L'ASSOCIATION TUNISIENNE DE BIOTECHNOLOGIE
THE TUNISIAN ASSOCIATION OF BIOTECHNOLOGY

ORGANIZES

IDB2019

THE 18TH INTERNATIONAL DAYS OF BIOTECHNOLOGY

December 18th - 21st 2019
Hotel Marhaba Palace El Kantaoui *****
SOUSSE, TUNISIA

TOPICS

INDUSTRIAL BIOTECHNOLOGY | ENVIRONMENTAL BIOTECHNOLOGY. | PLANT BIOTECHNOLOGY | HEALTH BIOTECHNOLOGY | BIOACTIVE MOLECULES AND APPLICATIONS

DEADLINES

- ABSTRACT SUBMISSION:
  - ORAL COMMUNICATIONS: OCTOBER 15th, 2019
  - POSTERS: OCTOBER 22nd, 2019
- REGISTRATION / CONFIRMATION:
  - OCTOBER 25th- NOVEMBER 15th, 2019

ONLINE ABSTRACT SUBMISSION AND REGISTRATION: www.atbiotech.org

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IDB 2019 – IPM-4-CITRUS
Programme & Meeting

The IDB 2019 full scientific programme is attached

Dec. 18th

10h30	**Departure from Tunis to Sousse for arrival at Palace el Kantaoui**
12h30	**Arrival & Registration to IDB 2019**

Dec. 19th

10h50-12h25	**Room 2: Industrial Biotechnology (IPM-4-Citrus) Oral communications**	
10h50-11h10	Sara FALVO	Visio conference: Exploitation of scientific results in the biotech field: the crucial role of intellectual property rights
11h10-11h25	Jihane SAAD	Mass balance and cells, spores and -endotoxine distributions during Bacillus thuringiensis kurstaki bioproduction with wheat bran based medium
11h25-11h40	Nouha ABDELMALEK	Monitoring of Bacillus thuringiensis kurstaki (Btk) growth, sporulation and -endotoxin production: insight of on-line alternative measurements with semi-synthetic and industrial media
11h40-11h55	Tayssir HAMMAR	Development of Biopesticide Formulations for New Strains of Bacillus thuringiensis Spores and Crystals to control citrus pests
11h55-12h10	WP3:	WP3: biocontrol activity / Exchange about biopesticides efficiency and innocuity (aroud the 3 IPM-4-Citrus posters)
Posters & informal talks 12h10 – 12h25	Hazar KRAIEM	Environmental toxicity assessment of NEW Bacillus thuringiensis kurstaki - based biopests on non-target organisms using ISO Standards and OECD Guidelines
	Zakaria BENLASFAR	, WP3 (poster ou oral) / Strategy for biosafety assessment of new strain-based biopests using laboratory animal toxicity models.
	Jerome CUBERO	Project IPM-4-Citrus at a glance
17h – 18h	**IPM-4-Health new project presentation & round table**	

Dec. 20th

17h – 18h30	**IPM-4-CITRUS update & meeting**

Dec. 21th

10h – 12h00	**Visit of the Plant Oil Mill & Factory « Huilerie KHALFALLAH" in Akouda, Sousse**
12h30 – 14h00	**Networking & Business Lunch**
14h00	**Departure from Hôtel Marhaba, Sousse to Tunis**

Exploitation of scientific results in the biotech field: the crucial role of intellectual property rights.

Dr. Sara Falvo, Technology Transfer & Business Development Coordinator, BioIndustry Park Silvano Fumero, Colleretto Giacosa (TO), Italy

Background and aim:
Advancements in the biotech sector represents a promise able to meet strong and unaddressed medical, social and environment needs. The biotech industry has the potential to develop new products and processes that may help to address some of the world's most pressing challenges. Consequently, it is fundamental to raise awareness about the proper management of the knowledge derived from R&D programs to correctly guide technological development, engage the right players, funders and stakeholders to ensure technology adoption and the consequent impact on our society.

The objective of this speech is to describe the innovation process, with a focus on the biotech sector and highlight the importance of the technology transfer process for the exploitation of scientific results.
The speech will focus on intellectual property management (IPRs) as an essential tool to manage innovation:

- The patent: essential tool to exploit a biotech solution
- The patent requirements
- Patentability and Prior Art analysis
- Freedom to operate
- Licensing opportunities

Basic concepts and tools will be mentioned to properly manage IPRs and thus foster the exploitation of scientific results making them interesting from the industrial point of view and contribute to the progress of our society.

Mass balance and cells, spores and δ-endotoxine distributions during *Bacillus thuringiensis kurstaki* bioproduction with wheat bran based medium

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Background and aim: IPM-4-CITRUS project, aim to produce *Bt kurstaki* based biopesticides with optimized processing pathway and cost in order to make them affordable to labor for a wider application. The objective of our work is to characterize the mass balance (dry matter, chemical composition) and cells, spores and δ-endotoxin distributions between supernatant and pellet during *Bt kurstaki Lip* production to evaluate fermentescible fraction and to select an adapted downstream processing (DSP).

Methods: Physico-chemical (granulometry, dry matter, minerals, total-sugars, organic and mineral nitrogen, proteins) and microbial (cells, spores) analysis were conducted on culture medium ([WB] = 66gdm/L) without sterilization, after sterilization without and with cell culture. Btk cultivation was conducted in flask cultures (50mL, triplicate) at 30°C, 250rpm during 48h. Prior to the biological and biochemical analysis, several physical treatments (decantation, centrifugation, filtration and sonication) of broth were carried out to screen d-endotoxin separation methods and mass balance between supernatant and pellet. .

Results:
The granulometry and composition of medium before/after autoclaving and the bacterium needs for growth, sporulation and δ-endotoxin production are reported. WB fermentescible fraction consumed by Btk is reported and compared in the light of biochemical analysis. For DSP, the sonication had no significant effect on solid and soluble fractions. Cells, spores and endotoxin distributions are studied as a function of broth post-treatment. The high concentration of endotoxin was found in the supernatant whereas Btk cells and spores were equally distributed between supernatant and pellets.

Conclusion: This preliminary study at flask scale highlights the endotoxin production (potential versus fermentescible substrate, distributions in supernatant and pellet versus broth DSP). From these data, decantation followed by supernatant filtration will be considered for scale-up strategy. In the future, these data should be confirmed with *Bt kurstaki HD1 and BLB1*.

Keywords: *Bacillus thuringiensis kurstaki* Lip, wheat bran, chemical and biochemical composition, post-treatment and separation.

Monitoring of *Bacillus thuringiensis kurstaki* (Btk) growth, sporulation and δ-endotoxin production: insight of on-line alternative measurements with semi-synthetic and industrial media.

Nouha ABDELMALEK[1], Joanna ABBOUD[2], Mireille KALLASSY[2], Nadia BEN SAID[1], Souad ROUIS[3], Slim TOUNSI[3], César Arturo ACEVES-LARA[4], Luc FILLAUDEAU[4] and Julien CESCUT[5].

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Background and aim: Alternative Integrated Pest Management (IPM) approach based on biological control looks to answer to multiple needs. Production of Bt-biopesticide ready for its use on the field is an identified issue (project IPM-4-Citrus). This study reports mains performances. It aims to identify and characterize the critical steps to control and understand cell physiology and δ-endotoxins production of Bt kurstaki, using classical and alternative technics and sensors with two different media.

Methods: Three Bt kurstaki BLB1, HD1 and Lip strains were cultivated in a 3L bioreactor (Biostat B plus) using a semi-synthetic (SS) and an industrial Wheat Bran (WB) media. Batch cultures were realized under controlled conditions. pO2 was monitored and maintained at 25% pO2sat with a constant aeration rate and under regulated mixing rate. During 48h, cultures were monitored through conventional (pH, Temperature, pO2, RPM…) and alternative in-situ sensors (OD600nm, FBRM, Dielectric permittivity), and physico-chemical off-line analysis (cell dry matter, OD600nm, cell and spore countings, δ-endotoxin proteins, HPLC, microscopy and granulometry) by sampling.

Results: Comparison between conventional and alternative sensors with both media highlighted on the successive phases of Btk cultivation and sporulation. With SS medium, three phases of Btk cultivation based on in-situ physico-chemical measurements and biokinetics description were identified. pH regulation was found to be a key indicator to identify sporulation phase. With WB medium, the biochemical and granulometric complexity of suspension should be explored. However, some conventional (dry matter and off-line OD) and alternative (on-line OD and DP) methods were found ineffective and non-reproducible in monitoring Btk growth and sporulation. Nevertheless, FBR measurements may help monitoring Btk consumption of the WB starch in real time and therefore correlate with gas analysis. In addition, mixing rate regulation alongside gas analysis were found to be more determining in monitoring sporulation occurring in WB medium.

Conclusion: Conventional microbial and biochemical analysis were reported and compared with literature. The complementarity between on-line, off-line measurements and alternative sensors was used to detect and identify the sporulation phase. Future cultures will initiate fed-batch mode considering this critical time, in order to increase the biomass in oxidative mode and consequently δ-endotoxins production.

Mot clés : *Bacillus thuringiensis kurstaki*, HD1, BLB1, Lip, sporulation, δ-endotoxin, in-situ alternative metrology.

Development of Biopesticide Formulations for New Strains of *Bacillus thuringiensis* Spores and Crystals to control citrus pests

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Background and aim:

Worldwide, the market size for biopesticides to control agricultural pests is growing. Within the framework of the EU funded project "IPM-4-Citrus" new environmental friendly biopesticides based on *Bacillus thuringiensis* should be developed to control insect pests, especially in citrus.
The formulation is one of the most challenging processes of biopesticides development that should be controlled and optimized in order to obtain a stable, effective, affordable and easy-to-use product. Therefore, the aim of this work is to develop a new sustainable formula of two new biopesticides based on two strains of *Bacillus thuringiensis* active against citrus pest, with improved physical and biological properties.

Methods: After liquid fermentation in an industrial medium under optimized conditions, the fermented broth was decanted. The liquid was then filtrated with a mesh of 100 µm to separate media particles, which may affect the final product quality, and concentrated to harvest the maximum of active ingredients (Proteins and spores) to be used afterward for the formulations. Different suspensions were prepared containing the active ingredients and additives, which were dried using the fluid bed drying system (granulation process) in order to get a wettable powder.

Results: In order to get the most effective formulation for each strain, different formulations were developed using the fluid bed drying. The different parameters of the process were optimized to get the best physical and biological properties of the final product. The moisture content was decreased under higher temperature. In laboratory bioassays final formulations of both strains were effective against *Spodoptera frugiperda* The final formulation for both strains is more effective against *Spodoptera frugiperda* than the standard product, has a moisture content of 2%, and stable under UV light.

Conclusion: In summary, the purification step allows to obtain a maximum of yield. The formulation is cost effective. The formulation was also optimized based on physical and biological properties of the fluid bed dried wettable powder of *B. thuringiensis*.

Keywords: Biopesticides, spores, crystals, formulation, *Bacillus thuringiensis*

Environmental toxicity assessment of NEW *Bacillus thuringiensis kurstaki* - based biopests on non-target organisms using ISO Standards and OECD Guidelines

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Background and aim: *Bacillus thuringiensis* (Bt) was the first biopesticide widely used since 1957 against lepidopteron pests. Toxicity is an important aspect of environmental impact and risk assessment to be monitored before biopesticide commercialization. The impacts on human and environment have to be evaluated, using toxicity assays according to the European guidelines. The aim of our research is to assess the biosafety of two new formulated Bt-based biopests on non-target organisms (i.e. *Bt kurstaki* BLB1 and LIP strains).

Methods: The toxicity assays have been carried out using rapid, simple and cost effective set of tests consisting of (i) freshwater microalgae microbiotests *on Pseudokirchneriella subcapitata* (Algaltoxkit F), (ii) freshwater crustaceans Daphnia magna (*Daphtoxkit F magna*) and (iii) *Aliivibrio fischeri* acute toxicity assays (luminescent bacteria test). All the tests complying with the ISO Standard protocols and OECD Guidelines were recorded in comparison to the commercial product (*Bt kurstaki HD1*).

Results: As results, the biosafety of the commercial HD1-based biopest as well as the of BLB1 and LIP formulation ones were assessed according to the expected results (i.e. 50% growth inhibition of growth (EC50), concentration), in comparison manner. All the data will be presented in details.

Conclusion: According to the guidelines, the expected toxicity results on non-target organisms help for decision making regarding implementation and commercialization of the new Bt-based biopests.

Keywords: *Bacillus thuringiensis kurstaki*, HD1, BLB1, non-target organisms, toxkit microbiotest.

Founding: European project IPM-4-Citrus (H2020-RISE-MSCA, EU project n°734921)

STRATEGY FOR BIOSAFETY ASSESSMENT OF NEW STRAIN-BASED BIOPESTS USING LABORATORY ANIMAL TOXICITY MODELS

Zakaria BENLASFAR [1], Hazar KRAÏEM [1,2], Rim EL JENI [1], Gül AYYILDIZ [3], Zeynep YURTKURAN-CETEREZ [3] & Balkiss BOUHAOUALA-ZAHAR [1,4]

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Background and aim: Even *Bacillus thuringiensis* (Bt) Kurstaki group, is generally recognized to be safe for environmental, human and animals, new formulations of biopesticides, have to be tested using toxicity assays for assessing the biosafety, according to European, American and international regulations. Nowadays, advantages of alternative methods are well recognized. However, until now most regulations believe that in vitro complementary methods could not be considered as equivalent to animal testing incites for evaluating the toxicity. For these reasons, the human risk assessment of the newly formulated biopesticides must be carried out on laboratory animals.

The aim of our research is the modeling of a comparative strategy for assessing the biosafety of two new Bt-based biopesticides, belonging to Bt *Kurstaki* formulations: Bt *Kurstaki* BLB1 and LIP from Tunisia and Lebanon, respectively.

Methods:

Our strategy to establish the pipeline of the most relevant toxicity tests is based on carefully considering their (i) relevance to the product usages, (ii) relative facility of achievement and (iii) especially the 3Rs principles respect "humane" animal experimentations (OECD Chemical Safety and animal welfare). In this respect, several international regulations as well as standards (CE, HSE, ISO, EPA, OECD) was examined and procedures of OECD guidelines recommended by ECHA (European Chemical Agency) have been checked. The final pipeline was set up according to the three main representative routes of possible impregnations and the most exposed tissues and organs.

Results:

Based on our strategy, with regards to the OECD guidelines and previous reported studies, three main toxicity assays were retained for assessing risks from digestive, eyes and skin contacts (i.e. Acute Oral toxicity, Eye and Dermal Irritation/Corrosion assays, respectively). In addition, the skin sensitization test is required in sub-chronic manner. The standards of protocols and an algorithm to support in decision-making were established. The potent toxic effects of BLB1 and LIP Bt-based formulations is compared to the equivalent "reference product" (*Bt Kurstaki* - HD1). The procedures and data analysis will be shown in details.
Conclusion: According to our pipeline, these minimum required methods assist in assessing the biosafety on the new biopests and human risk.

Keywords: *Bacillus Thuringiensis*, toxicity assessment, Laboratory animals, welfare.
Funding: European project IPM-4-Citrus (H2020-RISE-MSCA, EU project n°734921)