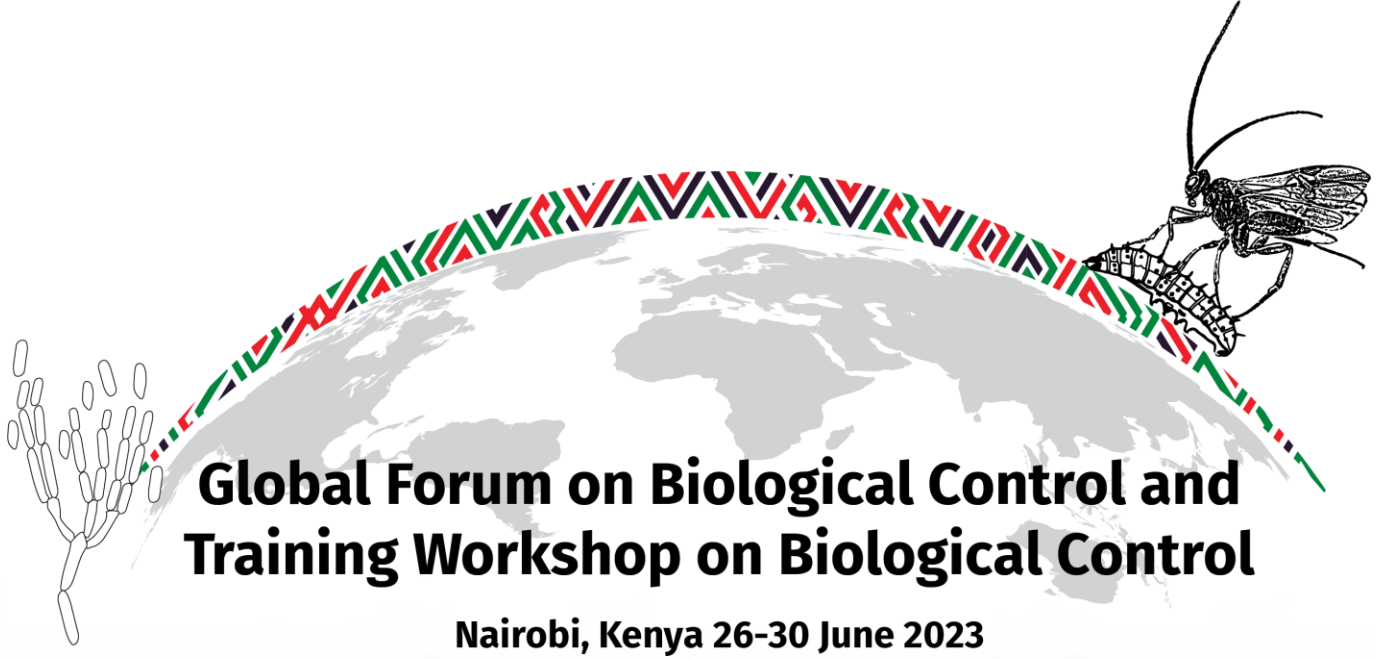

Global Forum on Biological Control and Training Workshop on Biological Control

Nairobi, Kenya 26-30 June 2023

Bioprospecting and characterization of microbials for pest management

Jane Kimemia

Arthropod Pathology Unit

Co-organized by

Food and Agriculture Organization of the United Nations

icipe

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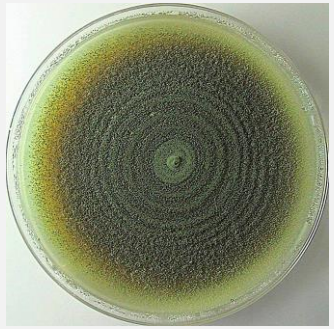
Diversity

Fungi

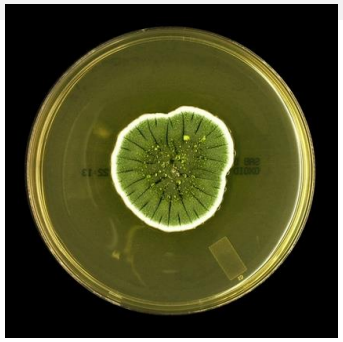


Metarhizium spp.

Beauveria spp.

Entomopathogenic fungi

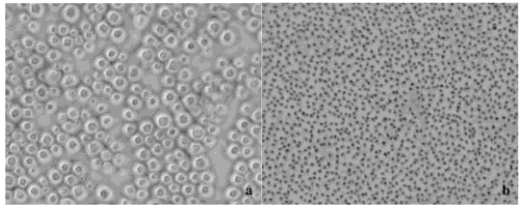
Trichoderma spp.



Penicillium spp.

Endophytic fungi

Viruses



Baculoviruses

Bacteria

Bacillus thuringiensis &

B. thuringiensis israelensis

Nematodes

Steinernema spp. & *Heterorhabditis* spp.

Collection of entomopathogens in icipe – Arthropod Pathology Unit germplasm

Type	Group	Number	Genera/species represented
Entomopathogens	Fungi	311	*Beauveria, Metarhizium, Verticilium, Isaria* etc
	Bacteria	157	*Bacillus thuringiensis, Serratia marcescens* etc
	Microsporidia	3	*Nosema, Melamoeba, Johenrea locustae*
	Nematodes	2	*Heterorhabditis, Steinernema*
	Viruses	2	Spodoptera littoralis NPV, Spodoptera exigua NPV
Endophytes			
	Fungi	10	*Hypocrea, Trichoderma, Clonostachys, Bionecteria*

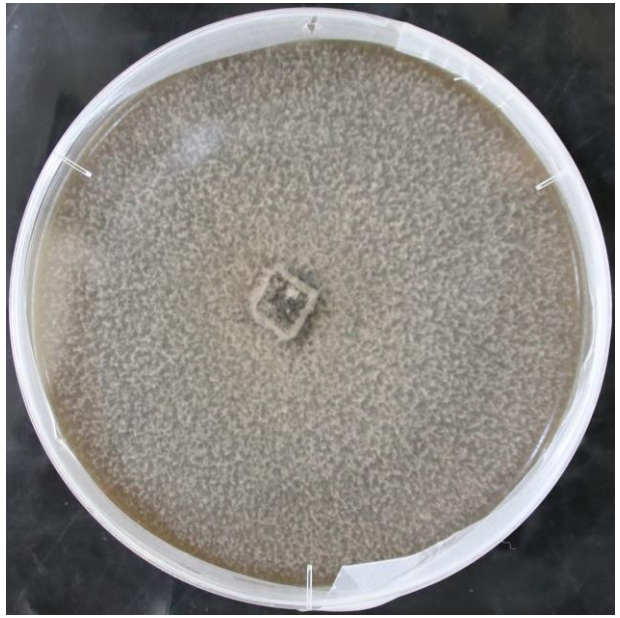
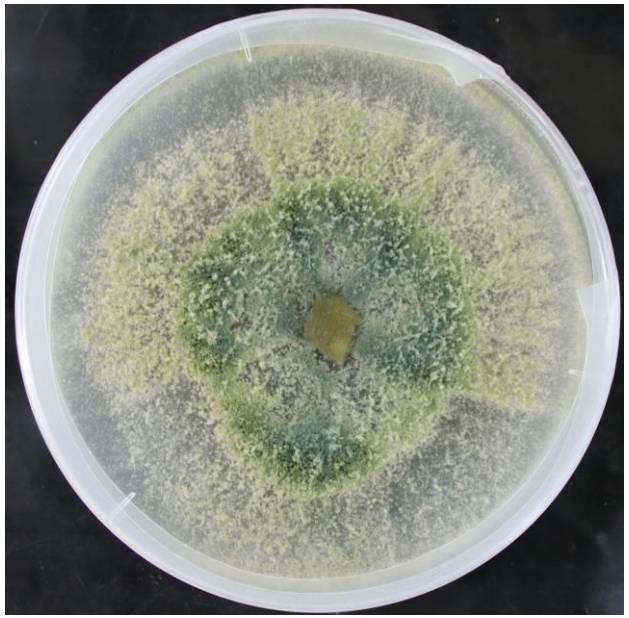
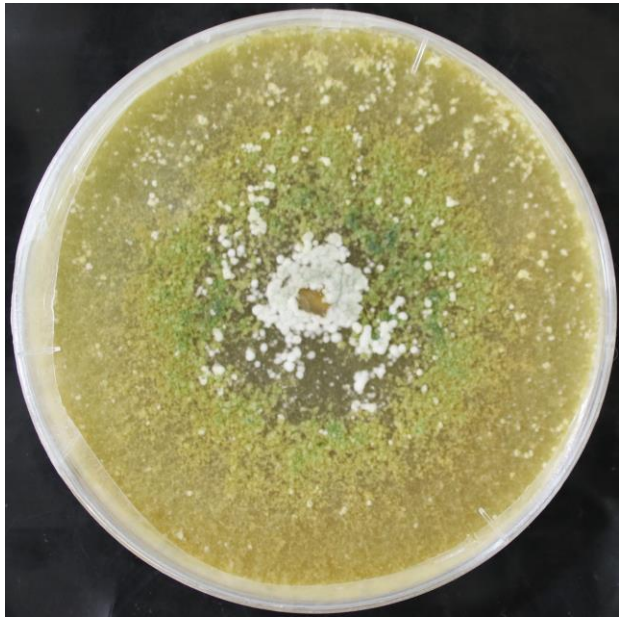
Characteristics of entomopathogens and their isolation methods

Type of pathogens	Mode of infection	Distribution	Types of isolation methods
Entomopathogenic fungi	Infection through surface integument	Ubiquitous	Dead insects, environmental sampling with selective medium, Baiting with wax moths
Entomopathogenic Bacteria	Oral infection	Ubiquitous	Dead insects, environmental sampling with selective medium
Endophytic fungi	Systemic induction	Mostly in different plant tissues	Isolation from roots, stem, leaves, seeds and fruits with selective medium
Entomopathogenic viruses	Oral infection	Mostly obligate with the host	Isolation from dead insects
Entomopathogenic nematodes	Infection through surface integument	Ubiquitous	Isolation from dead insects and baiting with wax moths

Plants collection and isolation of endophytes

- Collect healthy plants.
- Wash the plant samples to remove any external contaminants.
- Surface sterilization using 1.5% sodium hypochlorite and rinsed with sterile distilled water.
- Under sterile conditions, dissect the plant samples into smaller segments e.g., leaves, stem or roots.
- Place the dissected plant segment onto PDA agar plates supplemented with appropriate antibiotics to suppress bacterial growth.
- Seal and incubate the agar plates in an incubator at 25 ± 2°C for 2-7 days and monitor them regularly for fungal growth.
- In case of the fungal growth, transfer a small piece of the fungal colony onto fresh PDA agar plates, which is repeated until pure cultures are obtained.

Isolated endophytes

Soil sampling and insect cadaver collection for isolation

Cultivated fields, forests, unploughed fields, grazing fields, homesteads etc.

Soil sampling

Diseased or Dead insects

The procedure of soil sampling

Divide the sampling field into four equal units.

Remove the surface litter at the sampling spot.

Drive your spade /panga to a depth of 15cm and draw the soil sample.

Place the soil sample in a clean sampling bag (a Khaki bag).

Then label the bag well including location, date, and GPS coordinates then place it in a cooler box for transportation to the laboratory.

Procedure of isolation using selective media method

The soil is sieved, and 10 grams of the sample is weighed and added into a universal bottle containing sterile triton water.

Vortexed for 3-5 minutes to obtain a homogenous suspension.

0.1 mL of the suspension is spread onto SDA/PDA agar plate and using a sterile glass spreader

Agar plate is sealed using a parafilm and placed in an incubator at 25 ± 2°C, for 7-10 days and monitored for any growth.

If different colonies are observed each colony is sub-cultured onto a separate agar plate until a pure culture plate is obtained.

Wax moth *Galleria mellonella* larvae

Soil inoculated with *Galleria mellonella* larvae

Mycosed *Galleria mellonella* larvae

Baiting technique method

Galleria mellonella larvae are used because they are most susceptible to entomopathogens.

The soil is sieved and placed into sterile petri dishes and moistened by spraying sterile water.

Five to ten 2nd or 3rd instar *Galleria* larvae are placed over the soil in the petri dish, then sealed.

The petri dishes are kept in the dark for 7-10 days and monitored for cadavers after every 3 days.

The cadaver is rinsed 3 times with distilled water to remove any soil which might have attached to it.

Cadaver is then placed in Petri dish lined with moistened filter paper, sealed and kept to in dark to produce fungal conidia which are then harvested

Procedure for pin isolation of fungi from the cadaver

- These are required for pin isolation
  - ✓ Dissecting microscope, insect cadaver, flame
  - ✓ A pair of soft forceps, a pin or needle, 70% ethanol, SDA or PDA agar plates
- Under a dissecting microscope with sterile forceps hold your cadaver and extract the spores with a sterile pin
- Place the fungal spores on a sterile SDA/PDA agar plate. Repeat this five times and place the spores at different points on the agar plate.
- Seal the agar plate and place them in an incubator in complete darkness for 7-14 days.

Procedure for pin isolation of entomopathogenic fungi from infected cadavers

إجراء عزل الدبوس للفطريات الممرضة للحشرات من الجثث المصابة

Arthropod Pathology Unit

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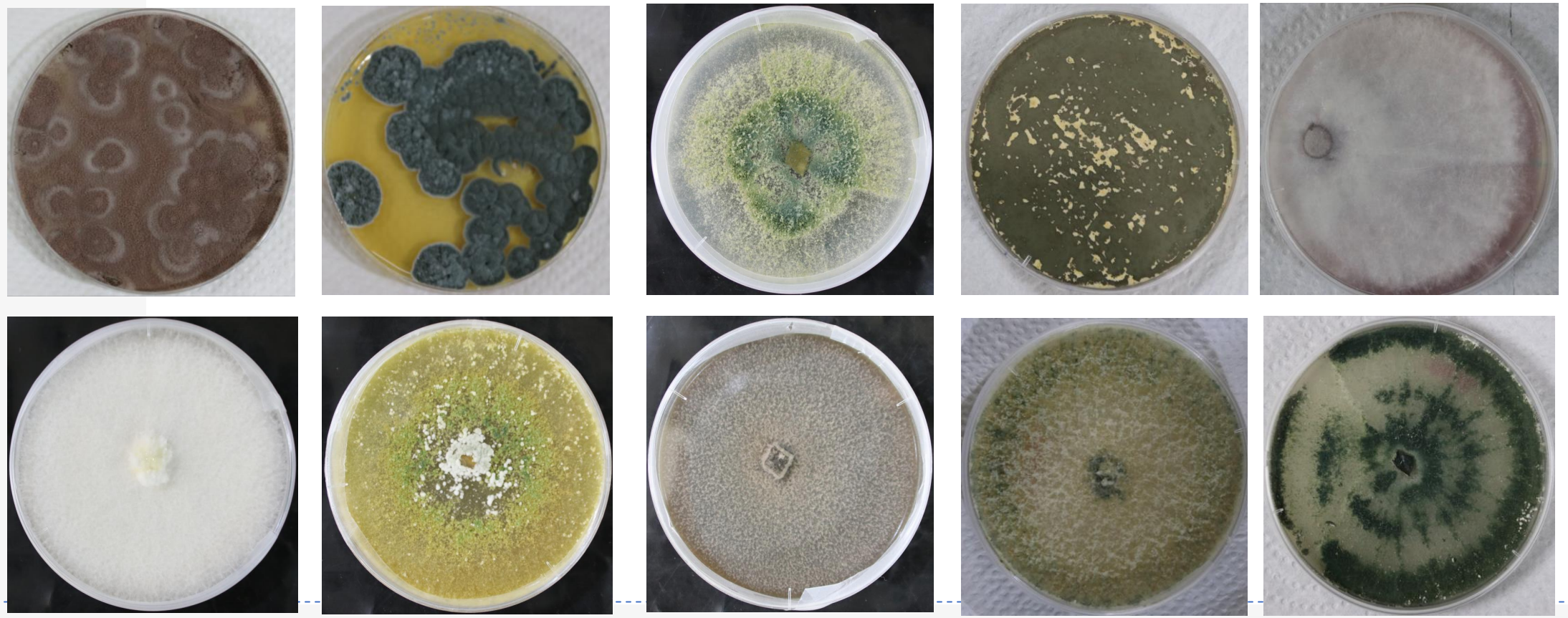
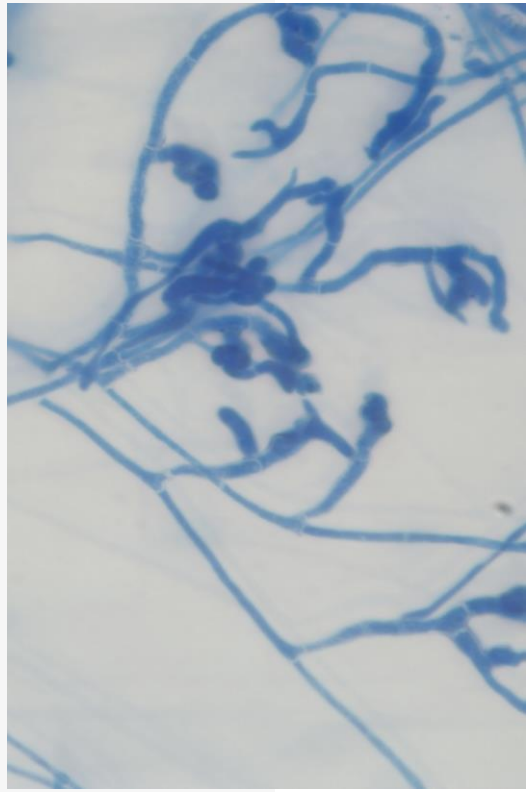
Morphological Identification

- Features used include growth patterns, colour of growing pathogen, spores shape, mycelial structures etc.

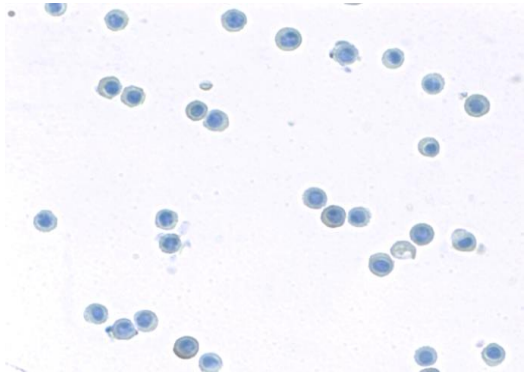


Morphological Identification (… *cont'd*)

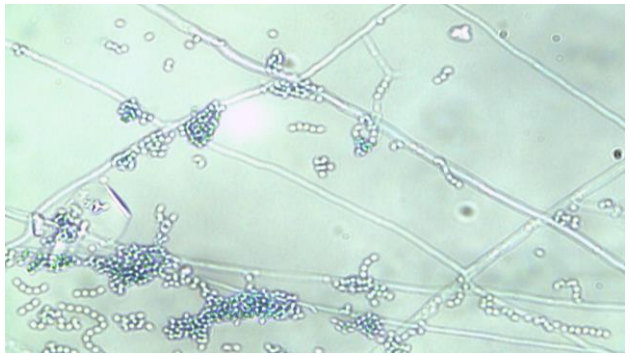
- The structures and shape of the spores are determined under a compound microscope



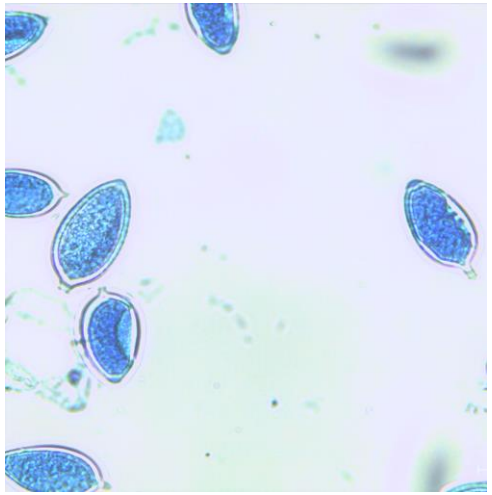
Hyphae network and sporangia



Globose-shaped spores (*Peyronellaea pomorum*)

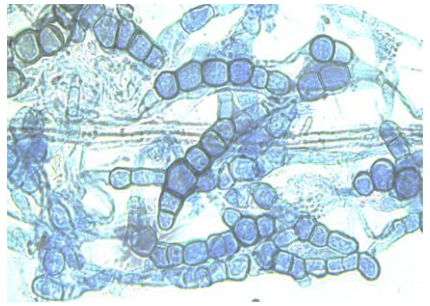


Oval-shaped spores (*Isaria* spp.)



Phytophthora infestans lemon-shaped asexual spores (sporangia)

Fussatium equiseti macroconidia (septate, sickle-shaped, and thick curvature)



Fussatium equiseti microconidia

Preparation of fungal slide cultures for morphological examination

تحضير المزارع الفطرية على الشرائح للفحص المورفولوجي

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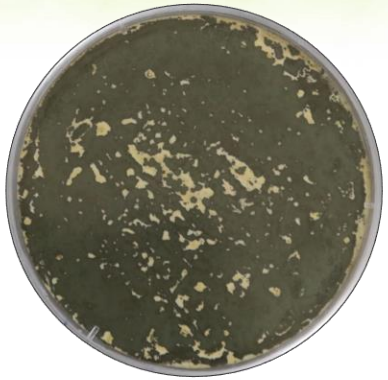
Morphological Identification (… *cont'd*)

- Bacillus thuringiensis strains growth features/growth patterns
- The colonies are rough, irregular, and white to off-white in colour, after overnight incubation at 37 °C.
- Gram staining is done to differentiate from Gram-negative organisms.
- Gram staining is not suitable to distinguish *B. thuringiensis* from *B. cereus*, hence the colonies are streaked on MYP (Mannitol egg York polymyxin) agar base medium and incubated at 30 °C for 24 h.
- *B. cereus* colonies are pink colour with yellow hallow
- *B. thuringiensis* colonies show no change in colour

Molecular identification

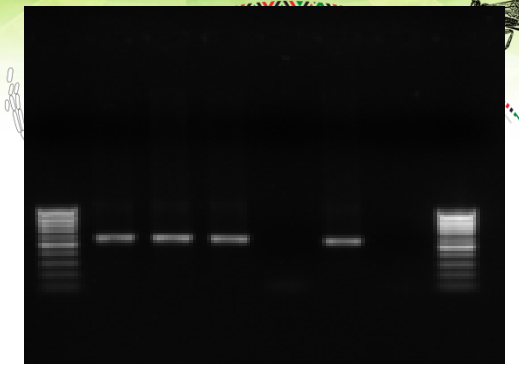


Pure culture of fungus

DNA extraction

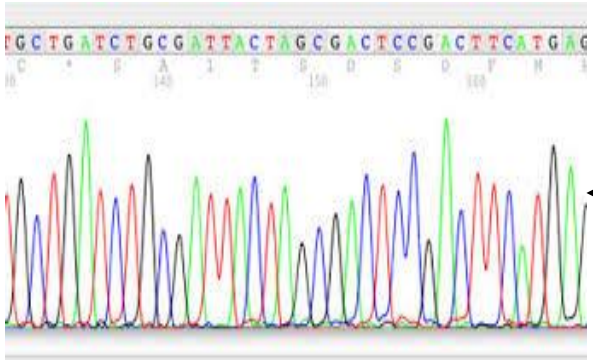
DNA quality checks

PCR amplifications and optimizations



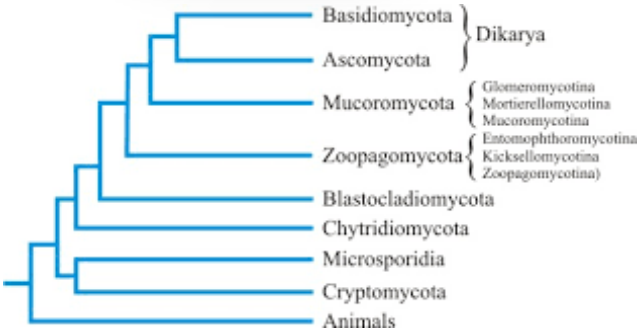
Gel electrophoresis (400 – 650bp)

Sequencing (Macrogen Inc)



Electrochromatogram

Sequence assembly, analysis, BLAST, Phylogenetic evolutionary analyses



Identity of the organism

Sample curation

Acknowledgements

- Dr. Subramanian Sevgan, Dr. Akutse Komivi Senyo, Dr. Fathiya Khamis, Dr. Saliou Niassy and Dr. Samira Mohamed.
- Technical team at Arthropod Pathology Unit – *icipe*.
- All of you for listening.

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