

# **A221 Microbiology**

## **Problem 7**

# Identity crisis

## 6<sup>th</sup> Presentation

Activity Owner: Lee Hui Cheng

Modified by: Ho Phui San (Dr)

Inputs By: Girija (Dr)

Approved By: Dr Serene Choo

Module Chair: Foo Toon Tien

## In today's problem

- ◎ Martin had been tasked to identify and classify a pure culture of Bacteria A, which was suspected to be the cause of a disease.
- ◎ You were asked how Martin should proceed to identify and classify the pure culture as well as the importance of classifying the pure culture.

# What do you recognize?

---

- ◎ Bacterium A might be the causative agent of disease
- ◎ Martin was tasked to identify and then classify the pure culture of Bacterium A
- ◎ Identification of bacterial cultures may be done by observing the colony morphology, using Gram staining, observation of the staining under microscope and some other tests
- ◎ Classification may involve categorization of groups of bacteria with similar characteristics

# An approach to the problem....

---

- ◎ How does the classification system work?
- ◎ How should Martin go about identifying an unknown bacteria sample?
- ◎ Classification via Physiological characteristics
- ◎ How should the bacteria be classified?
- ◎ Why is it important to classify the bacteria?

# How does the classification system works?

Kingdom - Eubacteria

E.g. *Escherichia coli*

Phylum - Proteobacteria

Class - Gamma proteobacteria

Order - Enterobacteriales

Family - Enterobacteriaceae

Genus - Escherichia

Ultimate aim is to  
identify the species



Species - *Escherichia coli*

# How should Martin go about identifying an unknown bacteria sample?

---

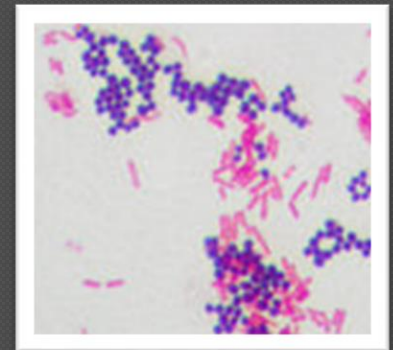
Observation of colony morphology  
(color, edge, size, elevation etc)  
Ensure that it is a pure culture



Perform Gram staining to identify its  
Gram status (Gram +ve/ -ve and shape)

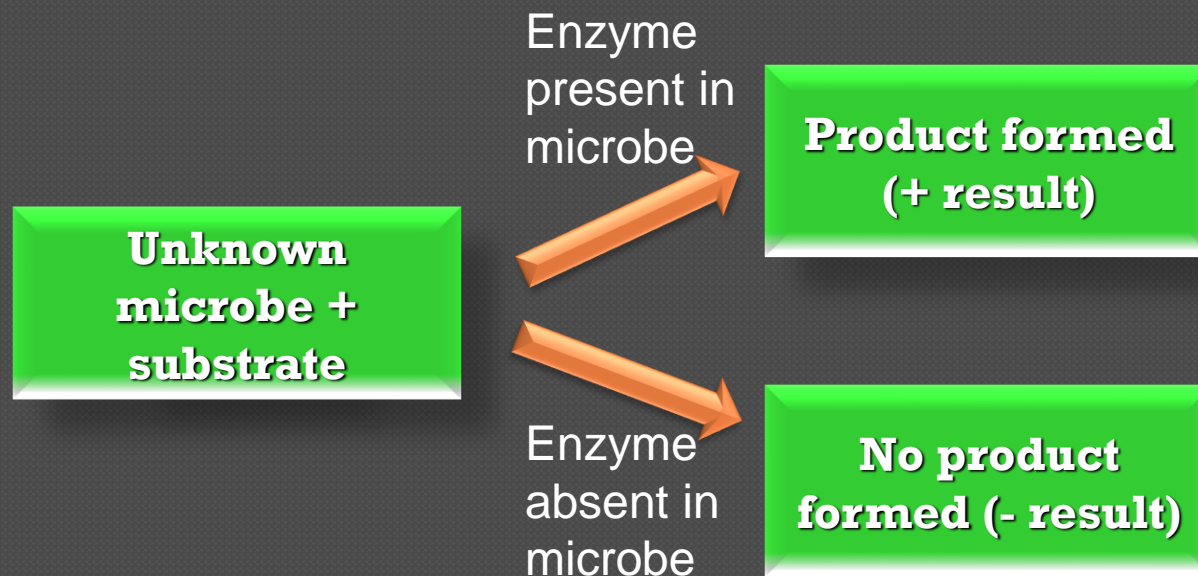


Additional test for identification of  
bacteria



# Classification via Physiological characteristics

Physiological reactions to nutrients and other substrates provide excellent indirect evidence of the types of enzyme systems present in a particular species





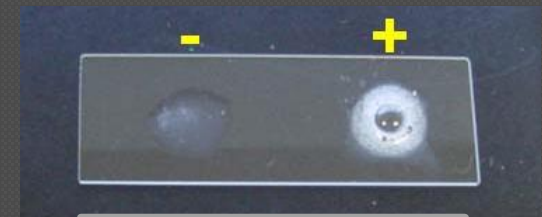
# Classification via Physiological characteristics

Biochemical tests for the differentiation of bacteria

- Carbohydrate fermentation (Acid/gas production)
- Hydrolysis of gelatin, starch and other polymers
- Enzyme actions such as catalase, oxidase, and coagulase
- Various byproducts of metabolism



Carbohydrate fermentation



Catalase test



# Identification of bacteria

---

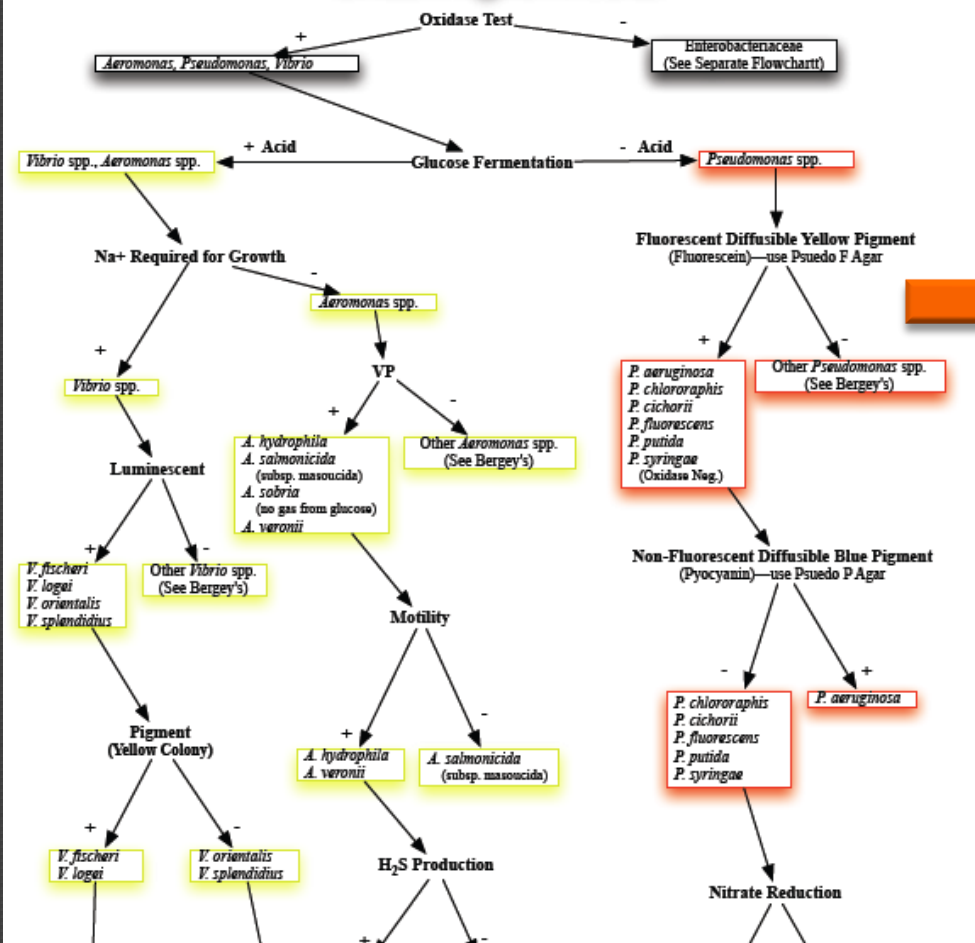
## ◎ Biochemical tests

- Oxidase test
- Indole test
- Citrate test
- Etc.

## ◎ Selective and Differential Media

# Classification via Physiological characteristics

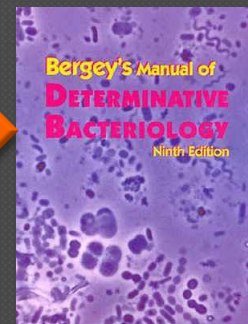
## Gram Negative Rods



Classification and identification of bacteria using

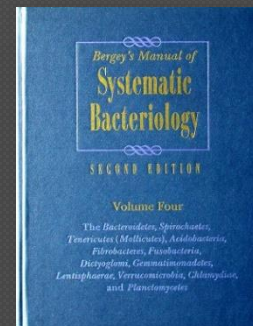
- Bergey's Manual of **Determinative Bacteriology**

(for identification based on morphologies, staining, biochemical tests results)



- Bergey's Manual of **Systematic Bacteriology**

(provides more information on bacteria based on gene sequencing for identification)



# Selective and Differential Media

---

- ◎ **Selective medium**

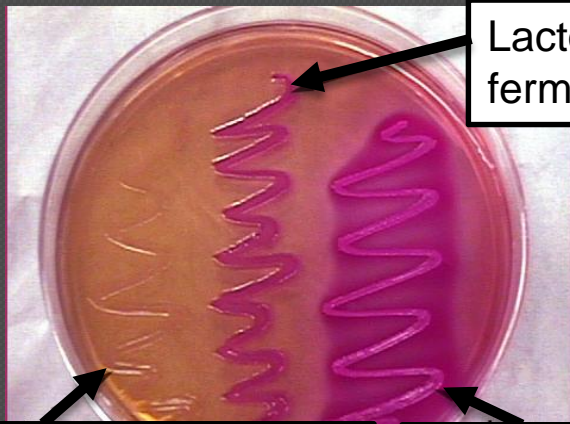
Allows the growth of only certain types of microbes while inhibiting the growth of others

- ◎ **Differential medium**

When specific microbes are present, the medium or bacterial colonies exhibit a colour change that provides information about their identity

# Selective and Differential Media

## MacConkey Agar



Lactose fermenter



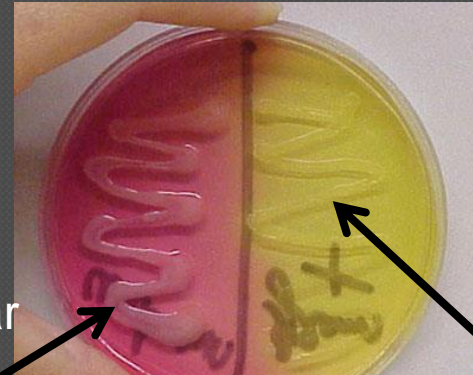
Uninoculated MacConkey Agar

Non-lactose fermenter (colonies did not turn red)

Lactose fermenter (colonies turned red)

- Crystal violet and bile salts: Selective for Gram-negative bacteria
- Lactose & Neutral Red: Differentiate between lactose and non-lactose fermenter

## Mannitol Salt Agar



Non-mannitol fermenter (agar did not turn yellow)



Uninoculated Mannitol Salt Agar

Mannitol fermenter (agar turned yellow)

- High Salt: Selective for *Staphylococcus* spp.
- Mannitol & Phenol Red: Differentiate between pathogenic (mannitol fermenters) and non-pathogenic *Staphylococcus* spp (non-mannitol fermenters).

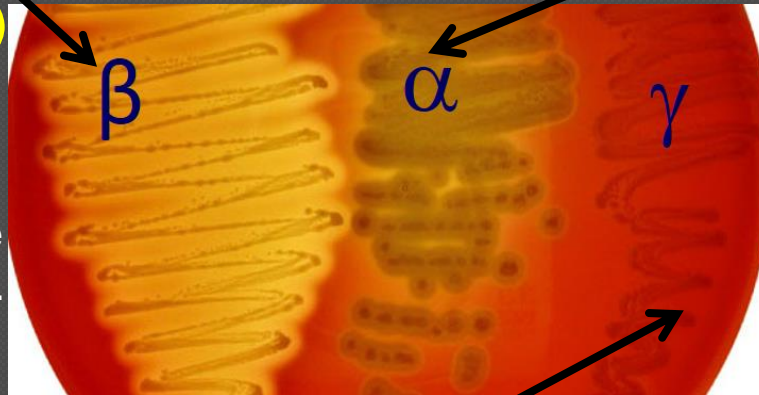


# Enriched and Differential Medium

## Blood Agar

### Beta ( $\beta$ ) haemolysis (Complete haemolysis)

A clear zone surrounds the colony. Many species of bacteria produce toxic by-products that are capable of destroying red blood cells.



### Alpha ( $\alpha$ ) haemolysis (Partial haemolysis)

Reduction of the red blood cell hemoglobin to methemoglobin in the medium surrounding the colony. This causes a green or brown discoloration in the medium.

### Gamma ( $\gamma$ ) haemolysis (No haemolysis)

Does not produce hemolysins and does not break down the red blood cells, no clearing will occur.

- Protein source (e.g. tryptone, soybean protein digest):  
Provide nutrients
- 5% sheep blood: Supports growth of most bacteria and differentiates between haemolytic and non-haemolytic bacteria

# How should the bacteria be classified?

---

- ◉ Colony morphology
- ◉ Bacterial shape (rods, cocci etc.) and cell arrangement (single, tetrad, cluster, chain etc.)
- ◉ Gram status
- ◉ Phylogenetic analysis via Physiological characteristic using
  - Bergey's Manual of Determinative Bacteriology (Bergey's Manual)
    - To know which test(s) to perform and identification based on test result(s)
  - Selective and differential media

# Why is it important to classify the bacteria?

---

- ◎ Help clinical microbiologist differentiate microorganisms
- ◎ Identification of microorganisms quickly through colony morphology and Gram results
- ◎ Organization of information from various researchers worldwide





# What have you learnt today?

- **List down the taxonomy ranking for all living organisms**
  - Name the different kingdoms to classify living organisms
  - List the specific hierarchy by which living organisms are classified
- **Explain how microorganisms are classified based on their cellular characteristics**
  - Explain the rationale for classifying bacteria
  - List the various ways bacteria can be classified
- **Describe how an unknown bacteria/ microorganism is classified**
  - Able to use the Bergey's Manual of Determinative Bacteriology
  - Explain how Gram staining acts a preliminary step to the identification of bacteria, after which further tests are required to identify
  - Identify a bacteria based on physiological reactions to nutrients and other substrates
  - Define the criteria for classification of selective and differential media
  - Compare and contrast between different selective and differential media that can be used to identify bacteria