# Bacterial diagnostics

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# BACTERIAL DIAGNOSTICS

## A: Symptoms and signs

(i) **Macroscopically**, look out for:

- ✓ Spots
- ✓ Lesions
- ✓ Blights
- ✓ Water-soaking
- ✓ Halos (chlorotic)
- ✓ Galls
- ✓ Wilting
- ✓ Rots (on fleshy tissues)

(ii) Microscopically, examine the samples for indication of bacterial infection, namely, bacterial streaming.

**Note:**

- ✓ Particulate material such as latex, starch granules and plastids might be mistaken for bacteria.

- ✓ In some cases, bacterial masses may not be seen due to low numbers of cells, as is likely with cankers.

## B: Identification of bacteria

There are a range of methods:

- ✓ Morphological examination
- ✓ Physiological and biochemical tests
- ✓ Fatty acid profiling
- ✓ Phage typing
- ✓ Serology
- ✓ Nucleic acid analysis and gene sequencing

## ISOLATION AND CULTIVATION OF BACTERIA

NB:*Pure bacterial cultures are vital, particularly where morphological, physiological/biochemical testing and fatty acid analysis are involved*

### (i) Disinfection of infected material

To remove saprophytic or epiphytic bacteria from plant surfaces. The selected portions of material are immersed in bactericidic solutions such as ethanol (70%) and sodium hypochlorite (0.5% NaOCl). The length of time for immersion depends on the thickness and type of material and the degree of contamination.

**Note:** Thin leaves should be washed under running distilled water to avoid bactericidal action of the commonly used sterilants.

## (ii) Culturing: Initial isolation may be achieved through:

- Direct streaking of bacterial ooze and exudates on agar medium.
- Direct streaking of plant material on agar medium.
- Streaking bacterial suspension onto agar medium after soaking in water.
- Streaking of bacterial suspension onto agar medium after macerating in water/buffer.

Sub-culturing is often necessary to purify the isolates

## Media:

- ✓ Culture medium is a liquid or gel that is used to support growth of microorganisms or cells.
- ✓ Two major types of growth media:
  - Cell culture which use specific cell types derived from plants or animals, and
  - Microbiological culture which are used for growing microorganisms, such as bacteria or yeast.
- ✓ Most common growth media for microorganisms are nutrient broths and agar plates.

NB: Isolation/culture media may be general (e.g. nutrient agar) or selective/semi- selective (e.g. YDC).

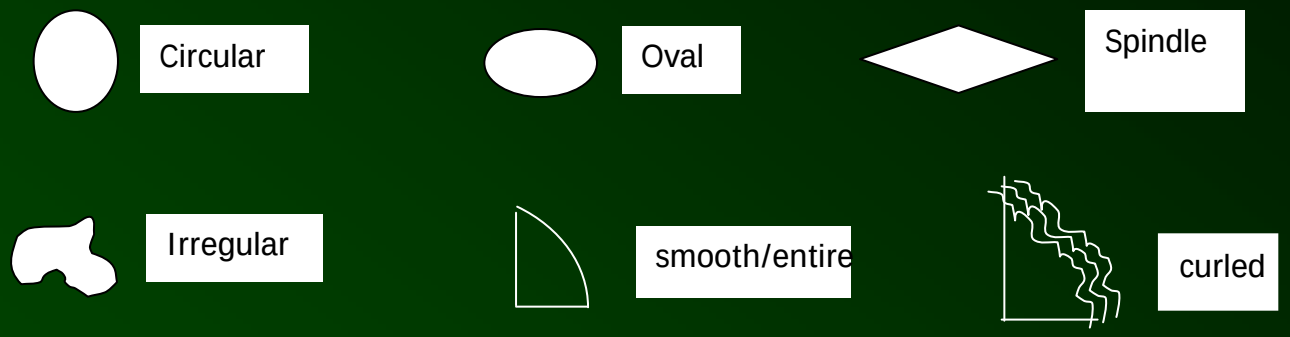
- ✓ Isolation (media and method of isolation depends on suspected disease).
- ✓ General isolation media is suitable and should be used if pathogen is unknown, however, specific semi-selective media are available for most phytopathogenic bacteria.
- ✓ Semi- selective media vary in complexity and usually contain antibiotics for suppression of non-target organisms, complex carbon sources utilized by a small group of MOs and on which target bacteria display diagnostic features.

## Key approaches for culturable bacteria

- ✓ Colony morphology
- ✓ Pigmentation/color
- ✓ Gram reaction (stain or KOH)
- ✓ Anaerobism/anaerobism test
- ✓ Growth at designated conditions e.g. temperature

# COLONY APPEARANCE AND GROWTH RATE

- ✓ On specific media, colony morphology, growth rate, color, appearance etc. are characteristic of specific bacteria species.




- ✓ On YDC for example Xanthomonads appear yellow.
- ✓ Pathogenic bacteria grow more slowly than saprophytes.
- ✓ Colonies of pathogenic bacteria usually visible after 36-72 hrs.

## Benefits of semi-selective media

- ✓ Media inhibit the growth of most saprophytes.
- ✓ Media allow contamination-free isolation of the pathogen from various plant parts.

## Disadvantages of semi-selective media

- ✓ SSM is only sensitive when isolating from sample with low levels of contamination.
- ✓ Isolation is done from symptomatic plants so is not useful during latent infection.
- ✓ Requires pathogenicity testing before conclusions can be drawn as to the identity of the isolated pathogen.

## Types of semi - selective media

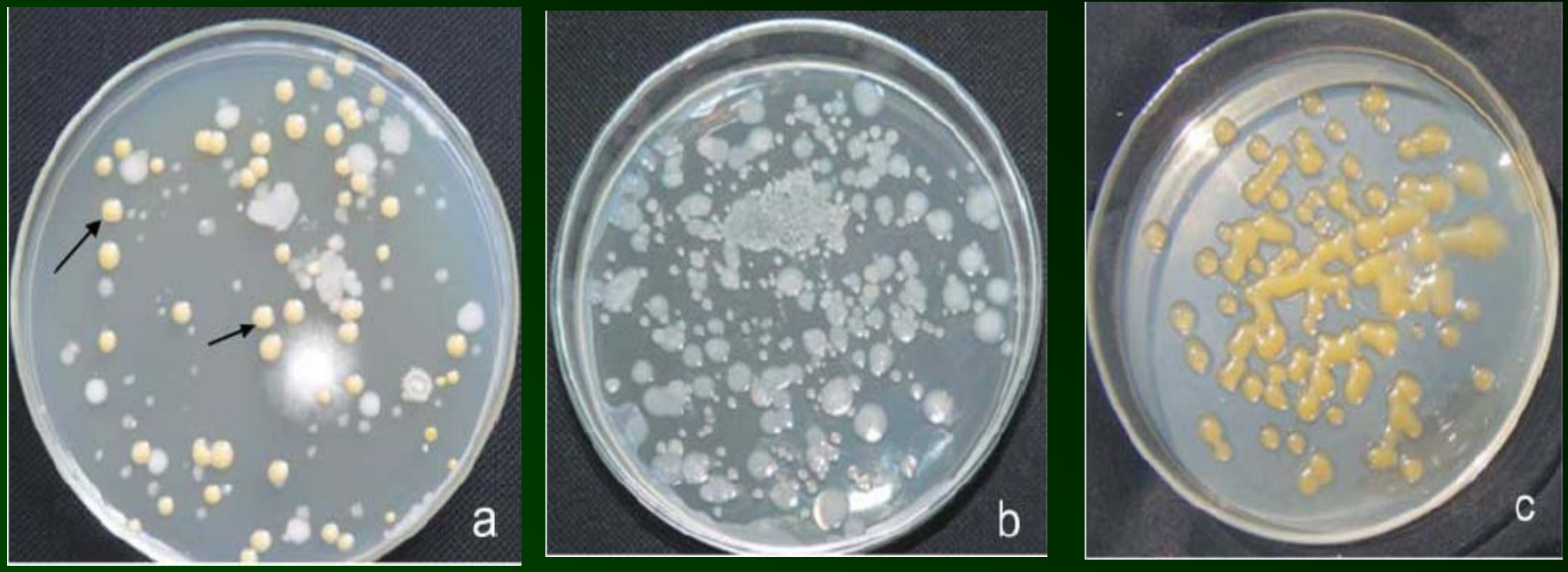
- ✓ CCA – Cellobiose Cephalexin Agar (Mwangi *et al*., 2007).
- ✓ YTSA-CC - Yeast extract, Trypton, Sucrose Agar, Cephalexin and Cycloheximide (Tripathi *et al.*, 2007).
- ✓ YPGA – Yeast Peptone Glucose Agar

# CCA – Cellobiose Cephalexin Agar

- ✓ Ingredients of CCA ($L^{-1}$): 1g yeast extract, 1g glucose, 1g peptone, 1g $NH_4Cl$, 1g $MgSO_4$. $7H_2O$, 3g $K_2HPO_4$, 1g beef extract, 10g cellobiose, 14g agar, 40mg cephalexin, 10 mg 5 - fluorouracil and 120mg cycloheximide.
- ✓ Contains cellobiose as the major carbon source.
- ✓ Cellobiose offers some degree of selectivity, especially against the soilborne saprophytes *Bacillus pumilus* and *Arcanobacterium* sp. and does not suppress *Xcm* growth.
- ✓ Amended with the antibiotics: cepahalexin and 5-fluorouracil.
- ✓ 5-fluorouracil eliminates fluorescent pseudomonads (Sijam *et al*., 1991), while cephalexin suppresses most Gram-positive bacteria, but is also reported to inhibit *Erwinia* spp. (Schaad *et al*., 2001).

# Conclusions on CCA media

- ✓ CCA is not superior to YPGA when isolating from less contaminated samples, but more selective when isolating from more contaminated sources, such as soil or rotting plant matter.
- ✓ Modifications of CCA to widen the range of organisms that are suppressed should be explored.
- ✓ The need to use the semi-selective medium should thus be based on the sampling source.
- ✓ Identification of more easily accessible and affordable compounds is required. Cellobiose, for example, is costly and tedious to incorporate.




(a): Six-day-old colonies of *Xanthomonas campestris* pv. *musacearum* from soil on the semi-selective cellobiose cephalexin agar showing yellow colour (arrows) and dome shape; (b): Non-selective yeast peptone glucose agar (YPGA) overgrown by contaminants within 48 h after spreading suspensions, making it difficult to enumerate *Xanthomonas* spp; (c): Colonies of pure *Xanthomonas* culture on YPGA with an intense yellow colour, visible after 72 h at 25°C and gradually merging.

## YPGA – Yeast Peptone Glucose Agar

- ✓ Ingredients of YPGA ($L^{-1}$): 5g yeast extract, 5g peptone, 10g glucose, 15g agar, 3.3ml$sl^{-1}$ cephalexine and 3.3ml$sl^{-1}$ 5-flourouracil.
- ✓ Contains glucose as the major carbon source.
- ✓ Amended with the antibiotics: cepahalexin and 5-florouracil.

## YTSA-CC - Yeast extract, Trypton, Sucrose Agar, Cephalexin and Cycloheximide

- ✓ Ingredients of YTSA-CC ($L^{-1}$): 1% yeast extract, 1% tryptone, 1% sucrose, 1.5% agar, 50mg$l^{-1}$ cephalexine and 150mg$l^{-1}$ cycloheximide at pH 7.0.
- ✓ Contains sucrose as the major carbon source.
- ✓ Amended with the antibiotics: cepahalexin and cycloheximide.
- ✓ Cephalexin inhibits most of the saprophytes and cycloheximide inhibits the fungal contaminants.

# Conclusions on YTSA-CC media

- ✓ YTSA-CCA is not superior to YTSA when isolating from less contaminated samples, but more selective when isolating from rotten fruits at advanced stages of disease development.

- ✓ A few saprophytes from soil samples may grow but are distinguished from *Xcm* based on colour and colony morphology.

## OTHER TESTS

- Gram stain
- Hypersensitive reaction
- Flagellation
- Oxidative use of carbohydrates
- Utilization of carbohydrates as sole carbon sources
- Levan production
- Urease production
- Arginine dehydrolase activity
- Oxidase test
- Potato soft rot
- Catalase production
- Production of poly-b-hydroxybutyrate (PHB) granules

## The Gram Stain

- ✓ The Gram stain procedure divides bacteria into 2 main groups: Gram + and Gram –
- ✓ Division based on the cell wall composition
- ✓ Gram +ve retain the stain while the Gram –ve lose the stain after washing
- ✓ The test can be done simply by vigorously mixing bacterial cells in 3% KOH solution. Gram -ve bacteria form a gelatinous thread on lifting. Gram +ve bacteria does not.
- ✓ Most plant pathogenic bacteria are Gram –ve.

## The Hypersensitive Reaction (HR)

- ✓ Important in distinguishing between pathogenic and non-pathogenic bacteria
- ✓ A bacterial suspension is infiltrated into between the cells of tobacco
- ✓ After 24 hrs cells collapse in a HR+ (for pathogenic bacteria)
- ✓ The plant's defensive apparatus responds by killing cells surrounding the infected area to stop further movement of the bacteria

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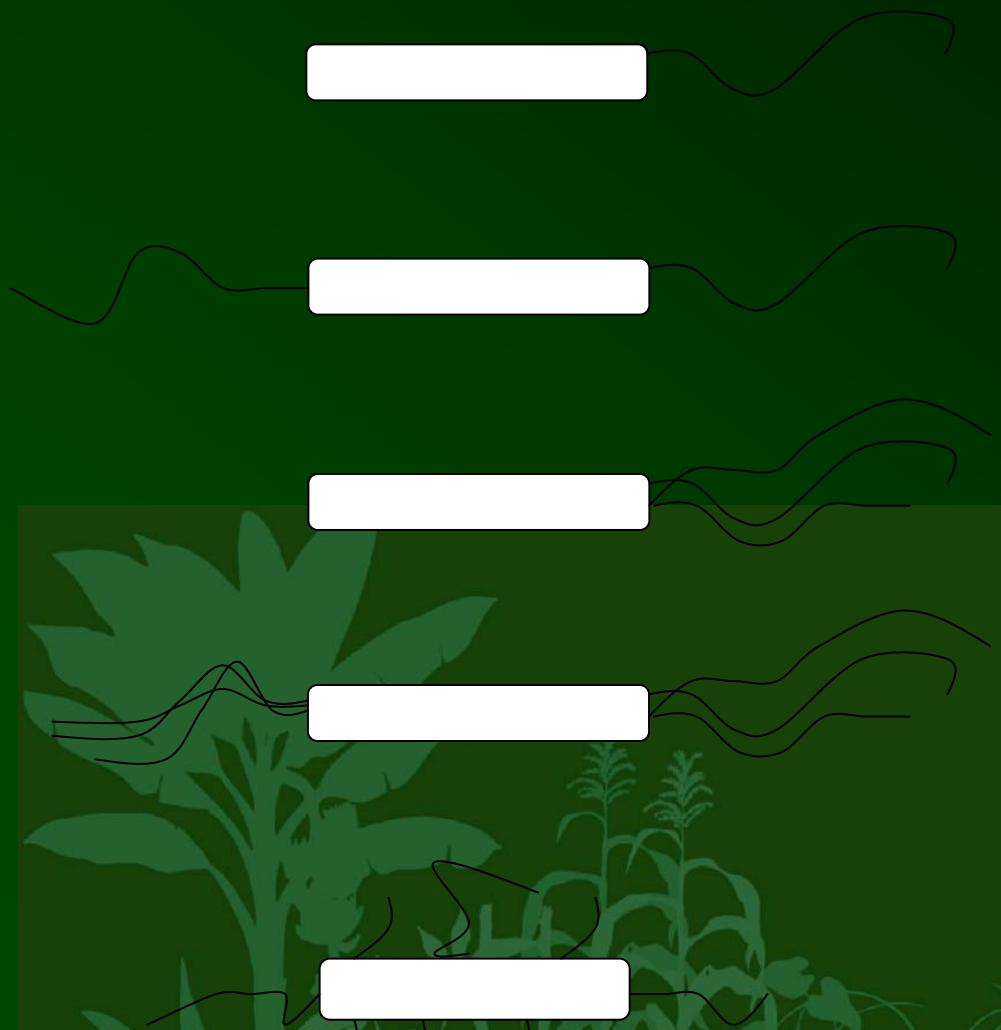
# Flagellation

Usually done using an electron microscope



Polar or monotrichous

Amphitrichous

Lophotrichous (2 or more flagella at one or both ends

Peritrichous

# Physiological and Biochemical characteristics of bacteria

- ✓ Oxidative or fermentative use of carbohydrates
  - Different bacteria utilize carbohydrates through oxidative or fermentatitive pathways.
- ✓ Utilization of carbohydrates as sole carbon sources
  - Some bacteria utilise specific carbohydrates as the only source of carbon.
- ✓ Levan production
  - On sucrose rich media, positive bacteria produce white mucoid dome-shaped colonies after 3-5 days incubation. These colonies contain levan, a fructose polymer produced after the enzyme levansucrase converting sucrose to glucose and fructose .
- ✓ Urease production
  - Tests for bacteria that produce urease, an enzyme that split urea $CO_2$ and $NH_3$. The pH of the media in +ve bacteria become alkaline and is indicated by a change in the indicator color from orange to dark pink.
- ✓ Arginine dehydrolase activity
  - Based on presence of enzymes that generate ATP by the degradation of arginine to ornithine with the generation of $CO_2$ and $NH_3$. Production of NH3 results in an alkaline reaction, and a color change of the pH indicator (Pink to Red).

- ✓ **Oxidase test**
  - A result of an enzyme cytochrome oxidase that oxidise tetramethyl-phenylenediamine – dihydrochloride and is detected as a purple coloration within about 10 seconds.

- ✓ **Potato soft rot**
  - For bacteria that produce pectinases, enzymes that degrade pectin and result into tissue disintegration.

- ✓ **Catalase reaction**
  - Tests the presence of catalase, an enzyme that convert the hydrogen peroxide into water and oxygen, thus causing bubbles in the slant culture to which $H_2O_2$ has been added.

- ✓ **Production of poly-b-hydroxybutyrate (PHB) granules**
  - PHB granules are reserves of carbon produced by some bacteria like *Pseudomonas* spp. These can be microscopically observed.

## Others

- ✓ Acid production from carbohydrates
- ✓ Salt tolerance
- ✓ Minimum and maximum growth temperature
- ✓ Hydrogen sulphide production
- ✓ Milk proteolysis
- ✓ Anaerobic growth
- ✓ Spore formation
- ✓ Nitrate reduction

## CHEMICAL AND PHYSIOLOGICAL TESTS for Xcm

- ✓ motile gram negative rods
- ✓ possess a single polar flagellum,
- ✓ oxidase and tyrosinase negative,
- ✓ does not reduce nitrates,
- ✓ does not hydrolyze starch or gelatin,
- ✓ does not accumulate poly β-hydroxybutyrate granules
- ✓ Non-florescent on kings B-medium

*THANK YOU.....QUESTIONS?????*