

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 bactericidal 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.



Circular



Oval



Spindle



Irregular



smooth/entire



curled

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

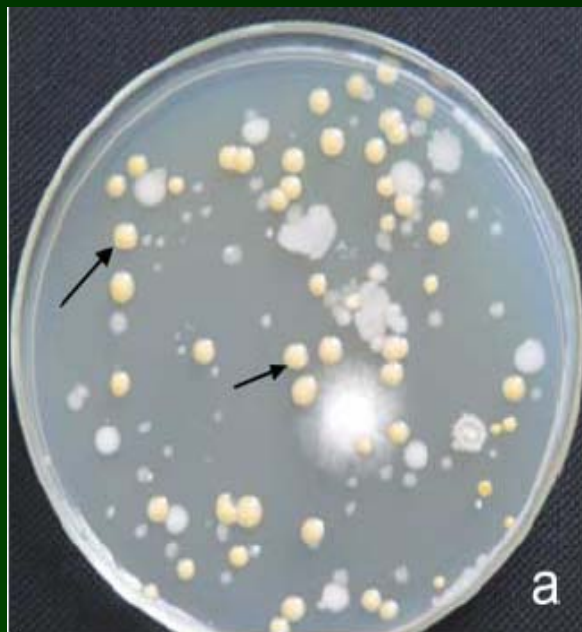
CCA – Cellobiose Cephalalexin Agar

- ✓ Ingredients of CCA (L^{-1}): 1g yeast extract, 1g glucose, 1g peptone, 1g NH_4Cl , 1g $MgSO_4 \cdot 7H_2O$, 3g K_2HPO_4 , 1g beef extract, 10g cellobiose, 14g agar, 40mg cephalalexin, 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 cephalalexin 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 cephalixin 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, 3.3mlsl^{-1} cephalaxine and 3.3mlsl^{-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, Cephalaxin and Cycloheximide

- ✓ Ingredients of YTSA-CC (L^{-1}): 1% yeast extract, 1% tryptone, 1% sucrose, 1.5% agar, 50mgl^{-1} cephalaxine and 150mgl^{-1} cycloheximide at pH 7.0.
- ✓ Contains sucrose as the major carbon source.
- ✓ Amended with the antibiotics: cepahalexin and cycloheximide.
- ✓ Cephalaxin 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- β -hydroxybutyrate (PHB) granules

The Gram Stain

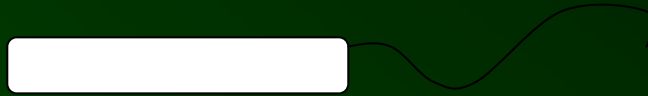
- ✓ The Gram stain procedure divides bacteria into 2 main groups: Gram + and Gram –
- ✓ Division based on the cell wall composition
- ✓ Gram +ve retain the stain while the Gram –ve lose the stain after washing
- ✓ The test can be done simply by vigorously mixing bacterial cells in 3% KOH solution. Gram -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

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 fermentative 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 NH_3 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 H₂O₂ 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?????