
Dear Participants!
親愛的參賽者

In the laboratory "**MICROBIOLOGY AND BIOTECHNOLOGY"** you will be given the
在「微生物與生物技術」的實驗部分，你必須完成以下兩項實作：
following two tasks:

Task 1. Identification of microorganisms.
第一題　微生物鑑定
Task 2. Study of Bacterial cultures expressing different genes.
第二題　檢測表現不同基因之菌種培養

Duration of the lab work is **60 minutes.**
實驗時間為 60 分鐘
Maximal number of points – **64** .
滿分為 64 分

You have to write down your results and answers into the **ANSWER SHEET** which will
你必須把實驗結果及答案填寫在答案卷中，答案卷將於實驗結束時由助教收回，你
be collected by an assistant when the time elapses. It is not necessary to write anything in the
無須在題目卷上做答。
task sheets.

Result sheets taken away from the laboratory will not be accepted!
答案卷嚴禁攜離實驗室

Please be careful when performing reactions and do not let the reagents and solutions
請小心各項實驗操作，不要讓勿讓藥品及溶液碰觸到皮膚及衣服。
to contact your skin and clothes!

GOOD LUCK!

Country_____

國家

First name_____ **Family name** _____

名 **姓**

Code_____

編號

Task 1. (46 points) Identification of microorganisms.

第一題（46 分） 微生物鑑定

Materials and equipment
材料及器材

1. Bacterial strains in:
   細菌菌種培養於
   - Petri dishes with solid media (plate "GCO" – 1, plate “protease” – 1, plate “amylase” – 1);
   固態培養基(GCO 培養皿 1 個、「蛋白酶」培養皿 1 個、「澱粉酶」培養皿 1 個)
   - tubes with solid medium (for “O/F-test”);
   試管固態培養基(O/F 試驗用)
   - tubes with broth (for “H_2S-test” и “NR-test”).
   試管液態培基(硫化氫試驗用或 NR 試驗用)
2. Wooden toothpicks for transfer of bacterial biomass from solid medium onto glass slides.
   將細菌從固態培養基挑取抹在玻片用的竹籤
3. Glass slides.
   玻片
4. Pipettes.
   吸管
5. KOH solution, 3 %.
   3 %的 KOH 溶液
6. H_2O_2 solution, 3 %.
   3 %的 H_2O_2 溶液
7. Dimethylparaphenilendiamine (DMPA) solution, 1 % .
   DMPA 溶液
8. Lugol's solution (Lugol).
   Lugol's 溶液(標示為 Lugol)
9. Griess solution, 1% (Griess).
   1% 的 Griess 溶液(標示為 Griess)

Identification of bacteria is based on the study of their certain biological properties, mostly
菌種鑑定是根據其生物特性，大多是形態的、生理的及生化的特性，你必須辨認五個
morphological, physiological and biochemical. You have to identify five bacterial strains
菌種，標示為№ 1-5。
labelled № 1-5. For this you'll have to perform five biochemical tests yourself (1.1, 1.3, 1.4, 1.6
為此，你將進行五種生化測試（1.1, 1.3, 1.4, 1.6 及 1.8），
and 1.8), and also use the results of the remaining tests given to you (tests 1.2, 1.5, and 1.7).
以及其他測試（1.2, 1.5, and 1.7）所得的結果來做判斷。
Some tests are followed with additional questions on the corresponding topic that you have to
有些測試之後，分別有對應的題目必須回答。
answer.

Please fill your results in the table "Identification of bacteria" in the answer sheet using the
在答案紙上的「細菌鑑定」表中，填入適當符號（如下）：
following symbols: "+" – positive reaction, "-" – no reaction. A sample table is given below.
"+" – 正（陽性）反應； "-" – 無（陰性）反應。表格如下頁所示。
Attention! In the column "Gram reaction" you have to put "+" for Gram-positive bacteria and "-"
注意！在「革蘭氏染色」一欄中，"+"表「革蘭氏陽性反應」； "-"表「革蘭氏陰性反

– for Gram-negative. In the column "O/F-test" put letter "F" for organisms with fermentative metabolism and letter "O" – for organisms with oxidative metabolism.
應」。在「O/F 測試」中，"F"表示有醱酵作用；"O"表示具氧化反應

Fill all columns of the table except for the last one. Then identify your bacterium using identification table in the end of the task sheet and put the letter corresponding to the identified species into the column " Result of identification".
填入所有欄的空格（最左欄除外），然後查對最後一頁的鑑定表，再將所對應的細菌名稱的英文字母代號，填入答案紙表格之「鑑定結果」一欄中。

Identification of Bacteria (30 points)
菌種鑑定（30）

Strain 菌種	Gram reaction 革蘭氏染色	O\ F-test O\ F 測試	The presence of: 是否有～								Result of identification 鑑定結果
			catalase 觸酶	oxidase 氧化酶	protease 蛋白酶	amylase 澱粉酶	H_2S production 產生硫化氫	nitrate reductase 硝酸還原酶			
1											
2											
3											
4											
5											

PLEASE BE CAREFUL WHEN PERFORMING REACTIONS AND DO NOT LET THE REAGENTS AND SOLUTIONS TO CONTACT YOUR SKIN AND CLOTHES!
請注意：小心操作實驗，勿讓藥品及溶液碰觸到皮膚及衣服。

PLEASE PUT USED PIPETTES, WOODEN TOOTHPICKS, GLASS SLIDES, FILTER PAPER, ETC. INTO A SPECIAL CONTAINER ON YOUR BENCH!
請將使用過的吸管、木籤、玻片、濾紙等放在實驗桌上的特定容器中。

Test 1.1. Gram reaction

革蘭氏反應

To perform this test you need:

操作此實驗你必須有：

1. Biomass of bacterial strains № 1-5 (from the GCO plate).
細菌菌種 № 1-5（從ＧＣＯ培養基中取出部分量）
2. KOH solution (3 % KOH).
3 % KOH 溶液
3. Five glass slides.
五片玻片
4. Wooden toothpicks.
木籤

Attention! You will need the «GCO» Petri dish later to perform tests 1.3 and 1.4. Please
注意！接下來你仍需要用到「GCO 培養基」（測試 1.3 至 1.4）
perform the tests in the suggested order: 1.1, 1.3, 1.4.
請依照 1.1→1.3→1.4 的次序操作

The method:

方法

Using a dropping bottle, put a small drop of the 3 % KOH solution onto a glass slide.
用滴瓶，在玻片上滴一小滴的 3 % KOH 溶液，
Using a toothpick, transfer some biomass (roughly 3-4 mm in diameter) of one strain to the KOH
分別以木籤上取少量的菌（直徑大約 3-4mm），加在 KOH 溶液滴上
drop, trying not to transfer the agar. Mix the bacterial mass with the KOH solution thoroughly. If
並避免取到洋菜膠， 將菌與 KOH 混合均勻
the mass sticks to the toothpick and moves behind it, the strain is Gram-negative, otherwise –
若菌體粘在木籤上並可隨木籤移動，則此菌為革蘭氏陰性，否則為革蘭氏陽性。
Gram-positive. You can repeat the test if results are not clear.
若結果不清楚，可重複此測試！
Using new toothpick each time, repeat the test with the remaining strains. Put the results in
操作不同菌時，用新的木籤。將結果填在答案紙上的「菌種鑑定」表格的對應格
the corresponding column of the "Identification of bacteria" table in the answer sheet using "+"
中。"+"：表示「革蘭氏陽性」；"-"：表示「革蘭氏陰性」。
for Gram-positive bacteria and "-" – for Gram-negative.

Test 1.2. (O/F- test).

測試 1.2. (O/F 測試)

O/F-test allows to determine the ability of bacteria to utilise glucose in aerobic (oxidative
O/F 測試可用以辨識細菌是否可在有氧狀態下利用葡萄糖(氧化反應)，以及在無氧狀
metabolism) and anaerobic (fermentative metabolism) conditions.
態下利用葡萄糖（醱酵作用）。

To determine the ability of your strains to utilise glucose aerobically and anaerobically,
為決定細菌菌種在有氧及無氧狀態下利用葡萄糖的能力，每個菌種
each strain was inoculated in advance into two tubes with agarised medium containing required
已預先接種在兩個含有基本的礦物鹽、葡萄糖及酸鹼指示劑（water blue and rosolic acid）
mineral salts, glucose and a pH indicator (water blue and rosolic acid) which is pink at neutral

的洋菜膠培養基中，該指示劑在中性時為粉紅色，酸性ｐＨ值時為藍色，鹼性時為紅
pH, blue at acidic pH and red at basic pH. To create anaerobic conditions, medium in the tubes
色。　　為了要製造無氧狀態，編號 1a – 5a 的試管在接
labelled 1a – 5a was covered with vaseline oil immediately after inoculation, while the tubes 1b
種細菌後隨即以凡士林封口，而 1b-5b 的試管則不塗凡士林。將這些試管在恆溫箱內培
– 5b remained without oil. The tubes were incubated in a thermostat for 24 hours.
養２４小時。

Analyse the colour change in the tubes for each strain. Put the results in the column "O/F-
分析每根試管內的顏色變化。在答案卷「菌種鑑定」表中的「O/F 測試」欄
test" in the table "Identification of bacteria" in the answer sheet. Use letter "F" for organisms
中作答。用字母"F"表示生物進行醱酵作用，以字母"O"表示生物進行氧化反應。
with fermentative metabolism and letter "O" – for organisms with oxidative metabolism.

1.3. Catalase test.
觸酶測試

To perform this test you need:
進行這個測試你需要：

1. Biomass of bacterial strains № 1-5 (on the GCO plate).
   細菌菌株№ 1-5（在 GCO 盤上）
2. Hydrogen peroxide solution (3 % H_2O_2).
   過氧化氫水溶液(3 % H_2O_2).
3. Five glass slides.
   五片玻片
4. Wooden toothpicks.
   木製牙籤
5. Pipettes.
   吸管

The method:
方法

Using a pipette, put a drop of hydrogen peroxide solution onto a glass slide. Using a
用吸管（pipette）滴一滴過氧化氫水溶液在玻片上。　　使用一根牙
toothpick, transfer some biomass of one strain from the GCO plate to the drop, trying not to
籤，自每一 GCO 盤中轉移部份細菌至過氧化氫溶液水滴內，儘量不要刮到洋菜膠。
transfer the agar. Mix bacterial mass with the hydrogen peroxide solution thoroughly. Register
把細菌與溶液充分混合。
the results while mixing the bacteria with the solution. Repeat the manipulation with the
在混合的過程中，記下實驗結果。　　按照相同的步驟處理其他編號的
remaining strains. Put the results in the corresponding column of the "Identification of bacteria"
菌株。　　把答案填入答案卷中「菌種鑑定」表的相關欄位中。
table in the answer sheet

Question 1.3.1. (2 points) Which reaction(s) is catalysed by catalase?
下列哪些是經由觸酶所催化的反應？

A. $3H_2O_2 + FADH_2 \rightarrow 3H_2O + O_2 + H_2 + FAD$
B. $2H_2O_2 \rightarrow 2H_2O + O_2$

C. $H_2O_2 \rightarrow 2HO^{\cdot}$
D. $H_2O_2 \rightarrow 2HO_2^- + H_2$
E. $2H_2O_2 + NADH + H^+ \rightarrow 2H_2O + NAD^+$

Put your answer code or codes into the line 1.3.1.
把你的一個或多個答案（代碼）填入 1.3.1 欄中

1.3.1. _____

Test 1.4. Cytochrome oxidase test.
細胞色素氧化酶測試

To perform this test you need:
進行此項實驗，你需要以下材料：

1. A Petri dish (GCO), with colonies of strains № 1-5.
一個標示為 GCO 的培養皿，內含編號№ 1-5 的菌株
1. 1 % solution of DMPA.
1 % DMPA 溶液

The method:
方法

Using a dropping bottle, put a drop of DMPA onto each colony. 30-60 seconds later the
使用一個滴瓶，在每一菌落中滴入一滴 DMPA 溶液，在三十至六十秒後，
colonies of oxidase-positive strains turn dark red (pink). Analyse the colony colour of each strain
對氧化酶呈現陽性反應的菌落為暗紅色（或粉紅色），分析每一菌株的菌落顏色變化，
and fill the results in the corresponding column of the "Identification of bacteria" table in the
把答案填入答案卷中「菌種鑑定」表的相關欄位中。
answer sheet.

Question 1.4.1. (4 points) Which of the following statements are true for cytochrome
下列哪些是對細胞色素氧化酶呈陽性反應細菌的
oxidase positive bacteria?
正確描述？

A. Capable of using O_2 as terminal electron acceptor in the respiratory chain.
可在呼吸鏈中利用 O_2 做為最終電子接受者。
B. All are capable of anaerobic respiration.
皆可進行無氧呼吸
C. All are strict aerobes.
全部屬於絕對的好氧菌
D. All are strict anaerobes.
全部屬於絕對的厭氧菌
E. All are facultative anaerobes.
全部屬於兼性厭氧菌（兼氣菌）
F. Cytochrome oxidase takes part in chemosynthesis in some strains.
在某些菌種中，細胞色素氧化酶參與化學合成反應

Put your answer code or codes into the line 1.4.1.
把你的一個或多個答案（代碼）填入 1.4.1 欄中

1.4.1. _____

1.5. Proteolytic activity test.

蛋白質水解活性測試

For determination of proteolytic activity you have to analyse a Petri dish with media
為了鑑定蛋白質水解活性的差異，你需要利用內含有酪蛋白的培養基，
containing casein, inoculated in advance with strains № 1-5. This plate is labelled "protease". Fill the
而後接種入不同的菌株（№ 1-5），此培養皿已標示為「蛋白酶」（protease），
results in the table in the answer sheet.
請把結果填入答案卷的表中

Test 1.6. Amylase test.
澱粉酶測試

The plate labelled "amylase" contains rich solid medium supplemented with 0.2% of starch
在標示為「澱粉酶」（amylase）的培養皿中，裝有 0.2%的澱粉之固態培養基
and has been inoculated with strains № 1-5 in advance. Cover the surface of this plate with
，並已預先接種上不同的菌株№ 1-5。 以 Lugol 溶液覆蓋培養皿表面，
Lugol's solution (Lugol) and determine which bacteria have the amylolytic activity. Fill the
若呈現紫色則為陽性反應。檢測哪些菌株具有澱粉酶活性。
reaction results into the corresponding column of the "Identification of bacteria" table in the
把答案填入答案卷中「菌種鑑定」表的相關欄位中。
answer sheet.

1.7. Test for hydrogen sulphide generation (H_2S-test).
H_2S 生成之測試

Here you have to analyse five tubes prepared before. The tubes contain meat broth and were
在此你須分析先前準備的五根試管，管中含有牛肉液態培養基，並已預先
inoculated with test strains some time before. The tubes also contain pieces of white indicator paper
接種菌種。 管中含有一片被醋酸鉛溶液浸潤（濕透）的白
saturated with the solution of lead acetate. Fill the results in the table in the answer sheet.
色試紙。若有 H_2S 生成則試紙會呈現黑色。將結果填入答案欄的表格內。
Write down in the answer sheet the single letter code for the correct answer to the two
並在答案卷上回答以下兩個問題，填入適當的英文字母代號。
questions below:

Question 1.7.1. (4 points) When bacteria capable of producing H_2S grow on meat broth
若細菌在牛肉液態培養基中可生成 H_2S，此 H_2S 是來自
medium, H_2S is generated from:
於

A. RNA.

B. DNA.

C. Arginine.
精胺酸

D. Methionine.
甲硫胺酸

F. Glycine.
甘胺酸

G. Thiamine.
硫胺酸（維他命 B_1）

H. Biotin.
生物素（維他命 H）

I. Taurine.
牛磺酸

E. Serine. 絲胺酸　　J. Cysteine. 半胱胺酸

Put your answer code or codes into the line 1.7.1.
把你的一個或多個答案（代碼）填入 1.7.1 欄中

1.7.1.: _____

Question 1.7.2. (2 points) Which reaction is responsible for the change of the indicator paper colour?
哪一個反應式表示試紙變色的過程

A. $2CH_3COOH + H_2S = (CH_3CO)_2S + 2H_2O$
B. $Pb^{2+} + S^{2-} = PbS$
C. $(CH_3COO)_2Pb + H_2S = 2CH_3COOH + Pb + S$
D. $2CH_3COOH + H_2S = CHSCOOH + 2H_2$
E. $2CH_3COOH + Pb + 2H_2S = 2C_2H_6 + PbSO_4 + S$

Write your answer code or codes down in the line 1.7.2. of the answer sheet
把你的一個或多個答案（代碼）填入 1.7.2 欄中

1.7.2.: _____

1.8. Nitrate reductase test (NR-test).
硝酸還原酶測試

For this reaction you need:
此反應需要：
1.Tubes with suspensions of cells of strains № 1-5 marked as "NR".
標有"NR"的№ 1-5 菌株細菌懸浮液之試管
2. Griess reagent, 1 % (Griess).
1 % Griess 試劑
3.Pipettes.
吸管

Add 1 ml of the 1% Griess (Griess) reagent to the to suspension of bacteria. The presence of nitrate reductase activity results in the appearance of red colour within 1 minute. Fill the results in the table in the answer sheet.
在細菌懸浮液中加入 1 ml 的 1 % Griess 試劑，若具有硝酸還原酶活性，則一分鐘內可見紅色反應，將結果填入答案卷的表中。

Question 1.8.1. (4 points) The presence of nitrate reductase allows:
硝酸還原酶可使細菌

A. To use nitrate as electron acceptor in the electron transport chain during chemosynthesis.
利用硝酸鹽作為化學合成作用中，電子傳遞鏈之電子接受者。

B. To use nitrate as electron donor in the electron transport chain during respiration.

利用硝酸鹽作為呼吸作用中，電子傳遞鏈之電子提供者。

C. To use nitrate as electron donor in the electron transport chain during chemosynthesis.
利用硝酸鹽作為化學合成作用中，電子傳遞鏈之電子提供者。

D. To use nitrate as electron acceptor in the electron transport chain during respiration.
利用硝酸鹽作為呼吸作用中，電子傳遞鏈之電子接受者。

E. Use nitrites as nitrogen source.
利用硝酸鹽為氮源。

Write your answer code or codes down in the line 1.8.1. of the answer sheet.
把你的一個或多個答案（代碼）填入 1.8.1 欄中

1.8.1.: _____

Use your results and the identification table to identify the species of your strains. **Fill the results in the table in the answer list.**
利用所得之結果及下列的鑑定表，來辨認你的細菌菌種。將結果填入答案卷的表格中。

Identification table

	Genus, species 屬、種	**Gram reaction** 革蘭氏染色	**O\F-test** O\F 測試	**The presence of:** 是否有～					
				catalase 觸酶	**oxidase** 氧化酶	**protease** 蛋白酶	**amylase** 澱粉酶	**H_2S production** 產生硫化氫	**nitrate reductase** 硝酸還原酶
A	Escherichia coli	–	F	+	–	+	–	+	+
B	Xanthomonas campestris	–	O	+	–	+	–	+	–
C	Lactobacillus delbrueckii	+	F	–	–	+	–	+	–
D	Erwinia herbicola	–	F	+	–	–	–	+	+
E	Clavibacter michiganensis	+	O	+	–	–	+	+	–
F	Staphylococcus saprophyticus	+	F	+	–	–	–	–	–
G	Pseudomonas mendocina	–	O	–	+	–	–	–	+
H	Pseudomonas putida	–	O	+	+	+	–	–	–
I	Sarcina lutea	+	F	+	–	+	–	–	–
J	Streptobacillus moniliformes	–	F	–	–	–	–	–	–
K	Agrobacterium tumefaciens	–	O	+	+	–	–	+	+

L	Pseudomonas fluorescens	–	O	+	+	+	–	–	+
M	Bacillus subtilis	+	F	+	–	+	+	+	–
N	Streptococcus lactis	+	F	–	–	+	–	+	+

Task 2. (18 points) **Study of Bacterial cultures expressing different genes.**
第二題（１８分）　檢測表現不同基因之菌種培養

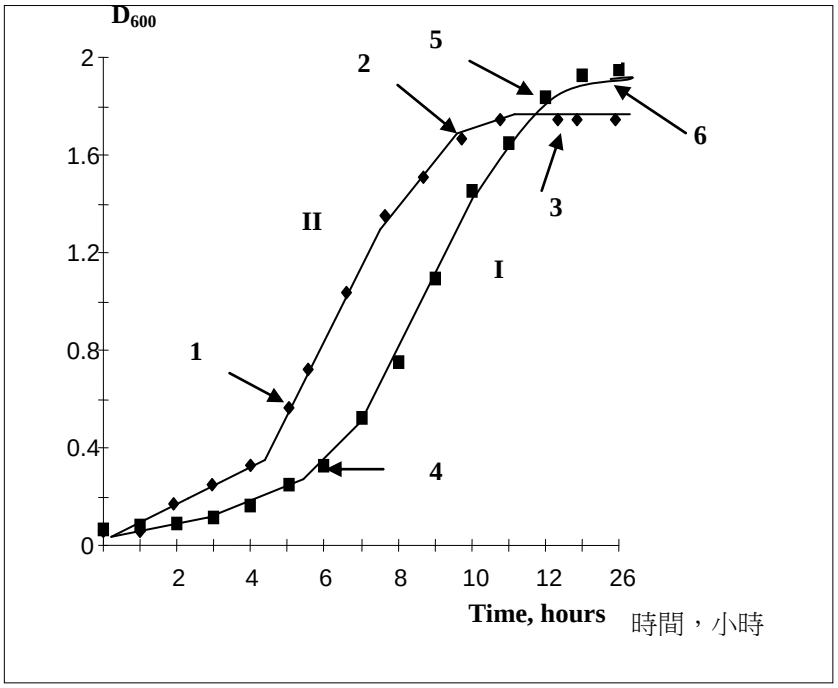
Materials and equipment
材料及器材

1. Six tubes with cells taken from cultures at different stages of growth. 6
   六個試管的細胞，代表不同生長時期
2. Distilled water (flask A). 1
   蒸餾水（燒瓶Ａ）
3. Dropping bottle with 0,5 M catechol solution (flask B). 1
   內含 0,5 M 兒茶酚溶液的滴瓶（燒瓶Ｂ）
4. Pipette. 1
   吸管

The *xylE* gene coding for enzyme catechol-2,3-dioxygenase is often used as an easy
兒茶酚－2,3－二氧化酵素的基因 *xylE* 常被用來不同基因表現之簡易分析。
assay reporter to study expression of various genes. This enzyme catalyses the conversion of
此酵素可將無色的兒茶酚轉成黃色的
colourless catechol into yellow coloured product - hydroxymuconic semialdehyde. Fusing
產物（氫氧黏液半醛）。
promoterless *xylE* sequence to the promoter of gene of interest allows to follow the expression of
將沒有啟動子的 *xylE* 序列與有特定目的且有啟動子的基因加以融合，可由黃色產物之出
this gene according to appearance and intensity of yellow colour of reaction products
現及其呈現黃色的深淺，來得知特定目的之基因的表現。

Two strains of *Escherichia coli* have been constructed experimentally in which the *xylE*
用大腸桿菌的二種菌株來作實驗，讓 *xylE* 基因和具有啟動子的兩種基因Ｃ及Ｄ
gene was fused to promoters of two different genes, gene C and gene D. The picture shows
融合，　下圖表示此二種菌
growth curves for these bacteria, labelled I and II (I - *E. coli* with *xylE* fused to gene C promoter,
株 I 和 II 的生長曲線（I 是 *xylE* 基因和啟動子Ｃ基因融合的大腸桿菌；
II - *E. coli* with *xylE* fused to gene D promoter). Arrows show when cell samples were taken
II 是 *xylE* 基因和啟動子Ｄ基因融合的大腸桿菌）。箭頭為細胞取出的時間，
from the cultures. Arrow number corresponds to tube number, in which the cells are located
箭頭的數字即代表細菌的試管編號，
now.

Determine the phases of culture growth in which genes C and D are expressed.
當基因Ｃ和基因Ｄ表現時，細胞之生長階段為何？



To do this you need to perform the following actions:
你需要進行以下的工作：

1) using pipette, add water (from flask A) to each tube. Fill the pipette with water to the mark,
用吸管吸水（從燒瓶Ａ）到每一試管中，加水至標示處。
2) using the dropping bottle (flask B), add one drop of catechol solution to each tube and mix
使用滴瓶（燒瓶Ｂ），各加一滴兒茶酚溶液至每一根試管中，搖晃混合均勻
the contents of the tube by shaking,

3) leave the tubes at room temperature for 3-5 minutes,
將試管置於室溫中三至五分鐘。
4) examine the appearance of yellow colour in each tube.
檢查每一試管是否有出現黃色。

Determine in which growth phases genes C and D are expressed and fill the table in the answer sheet, putting the "+" sign in the corresponding column.
決定哪一時期基因Ｃ及基因Ｄ會表現出來，並在答案紙的表格中的適當欄位填入"+"的代號。

Strain 菌株	**Gene** 基因	**The gene is expressed in** 此基因在_____時表現		
		early log phase 對數期早期	**late log phase** 對數期晚期	**stationary phase** 穩定期
I	**C**			
II	**D**			