2009 年臺灣國際科學展覽會

優勝作品專輯

編號： 070006

作品名稱

從麵包蟲體內分離出可分解保麗龍之菌種

得獎獎項

微生物學科大會獎第三名

美國正選代表: 美國第 60 屆國際科技展覽會

學校名稱： 國立臺中女子高級中學

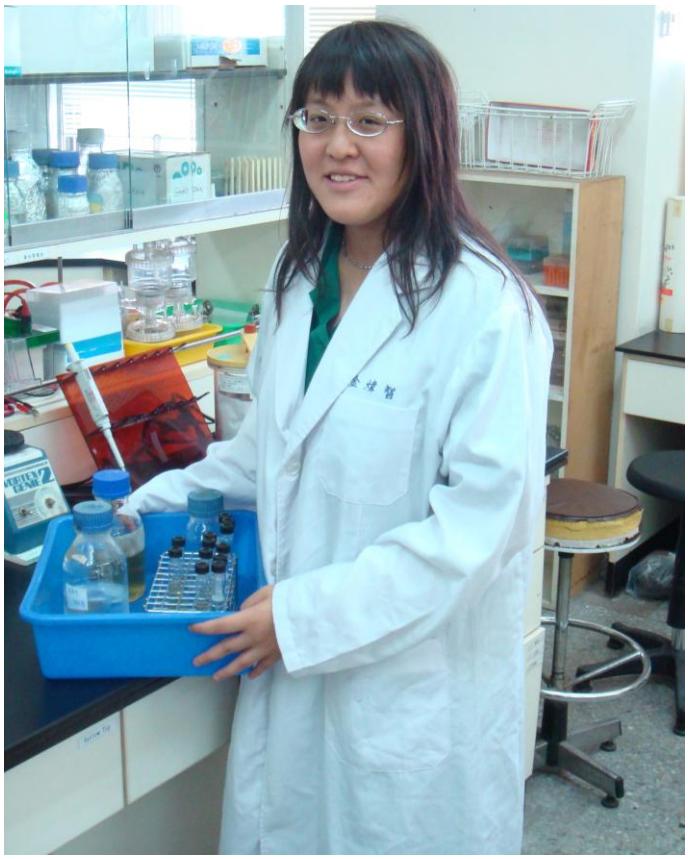
作者姓名： 曾依晴

指導老師： 邱伯勤　溫福賢

關 鍵 字： 麵包蟲、保麗龍、分解

Mealworm、Polystyrene、Decompose

作者簡介



我是曾依晴，興趣廣泛的高二學生。喜歡積極投入各式活動和探索科學，曾擔任社長，將一個迷你小社改造為人數爆滿的大社，參加過許多科學競賽，不太在意能否在各式活動中脫穎而出，而珍惜能從活動中結視同好和探索自己的機會。

在師長父母的支持下，申請中興大學與教育部合作的創意學院計畫，因此有幸進入實驗室學習 ；2008 年曾代表台灣赴美參加 ARML，並擔任 B 隊隊長，帶領台灣對獲得三項冠軍。

Isolation of Microorganisms Capable of Decomposing Polystyrene from Mealworms

ABSTRACT

In a previous science fair project “Polystyrene’s enemy—mealworms”, it was found that mealworms could feed on polystyrene. Based on this finding, this study aims at confirming whether or not mealworms can sustain life by feeding on polystyrene. The presumption is that mealworms feed on polystyrene and that this very fact has much to do with the microorganisms inside the worms’ digestive tracts. This study also attempts to make certain which kind of microorganism is responsible for the metabolization of polystyrene.

This study involved cultivating microorganisms from the digestive tracts on Luria-Bertani(LB)medium agar plates under anaerobic condition, which resembles the conditions in the mealworms’ digestive tracts. There were two kinds of bacteria isolated: one formed red colonies and the other formed white colonies. Different nutritional conditions were further designed to examine the ability of decomposing polystyrene of different bacteria. The results showed that the red bacteria from mealworms' digestive tracts could decompose polystyrene. Implication from the result of this study is that if we can find a way to mass-produce the red bacteria, the serious environmental pollution caused by polystyrene might be possibly solved.

摘要

在全國中小學科展「保麗龍的剋星麵包蟲」中，意外得知麵包蟲可能可以攝食保麗龍，也揭開本實驗的序幕。為了確定接續實驗的可行性，本實驗首先再次確認麵包蟲可因食用保麗龍而生長。我推測麵包蟲能攝食保麗龍而生長，可能和麵包蟲腸道內的特殊共生菌有關，因此本實驗嘗試找出麵包蟲腸道內的共生菌，並進一步確認是何種菌株和分解保麗龍有關。

一般而言腸道內氧氣含量極少且養分充足，所以本實驗選擇在厭氧與富含養分的條件下進行培養。實驗初期由麵包蟲的消化道內分離出兩種菌（暫且稱為紅菌和白菌），為了檢測分離出來的菌可否分解保麗龍，我設計營養條件不同的培養實驗，而結果顯示：紅菌可能為可分解保麗龍的菌種。

從麵包蟲體內分離出可分解保麗龍之菌種

Isolation of Microorganisms Capable of Decomposing Polystyrene from Mealworms

壹.前言

一.研究動機

全國中小學科展「保麗龍的剋星-麵包蟲」中，意外發現麵包蟲可由食用保麗龍長大，但他們的實驗主要在呈現麵包蟲可以食用保麗龍，並未進一步探討麵包蟲由保麗龍中攝取營養的原因。

由於保利龍所引發的生態問題令我十分憂心，又有鑑於保麗龍垃圾污染已成為現今重要的環保議題，所以我想若能找出生物方法有效分解保麗龍，甚至在擴展至各種塑化物上，必能達成垃圾減量，進而對環保造成貢獻。雖然國內外並無任何論文資料發現微生物可分解保麗龍，也幾乎無任何可參考的研究方法，但基於對環保的熱情和對科學的執著，於是我選擇這個題目，期許自己能發揮創意，為地球貢獻心力。

二.研究目的

先確認麵包蟲可經食用保麗龍而生長。猜測其原因為麵包蟲腸道共生菌，於是接著找出蟲體內消化道的共生菌，並進一步篩檢所得的共生菌可否分解保麗龍。希望能找到可分解保麗龍的菌種，為環境盡一份力。

三.研究材料簡介

1、麵包蟲簡介：

麵包蟲（如圖 1、圖 2 所示）俗稱黃粉蟲（英文俗名為 Mealworm），學名為 Tenebrio molitor。

分類上屬於：節肢動物門、昆蟲綱、鞘翅目、擬步行蟲科、粉甲屬，是一種完全變態的昆蟲，牠的生活史包括：卵→幼蟲→蛹→成蟲，卵大約 8~10 天孵化為幼蟲，成為幼蟲後約每隔 10 天脫一次皮，大約 45~55 天會蛻變為蛹，麵包蟲的總生育週期約 100 天。

麵包蟲原產於南美洲，為傳統的活餌，體內含有百分之三十一的脂肪和百分之五十六的蛋白質，人們常以它來飼養小鳥、青蛙、和魚。

圖 1 麵包蟲照片

圖 2 麵包蟲照片

2、保麗龍簡介：

保麗龍(如圖 3 所示)（Polystyrene，PS）是由聚苯乙烯(如圖 4 所示)（Styrofoam）發泡製成，為一種由石油提煉的塑膠原料，由碳和氫組成，化學式是$(C_8H_8)n$，可製成生活中各式各樣的用品。

保麗龍製品因具防水、保溫、使用方便等功能性，以致常常被氾濫使用；國內工業包裝及建材用保麗龍，每年的廢棄量高達 1 萬 2 千多公噸，再利用率雖然高達 67%，但因為回收過程中易被污染，以至回收後品質不佳；且扣除 67%的部份，每年仍有高達 33%約 4 千多公噸的廢包裝保麗龍流入垃圾掩埋場或焚化廠。

保麗龍使用過後，將成為一種頑固的廢棄物，若把保麗龍置於焚化爐中燃燒，需要 800℃才可完全燃燒；由於保麗龍易於急速燃燒，常造成缺氧燃燒，故燃燒大量會容易產生一氧化碳 （CO）、甲醛（HCHO）等氣體及生成有世紀之毒之稱的戴奧辛（Dioxins），在產生的廢氣之中，一氧化碳（CO）、甲醛（HCHO）之濃煙毒氣為酸性具腐蝕性之氣體，若吸入人體會有嚴重傷害。

若把保麗龍掩埋在垃圾場中，它會因在土中無法腐爛而永存於大自然；且保麗龍的體積蓬鬆，占用大量空間，容易縮短垃圾掩埋場的使用年限；此外保麗龍若浮在海面上易被風浪碎裂成小顆粒，海洋生物容易誤以為食物而吞食，所以也是海洋生物的致命殺手；若遇上颱風暴雨，更可能堵塞主要河川，影響排洪功能。

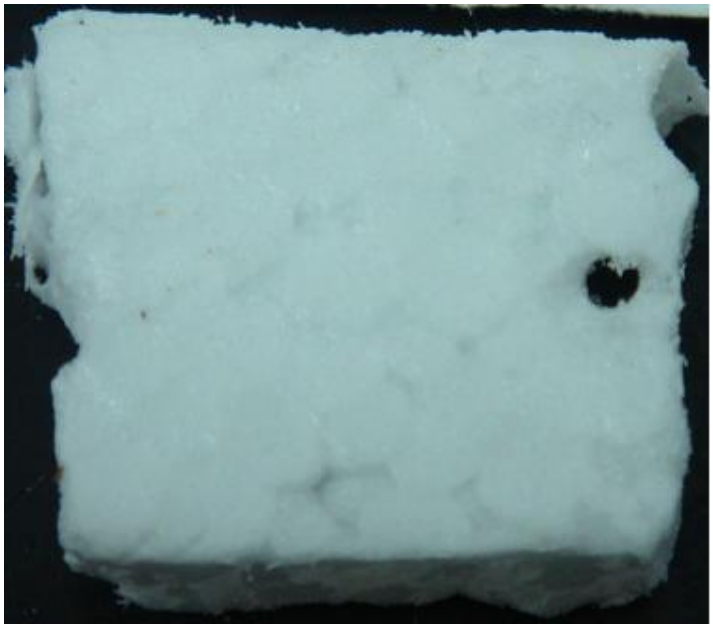


圖 3 保麗龍

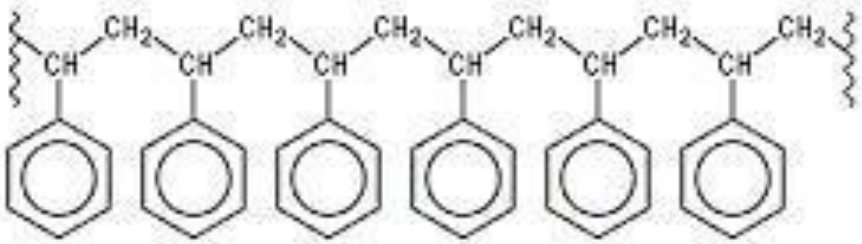
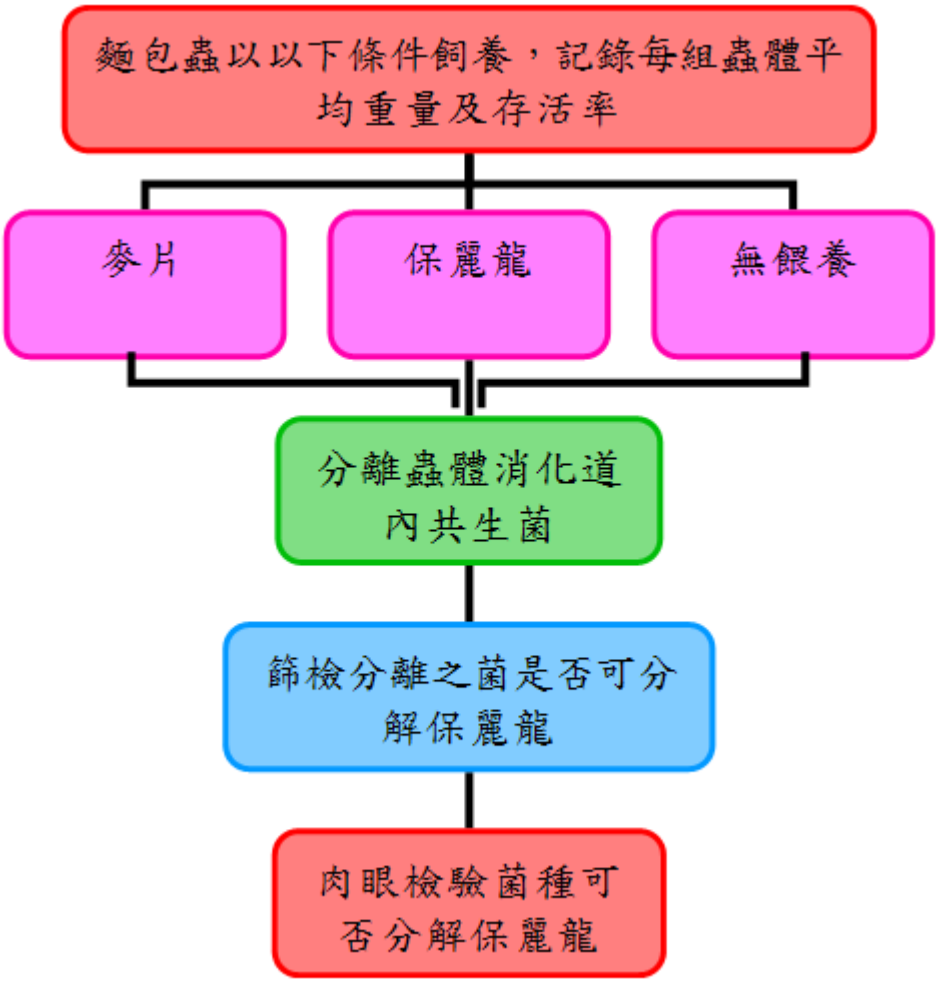


圖 4 聚苯乙烯

貳.研究方法和過程

一.研究流程



二. 研究材料

1、儀器與器材

①、滅菌釜

②、厭氧缸

③、無菌操作台

④、無菌針筒（接菌用，1ml）

⑤、硬試管（養菌用）

⑥、離心機

⑦、厭氧包

⑧、多功能工具盒（養蟲用）

⑨、光學顯微鏡

⑩、分光光度計

2、試藥

①、Sodium chloride（NaCl）

②、Magnesium sulfate （$MgSO_4$）

③、Ammonium dihydrogen phosphate

④、Dipotassium hydrogen phosphate

⑤、Nitrogen（N_2）

⑥、LB Broth（Peptone 10.0 g

Yeast extract 5.0 g

Sodium chloride 5.0 g）

⑦、聚苯乙烯（Polystyrene ；Styrofoam）

⑧、保麗龍（發泡聚苯乙烯）

⑨、食用麥片(桂格燕麥片)

3、實驗動物：麵包蟲（Tenebrio molitor，由鳥店購得）

三.研究方法

1、確認麵包蟲能經由食用保麗龍獲得能量並順利長成成蟲

(1) 在室溫（約 25℃）無太陽直射的條件下，用硬塑膠盒隔水養殖。盡量挑選體形相似、體重相近之麵包蟲，設計三種條件，每種條件各三組如表 1 所示：

表 1

	保麗龍	麥片	水（註）	每盒初始隻數	盒數
A	0 g	2 g	少量	15 隻	3 組 （A1、A2、A3）
B	0.08 g	0 g	少量	15 隻	3 組 （B1、B2、B3）
C	0 g	0 g	少量	15 隻	3 組 （C1、C2、C3）

(2) 每隔十天秤重，計算每組每隻蟲的平均重量，不把蛹及死蟲納入計算。

(3) 每隔十天記錄其存活隻數（含蛹）。

(4) 觀察麵包蟲生長情形。

圖 5 餵養實驗設計照片

圖 6 餵養麥片的麵包蟲

圖 7 餵養保麗龍的麵包蟲

2、尋找麵包蟲消化道內之共生菌

(1) a.將蟲以保麗龍飼養一段時間。

b.以兩次酒精 (70%) 將其表面洗淨後，再以一次無菌水洗去其表面酒精。

c.在無菌台內取麵包蟲之消化道，置於固態 LB 培養基上培養，並於培養基上以 劃線法 (streak plate) 劃單菌落。

d.放入厭氧缸 (GasPak system) 中培養 (37℃) ，過程中嚴防污染。

圖 8 解剖前的麵包蟲

圖 9 浸於酒精中的麵包蟲

圖 10 麵包蟲及其消化道

圖 11 麵包蟲之消化道

(2) 培養一個星期後將培養基從厭氧缸取出，判斷其中有兩種菌，依其菌落顏色暫稱其為紅菌和白菌，分別繼續以培養基（註二）在厭氧環境下培養以利接續實驗。

3、檢測所發現之共生菌是否為可分解保麗龍的菌種

(1) a.將紅菌接入液態 LB 培養基（10 ml）培養一天。

b.用無菌針筒將菌液抽出，置入離心管中離心，去除上清液。

c.在去除上清液的離心管中加入生理食鹽水(註一) ，以震盪器使之混合均勻， 離心後去除上清液，並重複此步驟。

d.用分光光度計測其吸光值，選用吸光值約為 0.06 O.D 時之稀釋倍率，取 0.1 ml 接入以下各管(註二) (如表 2 所示)，放入震盪培養箱培養。

表 2

分 組	保麗龍粉末	LB	合成培養基(註三)
R-A1	10 mg	0 ml	10 ml
R-A2	10 mg	0 ml	10 ml
R-B1	0 mg	0 ml	10 ml
R-B2	0 mg	0 ml	10 ml
R-C1	0 mg	10 ml	0 ml
R-C2	0 mg	10 ml	0 ml

f.接著換成白菌重複以上動作，同理，得 W- A1、W-A2、 W-B1、W-B2、W-C1、W-C2(如表 3 所示)，放入震盪培養箱培養：

表 3

分 組	保麗龍粉末	LB	合成培養基
W-A1	10 mg	0 ml	10 ml
W-A2	10 mg	0 ml	10 ml
W-B1	0 mg	0 ml	10 ml
W-B2	0 mg	0 ml	10 ml
W-C1	0 mg	10 ml	0 ml
W-C2	0 mg	10 ml	0 ml

(2) a.待培養一週後，從 R-A1 抽取 0.1 ml 液體，置於固態 LB 培養基上，以玻璃珠將其塗抹均勻。

b.將固態 LB 培養基放入厭氧缸培養，並將 R-A1 放回震盪培養箱繼續培養。

c.將其餘試管（R-A2、R-B1…… W-C2）重複上述 a~b。

(3) 一星期後取出觀察。

圖 12 三種培養基(由左到右依序為 LB、合成培養基 + 保麗龍、合成培養基)

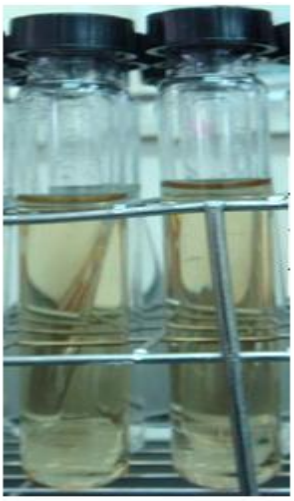


圖 13 LB

圖 14 合成培養基 +保麗龍
(紅圈處為保麗龍)

(註一)為與細胞濃度相似的 0.85 % NaCl 水溶液。

(註二)各管皆曝氮氣，以維持厭養狀態培養。

(註三)合成培養基（inorganic synthetic broth）其成份為（下為每 1 公升水其內所含成分）（以下成份無碳源）

Sodium chloride (NaCl)	5.0 g
Magnesium sulfate (MgSO4)	0.2 g
Ammonium dihydrogen phosphate (NH4H2PO4)	1.0 g

4、以肉眼檢驗紅菌可否分解保麗龍

(1) a. 將紅菌接入液態 LB 培養基（10 ml）中隔夜培養。

b. 用無菌針筒將菌液抽出，置入離心管中離心，去除上清液。

c. 在去除上清液的離心管中加入生理食鹽水，以震盪器使之均勻，離心後去除上清液並重複此步驟。

d. 將最後沉澱物(細菌主要位於沈澱物中)注入生理食鹽水溶液 0.5 ml ，用震盪器使之充分混合。

e.用無菌針筒將離心管內液體吸出，注入合成培養機加保麗龍的培養基內。

f. 另取一管未接菌的合成培養基加保麗龍培養基，將兩管一起放入震盪培養箱培養。

(2) 五日後觀察結果。

參. 研究結果

一. 麵包蟲飼養實驗

1. 每隔十天秤重，計算每組每隻蟲的平均重量，其結果如圖 15 所示：

（原始數據在附錄）

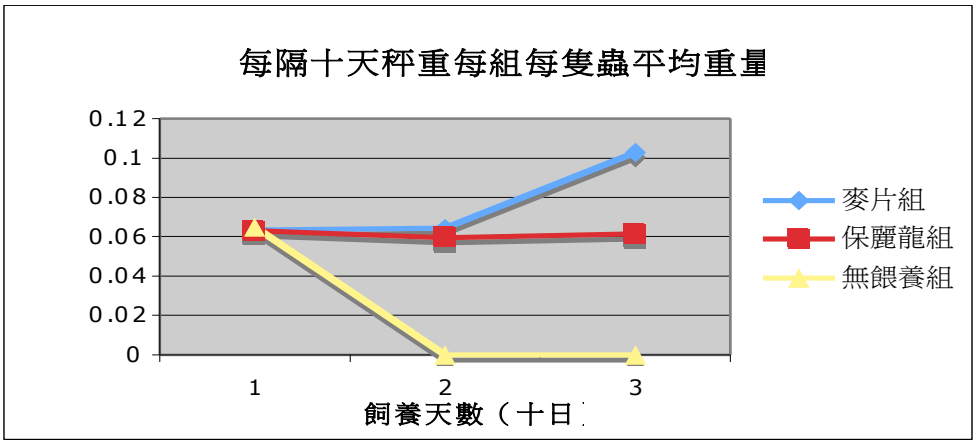


圖 15

2. 每隔十天記錄其存活隻數（含蛹）如圖 16 所示：

（原始數據在附錄）

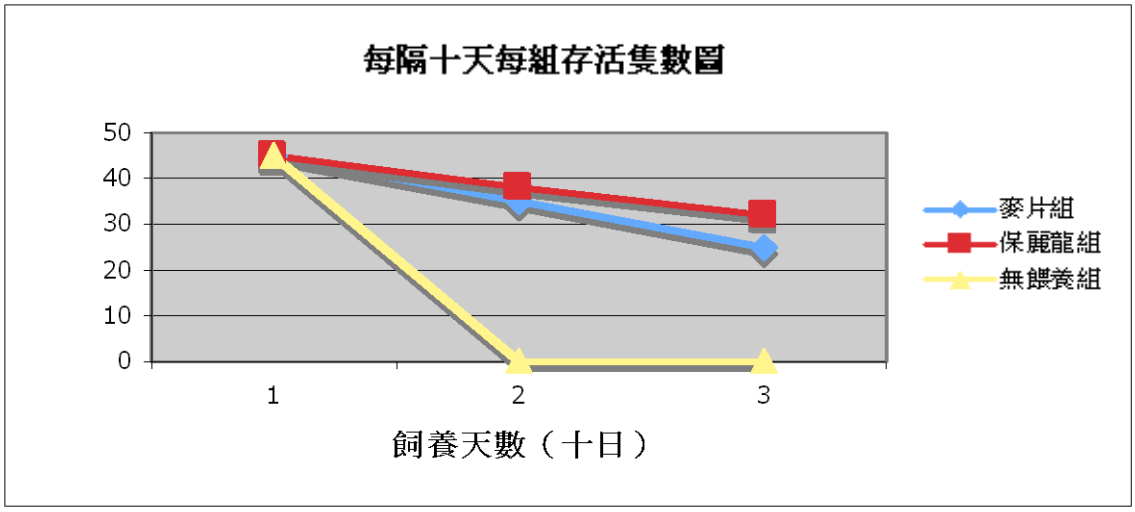


圖 16

3. 由肉眼可明顯發現保麗龍減少 0。

二. 由麵包蟲消化道中分離出兩種共生菌（暫命名為：紅菌與白菌）

三. 檢測所發現之共生菌是否真為可分解保麗龍的菌種

1、由實驗 3 - (2)，將紅菌、白菌以各種條件分別培養一星期後，再以玻璃珠將其均勻塗抹於固態 LB 培養基上，再放入厭氧缸培養一星期後之結果如表 4 所示。

表 4

分 組	菌落有無	分 組	菌落有無
R-A1	O	W-A1	X
R-A2	O	W-A2	X
R-B1	X	W-B1	X
R-B2	X	W-B2	X
R-C1	X	W-C1	O
R-C2	X	W-C2	O

圖 17 檢驗結果(R-A~C) 紅圈處即為紅菌菌落

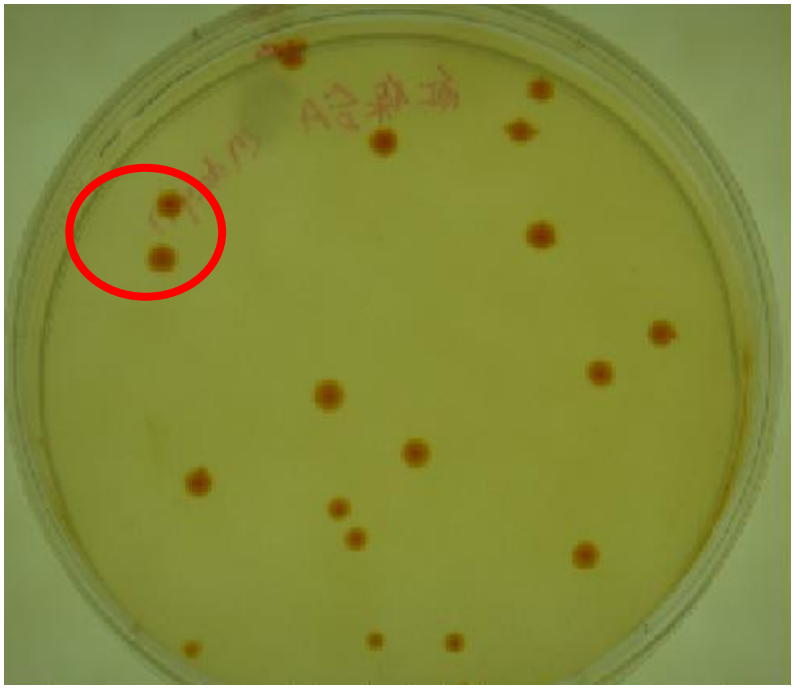


圖 18 R-A1 結果(紅圈處即為紅菌菌落)

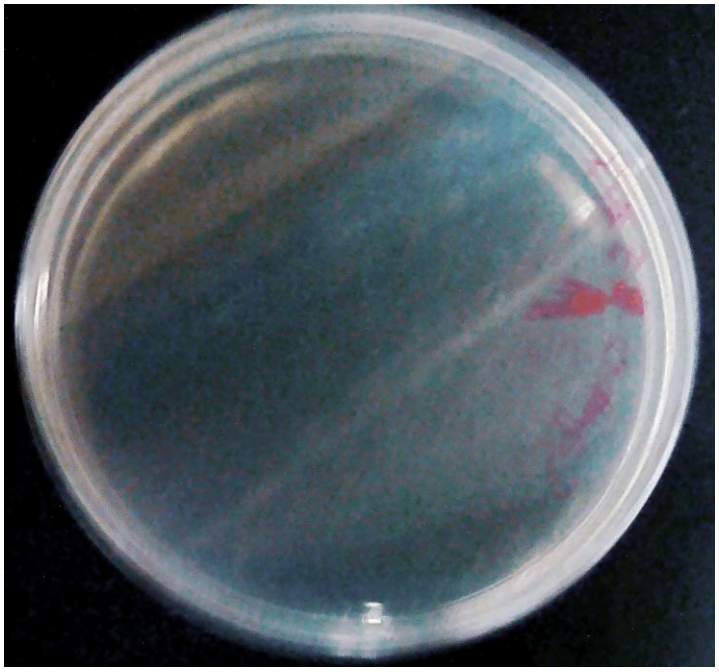


圖 19 W-A1 結果

四. 以肉眼檢驗紅菌可否分解保麗龍

結果如圖 20，圖 21，圖 22，圖 23 所示：

圖 20 未接菌前合成培養基 + 保麗龍

圖 21 一起放入震盪培養箱培養的兩管合成培養基+保麗龍，左管有接菌右管則無（圈起處為保麗龍）

圖 22　一起放入震盪培養箱培養的兩管合成培養基+保麗龍，左管有接菌右管則無

圖 23 接菌並放入震盪培養箱培養五日

肆、討論

一. 麵包蟲飼養與測量之條件討論

在實驗中為避免麵包蟲分解盛裝它的容器，所以須特別選用麵包蟲無法啃咬的硬塑膠盒。此外，因為在飼養的過程中，常常有螞蟻闖入飼養容器咬死麵包蟲，故以隔水養殖以避免這種情況。最後在計算每組每隻蟲平均重量時，不把蛹及死蟲納入計算，是因蛹和死蟲無法進食與生長，但在計算存活率時，因為蛹仍是活的生物體，所以納入計算。

在飼養過程中，無餵養組有發生蟲吃蟲的情況，即死亡數比所找到死掉蟲體數目還多，但麥片組和保麗龍組均無此現象，故可排除保麗龍組麵包蟲長大是因吃其他蟲所造成。此外，由於麵包蟲成為幼蟲後約每隔 10 天脫一次皮，且大約 45~55 天會蛻變為蛹，所以本實驗在測量麵包蟲體重時，選擇以每隔十天進行測量，而總測量時間為三十天。

二.　麵包蟲飼養之結果討論

從平均重量圖可知，保麗龍組（ B 組），重量只略少於麥片組(A 組)，其原因可能是麵包蟲以保麗龍獲得能量的效率較低。若麵包蟲可靠蟲體內貯存的養分繼續生長，那無餵養組應可繼續生長，但無餵養組在二十日前即全部死亡，因此排除麵包蟲完全只依靠體內貯存的養分繼而生長變態的可能性。

由存活數的圖可知，無餵養組（ C 組）在十日後，只剩下約三分之一存活，而在二十日前已全部死亡，但保麗龍組（ B 組）每隔十日都僅約死亡一隻，且存活率都高於麥片組（ A 組），此現象顯示餵食保麗龍組（B 組）與餵食麥片組（A 組）結果相近，而與無餵養組相差甚遠。因此可推斷麵包蟲可由食用保麗龍獲得生長所需之養分。此外由實際飼養麵包蟲發現，麥片組（ A 組）及保麗龍組（ B 組）皆可結蛹，並順利變態為成蟲，兩組成蟲的外觀並無明顯差異。

三. 培養麵包蟲消化道內共生菌之方法討論

在取消化道前先以兩次酒精一次無菌水，將其表面洗淨，目的為盡量使獲得的菌為麵包蟲消化道的菌，而非體表（或空氣中）的菌；在取消化道前， 因麵包蟲會蠕動增加取消化道的難度，所以實驗前先將蟲浸入無菌水中溺斃。經多次實驗後發現，取消化道時先除去頭部並夾掉一部分尾巴，再將麵包蟲之消化道拉出效果最好。此外，因腸道內應含氧較少，所以劃完單菌落的培養基選擇以厭氧條件培養。

四. 檢測共生菌可否分解保麗龍之實驗討論

本實驗因無其他相似研究方法可供參考，在此提出主要問題進行討論(如表 5 所示)：

表 5

失敗結果	須解決問題	解決方法
保麗龍懸浮於培養基上方造成混合困難（且若採固態培養會難以塗菌）	不能讓保麗龍浮在表面，且不能影響塗菌之進行	改採液態培養，且培養時置於震盪培養箱裡，使其充分搖晃，讓保麗龍均勻分布（採液態培養就無須擔心無法塗菌的問題）
LB 培養基本身養分已夠共生菌生長（難得知究竟它會不會分解保麗龍）	需確定菌株獲得的碳源為保麗龍而非培養基中其它材料	改採無碳源之合成培養基培養，強迫保麗龍為其碳之單一來源
無有效對照組	設計對照組以完整實驗	無碳源之合成培養基為對照組

需要洗菌步驟是因 LB 為富含養分的培養基，極易使細菌生長，若直接取已於 LB 中培養隔夜的紅菌接入合成培養基加保麗龍中培養，縱使紅菌可生長，也難以確定是因保麗龍所提供的養分，或是因菌體上殘留的 LB 所提供的養分。實驗中所加入

培養基中的保麗龍粉末為未發泡前之保麗龍（聚苯乙烯粉末），若採發泡後的保麗龍，因其太輕，甚至會在空氣中懸浮，容易造成操作困難及影響測量準確度。本推測發泡前與發泡後保麗龍的成份差異不大，但若實際進行產業研發，仍需比較兩者的差異。

由實驗結果，R-A1 、 R-A2（合成培養基＋保麗龍）有長出菌，且 R-B1、R-B2 (合成培養基) 沒長出菌，即只有合成培養基（B 組）不足以提供能量供紅菌生長繁殖，但合成培養基加保麗龍卻可以（A 組），由此可知紅菌可由保麗龍獲得能量。但實驗結果有一點卻令人惋惜，因為紅菌先前已在 LB 培養基中培養過， 確定可在其內生長，所以 R-C1、R-C2（LB）按常理推斷應要長出紅菌，但實驗結果卻無。此外，在 R-C1、R-C2 組中雖未長出紅菌，但有發現雜菌生長，推測可能是因為操作不熟造成污染，以致於有雜菌生長抑制紅菌。

五.以肉眼檢測紅菌可否分解保麗龍之實驗討論

從圖 20 所示可看出接菌前，保麗龍成片狀浮於合成培養基上，而由圖 22 所示可得知，接紅菌培養五天後，保麗龍明顯碎為小塊粉末狀。原本懷疑其碎裂可能是因震盪培養箱搖晃所造成，但與未接菌組進行比較（圖 21、圖 23 所示），未接菌組雖也經震盪培養，但明顯仍有大片保麗龍存在（紅線圈起處），因此推論紅菌可分解保麗龍。

六.總結

本實驗雖已初步證實麵包蟲中腸道的紅菌可分解保麗龍，但由於時間有限，未能測試紅菌分解保麗龍的最佳條件，或許詳細模擬麵包蟲的腸道環境，可提昇紅菌分解保麗龍的效率。此外，紅菌的培養過程中，似乎會受到其他菌種的抑制，若要大量培養紅菌仍須找到更有效的培養環境（培養基）及抑制其他雜菌的方法。

伍.未來發展

假使我們進一步將紅菌做菌種鑑定，研究其特性，並考慮量產的可能性。將來若能大量運用來解決保麗龍垃圾污染，必定為環保前進的一大步。

另外應研究麵包蟲消化道的環境，並依此調整所選用的培養基，希望找出大量培養紅菌的條件；其次應尋找紅菌最適宜分解保麗龍的條件，讓紅菌可以最有效率的分解保麗龍。

因已清楚觀察到，麵包蟲確實可由食用保麗龍生長，事出必有因，麵包蟲體內確實有一個東西（或機制），能將保麗龍轉化成麵包蟲可利用的能量，只要能找出原因，甚至推廣到其他塑化物上，必能為環保帶來莫大的助益。

陸.主要參考文獻

1. 張永仁 昆蟲圖鑑 第三版 台灣 遠流出版社 1998

2. 張碧芬、袁紹英、游呈祥 微生物學的世界 第一版 台灣 天下文化出版社 2004

3. 溫福賢 微生物操作手冊 96 年版 台灣 國立中興大學生科系 2007

4. 張雅晴、陳佳妤、田永筠、邱麟雅、江佳儒、白韻涵 保麗龍的剋星－麵包蟲 台灣 第 42 屆中小學科展 高雄市三民區博愛國民小學

5. Brasil 2008 Structure, processing and midgut secretion of putative peritrophic membrane ancillary protein (PMAP) from Tenebrio molitor larvae.

6. Goldring JP, Read JS. 1993 Insect acetyl-CoA carboxylase: activity during the larval, pupal and adult stages of insect development.

柒.附錄

一.原始數據

1、每隔 10 天秤重，計算每組每隻蟲的平均重量，其結果數據如表 6 所示：

表 6

	始日	十日	二十日
麥片組	0.06478	0.06435	0.1028
保麗龍組	0.06338	0.05938	0.06144
無餵養組	0.06278	0	0

（單位為克重）

2、每隔 10 天記錄其存活隻數（含蛹）其結果數據如表 7 所示：

表 7

	始日	十日	二十日
麥片組	45	35	25
保麗龍組	45	38	32
無餵養組	45	0	0

（為每種情況總隻數）

評語

本研究有新穎性及應用潛力，可惜部分菌種培養的結果一致性欠佳，此外紅、白菌的分類及紅菌是否確實具代謝保力龍之能力需有更適切的實驗結果加以佐證。

Abstract

Due to the properties of waterproof, light-weigh and low-price, styrofoam is now widespread used in our daily lives. However, overuse of styrofoam caused a serious environmental problem. The observation that mealworms might be able to feed on styrofoam sparked a curiosity to answer whether mealworms could digest styrofoam, and if so, how could they remain alive. First of all, these mealworms were fed in different kinds of food to insure the mealworms can digest styrofoam. The results showed that mealworms can indeed feed on styrofoam only. The hypothesis of this study is that microbes inside the digestive tracts of mealworms are responsible for the decomposition of styrofoam. To further test this hypothesis, microbes from the digestive tracts of mealworms were cultured at 37° C in LB medium under anaerobic conditions. There were many kinds of microbes isolated. From those microbes, a kind of bacteria forming red colonies was confirmed responsible for decomposing styrofoam. The growth conditions and strain characteristics of the bacteria forming red colonies were further examined. These results imply that the red bacteria isolated from the digestive tracts of mealworms may shed light on solving the serious environmental pollution caused by styrofoam.

A styrofoam-decomposing bacterium from mealworms

Introduction

1.Motivation

Styrofoam is usually oversed because it is light weight, water proof, and relatively inexpensive. When I bought mealworms for my cute little parrot, I used a styrofoam box to pack them. I was surprised to find that the mealworms had nibbled holes through the box. Because the fact that the serious pollution caused by styrofoam needs to be solved immediately, I decided to work on this topic out of concern for our environment and passion for science. What is better, this project can even be extended and probably applied to other pollutions like a variety of plastics. I sincerely hope that my research will provide an effective method contributing to environmental protection.

2. Goals

2.1 To study whether mealworms could feed on styrofoam alone.

2.2 To isolate styrofoam-decomposing microbes from the digestive tracts of mealworms.

3.Main material

3.1 Mealworm

Mealworm (*Tenebrio molitorz)* is categorized into Animalia, Arthropoda, Insecta, Coleoptera, Tenebrionidae. Its complete metamorphism includes four life stages - egg, larva, pupa and imago. Its life cycle spans about 100 days.

T.molitor originates from South America and is typically used as bait. Because of the high content of fat (31 %) and protein (56 %) in its body, it is often used to feed birds, frogs and fish.

Figure 1. Mealworm 5x.

3.2 Styrofoam

Styrofoam,a trademarked word, made from expanded polystyrene (PS), is a plastic material of refined petroleum. It is a polymer consisting of styrene.

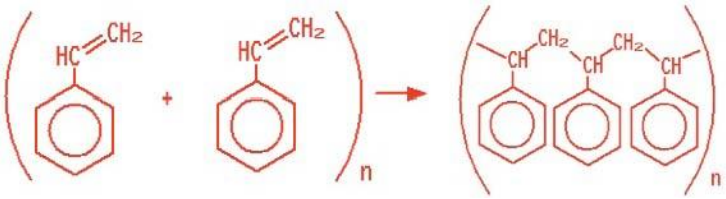


Figure 2. Chemical structural formula of styrofoam.

4. Media

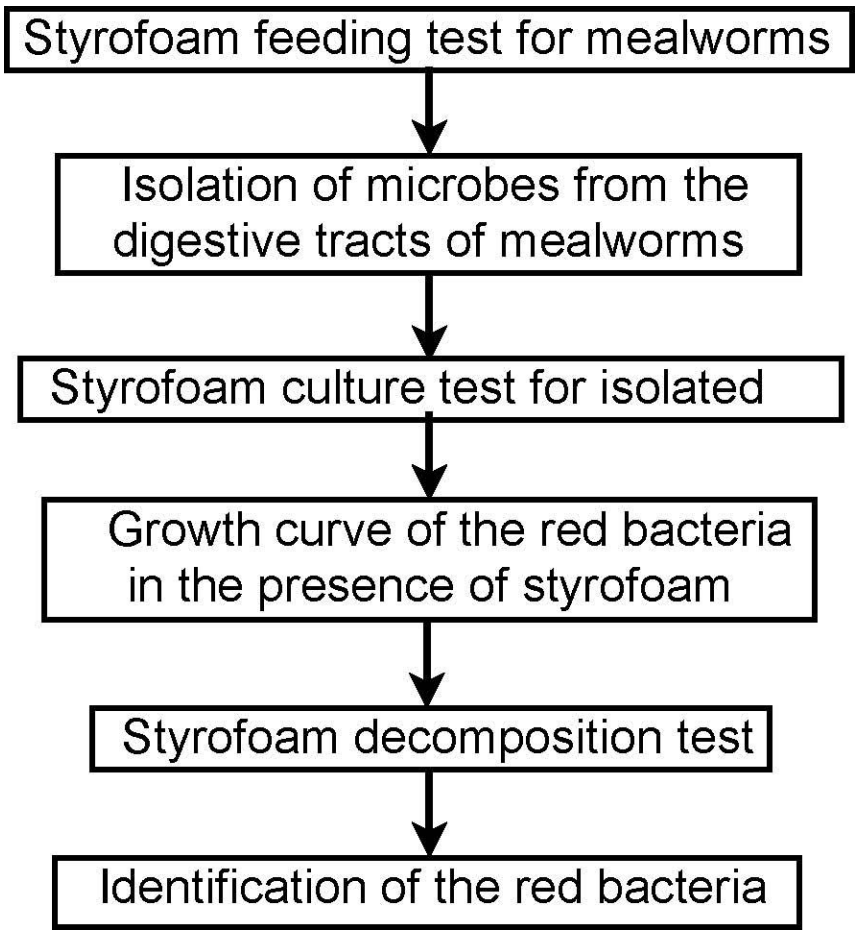
4.1 IS (inorganic synthetic) broth:

Sodium chloride	5.0 g / L
Magnesium sulfate	0.2 g / L
Ammonium dihydrogen phosphate	1.0 g / L
Dipotassium hydrogen phosphate	1.0 g / L

4.2 LB (Luria-Bertain) broth

Tryptone	10.0 g / L
Yeast extract	5 g / L
Sodium chloride	10 g / L

5.Research flow chart



Methods

1. Styrofoam feeding test for mealworms

(a) 10-day-old mealworms were divided into three feeding groups (Table 1.) and raised in three plastic boxes (15 mealworms per boxes) at 25° C .

Table 1. Three cultures were designed to feed mealworms.

Culture	Styrofoam	Oatmeal
A	-	+
B	+	-
C	-	-

(b) Counted the number of surviving mealworms (including pupae) every ten days for twenty days.

(c) Weighed all mealworms (pupae and dead worms were excluded) every ten days and recorded average body weight of each culture.

(d) Observed when mealworms grew.

(A)

(1)Feeding groups ofmealworms

(B)

(2)Mealworms fed with oatmeal

(C)

(3)Mealworms fed with styrofoam

Figure 3. Styrofoam feeding test formealworms.

2. Isolation of microbes from the digestive tracts of mealworms

(a) Chose mealworms fed with different culture.

(b) Soaked mealworms in 70% ethanol for two minutes and then washed with sterilized distilled water twice.

(c) Put mealworms in sterilized distilled water to drown them.

(d) In laminar flow, pulled out the digestive tracts of mealworms and mixed them with dd water.

(e) Spreaded the mixture on LB agar plates.

(f) Incubated the plates under anaerobic condition at 37 ° C.

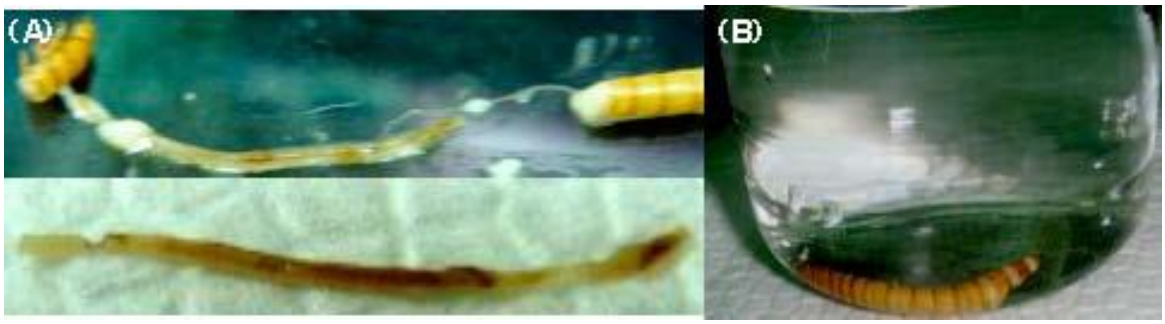


Figure 4. Digestive tracts of mealworms (A) Mealworms drown by water (B).

3. Styrofoam culture test for isolated microbes

(a) Cultured microbes isolated from the digestives tracts of mealworms and *Escherichia coli* in LB broth overnight.

(b) Centrifuged down bacterial cells and discarded supernatant.

(c) Resuspended cell pellets with sterilized distilled water.

(d) Inoculated cell suspension into three different media.

Table. 2 Three different culture media.

Condition	Styrofoam	LB broth	IS broth*
A	0	10 mg	0
B	10 mg	0	10 ml
C	0	0	10 ml

Figure 5. Three media(A,B,C).

(e) After growing for one week, 0.1 ml of culture fluid from different media was spreaded on LB agar plates.

(f) Incubated the plates under anaerobic condition at 37 ° C for one week.

4.Growth curve of the red bacteria in the presence of styrofoam

(a) Cultured the red bacteria in LB broth overnight.

(b) Diluted 100-fold into LB broth and IS broth with styrofoam.

(c) Cultured under anaerobic and aerobic condition.

(d) Number of the red bacteria was measured every four hours.

5. Styrofoam decomposition test

(a) Cultured the red bacteria in LB broth overnight.

(b) Centrifuged and discarded supernatant.

(c) Resuspended cell pellets with sterilized distilled water.

(d) Inoculated the cell suspension into IS broth supplementing with styrofoam disks (diameter : 7 mm thickness : 0.5 mm) for two days.

6. Identification of red bacteria

6.1 Gram's staining

(a) Prepared bacterial smears of the red bacterium and fixed by heat.

(b) Flooded the smear with crystal violet and let stand for 1 minute, and then washed with water.

(c) Flooded the smear with the Gram's iodine solution for 1 minute, and then washed with water.

(d) Decolorized with 95% ethanol.

(e) Washed with water to stop decolorization.

(f) Counterstained with safranin for two minutes.

6.2 Identification by 16s rRNA sequencing

(a) Cultivated the red bacteria in 3 ml of LB broth overnight at 37° C

(b) Centrifuged at 12000 rpm for 15 minutes

(c) Discarded supernatant

(d) Resuspended cell pellet with 550 µl TE buffer

(e) Added 30 µl of SDS (10%) buffer and 15 µl of proteinase K, mixed well.

(f) Added 100 µl of NaCl (5 mol/ L), mix well

(g) Incubated in a 65° C water bath for 10 minutes

(h) Added 100 µl of phenol, chloroform and isoamyl alcohol

(i) Centrifuged at 12000 rpm for 5 minutes

(j) Transfered the supernatant to another tube

(k) Added 70 µl of isopropyl alcohol

(l) Mix them softly until DNA precipitated

(m) Wash the pellet with 70% ethanol

(n) Dried and dissolved the DNA pellet in sterilized dd H2O

(o) Added 5 µl of chromosomal DNA into a tube as template

(p) Added 1 µl dATP, dTTP, dCTP ,dGTP (10 mM)

(q) Added 2 µl universal primer (35 p mole/ ml)

(r) Added 5 µl PCR buffer (10X)

(s) Added 2 µl Taq DNA polymerase

(t) Added sterilized dd H2O to 50 µl

(u) Started PCR (94 ^{o}C 30s, 55 ^{o}C 30s, 72 ^{o}C 30s =>35 cycles)(Perkin Elmer 2400)

(v) Sequenced the amplified 16s rRNA gene fragment.

6.3 Observed the red bacteria with microscopy.

Results

1. Styrofoam feeding test for mealworms

1.1 The mealworms of each culture were weighed every ten days and their average weight was recorded. The results are as following.

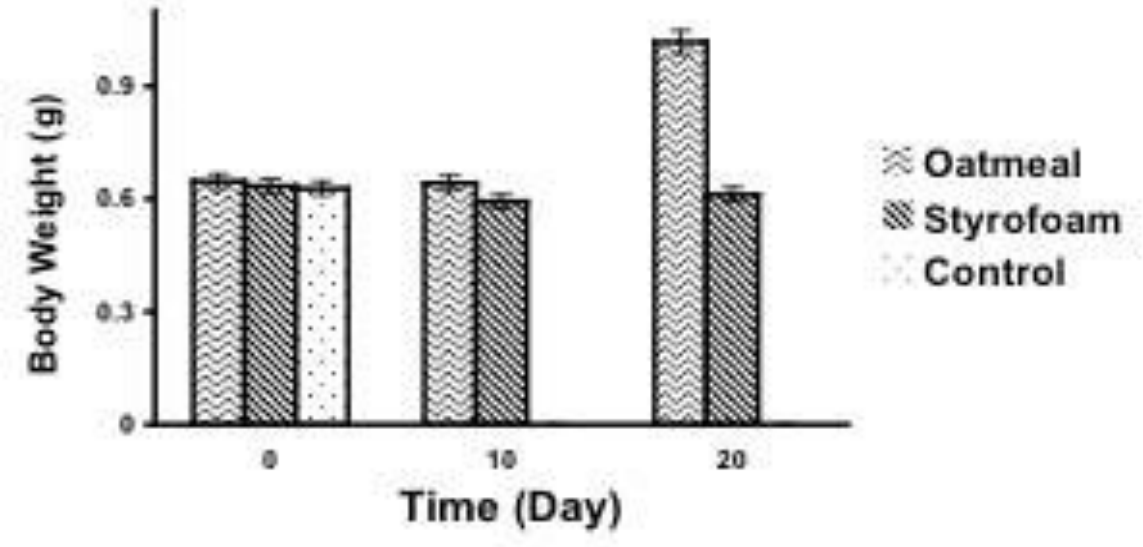


Figure 6. Average body weight of survival mealworms.

The average body weight of mealworms fed with styrofoam was similar to those with oatmeal, the control group could not grow without feeding.

1.2 Every ten days, the number of surviving mealworms were recorded (including pupae). The results are as follows.

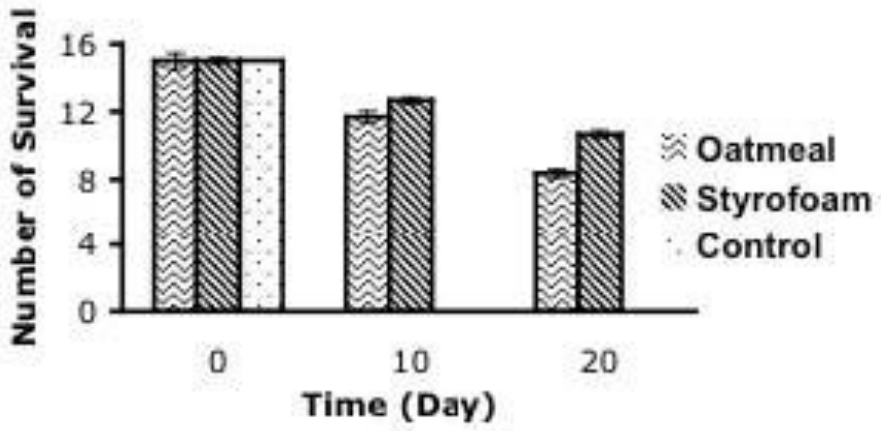


Figure 7. Average number of surviving mealworms.

The average numbers of surviving mealworms fed with styrofoam were similar to those with oatmeal. After twenty days, the control condition had no mealworms left.

2. Isolation of microbes from the digestive tracts of mealworms

Microbes isolated from the digestive tracts of mealworms fed with styrofoam contained a high percentage of the red bacteria, but microbes from mealworms fed with oatmeal did not.

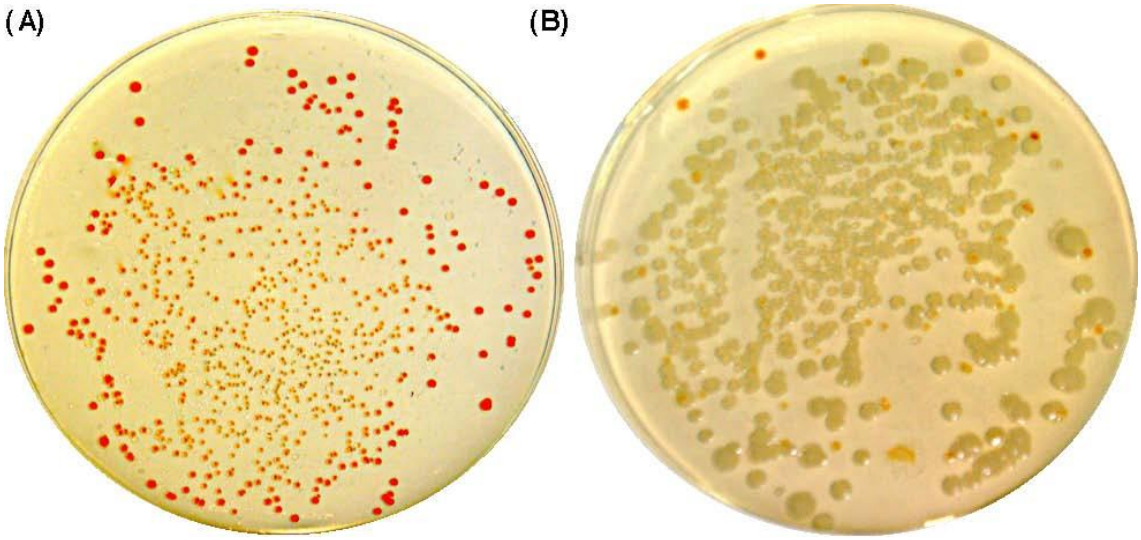


Figure 8. Microbes isolated from mealworms feeding on styrofoam (A) and oatmeal (B). (under anaerobic condition)

The majority of microbes isolated from mealworms feeding on styrofoam was a kind of bacteria forming red colonies, whereas those from mealworms feeding on oatmeal could not detect.

Figure 9. The streaking plate of the red bacteria.

When the red bacteria was streaked on LB agar plate, the same colonies ensure it is a pure isolate.

3. Styrofoam culture test for isolated microbes

The bacteria forming red colonies grew in IS broth with styrofoam, but did not grow in IS broth alone. *E. coli* and other bacteria isolated from the digestive tracts of mealworms did not grow in IS both even the styrofoam was added. Therefore, the red bacteria could use styrofoam as the only carbon source but the bacteria without styrofoam-decomposing activity could not.

Table 3. Growth of microbes with different medium.

Medium Microbe	A	B	C
R	+	+	-
E	+	+	-
O	+	-	-

A,B,C : see Table 2.
R : the bacteria forming red colonies
E : *E. coli*
O : the other bacteria isolated from

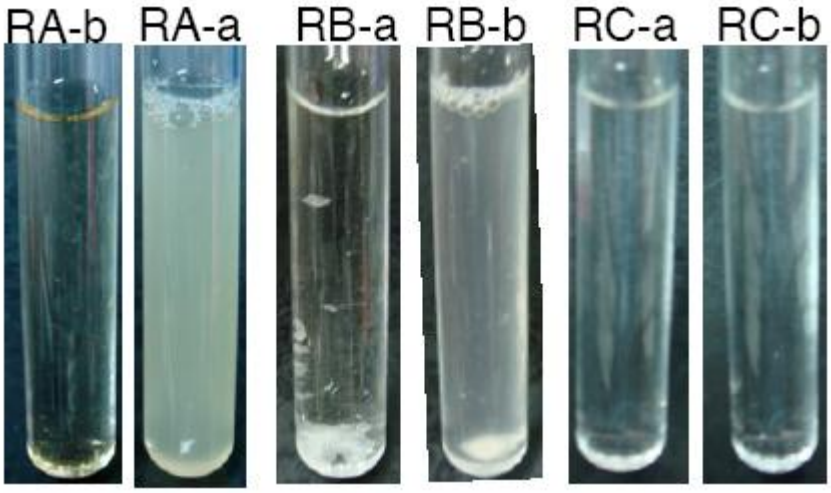


Figure 10. RA, RB, RC before cultured (b) and after cultured (a).

4.Growth curve of the red bacteria in the presence of styrofoam

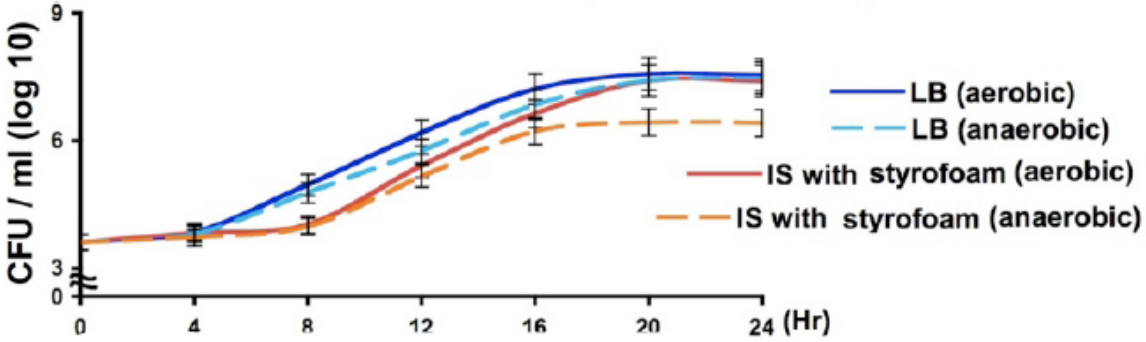


Figure 11. Growth curve of the red bacteria.

The red bacterium could surely grow in the presence of styrofoam under both anaerobic and aerobic conditions. The growth rate of the red bacteria under aerobic condition was higher than that under anaerobic condition

5. Styrofoam decomposition test

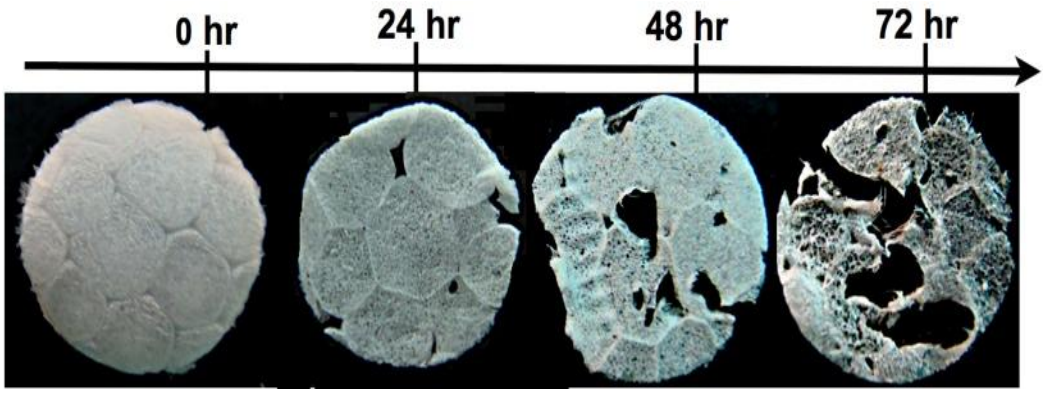


Figure 12. Styrofoam decomposed by the red bacteria.

Styrofoam can be decomposed by the red bacteria. The ability of red bacteria to decompose styrofoam is remarkable.

6. Identification of the red bacterium

6.1 Light and electron microscopy

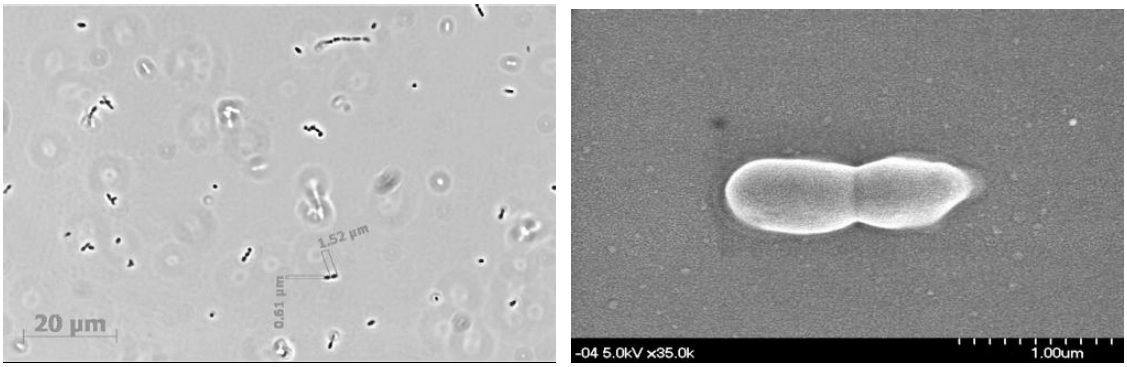


Figure 13. Morphology of the red bacterium.

The red bacterium is rod-shaped. Left is taken under a light microscope. Right is taken under a SEM.

6.2 Gram staining

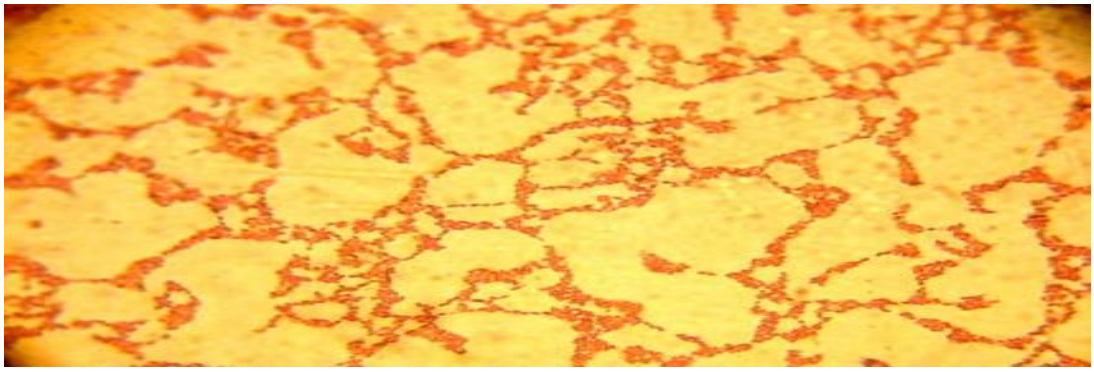


Figure 14. Gram staining of the red bacteria.

The red bacteria are Gram-negative.

6.3 The identification of 16S rRNA gene nucleotide sequence

The partial 16S rRNA gene nucleotide sequence of the red bacterium has 98 % identity to *Proteus mirabilis*. (Figure 15.)

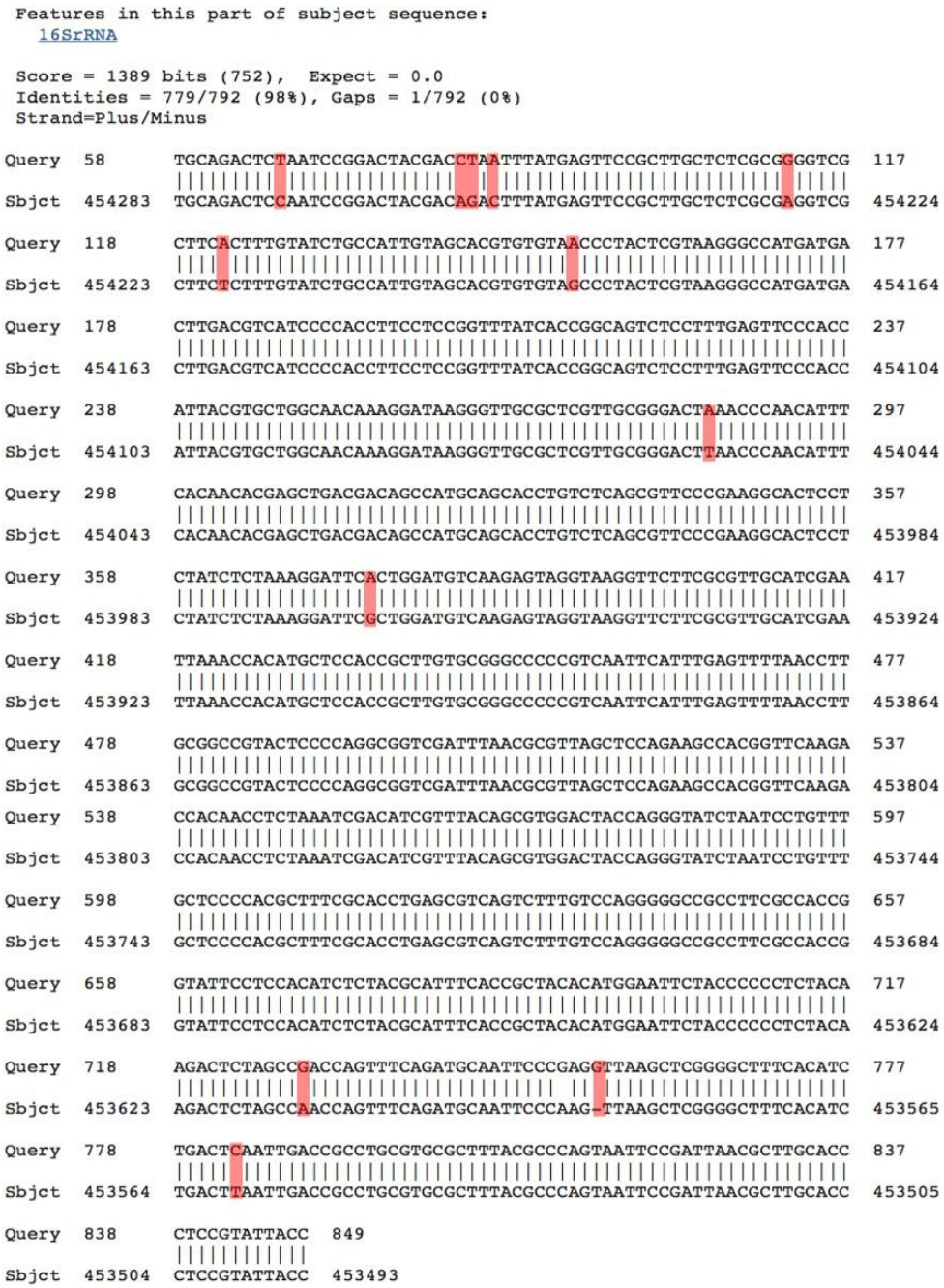


Figure 15. 16S rRNA sequence of the red bacteria (above) and Proteus mirabilis (below). (highlighted pairs have different bases, all others are identical)

Discussion

1. Styrofoam feeding test of mealworms

With ten days mealworms fed with nothing had all died. In this test, the survivals of mealworms fed with styrofoam was similar to those fed with oatmeal. The body weight of surviving mealworms (in terms of increasing or decreasing weight) of mealworms fed with styrofoam was similar to those fed with oatmeal and different from those fed with nothing. It indicates that mealworms could survive by using styrofoam as their only carbon source. In fact, It was also observed that mealworms fed with both styrofoam and oatmeal could complete their life cycle (includes egg, larva, pupa and imago).

2. Isolation of microbes from the digestive tracts of mealworms

Microbes isolated from the digestive tracts of mealworms fed with styrofoam contained a high percentage of the red bacteria. In other words, the red bacterium can survive by using styrofoam as their only carbon source.

3.Styrofoam culture test for isolated microbes

3.1 problems and solutions

3.1.1 Event: Styrofoam floated on medium, causing the trouble when mixing it. If solid medium is used, the styrofoam on the surface would be difficult for spreading microbes.

Solution: LB broth was used to replace solid medium. To mix medium uniformly, put culture tubes into shaking cultivation chamber.

3.1.2 Event: Nutrients in LB are enough for microbes to grow, so even though there are microbes can grow in LB with styrofoam. It is difficult to judge whether they can decompose styrofoam or not.

Solution: Using broth without a carbon source (inorganic synthetic broth).

4. Growth rate of red bacteria in the presence of styrofoam

At the begining, red bacteria in IS broth with styrofoam could not grow as well as in LB broth. However, at end the growth of the red bacteria in IS broth with styrofoam under aerobic condition is simillar to those in LB broth. The growth rate of the red bacteria with styrofoam under aerobic condition was higher than those under anaerobic condition, so pollution problems caused by styrofoam may be solved at a lower cost since anaerobic condition is not necessary for the red bacteria to decompose styrofoam.

5.Identification of red bacteria

There was no pervious reports showed that *P. mirabilis* could decompose styrofoam. Therefore, the strain found in this study might be a new strain due to its ability to decompose styrofoam.

Conclusions

- Mealworms could survive by feeding on styrofoam only.
- A predominant, facultatively anaerobic red bacteria was isolated from the digestive tract of mealworms fed with styrofoam.
- The red bacteria could decompose styrofoam, especially under aerobic condition.
- *Proteus mirabili*s has never been reported to decompose styrofoam. The red bacteria found in this study might be a new strain due to its ability to decompose styrofoam.
- This study may lead to the possibility of solving pollution problems caused by styrofoam.

Future work

- To study the mechanism of degradatiom of styrofoam by the red bacteria.
- Find the gene of decomposing styrofoam from the red bacteria.
- Find a way to mass produce the red bacteria.
- To use the red bacteria for decrease of the environmental pollution caused by styrofoam.

English Bibliography

- Food Industry Research and Development Institute. (2000). Media. Catalogue of fungi, (pp. 150). Hsin-Chu: Food Industry Research and Development Institute.
- Manual, D.Brenner.J and Bryant, P. (1986). Bergey's manual of Systematic bacteriology. Vol. 2: 327-330. USA: Williams and Wilkins.
- Krieg, N. R. and Holt, J. G. (1984). Bergey's manual of systematic bacteriology. Vol. 1: 587-591. USA: Williams and Wilkins.
- Rauprich, O., Matsushita, M., Weijer, C. J., Siegert, F., Esipov, S. E., and Shapiro, J. A. (1996). Periodic phenomena in *Proteus mirabilis* swarm colony development. *Bacteriol*, Vol.178, pp. 6525-38.
- Ayati, B. P. (2006). A structured-population model of *Proteus mirabilis* swarm-colony development. *Math Biology,* Vol. 52 pp. 93-114.