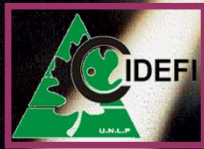


American Foulbrood Disease (AFB) General Introduction

OIE



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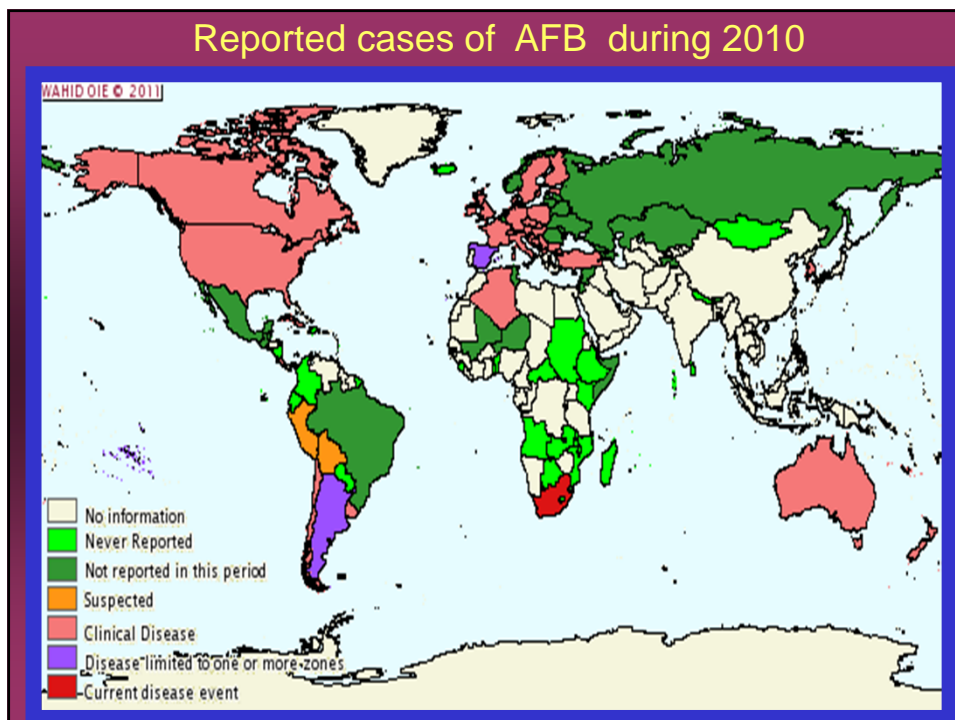
Laboratorio de Referencia para Loque Americana de la OIE
Unidad de Bacteriología, CIDEFI – UNLP
alippi@biol.unlp.edu.ar

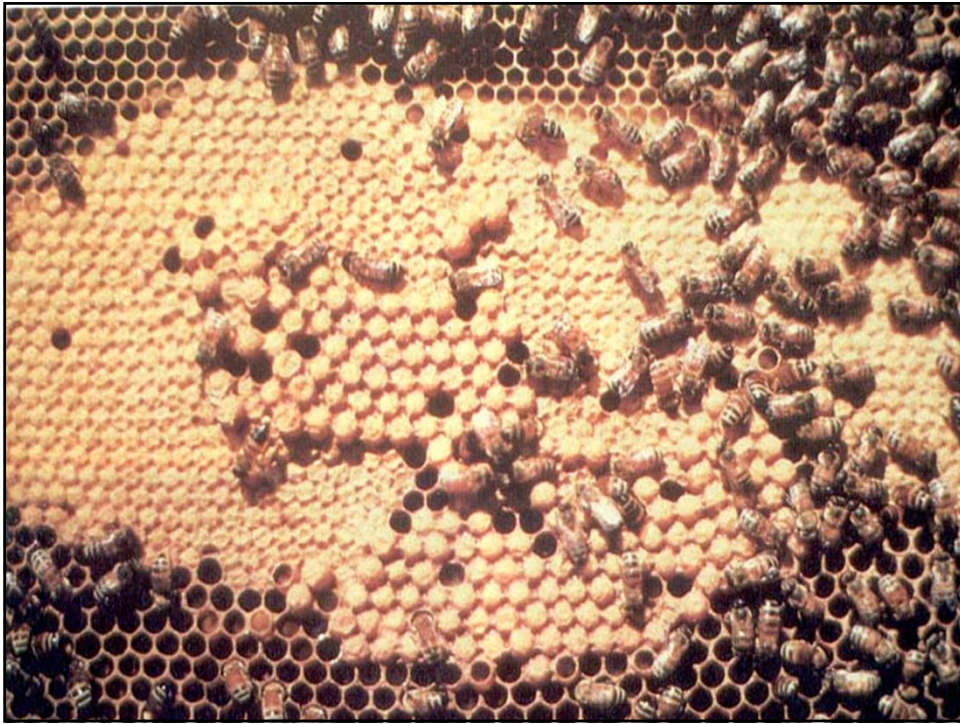
Bacterial species pathogenic to honey bees

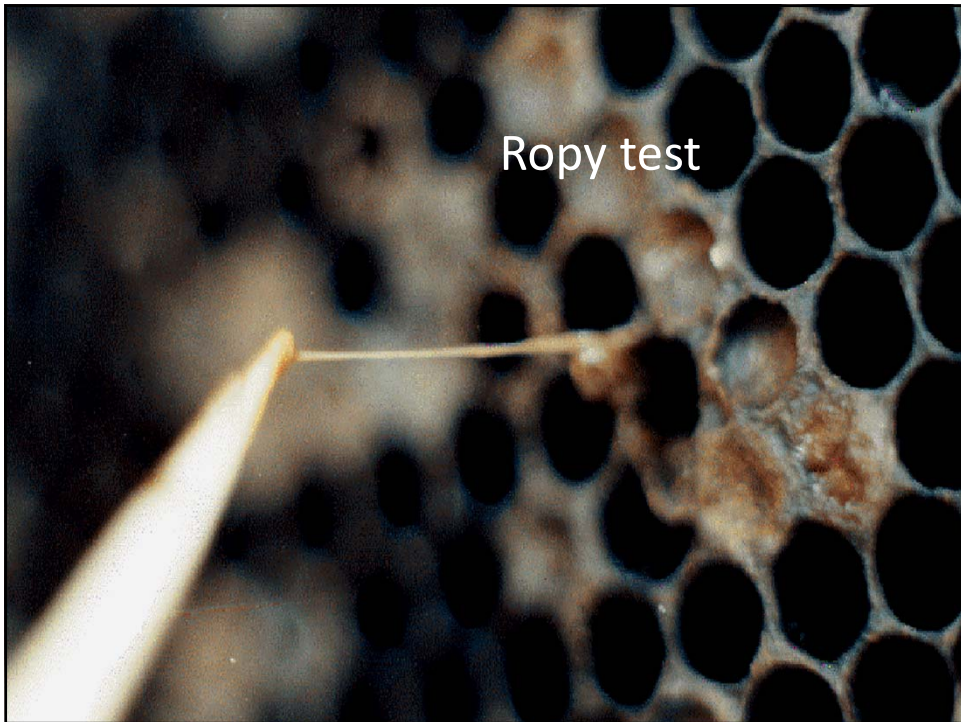
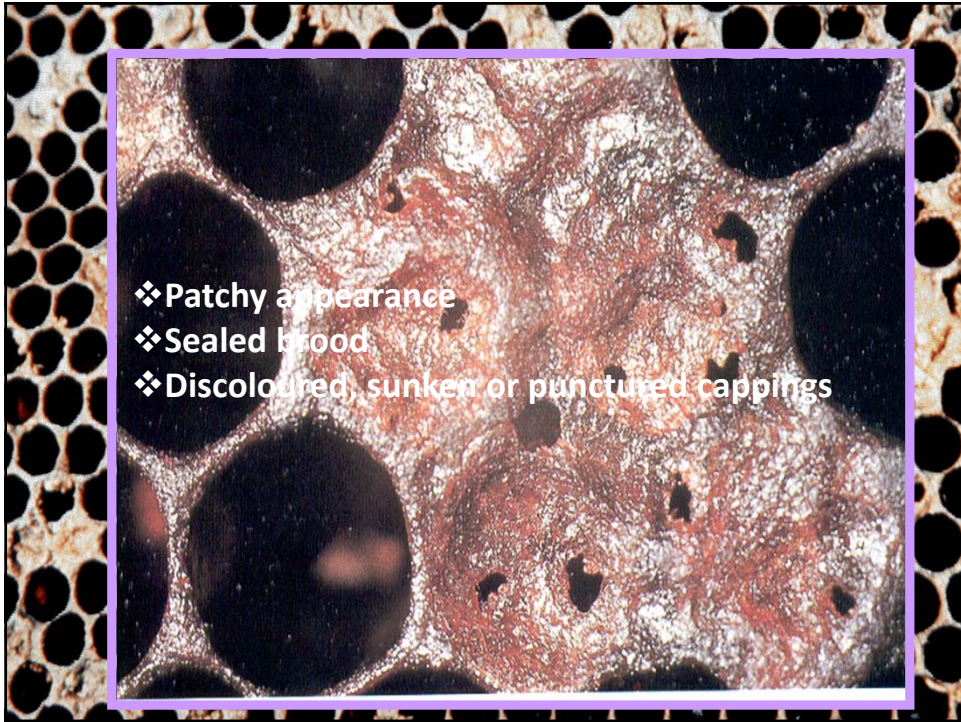
- ▶ *Paenibacillus larvae* American Foulbrood (AFB)
 - ▶ *Melissococcus plutonius* European Foulbrood (EFB)
 - ▶ *Paenibacillus alvei*
 - ▶ *Brevibacillus laterosporus*
 - ▶ *Paenibacillus apiarius*
 - ▶ *Enterococcus faecalis*
 - ▶ *Lactobacillus eurydice*
- ↻
- ▶ *Bacillus coagulans* Half-moon disorder
 - ▶ *Pseudomonas aeruginosa* Septicaemia
 - ▶ *Serratia marcescens - Hafnia alvei* Septicaemia
- ▶ Diseases associated to rickettsiae : genera: *Rickettsiella* and *Wolbachia*
 - ▶ Other diseases associated to Mycoplasmas and Spiroplasmas

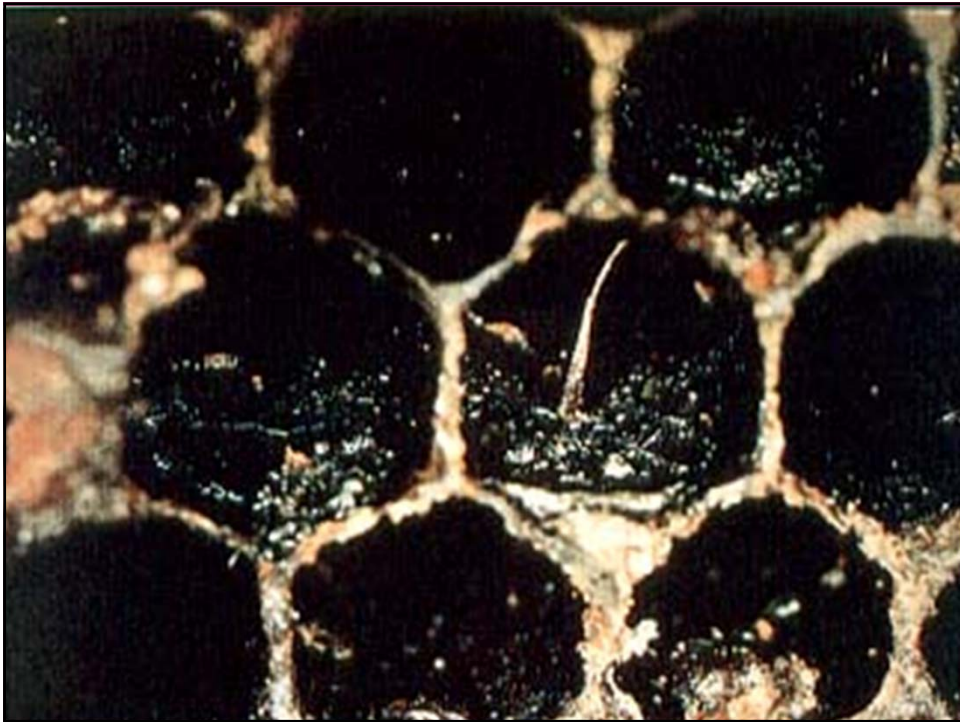
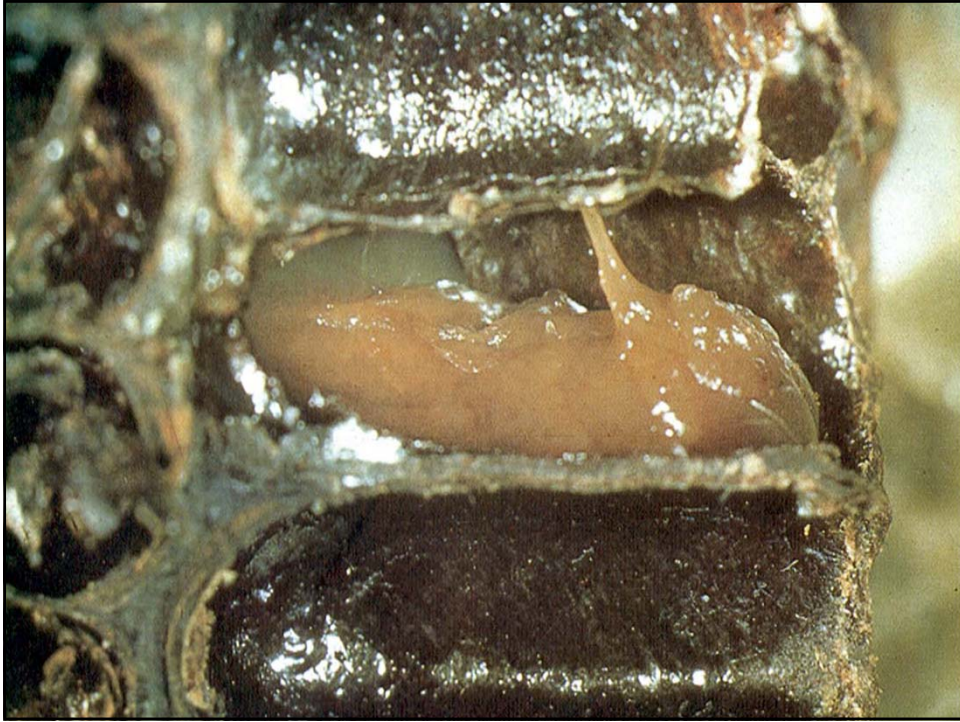
American Foulbrood of Honey bees

- American Foulbrood (AFB) is the most destructive infectious disease affecting larvae and pupal stages of honeybees
- Due to its highly contagious nature is one of the few bee diseases capable of killing a colony
- There is no seasonal outbreak of AFB → it occurs at any time of the year when brood is present
- Bacterial spores survive for decades remaining viable
- AFB occurs in temperate and sub-temperate regions throughout the world
- AFB is a notifiable disease in many countries
- AFB is classified on list B of the OIE → Animal diseases of socio-economic and/or public health importance









Comparative symptoms of the two major bacterial brood diseases of honeybees

(Adapted from Shimanuki and Knox, 1991)

Symptoms	American Foulbrood	European Foulbrood
Appearance of brood comb	❖ Patchy appearance ❖ Sealed brood ❖ Discoloured, sunken or punctured cappings.	❖ Patchy appearance ❖ Unsealed brood ❖ Some sealed brood in advanced cases
Age of dead brood	❖ Usually older sealed larvae or young pupae ❖ Upright in cells.	❖ Usually young unsealed larvae ❖ Occasionally older sealed larvae. ❖ Typically in coiled stage.
Colour of dead brood	❖ Dull white, becoming light brown, coffee brown to dark brown or almost black.	❖ Dull white, becoming yellowish white to brown, dark brown or almost black.
Consistency of dead brood	❖ Soft, becoming sticky to ropy. ❖ Stretching to a thin thread longer than 2.5 cm.	❖ Watery to pasty , rarely sticky or ropy. ❖ Stretching no longer than 2.5 cm ❖ Granular.
Odour of dead brood	❖ Slight to pronounced glue odour to gluepot odour.	❖ Slightly to penetratingly sour.
Scale characteristics	❖ Uniformly lies flat on lower side ❖ Adheres tightly to cell wall ❖ Fine tongue of dead pupae may be present ❖ Head lies flat; other adult characteristics like heads or legs may be present. ❖ Brittle, black.	❖ Usually twisted in cell ❖ Does not adhere tightly to cell wall ❖ Rubbery ❖ Dark brown to black.

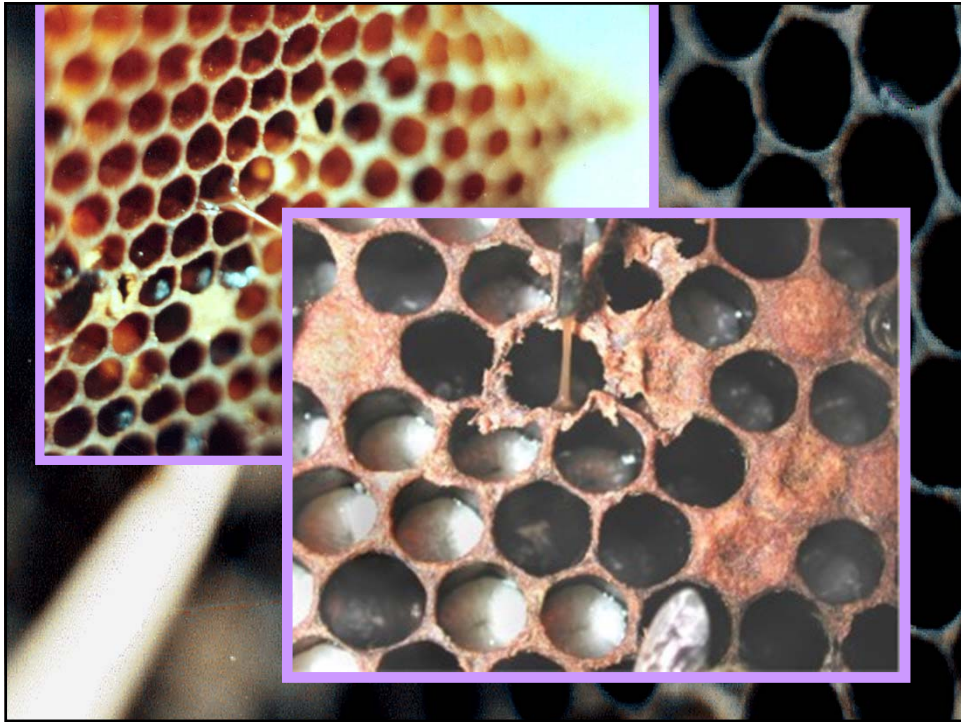
Detection of *Paenibacillus larvae*

➤ Clinical signs in brood combs:

- * Ropy test
- * Holst test
- * Lateral flow devise

➤ Microscopy from diseased larvae:

- * Single stain with carbol fuchsin
- * *Modified hanging drop*
- * Nigrosine stain



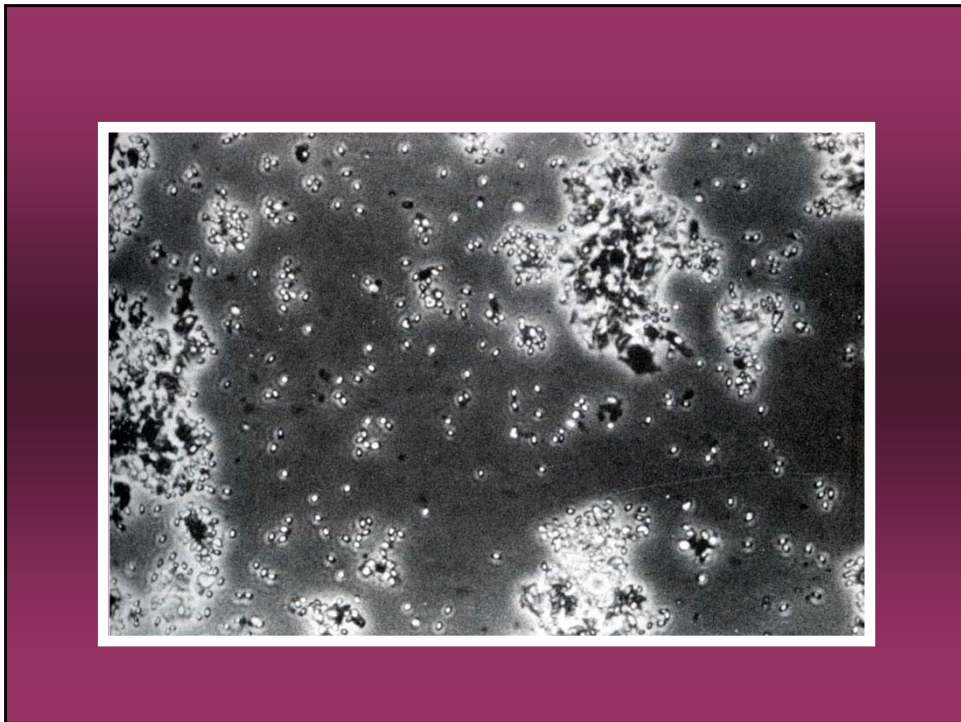
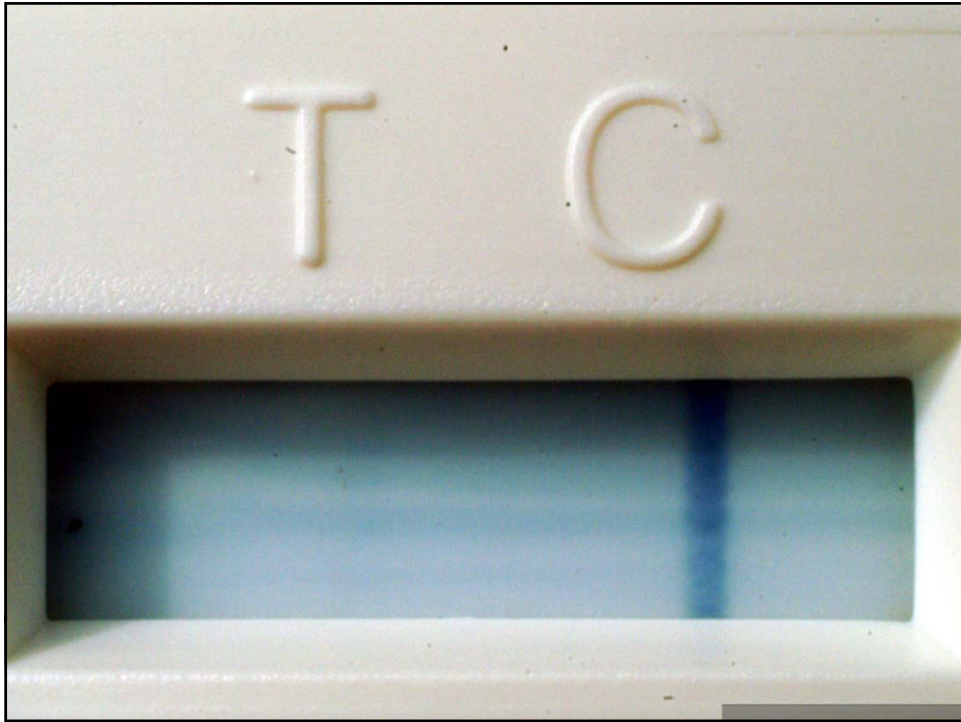
VITA Diagnostic Kit

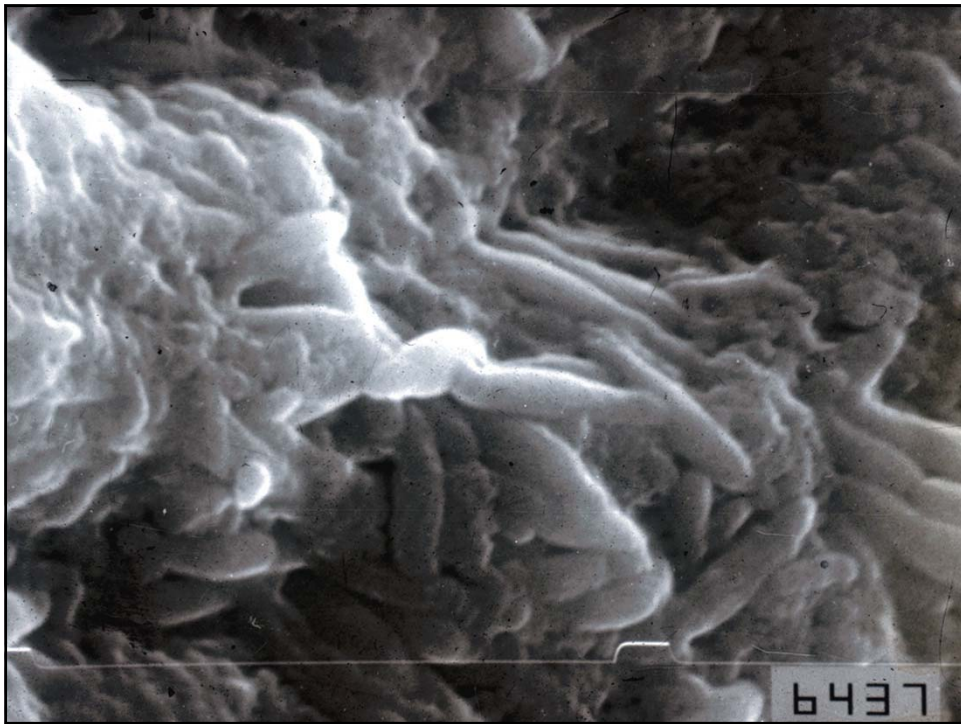
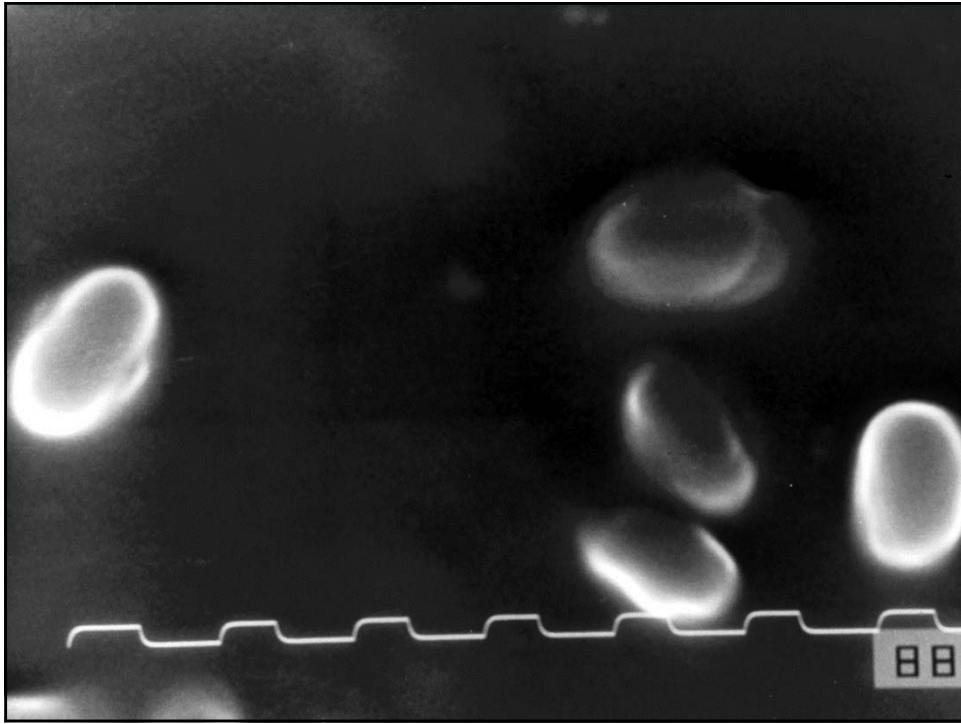


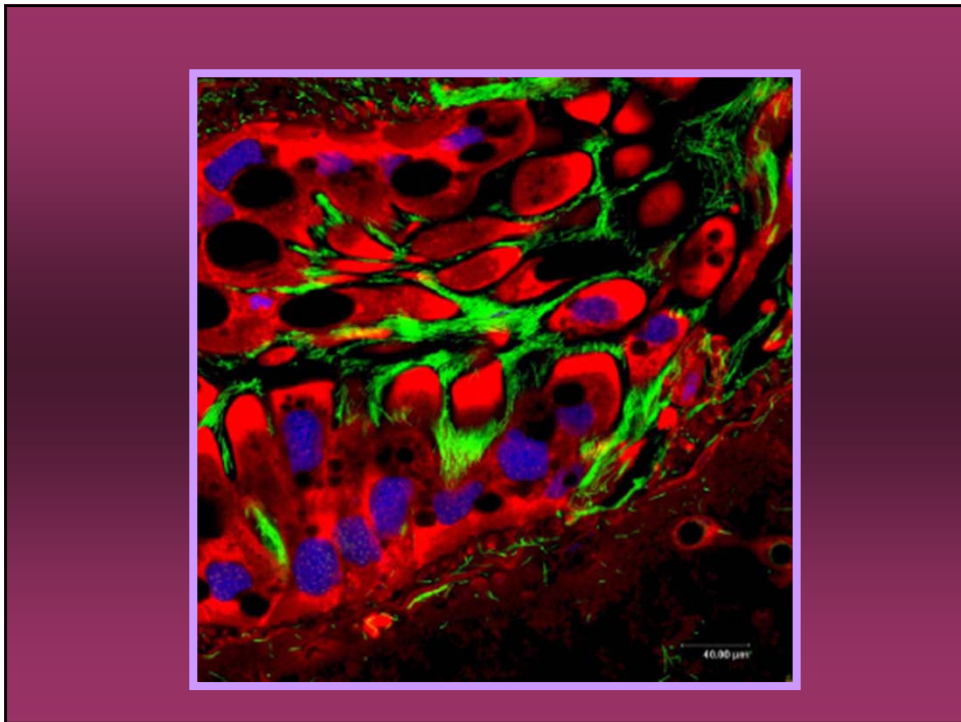
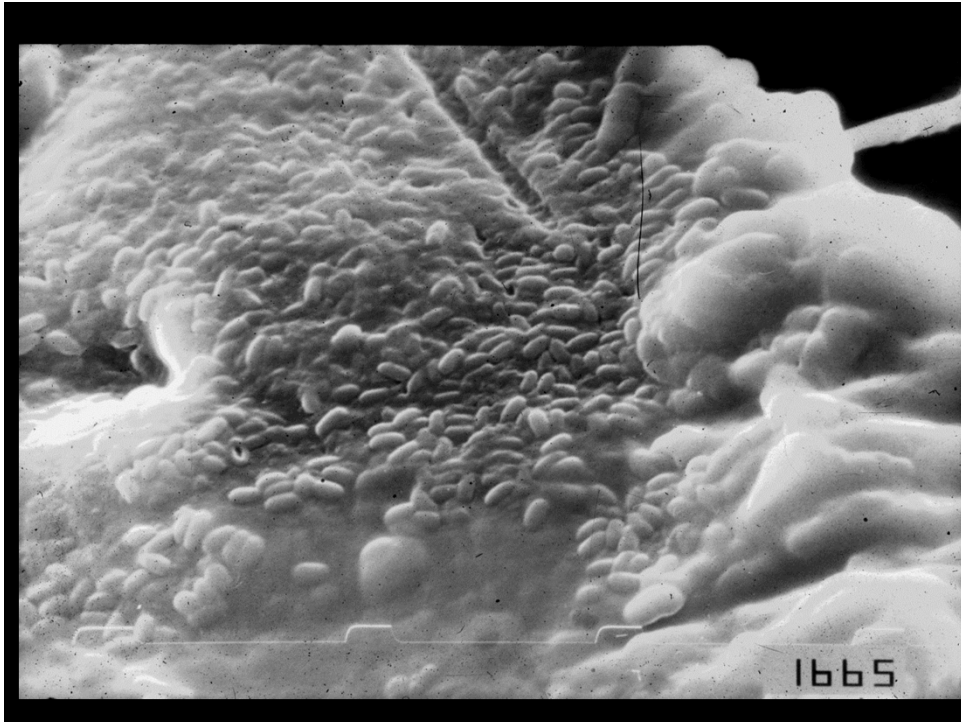
96% of correct diagnosis

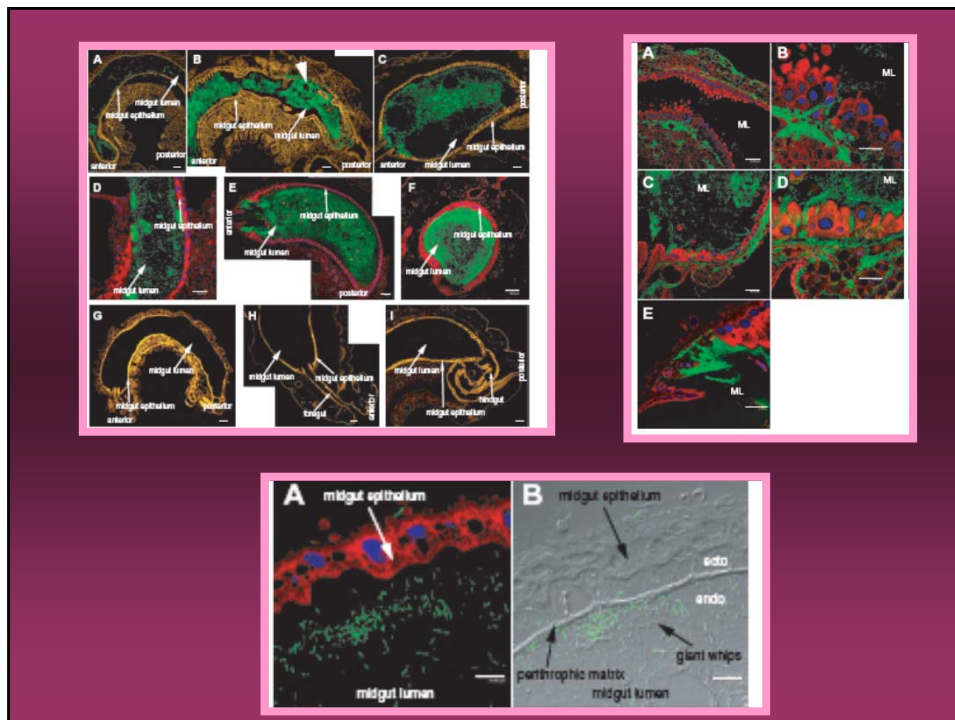












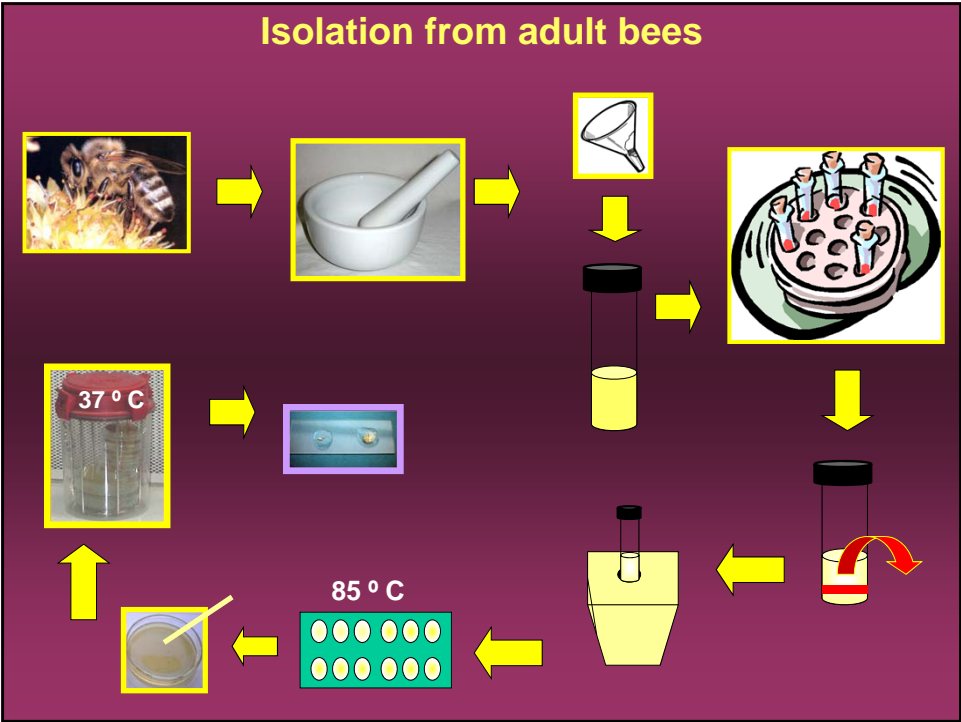
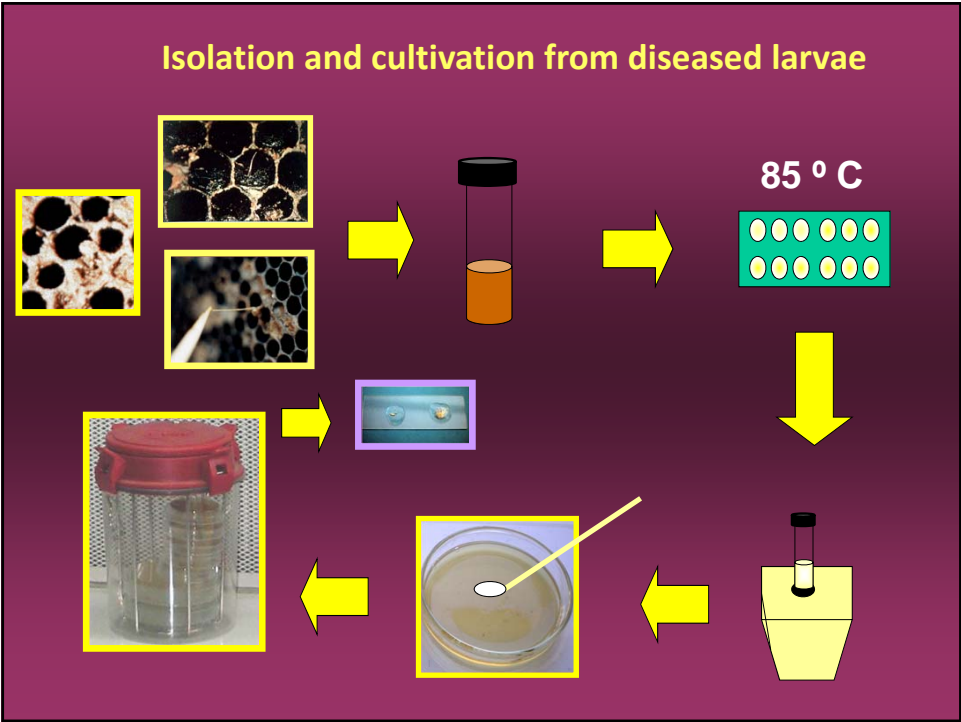
Paenibacillus larvae isolation and cultivation

Isolation and cultivation

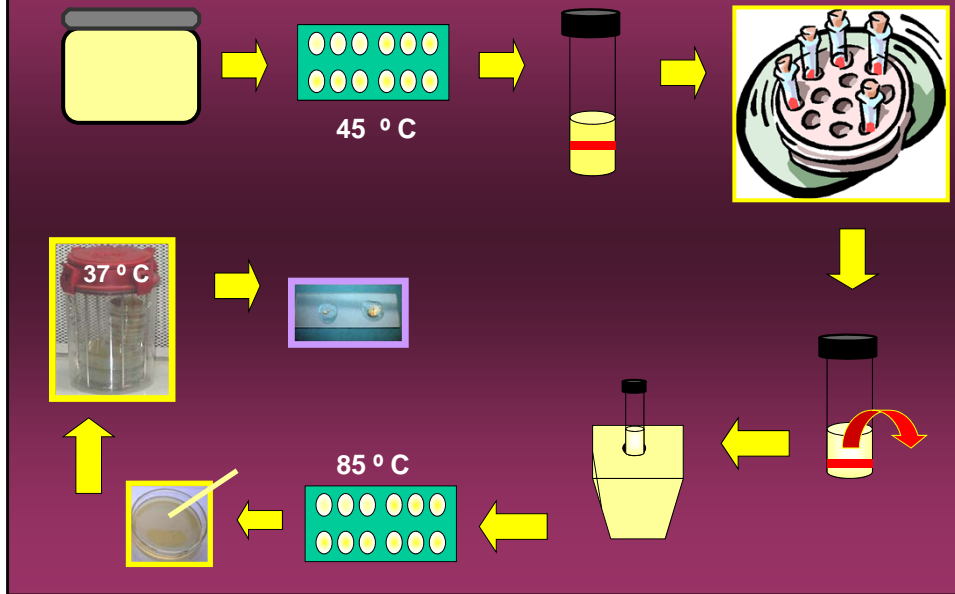
- J-agar
- MYPGP
- PLA (*Bacillus cereus* selective base + TSA + SNA)
- Brain Heart Infusion + thiamine (BHIT)
- Columbia base agar + supplemented with 5% horse blood

Confirmation

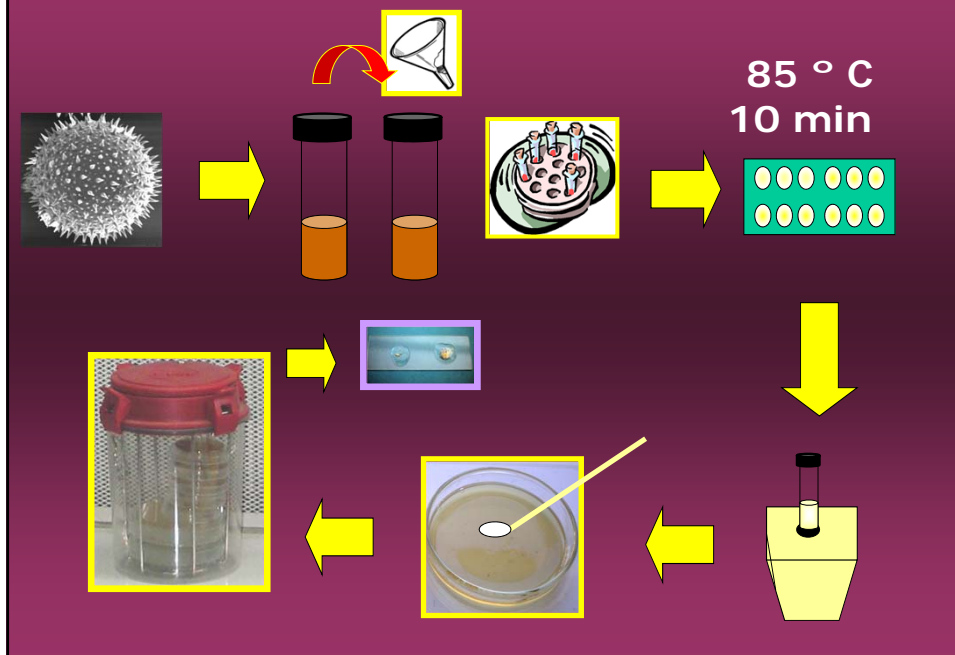
- Catalase
- Biochemical profiling
- Phage sensitivity test (Spot test)
- PCR

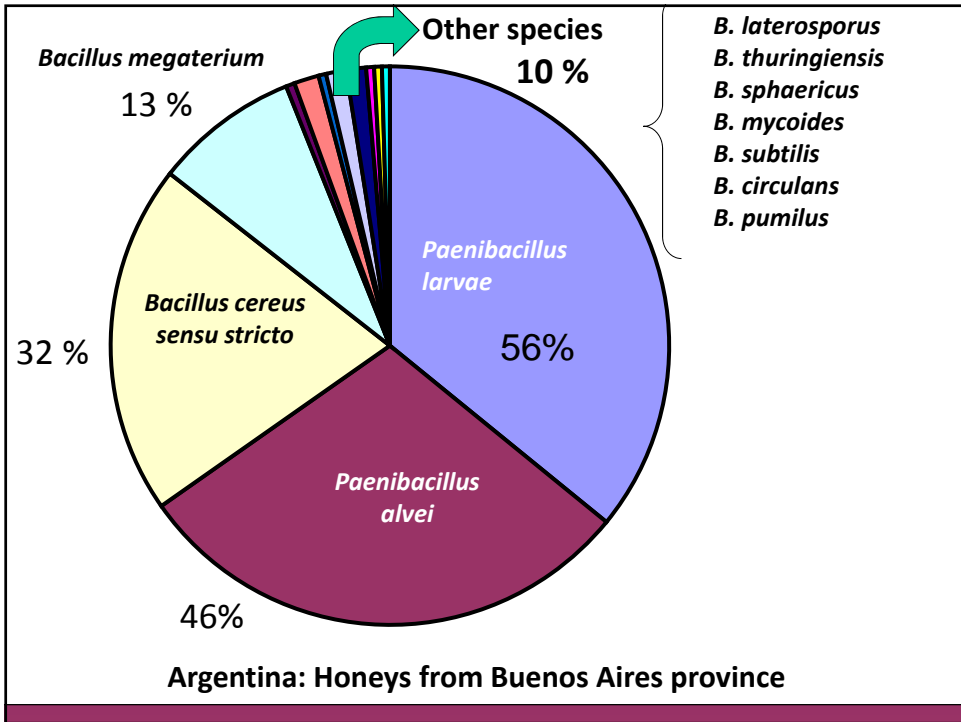


Isolation and cultivation from honey samples

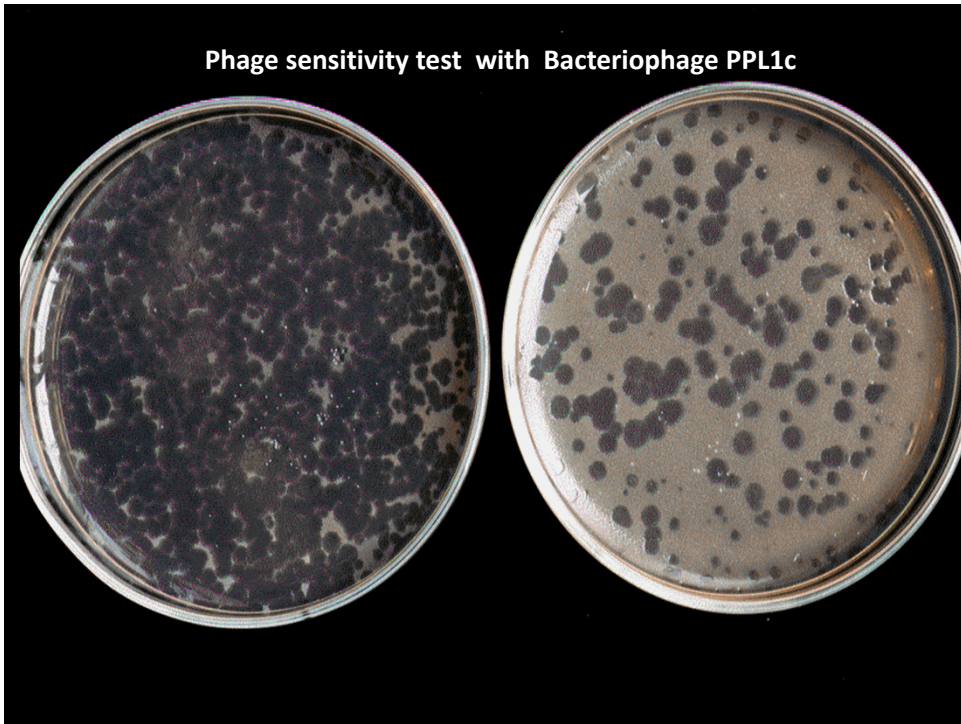


Cultivation from pollen

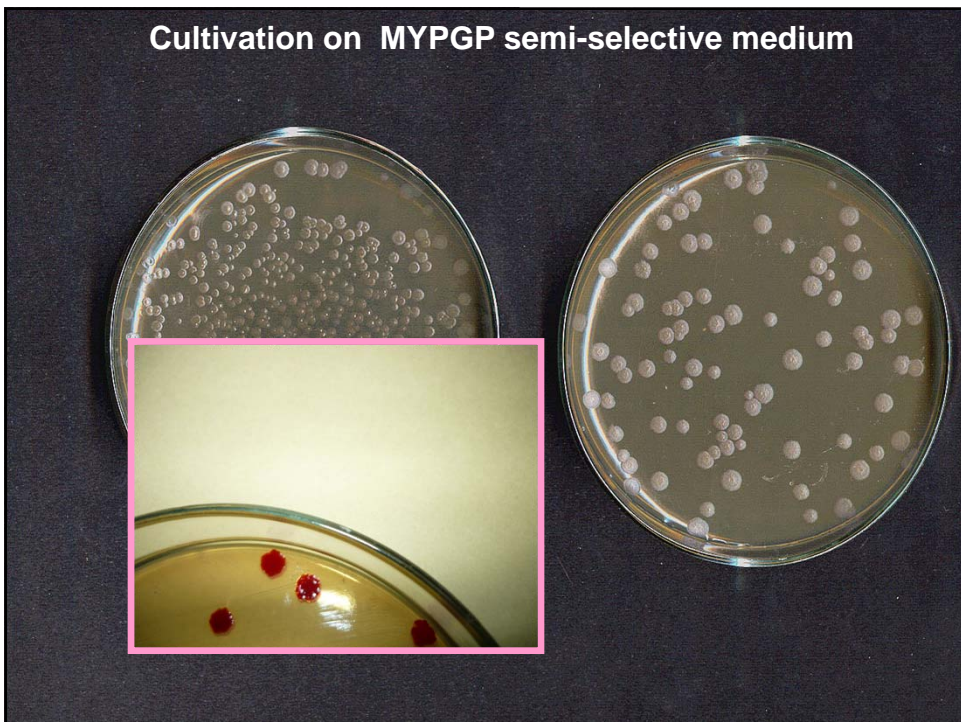




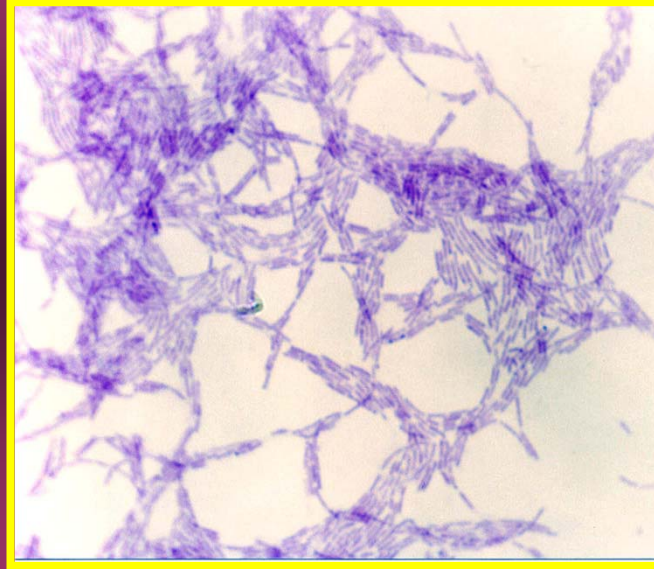
Phage sensitivity test with Bacteriophage PPL1c



Cultivation on MYPGP semi-selective medium

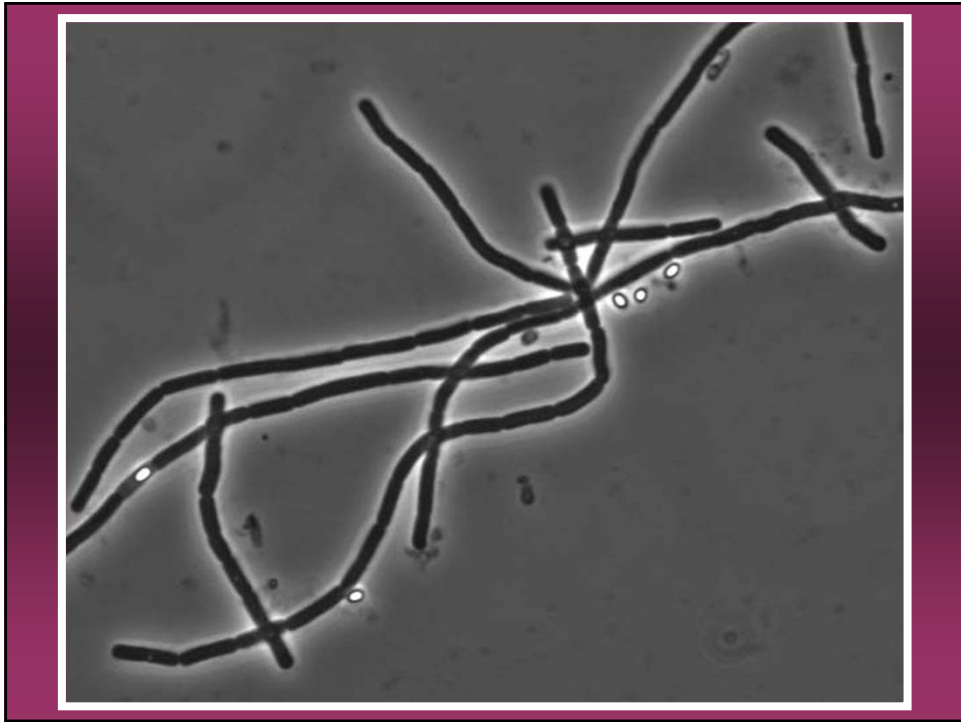


Gram reaction



Spores staining

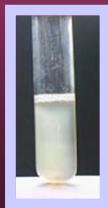




Reduction of nitrates to nitrites

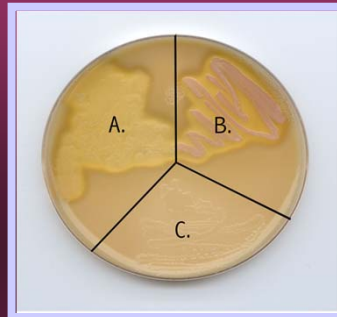


positive



negative

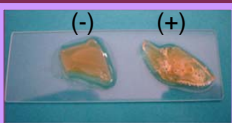
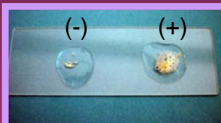
Casein hydrolysis

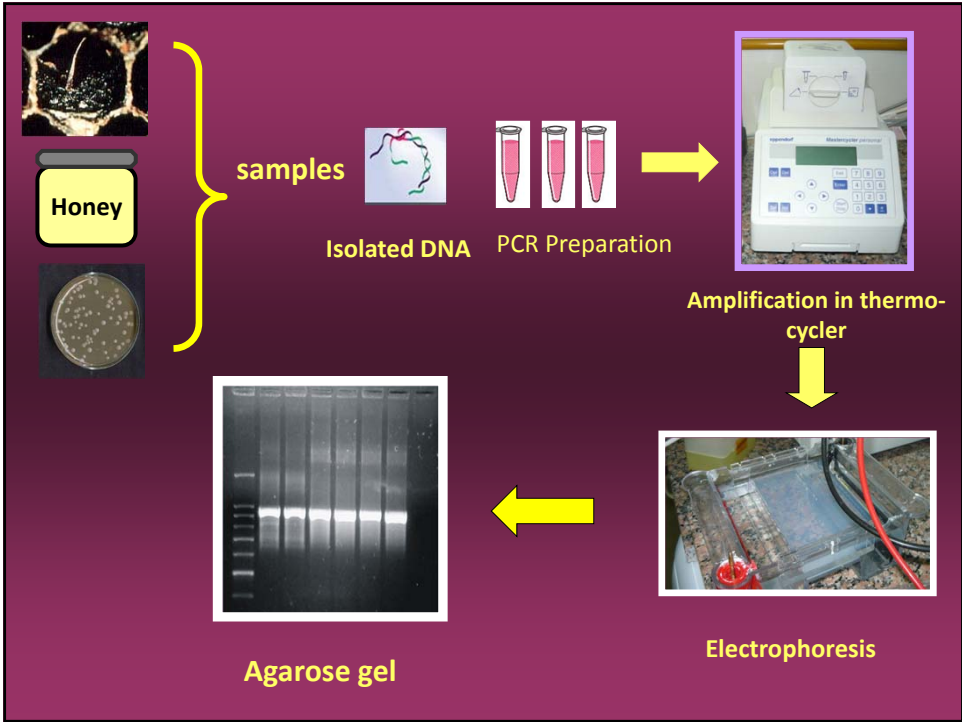
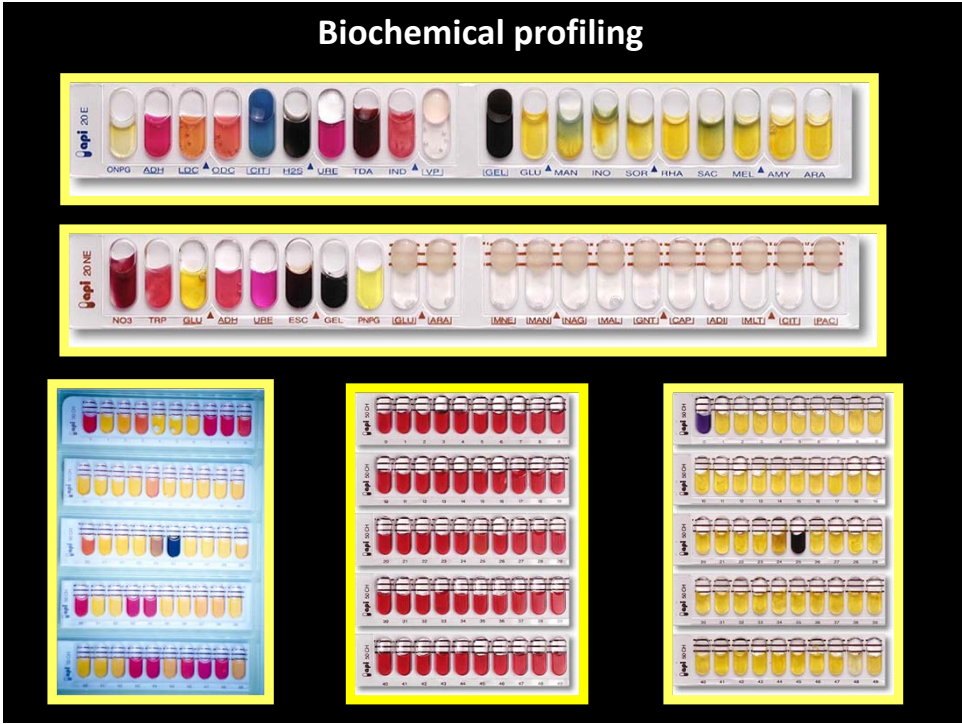


A and B: (+)

C: (-)

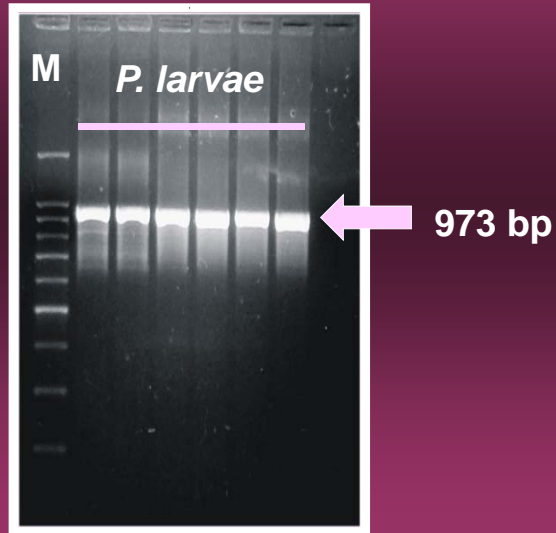
Catalase test





PCR amplification of specific bacterial DNA using a single primer set

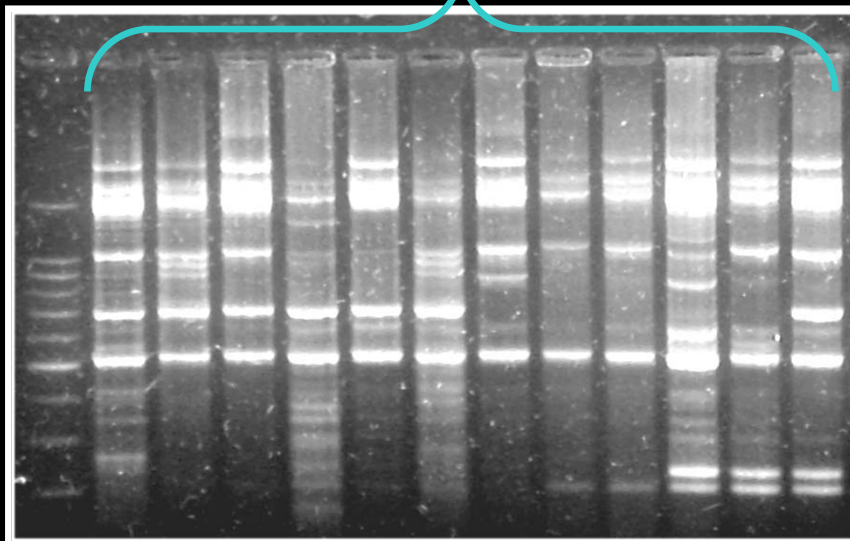
16S rRNA (Govan *et al.*)



Rep-PCR by using primers BOX

M

Paenibacillus larvae genotypes



Different techniques to identify *Paenibacillus larvae*

(Condensed from: de Graaf et al., Letters in Applied Microbiology 43: 583-590, 2006)

Technique	Principle	Samples	Advantages	Disadvantages
Cultivation	Germination and growth of <i>Paenibacillus larvae</i> spores on solid medium.	brood honey adult bees pollen wax hives debris.	*Detection of <i>P. larvae</i> in bee products facilitates tracing infection sources. *Very suitable for AFB detection programs. *Permits quantification of spore loads *Allows testing spore viability.	*Requires an additional identification step of suspect <i>P. larvae</i> colonies. *Semi-selective media usually required to avoid contamination with other bacteria.
Biochemical profiling	Identification of the species of bacteria based on the carbohydrate acidification profile, the catalase test and the casein hydrolysis plate test.	Bacterial colonies	*Traditional microbiological approach that can be performed in most microbiology laboratories. * Commercial kits -Biolog system, API strips- facilitates rapid full profile results.	*Requires a first step of isolation and cultivation of bacteria. * Results for a full profile by classic methods available after 2 to 3 weeks.
Phage sensitivity test	Plaque formation in a semi-solid medium as a result of bacterial cell lysis.	Bacterial colonies	*Easy and simple test to perform rapid diagnosis of AFB *Low cost	*Requires a first step of isolation and cultivation of bacteria.
PCR	Amplification of specific bacterial DNA using a single primer set.	Bacterial colonies, brood, honey, adult bees, pollen, debris.	*Fast * Permits rapid confirmation without cultivation step starting from different samples.	* Needs sophisticated equipment. * Can identify the presence of dead spores or spores that fail to germinate but not important for disease.
Real time PCR	Bacterial DNA is specifically amplified by PCR and after each round of amplification, the DNA is quantified by using fluorescent dyes that intercalate with double-strand DNA and modified DNA oligonucleotides that fluoresce when hybridized with a complementary DNA.	Brood honey adult bees pollen debris.	* Fast * Permits rapid confirmation without cultivation step starting from different samples. *Permits quantification of the spore-load. * No gel-basis analysis at the end of the PCR reaction * Because of its high sensitivity very suitable for AFB detection programs.	*Needs highly sophisticated and very expensive equipment. * Can identify the presence of dead spores or spores that fail to germinate but not important for disease. * The extreme sensitivity makes it vital to protect samples from contamination which would lead to false results

