蘇力菌——一種取之不盡的自然資源

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摘 要

利用蘇力菌製成生物殺蟲劑，防治農業害蟲的歷史已超過30年。蘇力菌是一種革蘭氏陽性能產生孢子的細菌，在芽孢生殖時，能產生伴胞晶體，在定常期被解離出來。結晶的形狀有雙金字塔形、立方形、扁平正方形、圓形和不規則形等。當被昆蟲取食時，受中腸的鹼性所溶解，釋放出27至140 kDa大小的原毒素。這些原毒素再被蛋白質酶活化成有毒的胜肽即爲毒素，毒素再與腸道細胞膜之受器結合，造成孔洞，擾亂了滲透壓，導致細胞的腫脹、解離。中毒的昆蟲迅速停止取食，終至死亡。

目前蘇力菌已廣泛地受到調查、研究並調製成商品，用來防治鱗翅目和鞘翅目的昆蟲。蘇力菌的分佈範圍很廣，可以自罹病昆蟲和其棲所，倉儲產品，土讓及某些落葉和針葉樹的葉片上分離出來。根據大庭(1991)之報導，依鞭毛(H)抗原，可將蘇力菌分成40個血清型。又根據殺蟲的特異性和核酸序列的相似性，可將蘇力菌殺蟲結晶基因分成五大類，現在有40個結晶基因被選殖和定性出來。

蘇力菌除了對昆蟲有活性外，其對植物和動物線蟲亦具活性，甚至對原生動物、寄生動物之肝蛭、蟎類有活性。截自目前爲止，共有 6 目的昆蟲，2 種節肢動物綱，和 4 個門的動物對蘇力菌具有感受性。照這種趨勢看來，未來可能會有更多有用的蘇力菌品系被發掘出來。本省地處熱帶和亞熱帶，地形複雜，耕作系統亦具多樣性，是微生物資源的溫床，有利於發現新的微生物包括蘇力菌。透過適當的篩選方法包括：晶體的形態觀察，血清型的鑑定，蛋白質的SDS-PAGE，ELISA分析，原生質體剖面分析，聚合酶連鎖反應和生物檢定，可分離出毒性强、殺蟲範圍廣的菌株。除可開發利用做爲蘇力菌製劑外，可借基因選殖、核酸定序及基因表現的研究，做爲爾後基因轉殖微生物或轉殖作物之基因來源。蘇力菌可以說是上天賦予取之不盡的自然資源。

（關鍵詞：蘇力菌，分離株，品系特徵化，殺蟲活性）

一、前 言

蘇力菌這種生物殺蟲劑上市用來防治農業害蟲的歷史超過30年(Feitelson, 1993)。蘇力菌是一種革蘭氏陽性細菌，在芽孢生殖時，能產生伴胞晶體，當被昆蟲取食時，被中腸之鹼性所溶解成原毒素，再受蛋白酵素活化成毒素，活化的毒素再和中腸表皮細胞作用，導致腸道腫脹，破裂而致死(Gill et al.,1992)。蘇力菌已廣泛地被調查、研究，並調製成商品，用來防治鱗翅目，雙翅目和鞘翅目的害蟲(Beegle and Yamamoto, 1992; van Frankenhuyzen,1993)。在田間使用的量約爲10-50克/英畝，當然我們考量到其主成分的分子量通常大於 50 kDa時，如此高的分子量，而用量如此低，顯然力價(potency)相對的高。故在以克分子(molar)爲基礎比較蘇力菌毒素之力價，遠大於傳統的化學殺蟲劑(Feitelson, 1993)。加上安全性高，又無環境污染之顧慮，是頗值得開發和應用的一種微生物資源。

二、蘇力菌之寄主範圍和分佈

蘇力菌的分佈範圍很廣，可以自罹病昆蟲和其棲所，倉儲產品，土壤及某些落葉和針葉樹的葉片上分離出來。

1901日本石渡繁胤，在大日本蠶絲會報上，報導自罹病家蠶分離出蘇力菌猝倒亞種(Ishiwata, 1901)。顯示蠶和蠶場是蘇力菌發源地之一。大庭等(1979)自98個蠶場分離出7種血清型之蘇力菌，其中4 a : 4 b, 4 a: 4c，7和10等H抗原爲主要血清型(Obha et al., 1979)。Dulmage (1970)自大量飼育的棉紅鈴蟲分離出庫斯塔基亞種HD-1品系。另外，在以色列，中北Negev沙漠之小池塘中罹病之熱帶家蚊中，分離出蘇力菌以色列亞種，H抗原爲14。對某些蚊、蚋之毒性頗高(Goldberg and Margalit, 1977; Margalit and Dean, 1985 ; de Barjac, 1978)。Chilcott and Wigley (1993)亦於昆蟲棲所及幼蟲上分離出蘇力菌來。

又,倉庫病爲害蟲和蘇力菌有緊密相關的生態系統(Burges and Hurst, 1977 ; DeLucca et al., 1982 ; Meadows et al.,1990, 1992)。幼蟲和含有蘇力菌孢子之蟲屍接觸感染。釋放出來的孢子和結晶，在倉庫的乾燥環境中存活相當長的時間。不同品系的蘇力菌在昆蟲體內生長亦可能會有原生質體的轉移(Jarrett and Stephenson, 1990)。故倉庫是一個提供細菌生長， 創造新品系有著新的毒素組成和孢子的長期生存等之理想環境 (Meadows, 1993)。Donovan et al.(1988)自倉庫中分離出EG2158之新品系蘇力菌對鞘翅目幼蟲具毒性。DeLucca et al.(1982)自穀物粉塵中分離出新的亞種B. t. colmeri。Kreig et al.(1983)及Huger et al.(1986)自罹病之麵粉甲蟲(Tenebrio monitor)分離出B. t. tenebrionis,對鞘翅目的幼

蟲具殺蟲效果。

最近的研究亦指出在北美洲的落葉和針葉樹之葉片上分離到蘇力菌 (Smith and Couche, 1991)。這些分離株爲自然發生者而非來自蘇力菌商品施用之殘留，與商品之結晶蛋白無交互抗體反應。在葉面分離之蘇力菌甚多，Smith和Couche (1991)認爲是葉面之表生菌。

土壤中存活著各種微生物，蘇力菌也不例外，早期的研究指出在土壤中找到蘇力菌的機率不高。在美國來自Bacillus cereus, B. t.之菌落形態組之分離株，只有0.5%爲蘇力菌(DeLucca et al., 1981)。在日本土壤中，相同的菌落形態組中有2.7%屬蘇力菌(Ohba and Aizawa, 1986)。在菲律賓54個土壤樣品中，只有12樣品含有蘇力菌(Padua et al.,1982)。這些研究之所以低估蘇力菌在土壤中之分佈，其主要原因是如何在含有其他微生物相當多的情況下，自土壤中區分蘇力菌。自醋酸鹽篩選技術開發之後，能更準確地決定蘇力菌之分佈(Travers et al., 1987)。此簡單的技術，有助於蘇力菌的分離。有一報告指出來自五大洲包括美國和其他29個國家的土壤1115個樣品中，有785個樣品中含有蘇力菌，顯示其爲一種普通的土壤細菌(Martin and Travers, 1989)。含有蘇力菌樣品的百分比，因來源不同而有差異。94%來自亞洲和中非洲及南非洲的土壤樣品含有蘇力菌，而來自歐洲則爲84%，最低回收率爲來自紐西蘭(56%)和美國(60%)。蘇力菌可在大草原、沙漠、農業地的土壤樣品中經常發現，但亦可在城市地區、森林、北極凍原地帶和無樹的大平原的土壤中發現。但較不易自海濱、洞穴、某些沙漠地點和雨林之土壤樣品中發現。自中國大陸西南、西北、中南地區土壤中分離到94株蘇力菌，經鑑定分屬於14個血清型及未知血清型，但僅管土壤中的蘇力菌資源豐富，要獲得高效菌株並不容易(李等，1992)。

然而妥善利用生物檢定技術可發現對無脊椎動物具毒性之新蘇力菌品系。 Wigley and Chilcott (1990)發現其收集之蘇力菌經篩選後有500個品系對紐西蘭牧草螬蟲，在高劑量時具有活性。Slaney et al. (1991)發現B. t. kumanotoensis之新品系對南方玉米根葉甲蟲和墨西哥豆瓢蟲有毒。B. t. japonensis之 Buibui品系對大綠金龜子和日本金龜子有高的殺蟲活性(Suzuki et al. 1992). Bradfisch et al. (1991)發現蘇力菌品系對根傷害線蟲具毒性。Zuckerman et al. (1993)利用蘇力菌CR-371品系來防治植物病原線蟲。蘇力菌以色列亞種對蕈蚊具毒性(Osborne et al., 1985)。Keil(1991)在實驗室和田間證實蘇力菌以色列亞種能防治菇蠅幼蟲。 Karamanlidou et al. (1991)自土壤中分離之蘇力菌中，發現有一品系能殺死油橄欖實蠅之幼蟲。 Bottjer et al. (1985)發現有20個血清型的蘇力菌對動物寄主線蟲具有活性。

三、新發現的蘇力菌

蘇力菌除了最近發現對鞘翅類有活性外(Kreig et al., 1983; Huger et al., 1986)，另外尚發現對寄生植物和動物線蟲有活性的蘇力菌(Edwards et al., 1988)。Feitelson (1993)更增加蘇力菌之寄主範圍，包括了原生動物病原，寄生動物之肝胚(Trematoda)和蟎類(Acari)。他認爲只要是：蘇力菌的蒐集具有多樣性；標的生物能取食或處理毒素；供試材料供應無缺；生物檢定方法已開發完成，我們就可以發現對任何標的生物具有特殊活性的蘇力菌品系，這些標的生物包括了最簡單的單細胞眞核生物(eukaryote) 到最進步的節肢動物。截自目前爲止，共有6目的昆蟲，2種節肢動物綱和4個門的動物對對不同的蘇力菌具有感受性(表一)。

發現包括對數種重要雙翅目害蟲如潛葉蠅(Agromyzidae)，家蠅成蟲，大蚊和蕈蠅有嶄新活力的蘇力菌。對頑抗的甜菜夜蛾(Spodoptera exigua)亦發掘出新的毒素出來，對鞘翅目幼蟲包括黃粉甲蟲，南瓜十二星甲蟲，日本金龜子和其他土壤蠐螬有活力的新品系和毒素亦被發現。甚至發現對膜翅目之螞蟻亦有活性(Feitelson, 1993)。

這些發現形成一個演化插入法(evolutionary interploation)假說：如果標的生物的演化是成線性或分叉的型式(如A→B→C→D),假使發現蘇力菌對某些標的生物有毒性(如A, B,和D)，我們就能發現一種蘇力菌對演化中間物(如C)具有毒性。且此假說已有實驗的支持(Feitelson et al. 1992, Feitelson, 1993).

四、蘇力菌之鑑定

至於蘇力菌之鑑定，可藉鞭毛(H)凝集做爲區分，可將之分成34個血清型(de Barjac, 1981, de Barjac and Frachon, 1990)。大庭(1991)則利用H-抗原和H-因子(factor)，將蘇力菌分成29個H抗原和40種血清型。結晶血清學亦可用來鑑定和分類蘇力菌菌株，使用多源抗原可比較對鱗翅類有效的品系中的結晶血清型(Krywienczyk et al., 1978, Lynch and Baumann , 1985; Smith,1987)。此外，亦可定殺蟲結晶蛋白質之量(Groat et al., 1990;段等，1993)。結晶的形狀可以光學或電子顯微鏡觀察(Ohba and Aizawa, 1986; Chilcott and Wigley, 1992; 李等, 1992)。SDS-PAGE可用來檢查和定量結晶蛋白質組成(Brussock and Currier, 1990; Chak and Young, 1993;段等，1993).亦有報告指出利用原生質體之剖面圖，依原生質體數目和大小，區分蘇力菌的品系(Gonzalez and Carlton, 1980; Chak and Young, 1993;高等1993). Hofte和Whiteley (1989)根據蘇力菌結晶蛋白質基因(cry genes)的表現型，結晶型狀和寄主範圍，將之分成五大類。而聚合酵素連鎖反應(PCR)可利用來鑑定和

表一· 蘇力菌之殺蟲範圍
Tablel. Examples of Bt Target Hits (Feitelson, 1993)

Target	Phylum	Class	Order	Family
Pharaoh and fire ants	Arthropoda	Insecta	Hymenoptera	Formicidae
Leafmining flies	Arthropoda	Insecta	Diptera	Agromyzidae
Houseflies and stable flies	Arthropoda	Insecta	Diptera	Muscidae
Sheep blowflies	Arthropoda	Insecta	Diptera	Calliphoridae
Mosquitos	Arthropoda	Insecta	Diptera	Culicidae
Craneflies	Arthropoda	Insecta	Diptera	Tipulidae
March flies	Arthropoda	Insecta	Diptera	Bibionidae
Black flies	Arthropoda	Insecta	Diptera	Simuliidae
Mushroom flies	Arthropoda	Insecta	Diptera	Sciaridae
Diamondback moth	Arthropoda	Insecta	Lepidoptera	Plutellidae
Armyworm, corn earworm	Arthropoda	Insecta	Lepidoptera	Noctuidae
European corn borer	Arthropoda	Insecta	Lepidoptera	Pyralidae
Codling and grape berry moth	Arthropoda	Insecta	Lepidoptera	Olethreutidae
Grapeleaf skeletonizer	Arthropoda	Insecta	Lepidoptera	Zygaenidae
Alfalfa weevil	Arthropoda	Insecta	Coleoptera	Curculionidae
Corn rootworm	Arthropoda	Insecta	Coleoptera	Chrysomelidae
Colorado potato beetle	Arthropoda	Insecta	Coleoptera	Chrysomelidae
Mealworm	Arthropoda	Insecta	Coleoptera	Tenebrionidae
Soil grub beetles	Arthropoda	Insecta	Coleoptera	Scarabaeidae
Aphids	Arthropoda	Insecta	Homoptera	
Sheep lice	Arthropoda	Insecta	Phthiraptera/ Mallophaga	
Mites	Arthropoda	Archnida	Acari	Tetranychidae
Animal health nematodes	Nemathelminthes	Nematoda	Strongylida	Trichostronglidae
Plant parasitic nematodes	Nemathelminthes	Nematoda	Tylenchida	Pratylenchidae
Plant parasitic nematodes	Nemathelminthes	Nematoda	Tylenchida	Heteroteridae
Flatworms	Platyhelminthes	Trematoda	Digenea	Fasciolidae
Protozoa	Sarcomastigorphora	Zoomastigophora	Diplomonadida	Hexamitidae

選殖嶄新的結晶基因。因聚合酵素連鎖反應可迅速的決定一標的的DNA序列是否存在(Saiki et al., 1988)。此法非常靈敏，少量的DNA即可分析，在很短的時間可分析大量的樣品。Carozzi et al.(1991)設計出能分辨出cry I, cryIII和cryIV基因的引子，這些基因分別對鱗翅目、鞘翅目和雙翅目有殺蟲效果。且經由聚合酵素連鎖反應所預測蘇力菌品系之殺蟲效果均與生物檢定相符合。Kalman et al. (1993)並設計8個forward引子和2個reverse引子，因以鑑定cry I基因。上述所有的方法均有助於蘇力菌品系的特徵化。然而殺蟲活性的篩選仍是找尋對新害蟲具有活性或對一特定害蟲具更强活性的蘇力菌品系之最好的方法(Beegle,1990； Chilcott and Wigley, 1992)。在本省也分別建立以埃及斑蚊、斜紋夜盜蛾和小菜蛾爲供試昆蟲的標準生物檢定法，可用以篩選具有殺蟲活性的蘇力菌分離株(高及羅，1990;高及段1992; 1993)。

五、結論和未來展望

在現代抗生素紀元(antibitics era)之早期，全世界都熱衷於找尋能生產嶄新的二次代謝產物而有抗微生物活性的生物。這種篩選的結果使得抗生素在製藥工業佔有鉅大而高獲利的地位，對醫藥和社會均有極大的貢獻。接著更努力去發展有著改良臨床活性的半合成衍生物，最近又利用分生生物學技術以發掘嶄新的化學結構。我們可以循著類似的軌跡，利用蘇力菌之殺蟲結晶和共同源基因，著手開發工作。

在化學殺蟲劑世紀，工業界已逐漸的學會並應用結構/活性之關係。面對數千種對許多門、目和種類的生物有衝擊的蘇力菌是不是有一種較佳的系統能夠瞭解蛋白質結構和功能之間的關係？每次只要新的蘇力菌被發現，打破了蘇力菌僅能殺少數鱗翅目幼蟲的神話，衍生的問題總是超過了答案。但是由於(1)利用X-射線結晶學技術(crystallography)的高解析度，瞭解了蘇力菌毒素的三度空間(Li et al., 1991); (2)許多基因被排序；和(3)發現許多新活性的蘇力菌。使得我們在瞭解蘇力菌毒蛋白結構和功能之關係，正面臨一種迅速發展的轉捩點。

若是更多的毒素被選殖和排序，我們可以預期爾後會有更有趣的發現。最後，由於這些研究所獲得的知識，可應用於對付抗蘇力菌之標的生物，利用嵌合毒素(chimeric toxin)之構築，增强活性和增廣殺蟲範圍，或者提供有用的探針，以發掘更多具有獨特性之毒素。當然亦可再造如同抗生素對科學和臨床方面的輝煌成就。

在過去22年中美國有53件蘇力菌專利案通過，其中39件是在過去5年中核發的。有兩個蘇力菌經過遺傳工程的產品已被美國環保署接受註冊，第三個產品已獲實驗使用許可(expermental use permit) (Feitelson, 1993).本省亦有防治小菜蛾用的蘇力菌遺傳工程

產品 MVP 接受登記。我們可以見到農業用蘇力菌產品在基礎研究、發明、應用和商品化，均有快速的進展。同時利用蘇力菌之殺蟲基因，經過選殖，育成含蘇力菌之基因轉殖作物或微生物亦是目前正在努力的方向，事實上含蘇力菌基因之轉殖作物產品亦將於最近問世。因此蘇力菌菌種之蒐集應多予重視、加强。我們應有種體認：這種來自自然，用於自然的自然資源是取之不盡，用之不絕的。

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Abstract

Kao, S. S. 1994. Bacillus thuringiensis– an inexhaustible natural resources. (Biopesticide Department, Taiwan Agricultural Chemicals and Toxic Substances Research Institute, Wufeng, Taichung,Taiwan, R.O.C.)

Among the microbial control agents (MPCAS), Bacillus thuringiensis (Bt) is the most promising agent for the control of insect pests. Hundreds of Bt isolates have been classified according the serotype of their flagellar antigens. Isolates representing these species show different toxicity level against given insect species. There has been much effort in the isolation of new Bt strains with the aim of finding strains with a new host range, or increased toxicity against a particular pest or pests recently. This is with a view to the production of microbial insecticides or transgenic plants expressing the Bt insecticidal protein. This has also reflected an increased interest in Bt, both as an environmentally safe alterative to many chemical insecticides and for the control of chemically resistant insect pests. Bt is widely distributed in the environment and can be isolated readily from insects, stored product material, sericultural environments, the phylloplane and it is also a ubiquitous soil bacterium. Despite the capacity to assemble a large number of Bt isolates into a collection the key problem is to distinguish new strains. Strains can be characterized by a number of techniques including serotyping, crystal serology, crystal morphology, crystal profiles, plasmid profiles, PCR and insecticidal activity. The tropical and subtropical climate, diversified topography and mosaic cropping system of Taiwan, provide a very rich microbial resources for discovering novel agents with high pathogenicity and broad spectra. The opportunity for finding new Bt strains will depend on screening isolates against new insect pests or large scale screening to find isolates with increased toxicity. And the finding of new Bt isolates having novel insecticidal activity and/or increased potency will add to the list of isolates that are specific for new groups of insect pests or serve as gene pool for further genetic manipulation.

Keywords : Bacillus thuringiensis , isolates, strain characterization insecticidal activity.