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AMERICAN FOULBROOD DIAGNOSTIC CAPACITY IN SOUTH AFRICA

TERESA GOSZCZYNSKA
Agricultural Research Council
Plant Protection Research Institute
Pretoria

THE LAB

Agricultural Research Council

- Plant Protection Research Institute
- Bacterial Diseases Unit In Pretoria

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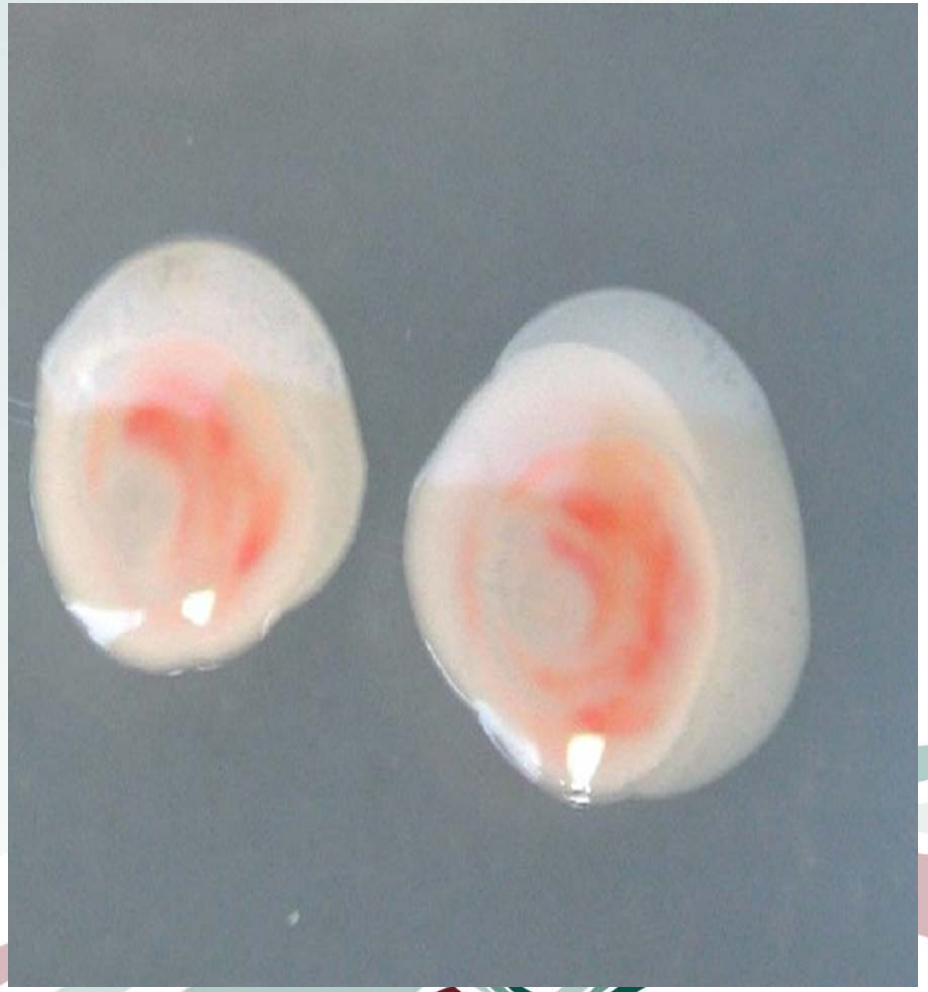
Bacterial Diseases Unit

- Diagnosis of bacterial diseases of agricultural crops
- Seed borne bacterial pathogens
- Detection systems
- Taxonomy
- National collection of Plant Pathogenic and Plant Protecting Bacteria



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Bacterial Diseases Unit

- Started to work with AFB in 2006 on a very small scale
- The only AFB positive samples for 3 years were that obtained from Europe and used as positive controls

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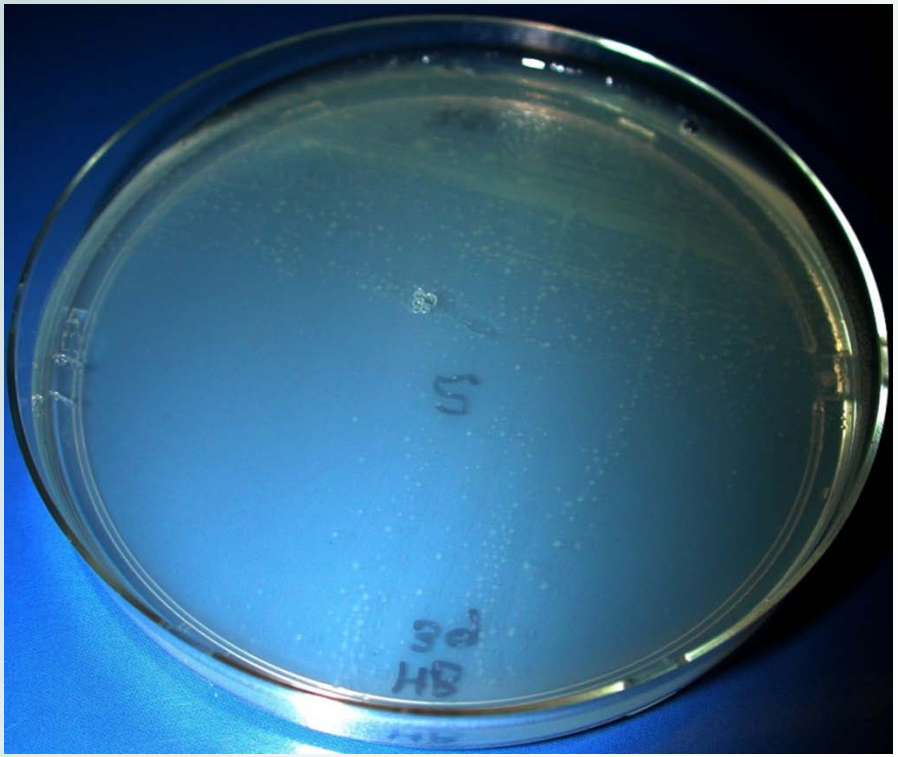
February 2009



Paenibacillus larvae **isolated and confirmed**
For the first time from the larvae collected
in the Cape Town apiary

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PROCEDURE

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HONEY EXTRACTION

- The jar with honey in a bag is placed in a water bath at 70^{o}C and incubated for 10-15 min
- 10 ml of honey is taken from the jar and placed in a centrifuge tube
- 15 ml of SDW is added and the sample is mixed
- Samples are centrifuged at 10000 rpm for 30 min

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Bacterial strains used as controls

- ***P. larvae* LMG 9820T (synonym *P. larvae* subsp. *larvae*, type strain)**
- ***P. larvae* LMG 15974T (synonym *P. larvae* subsp. *pulvifaciens*, type strain)**
- ***P. alvei* LMG , type strain**

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Additional controls

- **AFB positive honey**
- **AFB negative honey**

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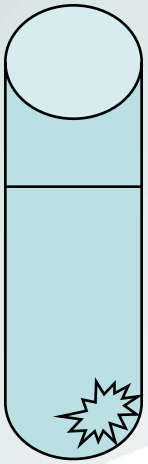
Honey extraction



Centrifugation

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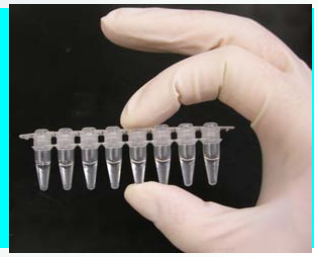
PLATING



- **Pellet is dissolved in 0.5 ml SDW and transferred into eppendorf tube**
- **Tubes are heated at 80^{o}C for 15 min**
- **Honey extract is plated on 4 plates of J-agar and 4 plates of MYPG agar**
- **Plates are incubated at 37^{o}C, 5% CO_2 incubator for minimum 10-11 days.**

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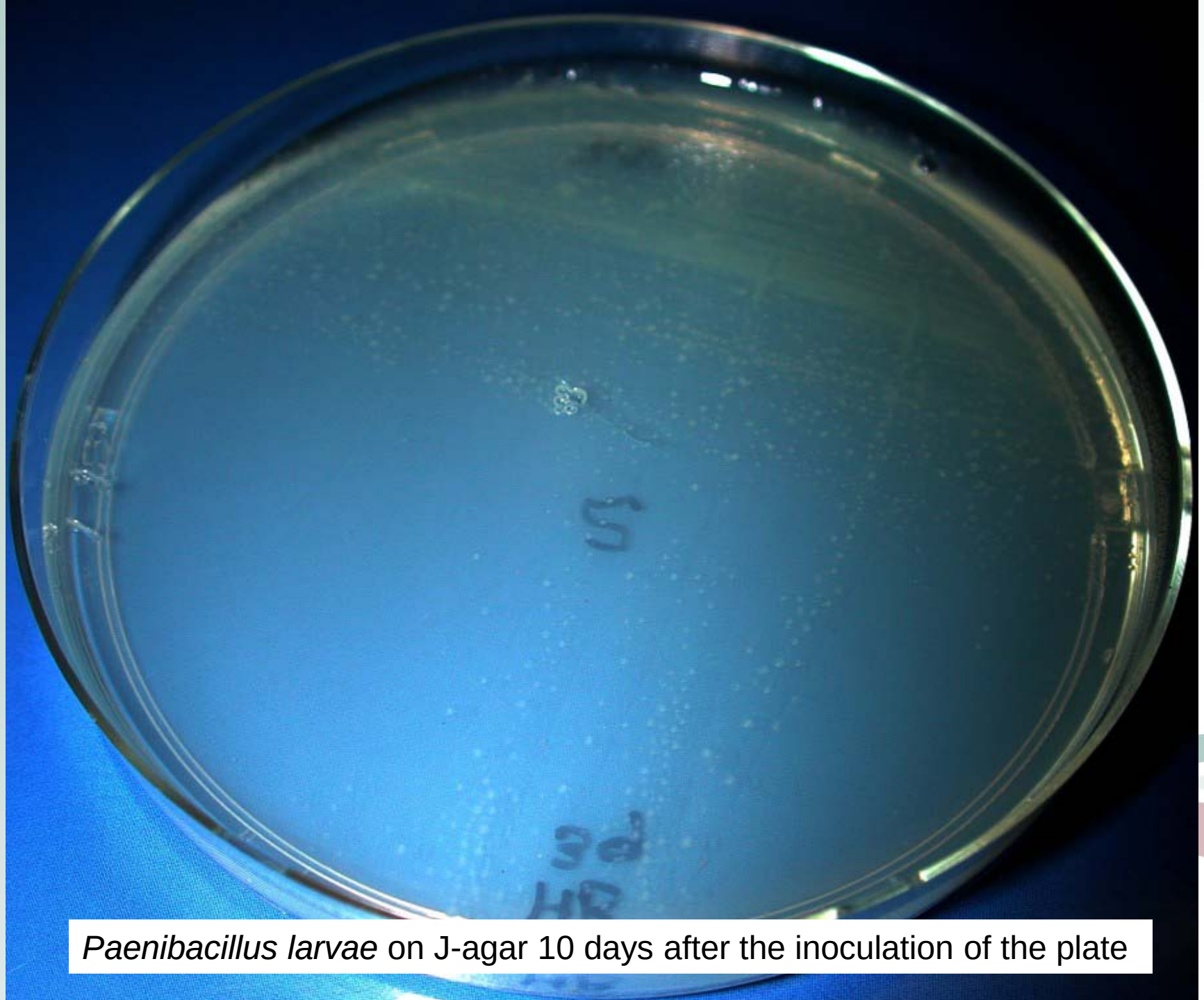
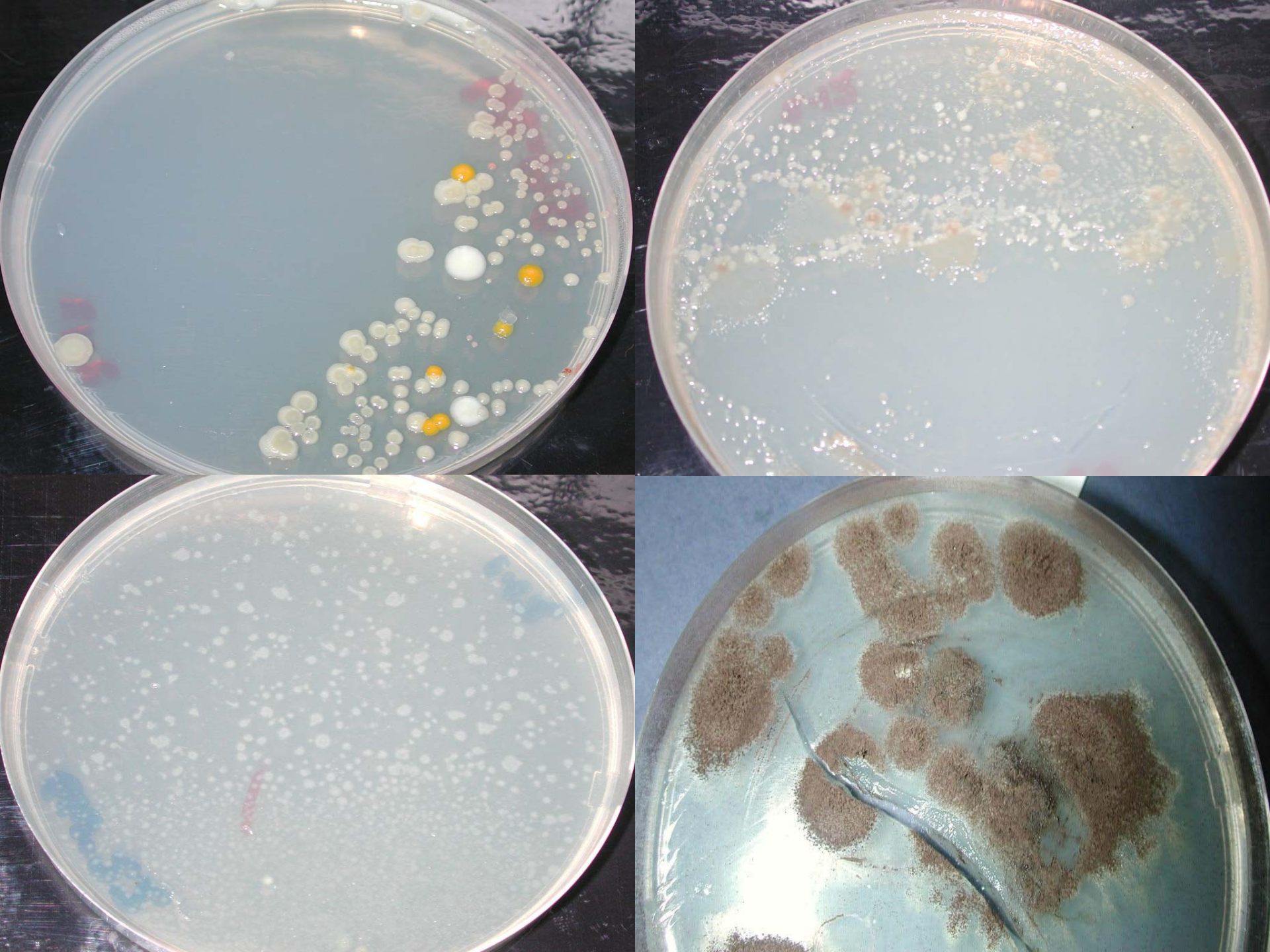


Paenibacillus larvae on J-agar 10 days after the inoculation of the plate



BIO-PCR

- **Schaad et al. (1995) developed the "Bio-PCR" to enhance the sensitivity of PCR reaction. Bio-PCR detects living cells of pathogens, those that could cause a disease, as bacterial colonies are washed up from agar plates preceding the PCR reaction.**

- **A honey extract was plated on J-agar, plates were incubated for 10-14 days**

- **and then the bacterial growth was removed from the agar plates and suspended in sterile distilled water.**

- **This bacterial suspension is used as a template in the PCR with primers targeting *P. larvae*.**

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PCR

- **PCR conditions were adapted from:**

- **Bakonyi T., Derakhshifar I., Grabensteiner E. and Novotny N. 2003. Development and evaluation of PCR assays for the detection of *Paenibacillus larvae* in honey samples: comparision with isolation and biochemical characterization. Applied and Environmental Microbiology, vol. 69, pages 1504-1510.**

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PCR

- **PCR primers**
- **AF primers**
- **Forward AF6f 5’- GCA AGT CGA GCG GAC CTT GT -3”**
- **Reverse AF7r 5’- GCA TCG TCG CCT TGG TAA GC -3’**
- **PCR product size: 237 bp**

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PCR

- **Thermal profile programme**
- **Stage 1: 95°C for 5 min**
- **Stage 2: 94°C for 20s, 50°C for 20s, 72°C for 1 min (40 cycles)**
- **Stage 3: 72°C for 7 min**

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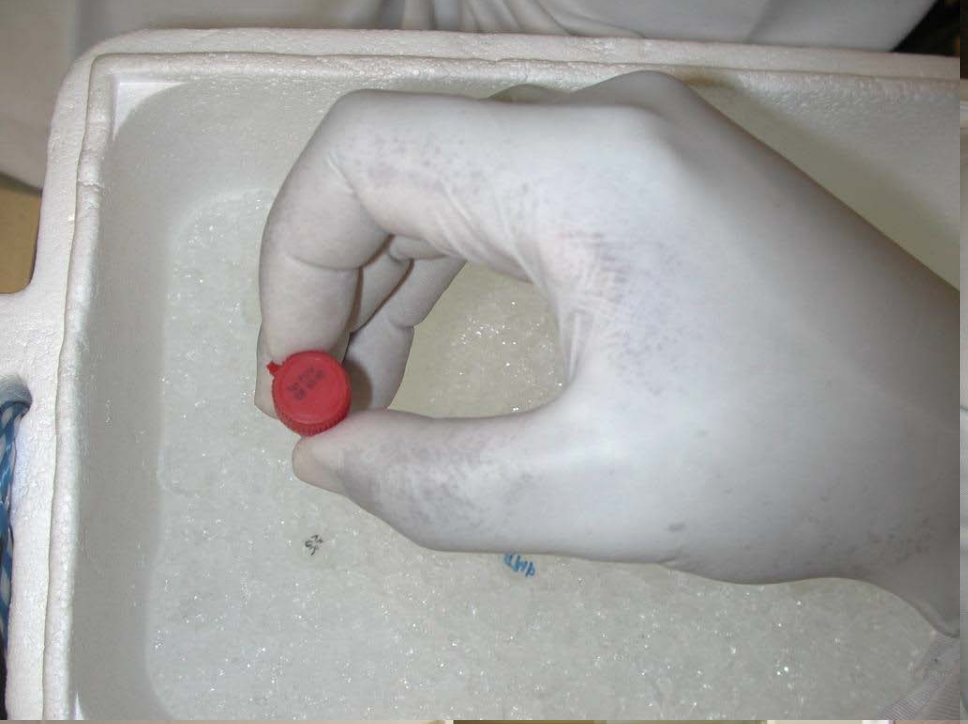


Wear
Disposable
Gloves


Wear
Disposable
Gloves

Electrophoresis and photograph

- **PCR products are loaded into the 1.5% agarose gel with ethidium bromide**
- **Electrophoresis is run for ~ 1h at 100V (in 1x TAE buffer)**
- **Gels are photographed under UV light**
- **Photographs are saved as jpg files and also printed**

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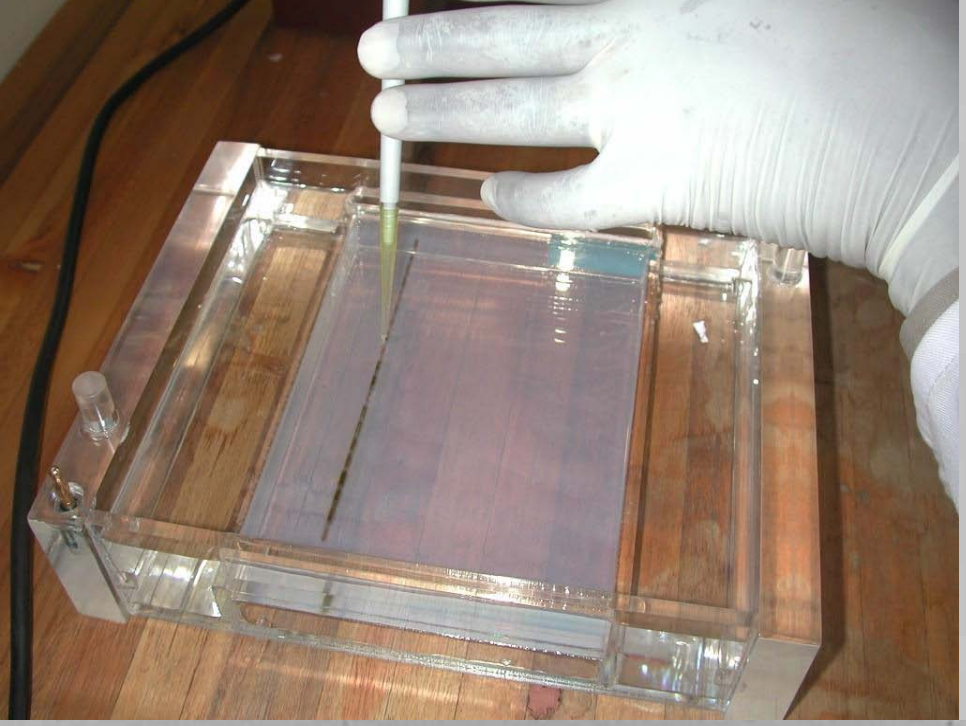

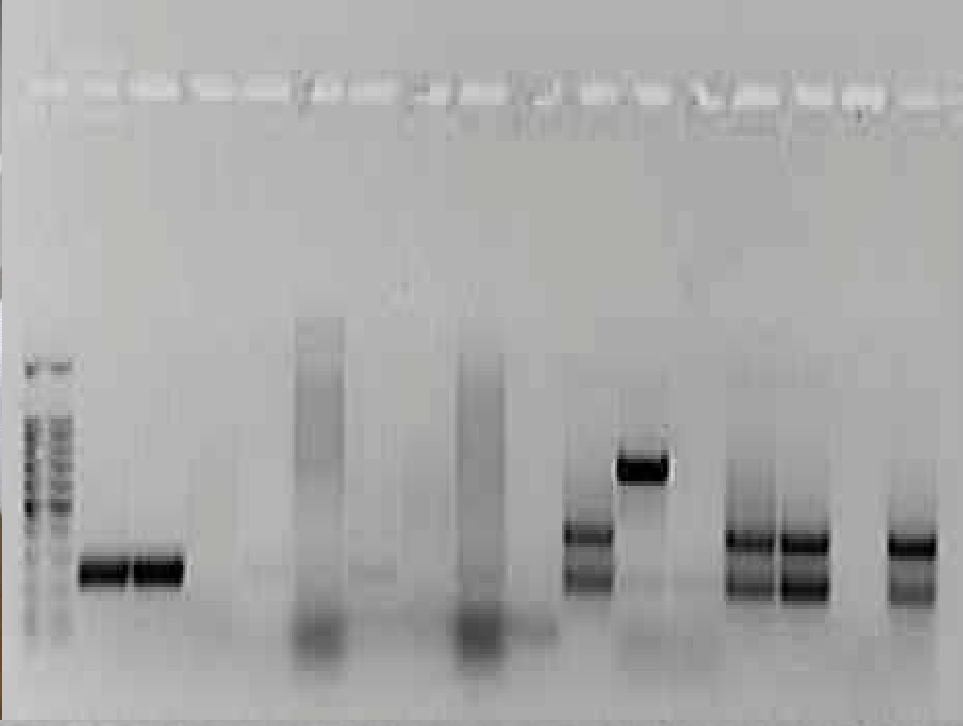

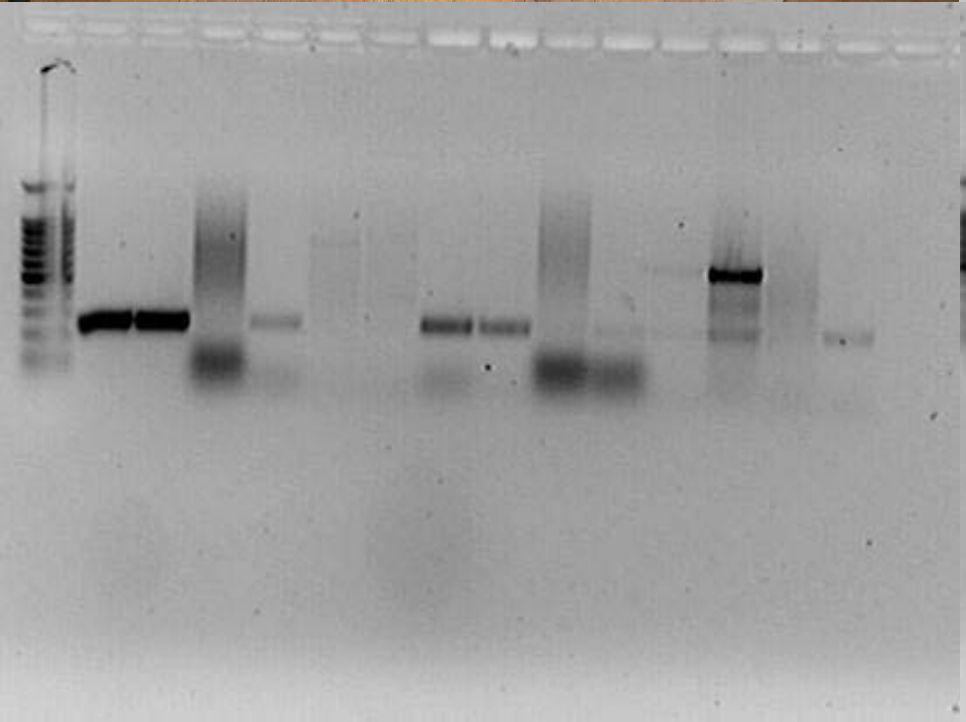

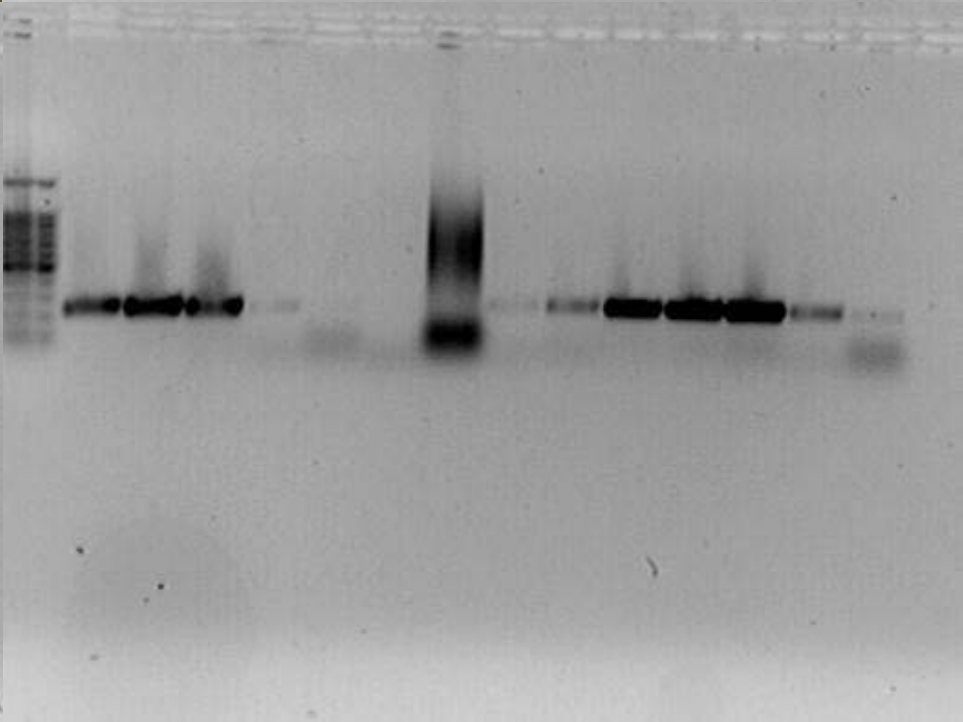

NUMBERS

- **Total NO of samples delivered**

  2032

- **NO of samples rejected**

  162

- **NO of samples tested in the first year**

1750

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08/12
08/12
9/12
08/12
9/12
9/12
9/12
9/12
9/12
10/12
10/12
10/12

PROBLEMS

Samples assessed in the field as AFB positive (clinical symptoms) came negative in testing

False positive results

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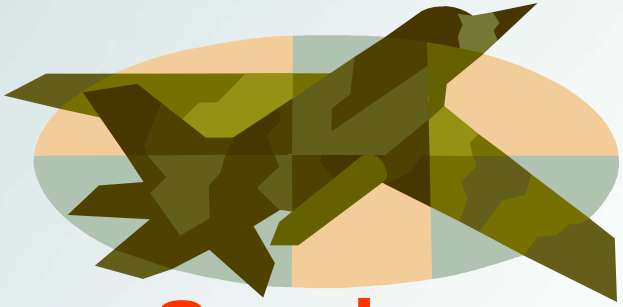
VERIFICATION

Samples in question were sent to 3 laboratories for verification of results



Sweden

Germany

Argentina

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VERIFICATION

Samples assessed as AFB positive in the field and negative in the lab testing were usually EFB positive

False positives – re-isolation and sequencing of the 16S rRNA gene fragment

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FUTURE

REGISTRATION OF THE ARC-PPRI LABORATORY WITH THE

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