17th INTERNATIONAL BIOLOGY OLYMPIAD
9 - 16 JULY 2006
Río Cuarto – República Argentina

PRACTICAL TEST 實作測驗
4

MICROBIOLOGY
微生物學

Student Code:學生代號	

17 th INTERNATIONAL BIOLOGY OLYMPIAD
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General remarks about the practical tests
關於實際的試驗的一般說明

DEAR PARTICIPANTS 參賽者

The practical tests are organized in four different laboratories.
實作試驗被在 4 間不同的實驗室進行

Nº 1- Plant Anatomy, Systematics and Physiology 植物解剖學，分類學和生理學

Nº 2- Animal Anatomy, Systematics and Ecology 動物解剖學，生態學和分類學

Nº 3- Biocheminstry 生物化學

Nº 4- Microbiology 微生物學

- You have **1 hour** to perform laboratories Nº 1 and Nº 2. 實驗室 1 及 2，1 小時
- You have **1 hour 30 minutes** to perform laboratories Nº 3 and Nº 4. 實驗室 3 及 4，1.5 個小時。
- You can score maximum of **40 points** in each laboratory, which means a total of **160 points** for the whole practical test. 每間實驗室最多可得 40 分，實作試驗共 160 分

Good luck !!!!!!

Practical test 4 實作 4

MICROBIOLOGY 微生物學

There are different systems of bacterium classification, but the most commonly used is the published in Bergey's Manual of Determinative Bacteriology.

細菌的分類有不同的系統，但最常用的為在 Bergey 的細菌學手冊中所記載的。

A working outline for the identification of a bacterial strain from the biochemical point of view is proposed below: 以下為一種由生化觀點鑑定細菌菌株的方法之概要：

1) Isolate the strain and obtain a pure culture. 分離菌株並取得純化培養

2) Carry out a microscopic examination of living cells and also of Gram stained smears. The morphology and type of Gram staining of the microorganism under study is thus determined. It is also important to determine the presence of clusters, spores and any other morphological characteristics that may be of interest.

取活細胞以革蘭氏染色後以顯微鏡觀察, 可得知微生物的形態及革蘭染色類型, 並根據其是否會形成菌團、孢子及其他形態學上的特性, 也是鑑定微生物種類的重要方式

3) Determine the nutritional characteristics (in general they come off from the methods used in the previous isolate and culture): photoautotrophs, photoheterotrophs, chemiautotrophs, chemiheterotrophs.

判斷營養特性(通常需先行分離、純化培養菌株)：光合自營、光合異營、化學自營、化學異營等

4) Conduce primary tests: The following group of tests, called primary tests, are used to determine the genus, group of genera or in some cases, the family to which an isolate belongs to. The primary tests are, beside Gram staining and morphology observation, the determination of catalase, oxidase, glucose fermentation, and motility, among others.

進行先期試驗：下列的試驗組稱為先期試驗，用來判斷此種純化菌株的屬及科分別為何。除了革蘭染色及形態觀察之外，主要的先期試驗包括觸酶活性、氧化酶活性、葡萄糖發酵活性和活動性等

Reagents and Equipment 試劑和設備

1. Dropping bottle with Gentian Violet (ready to use) 滴瓶-裝有龍膽紫
2. Dropping bottle with Lugol (ready to use) 滴瓶-裝有 Lugol
3. Dropping bottle with Gram decolorizer (ready to use) 滴瓶-裝有革蘭去色劑
4. Dropping bottle with Safranin (ready to use) 滴瓶-裝有 Safranin
5. Dropping bottle with Distilled water 滴瓶-裝有蒸餾水
6. 1 tube rack 一個試管架
7. 2 Kahn tubes containing a culture grown in Luria-Bertani medium of organisms A and B.
   二支試管-含有生長在 Luria-Bertani 培養液培養的 A 和 B 二菌種
8. 2 Lab gloves 二副手套
9. Respiratory mask 口罩
10. Marker pen 油性標示筆
11. Paper napkin 紙巾
12. 1 Bunsen burner 一支本生燈
13. Microscope 顯微鏡
14. Loop 接種環
15. 4 Slides 4 片玻片
16. Tray with slide holder 玻片盤
17. 1 plastic bottle with water for rinsing 1 個塑膠瓶-裝有洗滌用的水
18. 1 disposable glass 一個拋棄式小杯
19. 1 dropping bottle with immersion oil 一滴瓶礦物油
20. 1 dropping bottle with 3% H_2O_2 一滴瓶 3% H_2O_2
21. 2 Luria-Bertani agar plates inoculated with organisms A and B.
    二個 Luria-Bertani 洋菜培養盤-含有菌種 A、B
22. 1 Eppendorf tube with 2 oxidase disks 一微量離心管-含有 2 片氧化酶圓片
23. 1 pair of tweezers 一支鑷子
24. 2 Kahn tubes 二支試管
25. 1 Kahn tube with a stopper containing sterile distilled water.

一支有蓋的試管-含有無菌蒸餾水

26. 1 plastic Pasteur pipette. 一支塑膠巴斯德吸管
27. 3 plates with eosin methylene blue agar (EMB) medium (one of them inoculated with organism A, another inoculated with organism B and the last one without inoculation)
    三盤含有伊紅甲基藍洋菜培養基的培養皿(一盤裝有菌種 A, 一盤裝有菌種 B, 另一盤無菌)
28. 3 tubes with phenylalanine (one of them inoculated with organism A, another inoculated with organism B and the last one without inoculation). 三管苯丙胺酸(一管裝有菌種 A, 一管裝有菌種 B, 另一管無菌)
29. 1 Dropper containing 10% ferric chloride 一個滴瓶含 10%氯化鐵
30. 3 Kahn tubes with SIM (hydrogen Sulfide Indole Motility) medium (one of them inoculated with organism A, another inoculated with organism B and the last one without inoculation)
    三根試管-含有 SIM (可測硫化氫, 吲哚,及活動性之試劑) 培養基(一管裝有菌種 A, 一管裝有菌種 B, 另一管無菌)
31. 1 Dropping bottle containing Indole reagent 一滴瓶-含有吲哚試劑
32. 3 Kahn tubes containing UREA broth (one of them inoculated with organism A, another inoculated with organism B and the last one without inoculation)
    三試管-含有尿素培養液(一管裝有菌種 A, 一管裝有菌種 B, 另一管無菌)
33. 3 Kahn tubes with motility indole ornitine medium (MIO) (one of them inoculated with organism A, another inoculated with organism B and the last one without inoculation)
    三試管-含有 MIO (可測活動性,吲哚,及鳥胺酸)培養基(一盤裝有菌種 A, 一盤裝有菌種 B, 另一盤無菌)
34. 3 Kahn tubes containing Simmons citrate (labeled SC-A and SC-B and another one)
    三試管-含有 Simmons 檸檬酸 (標示 SC-A 、SC-B 及未接種 SC)
35. Clock located in view of all the students in the laboratory. 時鐘-置於學生可看到處

Caution: 注意

You must be careful in the manipulation of media and reagents since the quantities provided allow performing this practical test only once.

小心操作培養基和試劑, 這些試劑只能提供一份!!

If you work carelessly, with abrupt movements, far from the burner, you will contaminate the medium thereby preventing you from obtaining good results.

如果不小心操作- 如動作太突然, 或操作時離開本生燈太遠, 你就會因培養基污染而無法得到好的結果

You will perform the biochemical tests which basis and interpretation are detailed below by using the media, reagents, and the given bacteriological information (charts and diagrams)

生化試驗結果的呈現需根據操作培養基, 試劑所得之結果, 或根據題目所提供的細菌資訊(圖和表)

Note: Do not discard the tubes with organisms A and B. You will use them in task 2.
注意: 不要丟棄含有菌種 A 和 B 的管子, 在實驗二中仍會使用

TASK 1: Perform Gram-staining in organisms A and B.
實驗一: 對菌種 A、B 進行革蘭氏染色

EXPERIMENTAL PROCEDURE 實驗步驟

Introduction: 操作指引

Gram stain differentiates between two major bacterial cell wall types. Some bacterial species, because of the chemical nature of their cell walls, have the ability to retain the crystal violet even after the treatment with an organic decolorizer such as a mixture of acetone and alcohol.

革蘭氏染色可以區分二種類型的細菌之細胞壁, 有些細菌因其細胞壁的化學特性, 能在如丙酮或酒精等脫色劑的處理後, 仍能夠保留結晶紫的顏色

Gram stain technique 革蘭氏染色操作技術

1. Make a thin smear of the material to study and allow to air dry.
   將待研究的材料於玻片上製成薄的抹片標本,並風乾
2. Fix the material to the slide so that it does not wash off during the staining procedure by passing the slide three or four times through the flame of a Bunsen burner.
   將玻片於本生燈上過火三、四次以固定材料, 避免在染色過程中被洗去
3. Place the smear on a staining rack and overlay the surface with Gentian Violet solution.
   將玻片放置於玻片染色架上, 並以 Gentian Violet (龍膽紫)染劑蓋過材料
4. After 30 seconds of exposure to the Gentian Violet solution, wash thoroughly with running water. 以龍膽紫染色三十秒後, 以流水沖洗
5. Next, overlay the smear with Gram's iodine solution (lugol) for 30 seconds.
   以 Gram's iodine solution (lugol, 媒染劑)覆蓋材料 30 秒
6. Hold the smear between the thumb and forefinger and flood the surface with a few drops of the acetone alcohol decolorizer until no violet color washes off. This usually requires 10 seconds or less time.
   以拇指和食指握住玻片, 並以數滴丙酮或酒精清洗材料, 直到脫色劑不再呈現紫色為止, 此過程約需 10 秒鐘
7. Wash with running water and again place the smear on the staining rack. Overlay the surface with safranin counterstain for 20 seconds. Wash with running water.
   以流水沖洗後, 再將玻片放置於染色架上, 以 safranin(番紅染劑)覆蓋 20 秒以進行對比染色, 再以流水沖洗
8. Place the smear in an upright position in a staining rack, allowing the excess water to drain off and the smear to dry.
   將玻片豎立於染色架上, 流去多餘的水分, 並將玻片風乾
9. Examine the stained smear under the 100 x (oil) immersion objective of the microscope.
   於 100 倍油鏡下以顯微鏡檢查染色完成的玻片樣本
10. When you focus the microscope call the assistant.
    當對焦完成後, 請監試人員確認

Results

SELECT THE CORRECT ANSWER, FILLING THE CORRESPONDING BOX

將正確的答案填入正確的位置中

Organism 菌種	Gram staining		Assistant revision 監試人員確認
A	☐ Positive 陽性	☐ Negative 陰性	
B	☐ Positive 陽性	☐ Negative 陰性	

~~**Question 問題**~~

~~**Gram-variability 革蘭氏變異**~~

本題刪除

~~**A)**~~ ~~is a term which can be used where two Gram reactions are seen due to an error in the staining procedure.~~

~~是一個名詞用來~~

~~**B)** applies to an organism which changes its cell wall structure from the Gram-positive type to the Gram-negative type as the culture ages.~~

~~**C)** applies to what is ultimately seen when cells in a culture of gram-positive bacteria lose the ability to retain the primary stain during the decolorization process.~~

~~**D)** indicates a mixed (i.e., impure) culture.~~

Write the letter corresponding to the correct answer on the dotted line below:

將答案以英文字母寫於下方虛線處

……

TASK 2: Biochemical characterization of organisms A and B. 菌種Ａ和Ｂ的生化特性

In this part of the practical work you will determine, by means of metabolic tests (already provided or performed by you), the family and genus of the two organisms labeled as A and B.
在這部分請利用前述提供的資訊或以下操作的實驗, 來判定 A、B 兩菌種所屬的科及屬

Catalase Reaction: 觸酶反應

Some bacteria contain flavoproteins that reduce the oxygen resulting in the production of hydrogen peroxide (H_2O_2) or superoxide (O_2 -), which are extremely toxic since they are powerful oxidizers able to destroy the cellular constituents in a short time. Many bacteria possess enzymes that offer protection against these toxic compounds.
有些細菌具有黃素蛋白, 可還原氧氣產生具有毒性的過氧化物(H_2O_2 或 O_2^-), 因它們是強氧化物, 會破壞細胞的構造. 因此許多細菌會產生酵素來分解這些有毒物質, 以保護自己

Technique 操作

Perform the catalase test to organisms A and B by adding two drops of H_2O_2 to a bacterial suspension (3 loopfuls of the liquid culture labeled as LB-A and LB-B) placed on the slides.
對菌種 A 和 B 進行觸酶測試, 以接種環分別取二種液態培養基中的細菌懸浮液(標示 LB-A 和 LB-B)水膜三次, 放置於載玻片上, 分別加兩滴過氧化氫於玻片上的細菌懸浮液中

Note: Write the obtained results in the Biochemical test table using + (for a positive reaction) or – (for a negative reaction)
注意: 將所得的結果, 以＋(表示有反應)、－(表示無反應)寫於生化反應的表格中

Question

Which of the following reactions is carried out by the catalase enzyme?
觸酶可催化下列何項反應?

1) $H_2O_2 + NADH + H^+ \rightarrow 2\ H_2O + NAD^+$

2) $2H_2O_2 \rightarrow 2H_2O + O_2$

3) $O_2^- + O_2^- + 2H+ \rightarrow H_2O_2 + O_2$

4) $4\ O_2^- + 4H^+ \rightarrow 2H_2O + 3\ O_2$

Write the number corresponding to the correct answer on the dotted line below:
將答案以數字寫於下方虛線處

……

Oxidase Reaction:氧化酶反應

Test used for the detection of the cytochrome-c-oxidase enzyme, which is present in different genera, e.g. *Pseudomonas* spp., *Neisseria* spp., *Moraxella* spp., *Vibrio* spp., *Aeromonas* spp.
測試細胞色素 c 氧化酶(存在於不同屬細菌中, 如 *Pseudomonas* spp., *Neisseria* spp., *Moraxella* spp., *Vibrio* spp., *Aeromonas* spp.),

Oxidase discs contain dimethyl-para-phenylene-diamine, which is the substrate of cytochrome-c-oxidase enzyme.
氧化酶圓片(Oxidase discs)中含有細胞色素 c 氧化酶的受質(dimethyl-para-phenylene-diamine)
Organisms possessing this enzyme, in the presence of atmospheric oxygen and the substrate contained in the oxidase discs give a red-fuchsia color.
若細菌具有此種酵素, 在有氧氣的環境中, 將使氧化酶圓片呈現紅紫色變化.

Technique 操作

Perform the oxidase test to organism A and B according to the following instructions:

依據以下指示, 進行氧化酶測試於菌種 A 和 B 上

Oxidase test will be carried out using tubes. From a pure culture, prepare a heavy suspension in 0.2 ml of sterile distilled water, and add one oxidase disc.

以試管進行氧化酶測試. 將純化培養的細菌, 以 0.2ml 無菌蒸餾水製成懸浮液(懸浮液製法如下), 並加入一片氧化酶圓片

Note: Prepare the bacterial suspension starting from 3 colonies of each one of the plates labeled as LB-A and LB-B respectively.

懸浮液製法: 分別從標示 LB-A 和 LB-B 的培養皿中, 以接種環刮取三個菌落, 分別配置成細菌懸浮液

Results 結果

Generally, within the first minute and at room temperature, positive results are detected. A delayed reaction, evidenced after 2 minutes must be considered a negative result.一一般而言, 室溫下, 多可於一分鐘內呈現陽性反應, 若超過二分鐘才有變化, 則視為無反應(陰性反應)

Positive 陽性反應: discs show a red-fuchsia color.圓片呈現紅紫色

Negative 陰性反應: no changes in disc color.圓片無變化

Note: Write the obtained results in the Biochemical test table using + (for a positive reaction) or – (for a negative reaction)

注意: 將所得的結果, 以＋(表示有反應)、－(表示無反應)寫於生化反應的表格中

EOSIN METHYLENE BLUE (EMB) AGAR 伊紅甲基藍(EMB)洋菜培養基

This medium, is used for the selective isolation of fast growing and with scarcely nutritional requirements Gram-negative bacteria.

此種培養基可用來篩選生長快速、及對營養需求極少的革蘭氏陰性菌

It allows the growth of all Enterobacteriaceae members.

它可培養所有種類的腸內菌

Purpose 目的

This medium combines the Holt-Harris formulation with the Levine´s one to improve the selective isolation of Enterobacteriaceae and other Gram-negative bacterium species. The differentiation between lactose and/or sucrose fermenter organisms from those organisms which do not ferment them is possible due to the presence of the indicators eosin and methylene blue. Also, these indicators inhibit the growth of several Gram-positive bacteria.

此培養基結合了 Holt-Harris 及 Levine´s 兩種配方, 來增進腸內菌及其他革蘭氏陰性菌的篩選效果. 要分辨能夠進行乳糖及(或)蔗糖發酵的菌種、及無法進行發酵的菌種, 可藉由伊紅(eosin)及甲基藍(methylene blue)來辨識, 而這些指示劑也會抑制某些革蘭氏陽性菌生長.

Many strains of *Escherichia coli* and *Citrobacter spp.* show colonies with a greenish metallic sheen in this medium.

培養許多品系的大腸桿菌屬和檸檬酸菌屬的菌落時, 培養基中會呈現出綠色金屬光澤.

Lactose and/or sucrose fermenter organisms show colonies with a dark center surrounded by a blue or pink color, while lactose and/or sucrose non fermenter organisms show colorless colonies.

能夠進行乳糖及(或)蔗糖發酵的菌種, 菌落的中央會呈現深色, 而周圍則為藍色或粉紅色; 而無法進行發酵的菌種, 菌落則呈現無色.

This medium also allows the growth of different organisms in addition to the growth of the Enterobacteriaceae members, and may be generally differentiated by the appearance of their colonies.

這種培養基除了可以培養腸內菌外, 也可培養其他菌種, 但不同菌種所形成的菌落外形不同, 可用來區分之.

Instructions 指示

Using the EMB plates provided (labeled as EMB-A and EMB-B for organisms A and B respectively), determine the sucrose and/or lactose utilization for organisms A and B.
使用 EMB 培養基 (分別標示 EMB-A 和 EMB-B 表示菌種 A 和 B), 來判斷菌種 A 和 B 對蔗糖和(或)乳糖的利用狀況

Note: Write the obtained results in the Biochemical test table using + (for a positive reaction) or – (for a negative reaction).
注意: 將所得的結果, 以＋(表示有反應)、－(表示無反應)寫於生化反應的表格中

Question 問題

Fermentation 發酵作用…

A) results in production of acid and possibly gas from the breakdown of sugars.

結果可能造成蔗糖的分解, 並產生酸及氣體

B) is associated with the type of growth of facultative anaerobes in Thioglycollate (a reducing agent) Medium where growth is less dense in the anaerobic region.

為兼氣菌於硫醣(Thioglycollate, 一種還原劑) 培養基中的生長方式; 在無氧環境中, 菌落的生長狀態較稀疏.

C) is generally associated with a positive catalase reaction for an organism.

通常與菌體中觸酶表現陽性反應有關

Write the letter/s corresponding to the correct/s answer/s on the dotted line below:
將答案以英文字母寫於下方虛線處

……

Phenylalanine agar (Tubes labeled as Ph)苯丙胺酸洋菜培養基(試管以 Ph 標示)

Phenylalanine agar is recommended for the detection of production of phenylpyruvic acid from phenylalanine by deamination. A positive reaction results in a green coloration after the application of 10% ferric chloride.

苯丙胺酸培養基可用來測定 **苯丙胺酸** 經 **去胺反應** 所產生的 **苯丙酮酸**, 在滴加 10%氯化鐵(ferric chloride)溶液後, 如呈現綠色, 即表示有反應(陽性反應).

Instructions 實驗指引

Add 4 or 5 drops of the ferric chloride solution to the phenylalanine slants agar tubes (labeled as Ph-A and Ph-B for organisms A and B respectively). As the reagent is added rotate the tubes. An intense green color appearing within 10 minutes indicates the presence of phenylpyruvic acid.

加 4~5 滴氯化鐵溶液(ferric chloride)於含有苯丙胺酸的斜面培養基中 (試管標示 Ph-A、Ph-B 代表菌種 A、B), 當試劑加入後, 旋轉試管使溶液接觸培養基, 若在十分鐘內呈現深綠色, 即表示反應產生了苯丙酮酸.

Note: Write the obtained results in the Biochemical test table using + (for a positive reaction) or – (for a negative reaction).

注意: 將所得的結果, 以＋(表示有反應)、－(表示無反應)寫於生化反應的表格中

Hydrogen Sulfide indole motility medium (SIM) (Tubes labeled as SIM A and SIM B)

SIM 培養基(為測定硫化氫、吲哚的產生, 以及菌體活動性的培養基, 試管分別以 SIM A 和 SIM B 表示)

This medium is used for the detection of hydrogen sulfide, indole production, and motility in the same tube. Hydrogen sulfide production in this medium is originated from thiosulphate or sulphate reductases and not by cysteine desulfhidrases. Any blackening along the line of inoculation is considered as a positive hydrogen sulfide reaction, and it usually appears between 18-24 hours of inoculation. Motile cultures in SIM medium show diffuse growth away from the line of inoculation. This is an appropriated medium for the detection of Listeria's characteristic "umbrella-like" movement. The high content of tryptophane in this medium makes it very suitable for detection of indole production.

此種培養基可用來測定硫化氫及吲哚的產生, 及菌體的活動性, (I)硫化氫產生為硫代硫酸或硫酸還原酶(thiosulphate or sulphate reductases)的作用, 在接種線周圍產生黑色, 表示產生硫化氫(為陽性反應), 一般而言, 需要在接種 18~24 小時後, 才會出現. (II)若菌體在 SIM 培養基中具有活動性, 則菌落會在接種線附近呈現輻射狀的分布, 因此這種培養基可用來測試菌種是否具有 Listeria's(一種桿菌)的特徵(傘狀活動性). (III)培養基中含有高濃度的 trypteine, 也可用於測定吲哚(indole)的產生.

Instructions 實驗指引

Using the SIM tubes provided (labeled as SIM-A and SIM-B for organisms A and B respectively), determine the production of hydrogen sulfide and indole, as well as the motility for organisms A and B.

使用 SIM 試管(分別標示 SIM-A 和 SIM-B 表示菌種 A、B), 用以測定 A、B 二菌種產生硫化氫、吲哚的情形及其活動性

For indole production detection, add 5 drops of the reagent (labeled as indole) to the heavy growth obtained in SIM tubes. A pink color promptly developed indicates the presence of indole.

測定吲哚的產生, 可加五滴試劑 (於管上標示吲哚 indole)於含有高濃度細菌的 SIM 培養液試管中, 若立即變為粉紅色, 則表示有吲哚存在.

Note: Write the obtained results in the Biochemical test table using + (for a positive reaction) or – (for a negative reaction).
注意: 將所得的結果, 以＋(表示有反應)、－(表示無反應)寫於生化反應的表格中

Question:問題

A negative result for motility 活動性的陰性反應(即無活動性)…

A) is indicated if growth occurs only along the line where the medium was stab-inoculated.

表示以穿刺接種於培養基時, 菌種只會沿線生長

B) should be confirmed by a wet mount of a young culture of the same organism.

將菌液滴於固態培養基上, 可根據其生長過程來判斷

C) may exhibit growth over the surface of the medium.

可呈現出覆蓋於培養基表面的生長情形

D) may occur for strictly aerobic, motile organisms.

好發於絕對好氧性及活動性的菌種

Write the letter/s corresponding to the correct/s answer/s on the dotted line below:
將答案以英文字母寫於下方虛線處

……

UREA BROTH 尿素培養液

This medium is suitable for the differentiation of urease producing organism.
此種培養基可用於分辨產生尿素酶的菌種

Instructions 實驗指引

Using the urea broth tubes provided (labeled as UREA-A and UREA-B for organisms A and B respectively), determine the production of urease for organisms A and B.
使用含有尿素培養液的試管(分別標示 UREA-A 和 UREA-B 表示菌種 A、B), 判斷菌種 A 和 B 產生尿素酶的情形

Note: Write the obtained results in the Biochemical test table using + (for a positive reaction) or – (for a negative reaction).
注意: 將所得的結果, 以＋(表示有反應)、－(表示無反應)寫於生化反應的表格中

SIMMONS CITRATE AGAR 西門氏檸檬酸洋菜培養基

It is a medium capable to differentiate between bacteria harboring citrate permease enzymes and those that do not harbor such enzymes.
這是一種用來判斷菌種能否利用檸檬酸滲透酶的培養基

Instructions 實驗指引

Using the SIMMONS CITRATE tubes provided (labeled as SC-A and SC-B for organisms A and B respectively), determine the presence of growth for organisms A and B.
使用西門氏檸檬酸培養基試管 (分別標示 SC-A 和 SC-B 表示菌種 A、B), 來判斷菌種 A 和 B 是否生長.

Note: Write the obtained results in the Biochemical test table using + (presence of growth) or – (absence of growth).
注意: 將所得的結果, 以＋(表示有生長)、－(表示無生長)寫於生化反應的表格中

Motility Indole Ornitine (MIO) Medium 活動吲哚鳥胺酸(MIO)培養基

The reactions in this medium are observed as follows:

於此培養基中可觀察到以下的反應.

- Ornithine Decarboxylation (ODC). Observe the lower three-quarters (anaerobic region) of the medium for change in color of the pH indicator; growth must be present in this part of the tube for correct analysis of result:

  鳥胺酸去碳酸反應(ODC): 藉由酸鹼指示劑觀察試管底部培養基(下方 3/4 的無氧區域)變色情形, 細菌可於此區生長, 而其能否產生 ODC 酵素可由以下結果判斷:

  - Gray, blue or purple color: Positive reaction for ornithine decarboxylation – formation of a highly alkaline product, over-neutralizing the acid produced from glucose fermentation.

    呈現灰色、藍色或紫色: 陽性反應, 表示發生 ODC 反應, 產生強鹼性產物, 可過度中和由葡萄糖發酵所產生的酸, 並使溶液呈現鹼性.

  - Yellow color: Negative reaction. Yellow color is due to the "default" acid production from glucose fermentation.

    黃色: 陰性反應, 呈現原本的顏色, 由葡萄糖發酵產生酸所造成

    .

Instructions 實驗指引

Using the MIO tubes provided (labeled as MIO-A and MIO-B for organisms A and B respectively), determine the production of ornithine decarboxylase enzyme for organisms A and B.

使用含有 MIO 培養基的試管(分別標示 MIO-A 和 MIO-B 表示菌種 A、B), 判斷菌種 A、B 產生 ODC 酵素的情形.

Note: Write the obtained results in the Biochemical test table using + (for a positive reaction) or – (for a negative reaction).

注意: 將所得的結果, 以＋(表示有反應)、－(表示無反應)表示, 填入生化反應的表格中

Results:實驗結果

Write the results of the biochemical tests in the following table (11 points)

填入生化反應的結果於以下的表格中 (11 分)

Organism	Catalase 觸酶	Lactose 乳糖	Sucrose 蔗糖	Motility 活動性	Indole 吲哚	H_2S 硫化氫	Phenyl-alanine 苯丙胺酸	ODC	Urease 尿素酶	Citrate 檸檬酸	Oxidase 氧化酶
A											
B											

Using the tables in the annex indicate (9 points)

利用附表中的資訊判斷菌種 A、B 之科及屬分別為何? (9 分)

	Family 科	Genus 屬
Organism A		
Organism B		

Questions 問題

1. You have cultures of five organisms as listed below. However, the labels of the tubes have come off and you need to re-label the tubes correctly! First, you must consider the various reactions you know for the organisms in question:

若你培養下列五種細菌, 但含有培養基的試管上的標籤脫落, 需要重新標示. 因此, 請根據下表所列之試劑反應, 回答下列問題.

Genus 屬	Gram stain 革蘭染色	Shape 形狀	Catalase reaction 觸酶反應	glucose fermentation 葡萄糖發酵	lactose fermentation 乳糖發酵	phenylalanine deaminase 苯丙胺酸去胺酶	Citrate utilization 檸檬酸利用
Bacillus	+	Rod 桿狀	+	+ or –	?	?	?
Staphylococcus	+	Coccus 球狀	+	+	?	?	?
Enterobacter	–	Rod	+	+	+	–	+
Morganella	–	Rod	+	+	–	+	–
Pseudomonas	–	Rod	+	–	–	–	?

a. The results obtained from what specific laboratory procedure will differentiate *Bacillus* and *Staphylococcus* from each other and also from the remaining three genera?

以下何種測試可區分 *Bacillus* 和 *Staphylococcu* 兩菌屬, 並能藉此區別此兩屬與其他三菌屬

A) Glucose fermentation 葡萄糖發酵

B) Citrate utilization 檸檬酸利用

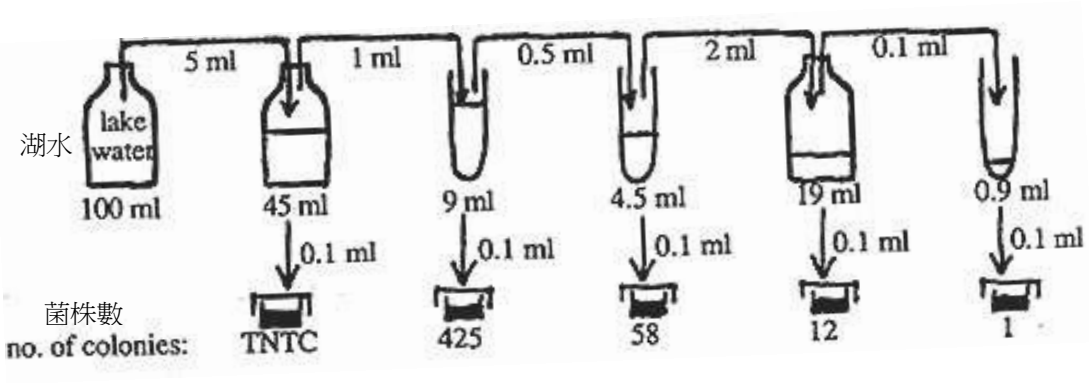
C) Catalase reaction 觸酶反應

D) Gram stain 革蘭氏染色

Write the letter corresponding to the correct answer on the dotted line below:
將答案以英文字母寫於下方虛線處

……

2. Consider the following dilution scheme:應用以下的稀釋方法



a. Report the total number of CFUs (colony forming units 形成多少個菌株) in the entire 100 ml amount of the original lake water sample. (TNTC=too numerous to count.數量太多以至於無法計數)

請問原本 100ml 湖水中之總菌株數(CFU)為何?

A) 5.8×10^{7} cfu / 100 ml

B) 4.25×10^{8} cfu / 100 ml

C) 1.2×10^{9} cfu / 100 ml

Write the letter corresponding to the correct answer on the dotted line below:
將答案以英文字母寫於下方虛線處

……

b. Would you expect any change in the answer of the above problem if the first dilution was made by adding one ml of sample to 9 ml of diluent?

若於第一次稀釋時, 加 1ml 樣本於 9ml 稀釋液中, 你認為上述的答案是否可能改變?

A) Yes

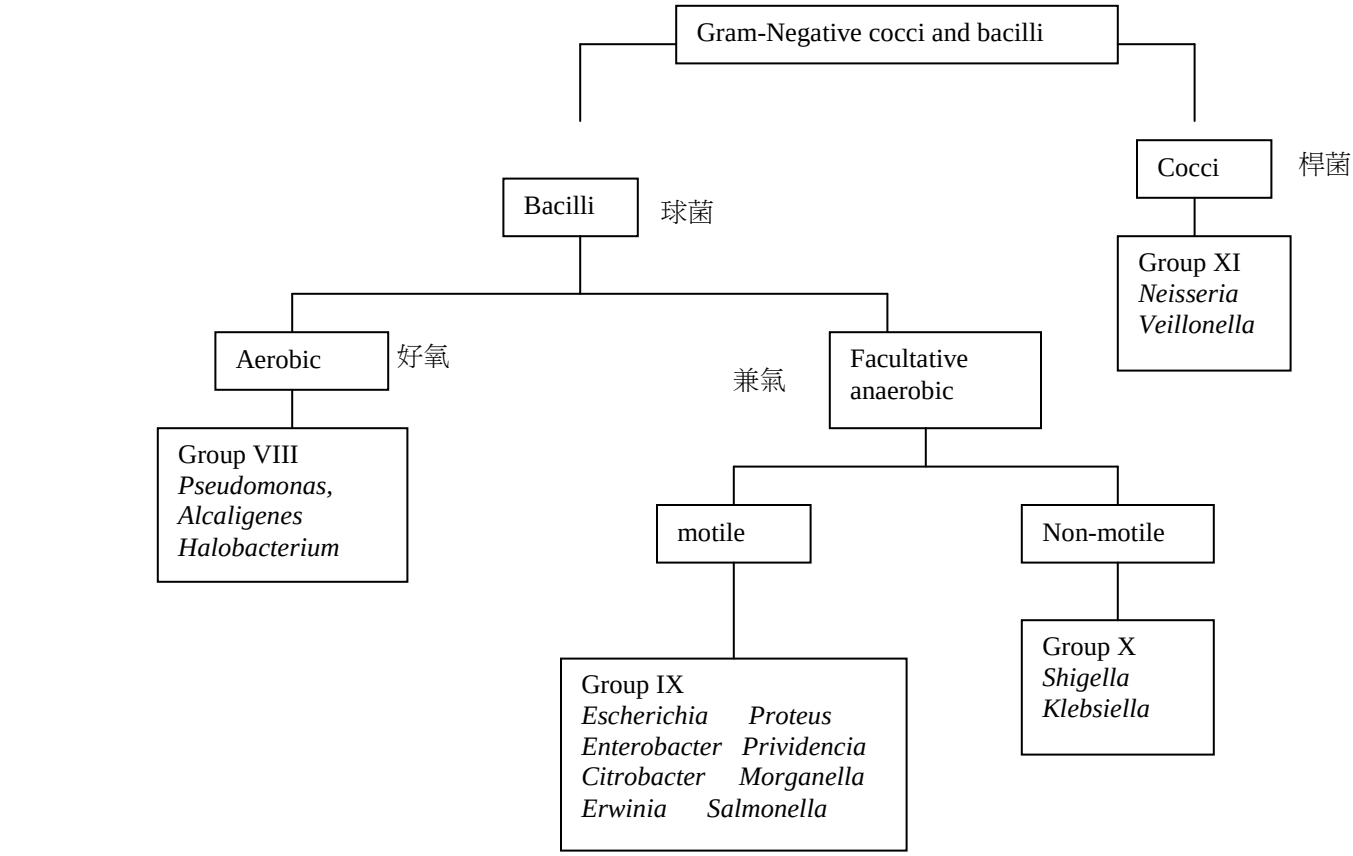
B) No

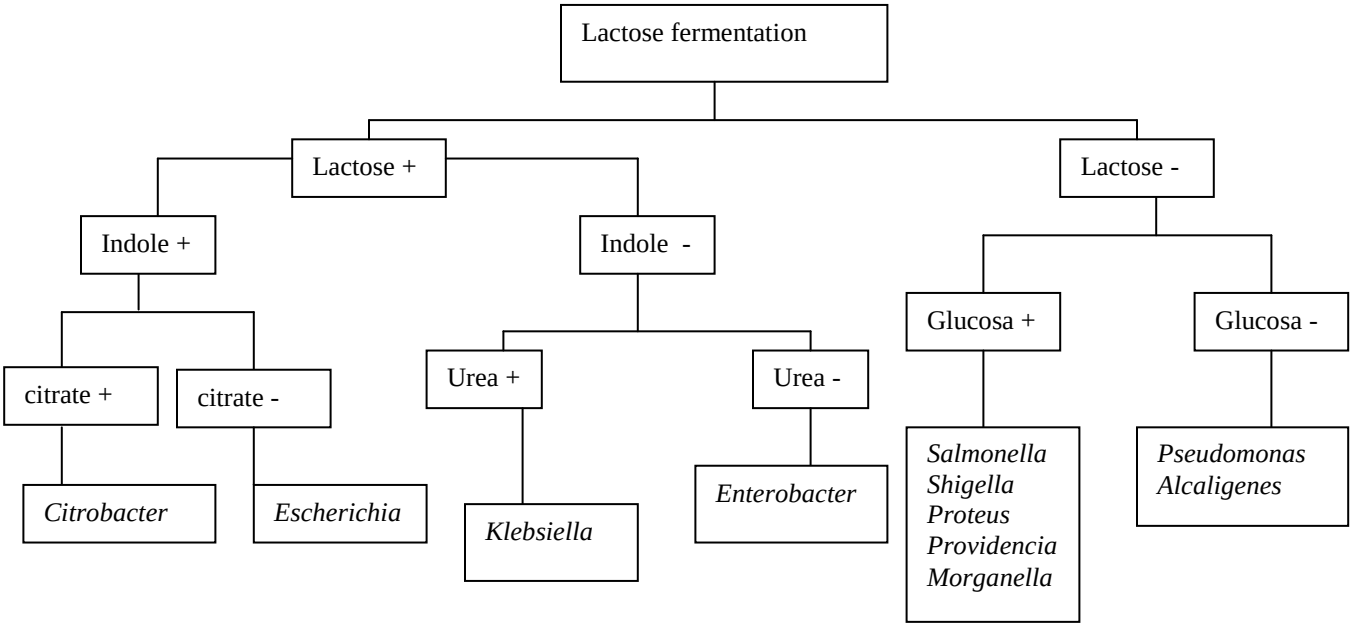
Write the letter corresponding to the correct answer on the dotted line below:
將答案以英文字母寫於下方虛線處

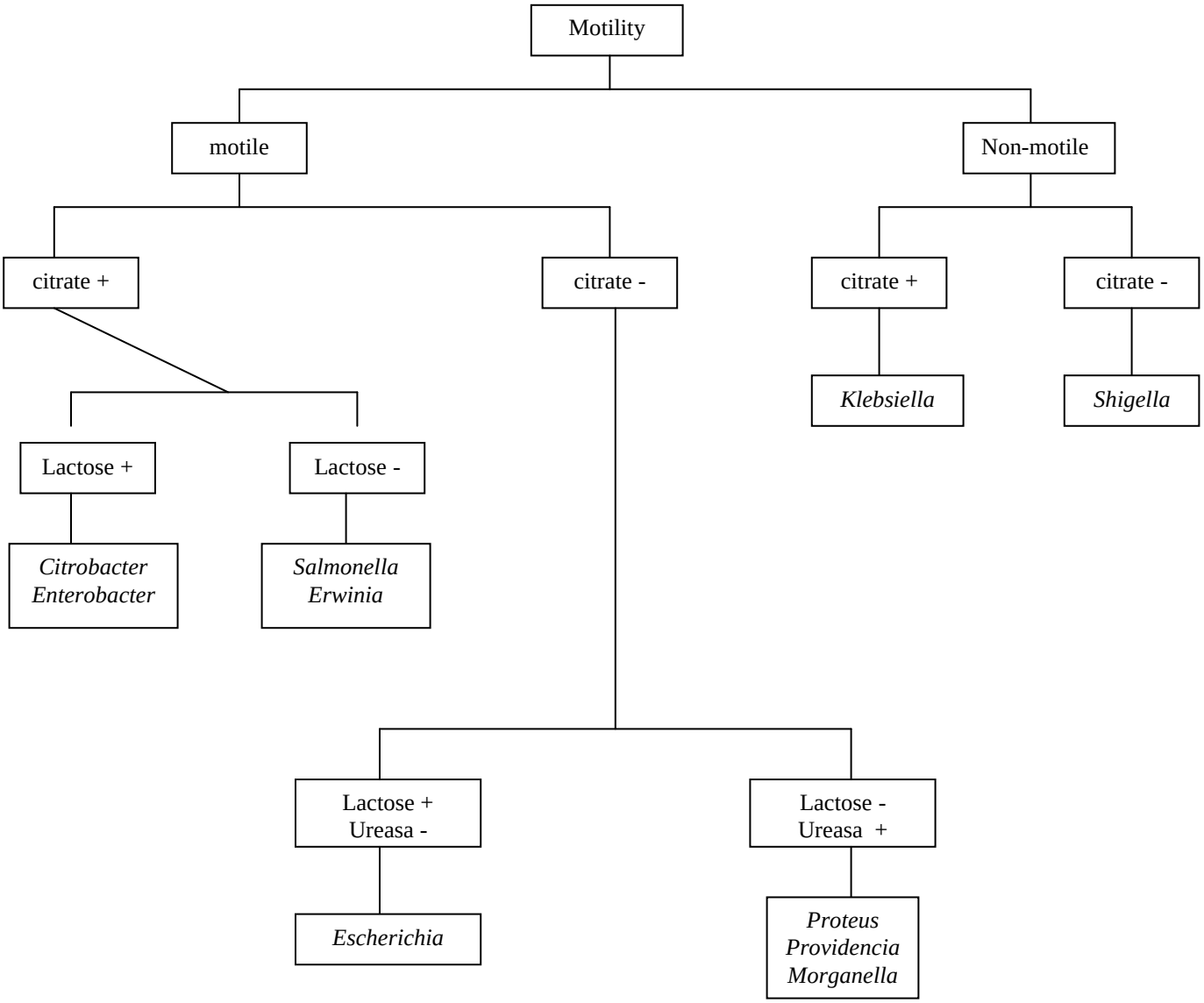
……

Annex 1 附表 1

Gram stain (fresh culture) 革蘭染色	+	+	+	+	+	+	+	+	–	–	–	–	–
Shape 形狀	coccus	coccus	coccus	coccus	rod	rod	Rod	rod	rod	rod	rod	rod	coccus
Grouping 聚合方式	Clusters 群聚	Clusters 群聚	Chains 鏈狀	Tetrads 四聯體									Pairs 成對
aerobic growing 有氧生長情形	+	+	+	+	+	–	+	+	+	+	+	+	+
anaerobic growing 無氧生長情形	–	+	+	+	+	+	+	–	–	–	+	+	–
motility 活動性	–	–	–	–	–	+ or –	+ or –	+ or –	+ or–	+ or –	+ or -	+	–
catalase 觸酶	+	+	–	–	–	–	+	+	+	+	+	+	+
oxidase 氧化酶									+	+	–	+	+
fermentation of glucose to acid or acid+gas 葡萄糖發酵產生酸	–	+	+	+	+	+ (or –)	+	–	–	–	+	+	–
Micrococcus	**X**												
Staphylococcus		**X**											
Streptococcus			**X**										
Lactococcus			**X**										
Enterococcus			**X**										
Clostridium						**X**							
Bacillus							**X**	**X**					
Alcaligenes									**X**				
Pseudomonas										**X**			
Enterobacterias											**X**		
Aeromonas												**X**	
Chromobacterium												**X**	
Neisseria													**X**


Gram-Negative cocci and bacilli
Cocci
桿菌
Bacilli
球菌
Group XI
Neisseria
Veillonella
Aerobic
好氧
兼氣
Facultative anaerobic
Group VIII
Pseudomonas,
Alcaligenes
Halobacterium
motile
Non-motile
Group IX
Escherichia Proteus
Enterobacter Providencia
Citrobacter Morganella
Erwinia Salmonella
Group X
Shigella
Klebsiella


Lactose fermentation
Lactose +
Lactose -
Indole +
Indole -
Glucosa +
Glucosa -
citrate +
citrate -
Urea +
Urea -
Citrobacter
Escherichia
Klebsiella
Enterobacter
Salmonella
Shigella
Proteus
Providencia
Morganella
Pseudomonas
Alcaligenes


Motility
motile
Non-motile
citrate +
citrate -
citrate +
citrate -
Klebsiella
Shigella
Lactose +
Lactose -
Citrobacter
Enterobacter
Salmonella
Erwinia
Lactose +
Ureasa -
Lactose -
Ureasa +
Escherichia
Proteus
Providencia
Morganella

Family	Genus		Oxidation 氧化									
		catalase	lactose	sucrose	motility	indole	SH_2	Phenyl alanine	ODC	Ureasa	citrate	Oxidase
Enterobacteriaceae	*Escherichia*	+	+	+	+	+	-	-	-	-	-	-
	Shigella	+	-	-	-	+	-	-	-	-	-	-
	Salmonella	+	-	-	+	-	+	-	+	-	-	-
	Citrobacter	+	+ / -	-	+	+	+	-	+	-	+	-
	Proteus	+	-	+	+	+ / -	+	+	+	+	+ / -	-
	Morganella	+	-	-	+	+	-	+	+	+	-	-
	Enterobacter	+	+	+ / -	+	-	-	-	+	+ / -	+	-
	Serratia	+	+ / -	+ / -	+	-	-	-	+ / -	+	+	-
	Klebsiella	+	+	+	-	-	-	-	-	+	+ / -	-
Pseudomonaceae	*Pseudomonas*	+	?	-	+	-	-	+ / -	+ / -	+ / -	+ / -	+