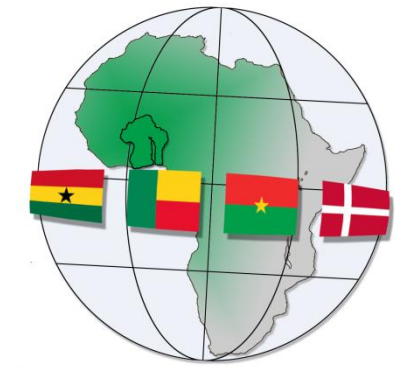

REPORT:

BCCM TRAINING ON PRESERVATION OF MICROORGANISMS

18-22 September 2017, Belgium

By: Clarisse COMPAORE and Margaret OWUSU

Introduction

The overall objective of the DANIDA Green Growth project is to enable the West Africans to preserve and fully utilize their natural microbial resources, and to up-grade the food sector in an environmentally friendly way assuring the quality, safety and marketability of food products. The different African countries partners of the project were provided with a -80°C freezer and one activity of the WP2 was the training of their staff members in management of culture collections. Therefore one scientist responsible of the management of culture collections in each country was registered for the BCCM training on preservation of microorganisms which took place from 18 to 22 September 2017 in Belgium. The participants were: Dr Margaret OWUSU from FRI Ghana, Dr Clarisse COMPAORE from DTA, Burkina Faso and Dr Yann MADODE from UAC, Bénin. This report summarizes the course of the training session in Belgium.

1. Report on activities of Monday, 18th September, 2017

Monday 18th September was the first day of the training programme. The venue for the day's programme was at the office of the Belgian Science Policy, Belspo in Brussels.

Participants

Participants for the course were about eighteen in number and came from different countries and institutions mostly in Europe, apart from the three of us on the Green Growth project from Africa (Ghana, Burkina Faso and Benin). There were also two people (a couple) from Ecuador. Participants were either taking a major in bacteria and a minor in fungi preservation or vice versa.

Presentation 1: Opening and Introductory Presentation on BCCM

The program started with a self-introduction of participants and presenters. Participants were then introduced to the consortium of laboratories/institutions that form the Belgian Culture Collection of Microorganisms (BCCM) (Fig 1), their operations and an over-view of laws that regulate their modus operandi.

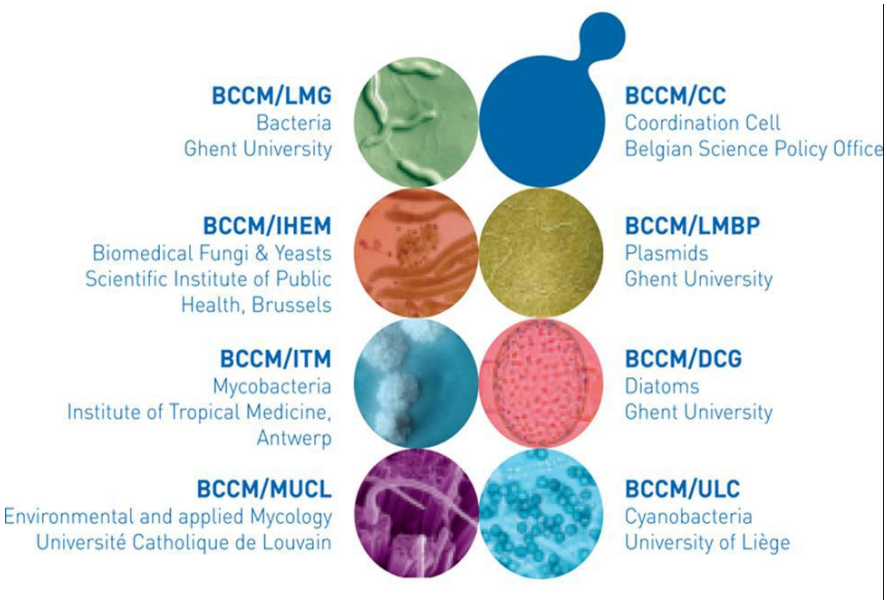

Fig.1: The institutions that form BCCM and their expertise

Presentation 2: Information Management

The presenter for this session was Zayid Benyahia, BCCM Information Technology Cell Manager. He took participants through the structure and data management of the Laboratory Information Management System (LIMS) that BCCM uses. His presentation also dealt with the objectives of the LIMS, its relevance and responsibilities of an information technology expert for a culture collection, among others. In his conclusion, he mentioned the importance of involving an IT expert in the management of a culture collection. He also mentioned the need to look for commercial LIMS software that is suitable for one's situation or a combination of a commercial and a self-made one to suit each situation.

Presentation 3: Quality Management

Participants were next given a presentation on quality systems by the Quality Manager of the BCCM Coordination Cell at Belspo, Dr. V. Van de Perre. His presentation dealt with:

- Why implement Quality Management System (QMS)
- Different standards
- ISO 9001:2015
- ISO 20387
- How to implement QMS

He stressed the importance of implementing QMS in institutions/laboratories to improve

- organizational effectiveness
- customer satisfaction

- compliance
- organisational culture
- documentation

The Coordination Cell at Belspo runs a QMS under ISO 9001:2015.

Presentation 4: Regulations

This section was presented by Philippe Desmeth, the legal expert of the BCCM Coordination Cell. The presentation centred on laws and treaties that regulate the operations of a culture collection. He stressed the importance of incorporating legal obligations into the daily activities of a culture collection.

According to the presenter, laws and regulations that one needs to consider in running a culture collection include among others, the Budapest Treaty, Nagoya Protocol, Intellectual Property Rights, Patent and biosafety.

Aside the laws, a number of international agreements have been ratified which also needs consideration. The following agreements/organizations may be relevant as they deal with different aspects of the operations of a culture collection:

- Convention on Biological Diversity
- World Health Organization
- World Trade Organization
- The Biological and Toxin Weapons Convention
- United Nations Office for Disarmament Affairs

Presentation 5: Cost Calculation

Participants were next taken through the principles of cost calculation for cultures requested by clients. This aspect of the course was presented by Virginie Storms, the Manager/Curator of BCCM Coordination Cell.

Through her presentation, it became evident that four cost centres have to be considered in determining the cost of a preserved culture:

- Personnel
- Equipment
- Consumables
- IT

She mentioned that although cost determination can be a daunting task, the use of an Excel spreadsheet makes it easier.

Social event

The evening of Monday, 18th September 2017 was reserved for social activities. The first event was a trip to the Chocolate Museum, Brussels. The Guide took participants through the history of cocoa down to cocoa processing and chocolate production. He demonstrated how different chocolate products such as fondants are made. Participants had the pleasant opportunity of tasting chocolate prepared with cocoa beans of different origins.

Outside the Museum, the Guide explained in details the long history of different buildings and avenues in the area.

The day ended late with a three-course dinner at a restaurant.

Fig.2: Participants and facilitators at the BCCM course on preservation of microorganisms at Belspo.

2. Reports on activities from Tuesday 19 to Thursday 21 September 2017: Major (Myco-) bacteria:

The venue for this three days training programme was at the Institute of Tropical Medicine (ITM) in Antwerp. The training consisted of several presentations, visit of laboratories and practical training on mycobacteria preservation. ITM team for this training session were Dr Leen Rigouts, Ms Maren Diels and Ms Amalya Avakymian.

Presentation 1: Introduction to mycobacteria and biosafety

This presentation was done by Dr Leen Rigouts. She gave us general information on mycobacteria with focus on *Mycobacterium tuberculosis* (diagnostic tests, treatment, drug resistance, molecular epidemiology…).

Presentation 2: Processes and related documentation as per quality management system.

Made by Dr Leen Rigouts, this presentation explained how to check quality of mycobacterium before and after freeze drying; the registration of quality control check; the checking of lyo process. She also presented General good laboratory practices, the validation of lyo process and how to efficacy heat killed mycobacterium.

Presentation 3: Freeze drying of bacterial cultures as long term preservation technique

During this presentation, Dr Leen Rigouts explained the principle of freeze-drying, the goal of freeze-drying, applications. She showed the physical state diagram of water, gave an overview of different types of freeze-dryers and the freeze-dryer components. She also explained the different steps of freeze-drying process. Dr Leen ended this communication by presenting the freeze-drying procedure at BCCM/ITM, the quality control done after freeze-drying, the spark coil leak test and determination of residual moisture content. It was clear that the main storage process at ITM is freeze-drying using a sucrose based protectants. 23 ampules are prepared for each strain, 20 are use as standard stock, 2 for quality control and 1 for security stock. Ampules are store at ambient temperature (about 20°C) for at least 20 years. News ampules are prepared when two is left in the stock.

Presentation 4: Cryopreservation of bacteria: theory and applications

The content of this presentation was: terminology of cryopreservation, freezing of water and solutions, influence of freezing rate on cells, cryopreservation, cryoprotectants, revival of cryopreserved strains and protocols of cryopreservation at MG and ITM.

During these three days training, we were able to practice a little freeze-drying and cryopreservation of *Mycobacterium tuberculosis*. We also visited ITM mycoBiobank and learnt how to ship dangerous goods. However, we were not allowed to take pictures in the laboratory because of confidentiality. In addition, we could not handle a lot the different preservation methods because the mycobacteriology Unit is a class III laboratory with high risk of contamination.

Fig. 3: Photo with the team of Mycobacteriology Unit at ITM

3. Reports on activities of Friday 22 September 2017: Minor fungi

Friday 22nd September was the last day of the training program and consisted on Minor fungi. The venue for this training was the Institute of Hygiene and Epidemiology-Mycology (IHEM), Scientific Institute of Public Health, Section Mycology and Aerobioloy in Brussels.

Several presentations were done by the staff of IHEM and also by Sylvie Cranenbrouck from MULC of the Catholic University of Louvain.

Presentation 1: BCCM Training on fungi preservation

This communication was given by Sylvie Cranenbrouck. Her presentation dealed with the history of the BCCM consortium to set a training course on fungal preservation. She presented the program of the day and the team of MUCL and IHEM. She finally explained the different preservation methods with the advantages and inconvenient of each method.

Presentation 2: Mycology laboratory guidelines and recommendations

Derick Stubbe presented this session and showed the different material necessary to manipulate cultures with the advantage of each material. He indicated the optimal conditions for culturing and incubating cultures of fungi. He also explained how to avoid contamination during manipulation and the importance of regular control of surfaces. He ended his

presentation by recommendation for an efficient cleaning of surfaces and how to verify the efficiency of the cleaning.

Presentation 3: Cryopreservation in straw

During this second communication Sylvie Cranenbrouck explained cryopreservation in straw methodology and indicated that the agar slant culture is indicated for filamentous fungi (preferably sporulating) and yeasts while petri dish is suitable for non sporulating fungi culture. She mentioned that the cryoprotectant used in this method is glycerol at the concentration of 10%. She indicated that cells stored in straw can be kept for at least 20 years, but a new batch must be prepared each year. She also indicated that for fungi preservation, it is important to cool cells very slowly and to use water-bath (35-40°C) for thawing). Ms Sylvie finished her presentation by showing how to revivify a microorganism from a preserved straw.

Presentation 4: Freeze-drying of moulds and yeasts

Presented by Derick, he explained the general principle of the freeze-drying process; presented the different types of freeze-dryer, the preparation of samples for freeze-drying and how to seal ampoules after the freeze-drying. He also gave informations regarding the batch management (storage, quality control, back up) and ended with the revival of the lyophilisate.

Presentation 5: Cryobeads: a user-friendly alternative?

This communication was presented by Pierre Becker from IHEM. After an introduction, he presented the advantages of cryobeads and described the composition of each bead. He detailed the using of cryobead and mentioned that the cryobead method of preservation does not work very well with non sporulating fungi. He also talked about the stock management and how to start a new culture from a cryobead. He finished his presentation by presenting many studies which confirmed the efficiency of cryobeads.

Presentation 6: Preservation under water and oil

This presentation was done by Ms Sylvie Cranenbrouck who explained the preservation under water and mineral oil.

Presentation 7: Quality controls in biomedical fungal collection

During this session, Dirk Stubbe mentioned the importance of quality control for preserved batches. He also indicates that the mains procedures for quality control are to check microorganisms for Viability-Identity and Purity (VIP) and explained the different steps.

Mr Pierre Becker ended this session by explaining the Maldi-Tof-MS method used to check species identity.

The last day training ended around 5 pm.

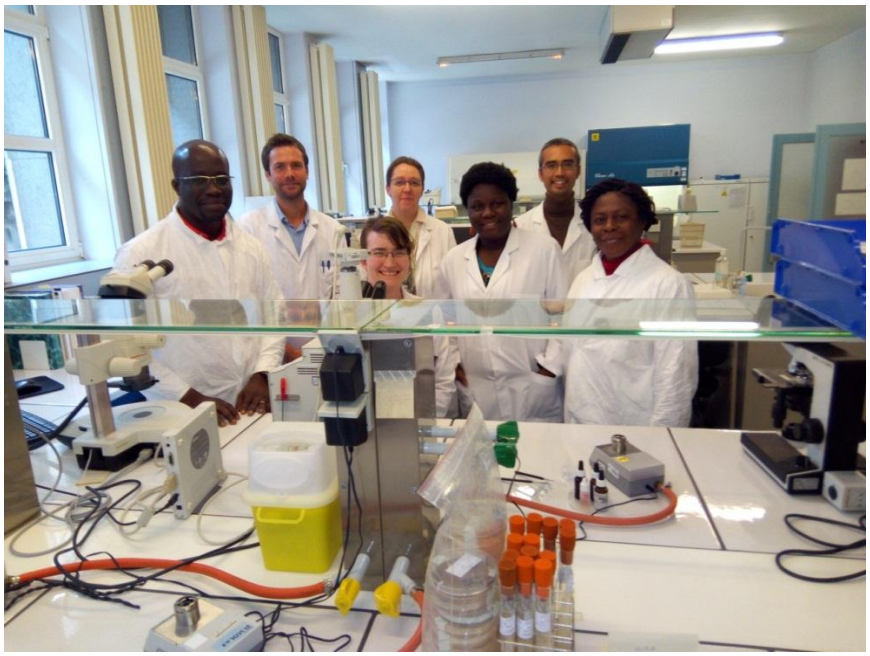

Fig. 4: Photo with the minor fungi trainer

Conclusion

The training at BCCM went very well and allowed us to reinforce our skills in preservation of microorganisms. We would like to thank DANIDA Green Growth project for funding our trip and supporting the training fees. Our thanks go also to Pr Lene JESPERSEN and Pernille JOHANSEN for helping in the preparation of the trip and visit in Belgium.