜，經影像分析比對結果險示各蛇毒蛋白具有其特有之蛋白圖譜，其中雖有相互間相似之蛋白質，但仍有許多蛇種間的差異蛋白，顯示嘗試由台灣六種常見毒蛇所分泌毒液來區分是何種毒蛇之策略是相當可行的途徑。在毒蛇蛋白質鑑定方面：利用先進質譜法鑑定特定蛋白之物種是目前最靈敏且快速的方法，我們以蛋白質體學 MALD-TOF 質譜分析法進行蛇毒蛋白在二維凝膠電泳上的鑑定，首先將蛋白質點挖出，蛋白質的 trypsin 酵素水解，經萃取濃縮後點於質譜樣品板上以質譜儀收集 peptide fragment fingerprint 資料後進行生物資料庫搜尋及比對以鑑定出此蛋白質之身分。在蛇毒蛋白晶片(protein chip)之模擬系統方面：利用蛇毒抗體與蛇毒的專一性接合特性，先將蛇毒抗體點於晶片上，再與蛇毒進行專一結合，再以螢光修飾之抗體進行第二次結合，藉此以區分出不同蛇毒。結果顯示蛇毒蛋白晶片模型系統確實初步鑑別出不同蛇毒蛋白，一旦未來研究計畫找出特有蛇種蛋白標的物後，配合目前蛇毒蛋白晶片模型系統的初步成果，將可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、Proteomic、protein chip

英文摘要

In the first year of the project, the protein expression maps of Taiwan snake venoms were established by two dimensional gel electrophoresis analysis. There are some snake-specific proteins discovered in certain snake venoms as analysis by gel image analysis. This result indicates it is feasible that the species of snakes can be identified only on the basis of the specific venom protein patterns. To further identify the specie-specific venom protein, the proteomic technology has been used to characterize these proteins. Protein spots were picked from 2D-PAGE gel and in-gel digested by trypsin. Peptide fragments were extracted and identified by MALDI-TOF and database searching to determine the protein identification. On the other hand, protein chip technology has been served as a model to rapidly identify the snake venom protein species. Our results show that the tested snake venom protein chip model has been successfully installed to discriminate the differential specie snake venoms. Combination of proteomics and protein chip technologies, rapid detection kit for Taiwan snake venoms will be further studied to produce a practically useful diagnostic product for polular availability in Taiwan as well as other countries.

(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，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、赤尾鮐及鎖鏈蛇)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，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, immobiline 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 CHAPS 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.

1.收集台灣六大毒蛇(眼鏡蛇、雨傘節、百步蛇、龜殼花、赤尾鮶及鎖鏈蛇)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 20 KVh，經由平衡溶液作用後，跑第二維 SDS-PAEG(10%-20% acrylamide)。跑完後利用 SYPRO Ruby™ 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)，於 20°C 下高電壓下(8000 V)跑 20 KVh，經由平衡溶液作用後，跑第二維 SDS-PAEG(10%-20% acrylamide)。跑完後利用 SYPRO Ruby™ 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 mM ammonium bicarbonate buffer pH 8.0 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°C for at least 16 h. The gels were extracted with 50% ACN, 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. 1.5 μl of the samples were applied onto spot matrix (1.0μl). The matrix was consisted of α-cyano-4-hydroxycinnamic acid (Sigma) dissolved in 60% ACN containing 0.1% TFA. The digested mixtures were analyzed by matrix assisted laser desorption ionization time of flight (MALDI-TOF) MS on Voyager Elite (Perseptive Biosystems, Framingham, MA, USA). The monoscopic peptide masses were matched with the theoretical peptide masses using Protein Prospector Software. Mass tolerance windows under 50 ppm were allowed.

8. Specificity of snake venoms on antibodies

The snake venoms used for this experiments were obtained from *B. multicintus*, *N. naja atra*, *D. acutus*, *V. russelli formosensis*, *T. mucrosquamatus* and *T. stejnegeri*. They were tested the specificity with two polyclonal antibodies that were anti-*Bungarus* and *Naja*, and anti-*Trimeresurus*. The snake venoms were applied approximately 10 μg per well on the PVDF membrane and then blotted on to PVDF membrane using BioDot (BioRad). The membranes were incubated in blocking buffer pH 7.6 containing 5% BSA, 50 mM Tris, 150 mM NaCl and 0.1% Tween 20 for 1 h at 4°C, and washed with the same buffer

twice times. The membranes were then incubated in 10 ml of FITC-conjugated antibodies that resuspended in the same buffer (1:1000) for 1 h at the room temperature. After that, they were washed with the basal buffer and visualized using CCD camera.

9. **Protein chip model test of snake venoms**

For protein chip preparation, two kinds of polyclonal antibodies, *Bungarus* and *Trimeresurus* antibodies, were used for protein chip test. SuperAmine substrates (ARRAYIT, USA) were used as supporter for spotting the antibodies. The slides were washed and made to be an active form. Antibodies (0.25-0.5 μg/μl) were spotted onto the active slides and let it stand for 1-2 h and then rinsed with PBS buffer for three times. The reactions of antigen-antibody on slides were blocked with 5% BSA for 30 min and the slides were final rinsed with water and PBS buffer for three times. After protein chip preparation, the two snake venoms: *B. multicintus* and *Tr. Mucrosquamatus* venoms (1 μg/μl) in buffer A were added on the slides containing antibodies and incubated at 37°C for 15 min. The slides were washed with buffer A and incubated one more time with FITC-conjugated antibodies. The slides were then washed with buffer A and visualized with CCD camera.

(3)結果：

1. 2-D PAGE analysis of snake venoms

Six samples of snake venoms in the families of Elapidae and Viperidae were separated on 2-D gels using non-linear gradient pH 3-10. In the first study, all of the snake venom samples were solubilized with lysis buffer without thiourea in the IEF step. The results of 2-D gels showed that the separation of snake venoms was not good and only a few proteins were detected on 2-D map of each kind of snake venom. Some of snake venoms 2-D maps appeared horizontal streaking lines and smearing area due to no completely solubilization of the venom proteins. The proteins on 2-D maps were not thus identified. Otherwise, the protein compositions in snake venoms were abundant and more complex that was easily to aggregate when they were run on 2-D PAGE. Moreover, we improved the separation of snake venoms on 2-D PAGE and succeed in use of thiourea to solve the problem of solubilization. Thiourea was used at concentration of 2 M in conjugate with 7 M urea and was sufficient to demonstrate greatly improve solubility compared with standard IEF solution without thiourea. The 2-D maps of the snake venoms presented more abundant of proteins and well separation (圖一至六). 2-DE revealed unique and complex patterns of venom proteins in each species. Snake venom compositions have pI values from acidic to highly basic. The morphology of the spots from the gels appeared to be circular-oval, line and tailing spot shapes.

In the family of Elapidae, the snake venoms in the same species such as *N. naja atra* and *N. naja kaouthia* had the nearly same patterns of 2-DE maps. The difference between the 2-DE maps was the number of protein spots and/or

abundance of proteins in the same area. At the molecular weight area about 30-40 kDa, the 2-DE map of *N. naja kaouthia* venom showed the different protein pattern that had more spots of proteins than *N. naja atra*. The 2-DE maps of *B. multicintus* and *B. fasciatus,* which were different in species of the genus *Bungarus,* were also similar and had some differences of proteins in a range of acidic pI value. At the acidic area, *B. multicintus* had many proteins more than *B. fasciatus* and the 2-D map was clearer. They have the lines of dark stain at the basic pI value nearby the low molecular weight that indicated the aggregation of some proteins. In contrast, they had no appearance of proteins in a range of molecular weight between 20 to 40 kDa. Otherwise, the 2-DE maps of *O. Hannah* venom showed the most abundance of protein compositions among the snake venoms in the same family. Its molecular weights and pI values of protein compositions were widely distributed than the other snake venoms but the 2-DE map also showed the aggregated peak areas indicating the aggregation of proteins.

In the family of Viperidae, *V. russelli siamensis* and *V. russelli formosensis* venoms had very similar in 2-DE maps but also showed the differences in some details such as the apparent of different proteins at the low molecular weight nearly to the basic area. The snake venoms in the genus *Trimeresurus*, *Tr. mucrosquamatus* and *Tr. Stejnegeri*, had significantly different patterns in 2-DE maps. The two snake venoms had many different kinds of proteins, which were varied to molecular weight and pI value. However, they had also the dense areas of aggregated proteins. For *D. acutus* venom, only few of proteins were separated and it had the dark line of proteins in the middle of 2-DE map because of underfocusing in IEF step. The separation will be better if

it was prolong the focusing time. However, its 2-DE map was different from the others n the same family. Therefore, the 2-DE patterns in the snake venoms showed a difficulty for separation when the same conditions for 2-DE were used.

The differences in protein profiles of snake venoms on 2-D gels may be caused by the genetically differences in different family, genus and species, or other factors. Snake venoms are composed of the extracellular proteins, which the synthesis is influenced by the change of environment conditions. So, the environmental stimuli had effects in the synthesis of some protein compositions. Otherwise, the geographical difference is one of the important factors that effects on variations in venom compositions, venom properties and biochemistry.

Trains of spots were noted in protein profile of 2-DE map of most kinds of snake venoms. The trains of spots presenting on 2-D gels may indicate the isomorphs, post-translational modification of some proteins or degradation components. There always have the identification of isoforms and new proteins in snake venoms such as various isoforms of phospholipase A_2 in the same venom. The isoforms of the same protein were usually resulted from the post-translational modification such as methylation or phosphorylation or the change of some low conserved amino acids in protein sequences. It is possible that the post-translational modifications occur to be a high rate in snake venom and all protein spots did not represent a unique protein. It is also possible that there are gradually change in the biological activity of gene expression of many proteins in snake venoms. These show the complex nature of snake venoms. The variation of biological properties of phospholipase A_2, which has highly in

sequence homology, is an example. 2-D PAGE is a present method of protein identification that allows to separate and analyzed protein isoforms.

All kinds of snake venoms also showed the complex clusters of spots on 2-D profiles indicating that the complex interaction of proteins had closely in molecular weights and pI values. The proteins had affinity to each other and did not separate well even in the strongly denaturing conditions. For the Elapidae snake family, there was evidenced that the venom proteins accumulated preferentially in the basic part of the gels and especially on the low molecular weight area. The separation of basic proteins had long been difficult via 2-DE and the basic proteins were under presented in almost referent mapping database. For the Viperidae snake family, the dark stained area was presented at high pI values with moderated molecular weight area of *Tr. mucrosquamatus* venom whereas *Tr. stejnegeri* and *D. acutus* venoms were found on the wide ranges of pI values with the moderated molecular weight areas. Therefore, the results of 2-DE gels showed a high throughput of the method in separating proteins mixture of snake venoms. However, it still has a limitation for the separation of some kinds of proteins such as a mixture of small proteins having highly hydrophobic and basic nature.

2. MALDI-TOF MS analysis and protein identification

For protein identification, we use the gel digestion and MALDI-TOF MS analysis to identify some of proteins in snake venoms from *N. naja atra*, *N. naja kaouthia*, *Tr. mucrosquamatus* and *Tr. stejnegeri* that separated on 2-DE maps. The mass fingerprints were used to search proteins using Protein Prospector

Software and some of proteins could be identified (圖七至九). From previous reports of the studied of proteins from snake venoms, many kinds of enzymes and toxins were noted. Proteins that have been purified to see the molecular weight, pI, and amino acid compositions as well as studied in their biological activities were identified at the low molecular weight. Data based of proteins identifying from snake venoms were still limited. From 2-DE maps of snake venoms presented in this study, it was found that the low molecular weight proteins which were presented as a limit number in protein data based not easily be identified because of their aggregation.

3. Specificity of snake venoms on antibodies and protein chip model test

The two polyclonal antibodies were used for testing the specificity of 6 kinds of venoms. The anti-*Bungarus* and *Naja* were used for defining the snake venoms that specified to the hemotoxic venoms and the anti-*Trimeresurus* was used for defining the snake venoms that specified to the neurotoxic venoms. The venoms from *B. multicintus* and *N. naja atra* in Elapidae family showing neurotoxicity had the high specificities with the anti-*Bungarus* and *Naja* whereas little cross-reactivity to anti-*Trimeresurus* (圖十). The venoms from *Tr. mucrosquamatus* and *Tr. stejnegeri* in Viperidae family had the specificity with the anti-*Trimeresurus* and also showed a little cross reactivity to anti-*Bungarus* and *Naja*. The venom from *D. acutus* had the specificity with the anti-*Trimeresurus* higher than anti-*Bungus* and *Naja* whereas the venom from *V. russelli siamensis* had a low specific binding to both antibodies. These results indicated that the snake venoms had the different toxic compositions even though in the same family. Although the snake venoms show a basic toxicity as

hemotoxic or neurotoxic but they also have cross reactivity to another toxic property. From the specificity of snake venoms and two antibodies, we are trying to create a biosensor chip of snake venom that is very important for the pharmaceutical use. In preliminary study of protein chip of snake venoms, we used only two antibodies and two snake venoms as antigens correspond to the antibodies for testing. The result showed that the antibodies had well specific to the low concentration of snake venoms as antigens (圖十一至圖十三). In pharmaceutical application, various kinds of antibodies from various kinds of snake venoms in different families have to be applied and varied in their concentrations. The sensitivities had good enough for detecting vary diluted snake venoms from biological samples such as blood from victims of snake envenomation. In addition, the array of antigen-antibody is very useful and being a high efficiency diagnostic method to generate in the formation about the kind of snake in any case of snake envenomation. Microarray data coupling with clinical information promise to accelerate and reduced the cost of drug development.

(4)討論：

本計劃執行一年來已經設立並尋找出以蛋白質體學技術(二維擬膠電泳分析、質譜分析與資料庫比對)分離並鑑定台灣特有蛇毒蛋白的最佳系統與條件，並且已初步測試完成開發蛇毒 protein chip 的系統，整體來說雖曾有許問題與困難，但均已再不斷試驗中逐步被克服，根據本年所累積的研究成果與經驗，後續計畫年度應能有效率且順利完成本計劃並獲具體成果。

(5)結論：

在這一年執行計劃期間，本研究室已完成台灣特有蛇毒之二維凝膠蛋白圖譜、蛋白質鑑定及蛇毒蛋白 protein chip 之模擬系統。在台灣蛇毒二維凝膠圖譜方面：台灣六種常見毒蛇之蛇毒蛋白以二維凝膠電泳分離染色後已建立出各蛇毒的蛋白質分佈圖譜，經影像分析比對結果險示各蛇毒蛋白具有其特有之蛋白圖譜，其中雖有相互間相似之蛋白質，但仍有許多蛇種間的差異蛋白，顯示嘗試由台灣六種常見毒蛇所分泌毒液來區分是何種毒蛇之策略是相當可行的途徑。在毒蛇蛋白質鑑定方面：利用先進質譜法鑑定特定蛋白之物種是目前最靈敏且快速的方法，我們以蛋白質體學 MALD-TOF 質譜分析法進行蛇毒蛋白在二維凝膠電泳上的身份鑑定，首先將蛋白質點從凝膠片挖出，進行蛋白質的 trypsin 酵素水解，digested peptides 經萃取濃縮後點於質譜樣品板上以質譜儀收集 peptide fragment fingerprint 資料後進行生物資料庫搜尋及比對以鑑定出此蛇毒蛋白質之身分。在蛇毒蛋白晶片(protein chip)之模擬系統方面：利用蛇毒抗體與蛇毒蛋白的專一性接合特性，先將蛇毒抗體點於晶片上，再與蛇毒進行專一結合，再以螢光修飾之抗體進行第二次結合，藉此以區分出不同蛇毒。實驗結果顯示蛇毒蛋白晶片模型系統確實初步鑑別出不同蛇毒蛋白間的差異，一旦後續研究計畫找出台灣特有蛇種蛋白標的物後，配合目前蛇毒蛋白晶片模型系統的初步成果，將可發展出具蛇種專一性的檢驗試劑來鑑別台灣毒蛇的種類。

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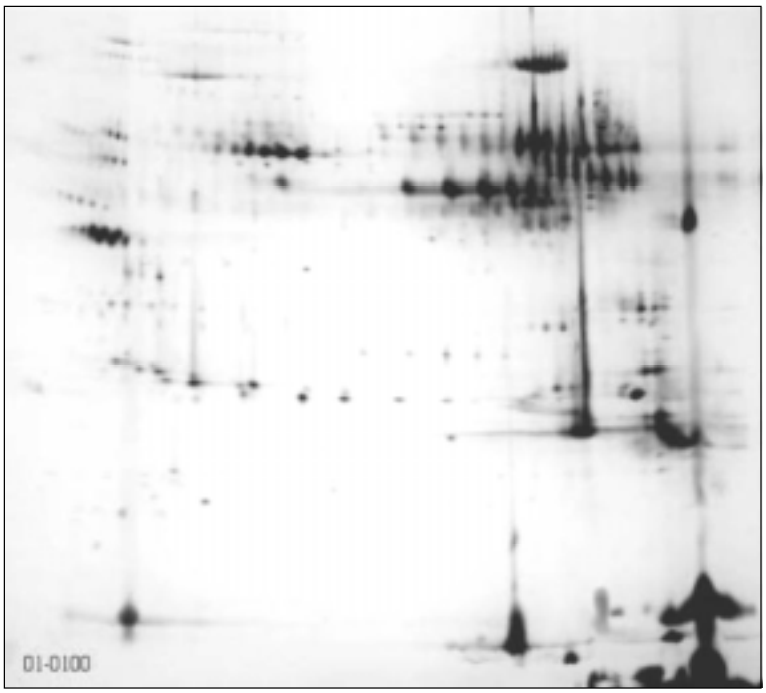
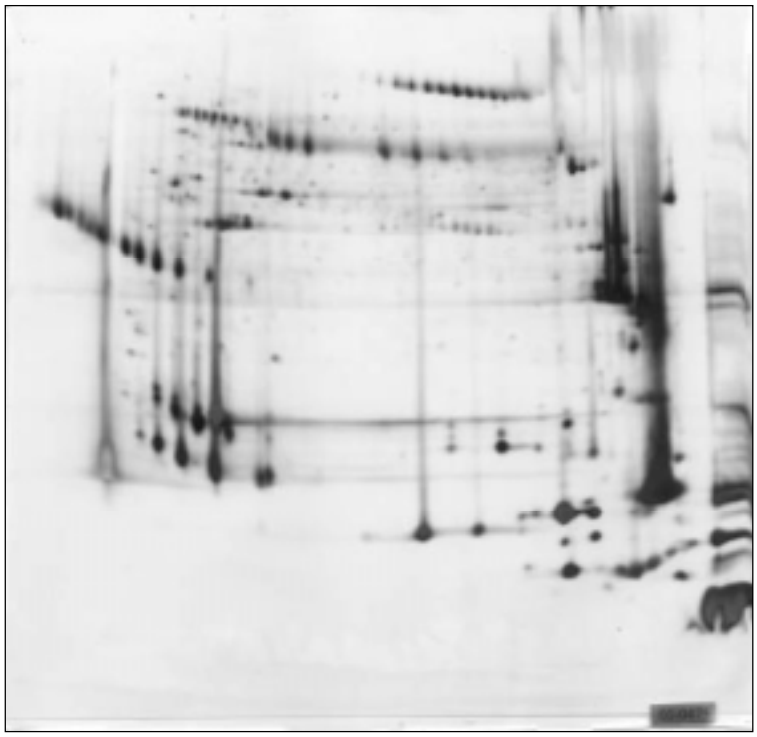
(7)圖、表：



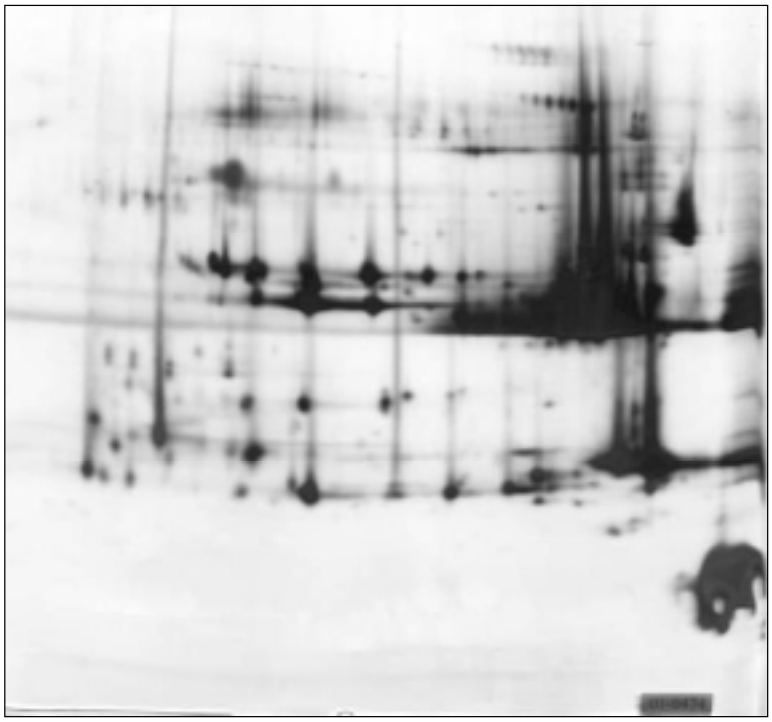
圖一

Naja naja atra



圖二

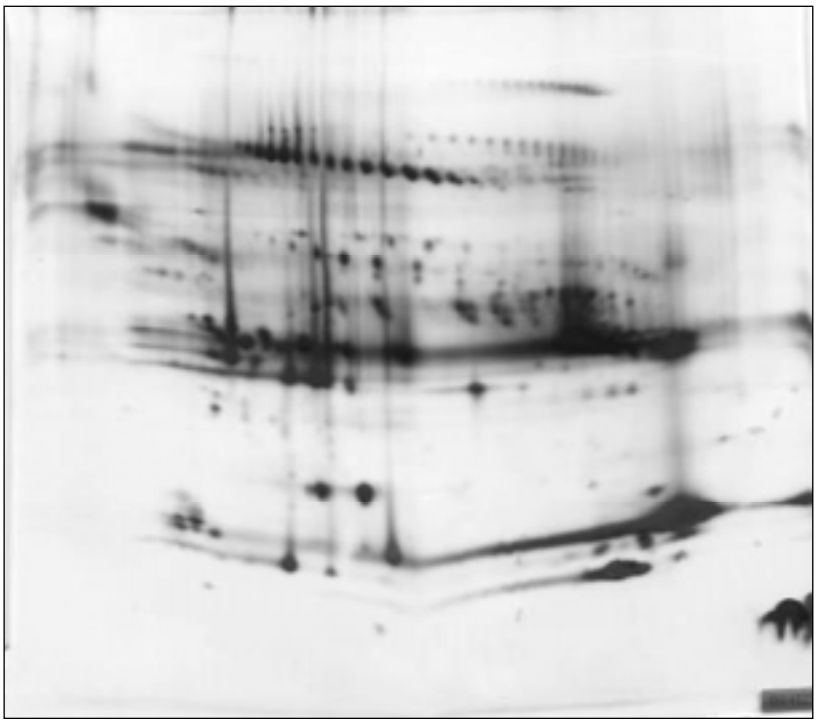
Vipera russelli formosensis



圖三

Trimeresurus mucrosquamatus

圖四

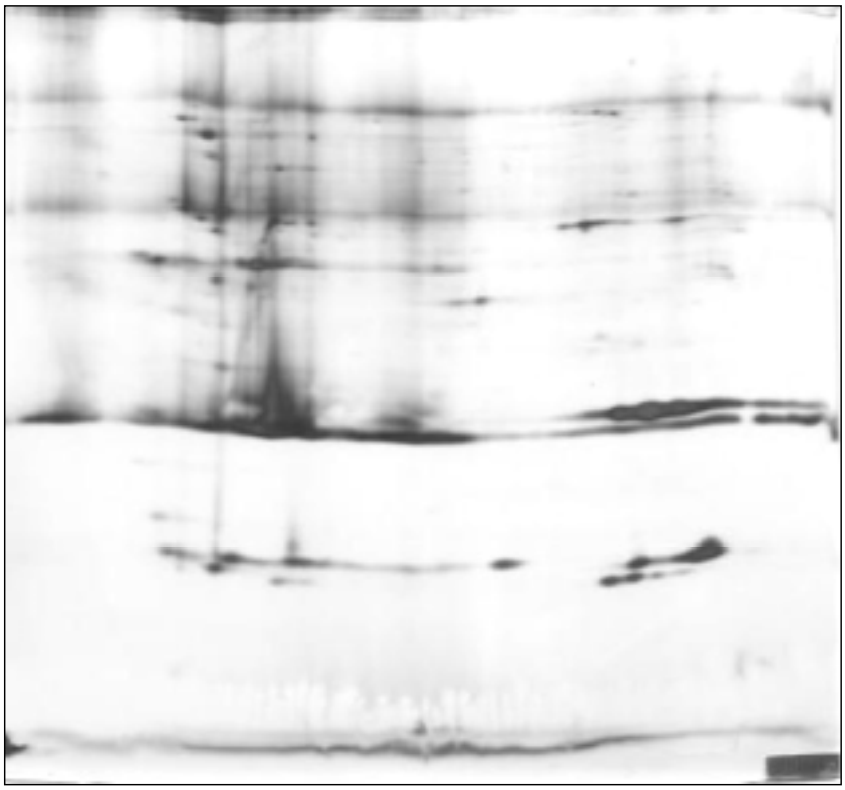


Trimeresurus stejnegeri

圖五

Bungarus multicintus

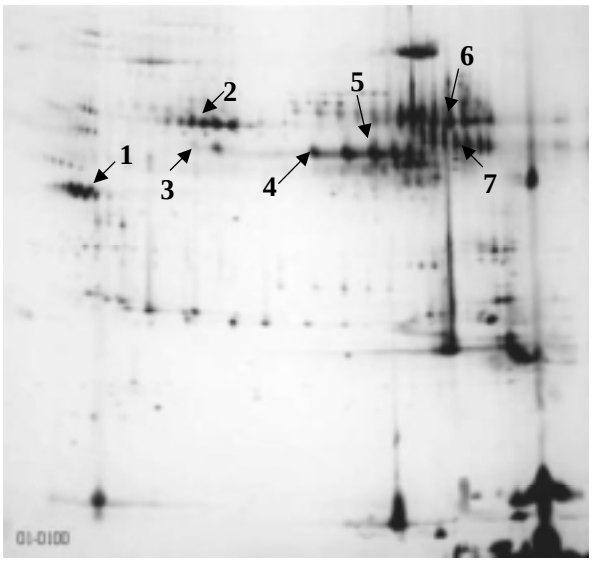
圖六



Deinagkistrodon acutus

Mass analysis of *Naja naja atra*

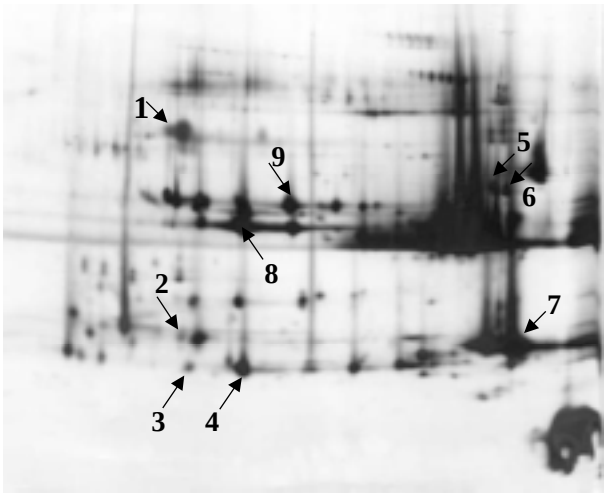
圖七



Sample	Naja naja atra											
Spot	Entry			Matches	MW	PI	Description				Sequences coverage	
1	gi|12723144	Lactococcus lactis		6	43100.24	5.28	amino acid amidohydrolase				27	
2	gi|323539	Dengue virus type 3		7	72318.3	6.66	L11430 polyprotein				24	
3	gi|3599392	Cenarchaeum symbiosum		6	63599.49	6.31	AF083071 glucose-1-dehydrogesase				21	
4	gi|2880052	Arabidopsis thalina		7	56152.2	7.25	Cytochrome P450 71A12				27	
5	gi|7463844	Helicobacter pylori		8	62813.25	7.63	ATP-dependent zinc metallopeptidase				28	
6	gi|7447142	Homo sapiens		6	54500.78	8.04	D10355 alanine aminotransferase				25	
7	gi|7448583	Helicobacter pylori		7	57127.16	8.54	sigma-54 interacting protein				28	

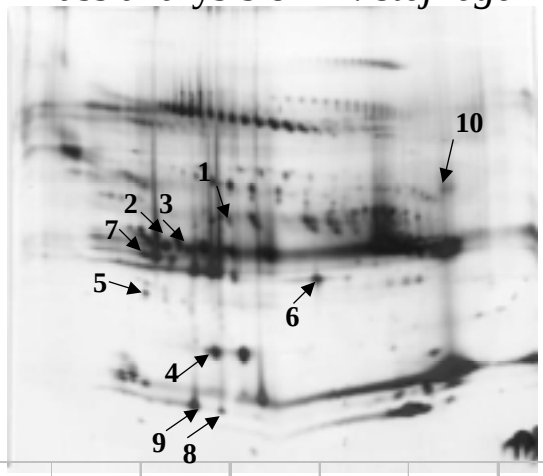
Mass analysis of *Tr. mucrosquamatus*

圖八



Sample	Tr. Mucrosquamatus											
Spot	Entry					Matches	MW	PI	Description			Sequence coverage	
1	gil748142	Streptomyces coelocolor				8	40036.9	5.35	probable solute binding lipoprotein			37	
2	gil 156493	Caenorhabditis elegan				4	11060.6	6.27	M10105 vitellogenin 2			50	
3	gil 7471082	Deinococcus radiodurans				4	9161	5.33	acyl-CoA-binding protein			86	
4	gil 13473088	Mesorhizobium loti				3	8418.76	6.03	hypothelial protein			36	
5	gil 14707	Xenopus laevis				6	24829.9	8.12	oocyte zinc finger protein XLCOF10			39	
6	gil 6226337	Rickettsia prowazekii				6	22602.7	9.35	hypothetical protein RP168			39	
7	gil 283786	Pseudopleuronectes amer				5	12537.6	9.51	multidrug resistence protein A			43	
8	gil 9665155	Arabidopsis thaliana				6	20769.7	5.89	unknown protein			47	
9	gil 297490	Helicobacter pylori				5	26172.4	7.74	uridylate kinase			46	

Mass analysis of *Tr. stejnegeri*



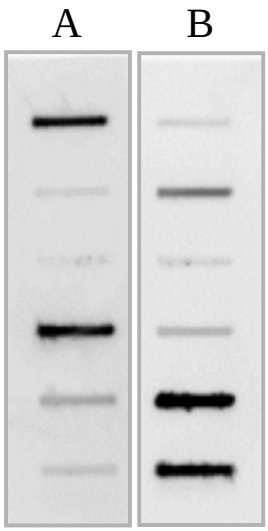
圖九

Sample	Tr. stejnegeri				
Spot	Entry	Matches	MW	PI	Description
1	gi|7432638 Aquifex aeolicus	7	67892.87	5.95	NADH dehydrogenase (ubiquinone) (EC 1.6.5.3) I chain nuoD2
2	gi|1266351 Neisseria meningitidis	5	39335.13	5.33	GTP cyclohydrolase II/3,4-dihydroxy-2-butanone-4-phosphate synthase NMB12
3	gi|401416 Xenopus laevis	7	39715.17	7.73	WNT-3A PROTEIN PRECURSOR (XWNT-3A)
4	gi|11280172 Vibriocholerae	5	26739.95	5.98	purine nucleside phosphorylase VCA0053
5	gi|1302634 Bacillus sphaericus	5	35916.43	5.34	35.8-kilodaton mosquitocidal toxin [Bacillus sphaericus]
6	gi|10954870 Agrobacteriumtumefaciens	7	33969.09	6.21	Hypothetical gene
7	gi|126036 Lactobacillus delbrueckii	7	36904.08	5.08	D-LACTATE DEHYDROGENASE (D-LDH)
8	gi|4099283 Mus musculus	4	13023.62	5.78	immunoglobulin heavt-chain gamma 1 variable region SZ-51
9	gi|3608132 Arabidopsis thaliana	5	13321	5.77	putative serpin [Arabidopsis thaliana]
10	gi|7479699 Streptomyses coes coelicolor	7	60264.41	9.65	hypothelial protein SC6G3.05-Streptomyses coelicolor

圖十

Characterization of anti-snake venom antibody

Antibody: A- anti-*Bungarus* and *Naja*
B- anti-*Trimeresurus*



Snake venom	
雨傘節 (*Bungarus multicinctus*)	neurotoxin
百步蛇 (*Deinagkistrodon acutus*)	hemotoxin
鎖鏈蛇 (*Vipera russelli formosensis*)	hemotoxin or mix
飯匙倩 (*Naja naja atra*)	neurotoxin
龜殼花 (*Trimeresurus mucroquamatus*)	hemotoxin
赤尾鮐 (*Trimeresurus stejnegeri*)	hemotoxin

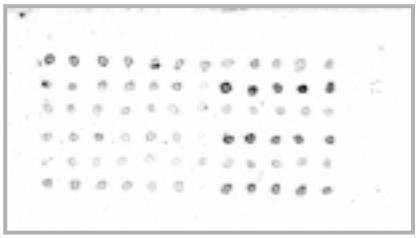
圖十一

Protein chip- snake venom detection system

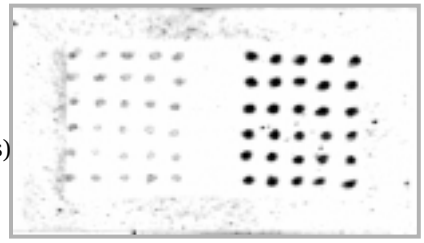
1 μg/mL

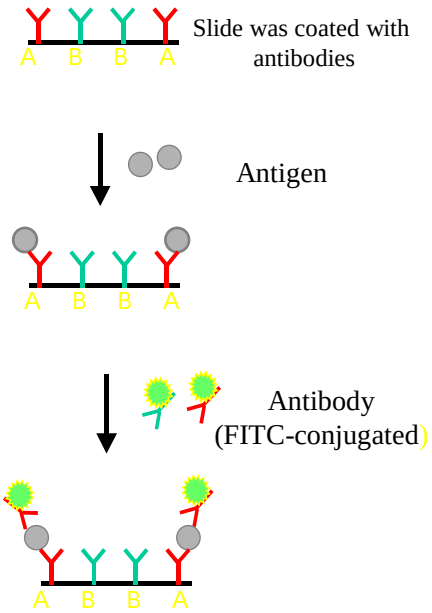
antigen

(B. multicintus)



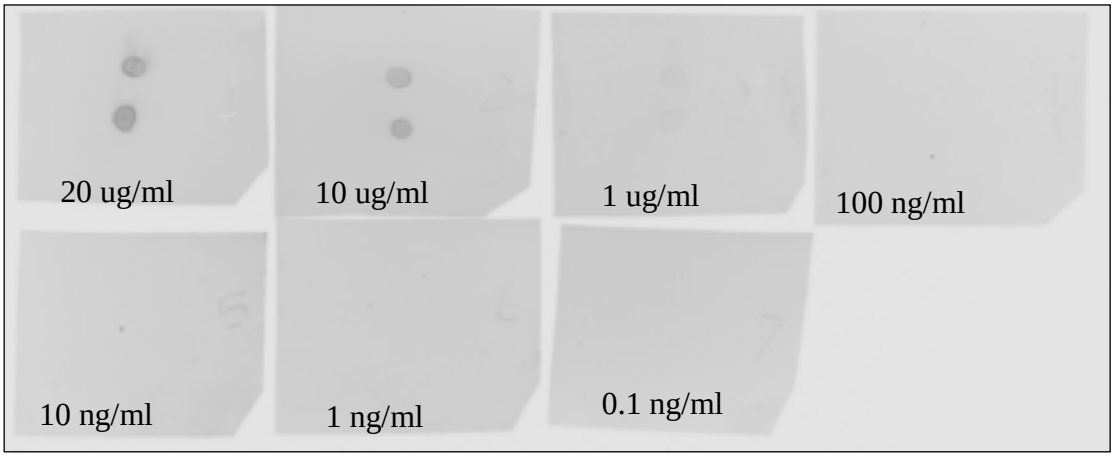
(Tri. mucrosquamatus)





PROTEIN CHIP OF SNAKE VENOM

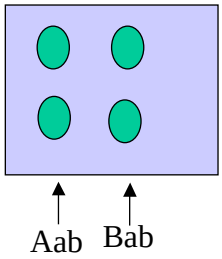
圖十二



Aab = *B. multicintus*

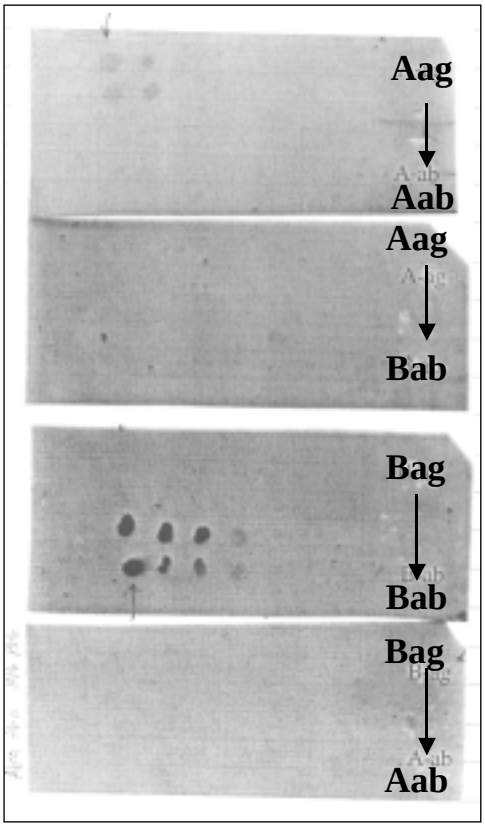
Bab = *Tr. mucrosquamatus*

Antigen = *Tr. mucrosquamatus*



PROTEIN CHIP OF SNAKE VENOM

圖十三



Aab = Bungarus fasciatus antibody

Bab = Trimeresurus mucrosquamatus antibody

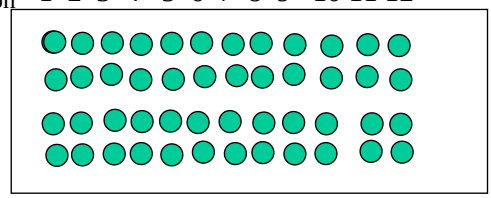
antibodies : 5% BSA in PBST = 1 : 1000

Aag = B. multicintus venom

Bag = Tr. Mucrosquamatus venom

Antigen conc. = 1 ug/ul

position 1 2 3 4 5 6 7 8 9 10 11 12



Ab spoted on membrane is 10 fold serial dilution of the first position (10 mg/ml)