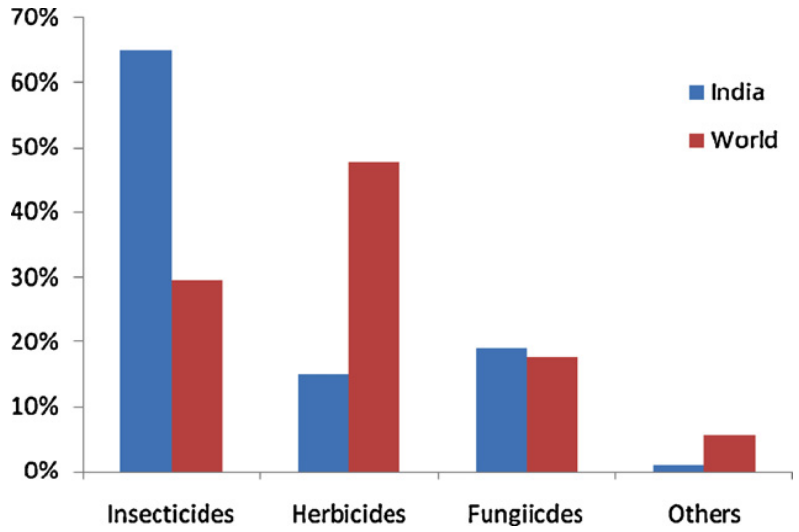
Entomopathogenic Fungi

Introduction

- Over the past decade the agricultural map of world has changed vigorously.
- Herewith, the use of pesticides is also increasing. Use of pesticide has increased 50 folds since 1950 leading to environmental problems like soil and ground water contamination with the pesticide residues
- Random uses of insecticides cause the insects to acquire resistance against the insecticides



Introduction

- Insect pest menace is a major factor that destabilizes crop productivity in agricultural ecosystems.
- Development of chemical pesticides – usage by farmers
- Disadvantages of chemical pesticides – development of resistance in pests, environmental persistence and toxicity.
- Association of fungi with insects is well known and some of these cause serious diseases in hosts.
- Safe towards humans, environment and non-target organisms.
- These can be used in conjunction with synthetic chemical insecticides.
- Most common entomopathogenic fungi are *Beauveria bassiana, Metarhizium anisopliae, Nomuraea rileyi, Paecilomyces sps. Verticillium lecanii, Hirsutella thompsonii*

Why Biocontrol

- Environmentally compatible, naturally occurring
- Broad or narrow targets depending on organism
- Less prone to resistance
- Low cost of development
- Integrated control possible, reducing chemical use
- Less expensive and Rapid registration

POTENTIAL AND STATUS OF EPF

- Contact mode of action.
- EPF have a broad host range and also cause epizootics.
- Resistance in pests - Single site of action of *Bt* toxins Vs multiple site/multiple step mechanism of action.
- EPF are ubiquitous to many environments – leads to the discovery of native-indigenous isolates.
- Interaction of the EPF – host depends on the occurrence of non-specific and specific events between the conidia and the insect cuticle.
- EPF can be easily grown on simple media and can be easily formulated.
- Toxins and enzymes produced can be exploited

Biological pesticides

"**Any molecules from the biological origin, whole organism or product derived from them**" (Villaverde *et al.*, 2014)

Bacteria

- *Bacillus thuringiensis* (*Bt*)

Cry, Cyt, Vip

Virus

- Baculovirus

Needs to be ingested to act

Fungi

- ***Beauveria, Metarhizium,***
- *Lecanicillium lecanii*
- *Isaria fumosorosea,*

Acts by direct penetration (or)

Contact infection

Entomopathogenic fungi

- *Metarhizium* and *Beauveria*, approved by USEPA (Wang and Leger, 2013)
- *B. bassiana*- Agostino Bassi, white muscardine fungi disease in silkworm
- *Metarhizum* – may not be from insect origin, soil root interphase (St. Leger *et al.*, 2011)- Green muscardine fungi
- Coleoptera (Williams *et al.*, 2013), Diptera (Kim *et al.*, 2014), Lepidoptera (Ramzi and Zibaee., 2014), Hemiptera (Lacey *et al.*, 2011), Thysanoptera (Wu *et al.*, 2013)




http://agritech.tnau.ac.in/crop_protection/crop_prot_crop_sto_insect.html

Entomopathogenic Fungi Life Cycle

2. Spore adherence

Hydrophobins and adhesins (Zhang *et al.*, 2011)

3. Appressorium formation and Epicuticule penetration

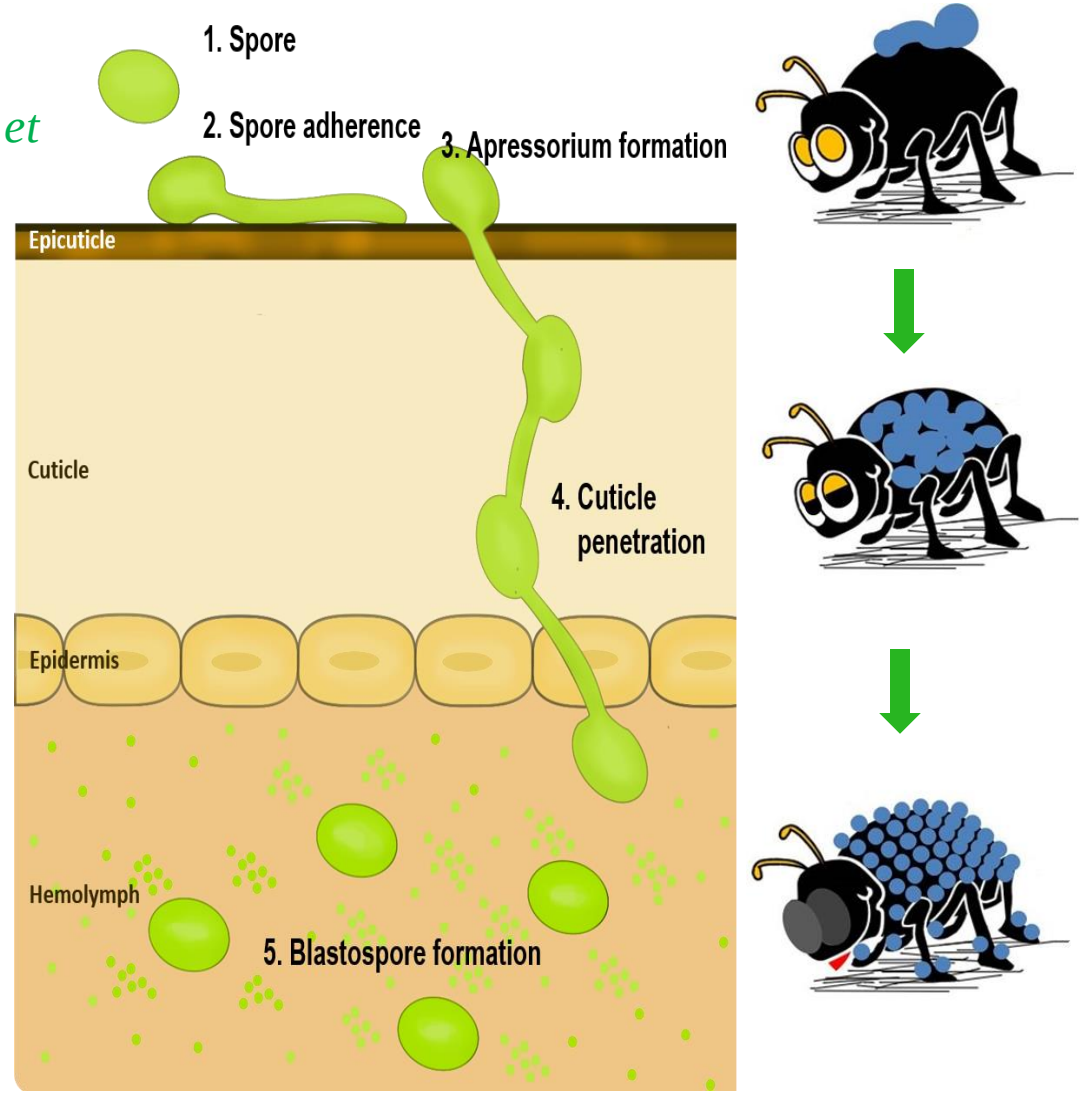
MAPK (Zhang *et al.*, 2010)

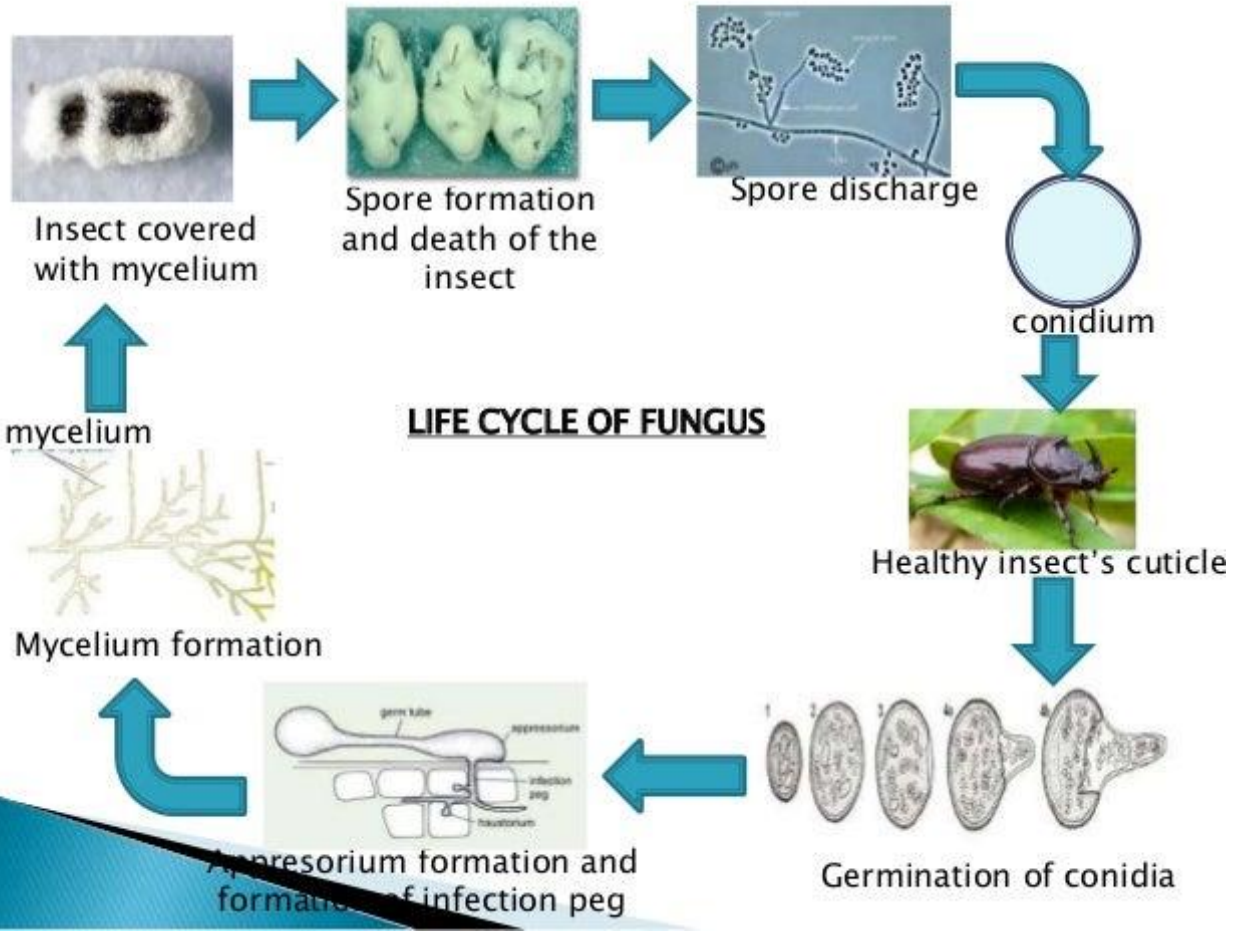
Cytochrome P450 (CYP52X1)

4. Cuticle penetration

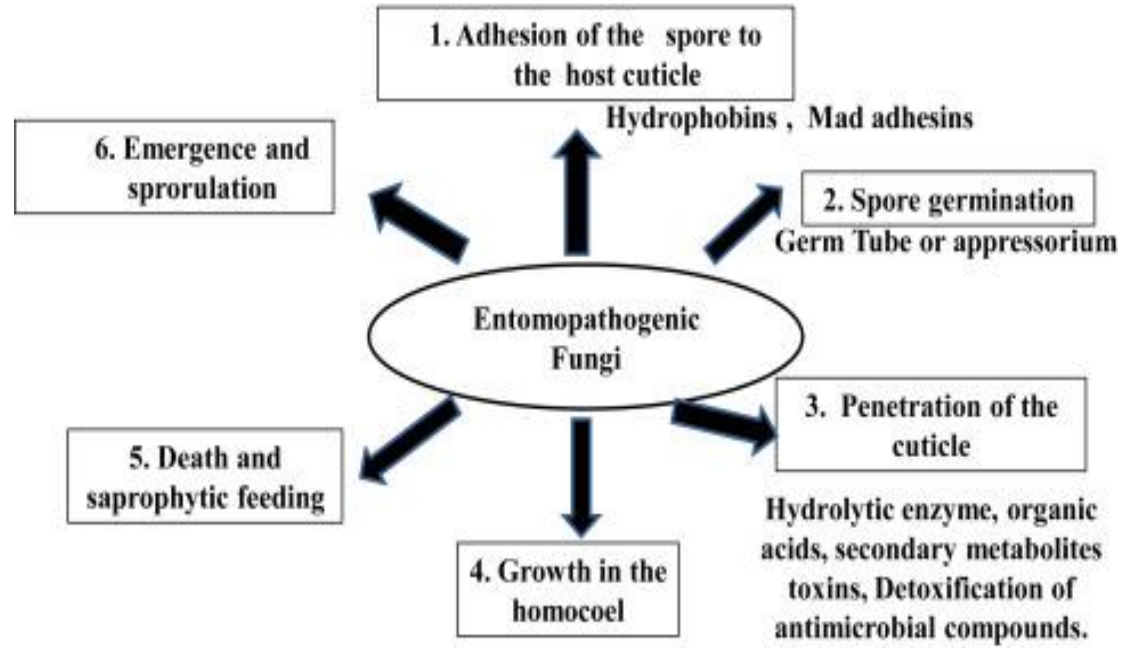
Chitinases (CHIT1), proteases (PR1 and PR2)

5. Blastospores formation- Hemolymph





Entomopathogenic fungi- Mode of Action on Host insect.



Entomopathogenic Fungi - Drawbacks

- Low efficacy in killing the host
- Sensitivity towards abiotic stress
- Major crop loss even after infection

?

Reduction in Lethal spore dosage

Reduction in Lethal time

Major areas

- Mass production, formulation and application.
- Studies on toxins produced by entomopathogenic fungi
- Strain improvement by following methods like
- Transformation
- Over expressing the extracellular enzymes
- Engineering entomopathogenic fungi to express neuro toxin genes in order to enhance the virulence.
- In vitro anticancer effects (Breast cancer)

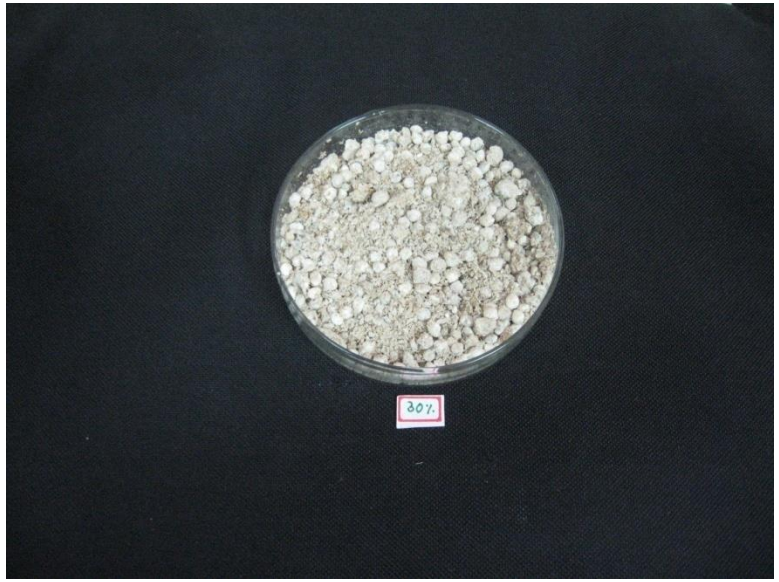
Mass production

- A considerable effort has been made to establish entomopathogenic fungi as a commercial mycopesticide by using Solid State Fermentation (SSF) as a suitable method for mass production
- Conidia produced by SSF are considered to be similar to those produced on insect cadavers
- A comparative study shows production of 10^{13} conidia, costs the same as chemical insecticides used per hectare. This high production efficiency leads to the development of consciousness to mass produce conidia of entomopathogenic fungi in a cost effective way
- It is preferred in order to produce many industrially important enzymes and antibiotics due to high productivity, low cost and easier downstream processing
- The maintenance of conidial viability in formulations during storage is crucial for obtaining effective insect control in the field

- Compatibility between production, formulation and application techniques is vital for the successful use of microbial biopesticides.

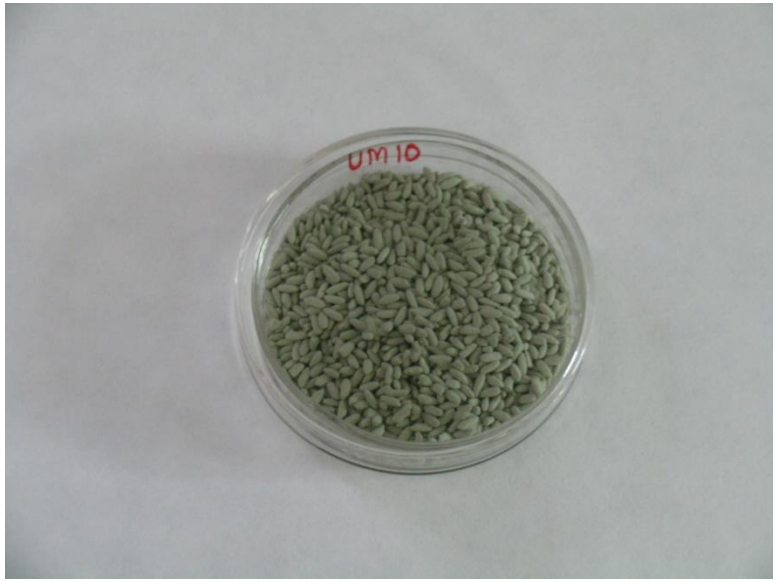
Mass production – *B. bassiana*

Rice – 40% Humidity
Spore output $5.6x10^9$



Sorghum+rice bran – 30% Humidity
Spore output $1.3x10^9$

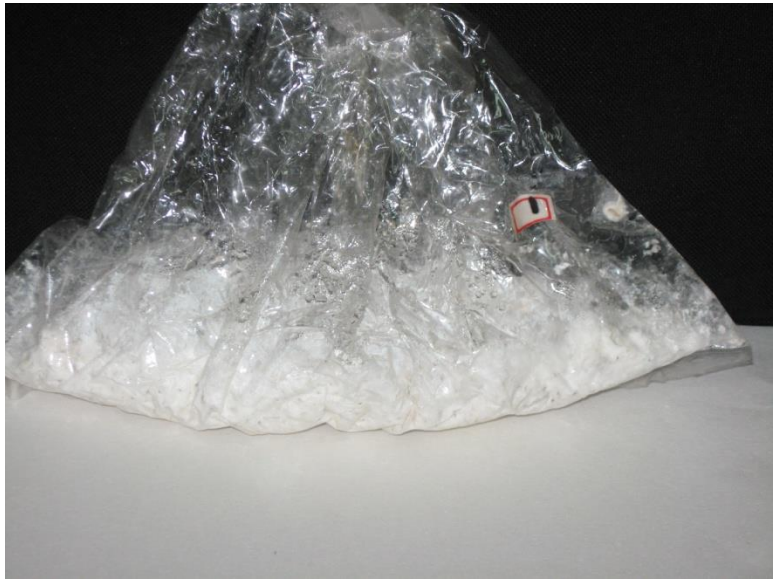
Mass production – *M. anisopliae*



Rice – 40% Humidity
Spore output - $1.1x10^{9}$

Rice – 65% Humidity
Spore output $1.99x10^{10}$

Mass production – *B. bassiana*



Rice with 1% yeast extract
Spore output $2.52x10^9$

Sorghum with 1% yeast extract
Spore output $3.6x10^9$

Formulation and Application of Mycoinsecticides

- Compatibility between production, formulation and application techniques is vital for the successful use.
- Formulation is important in terms of improved survival during storage, persistence in the field and ease of application.
- Can be formulated as wettable power and oil formulations.
- Powder formulations can be done in talc, silica gel, powdered rice and cornstarch. Algination is also reported.
- Viability retains for just 1-8 months when stored at room temperature but can retain for 7 years when stored under low temperatures.
- Ultra low volume (ULV) spraying, rotary atomizers, electrostatically-charged ULV sprayers, hydraulic spray systems etc have been used.
- Spray supplements like surfactants, emulsifiers, dispersing agents, antievaporants etc may be necessary for efficient application

Formulation of the mass produced conidia

- It is imperative to assess the shelf-life of the mass produced conidia and increase the shelf life during storage with proper formulations
  - Dry matrix:
    - Diatomaceous earth
    - Fullers earth
    - Kaolin
  - Oils:
    - Coconut oil
    - Mustard oil
    - Soybean oil
  - Oil and water emulsions:
    - Coconut oil and water emulsion
    - Mustard oil and water emulsion
    - Soybean oil and water emulsion
  - Sodium alginate encapsulation

- Conidial viability checked by ability of conidia to germinate in the agar-microscope slide assay by at the end on each month up to six months

Methods to Increase the potency

Overexpression of **endogenus genes** which are over expressed during host -pathogen interaction process

Endogenus gene(s)

Chitinases (BbChit1)

Proteases (Pr1)

Lipases

Reduction in LT_{50} values : 20-25 %

Overexpression of **Heterologous originated genes** with insecticidal properties

Heterologous gene(s)

Neurotoxic genes from arachnids and scorpions

LT_{50} 36.7 % reduction against

Dendrolimus punctatus

Bioactives from EPF

- Several metabolites produced by entomopathogenic fungi have shown toxicity to a number of insect pests.

- *B. bassiana* produces toxins like beauvericin, beauverolides, bassianolides and all cyclotetradepsipetides.

- On the other hand, M. *ansiopliae* also produces toxic metabolites, the best described of these are the destruxins and Swainsonine.

- Insect cell lines and human leukemic cell lines are being used in recent times to check the toxicity levels of these produced by EPF.

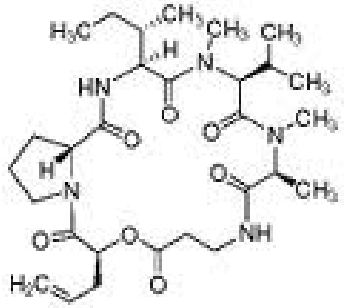
Mycotoxins from *Metarhizium* Species

Metarhizium anisopliae

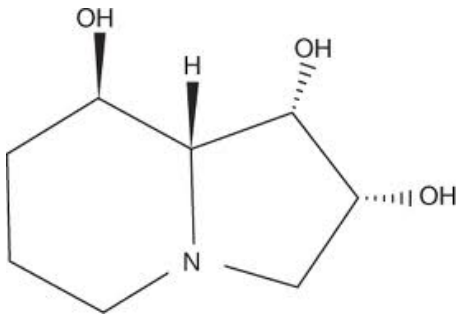
Swainsonine **inhibits the processing of asparagine-linked glycoproteins by impeding the action of Golgi α-mannosidase II** as well as lysosomal mannosidase.

Destruxin

Insecticidal
Antiviral
phytotoxic

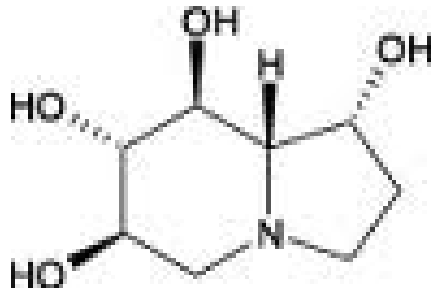


Swainsonine



Mannosidase -II inhibitor

Castanospermine



Glucosidase inhibitor

Natural Product Research ›
Formerly Natural Product Letters
Volume 28, 2014 - Issue 22

Short Communication

The antileukaemic cell cycle regulatory activities of swainsonine purified from *Metarhizium anisopliae* fermentation broth

Digar Singh & Gurvinder Kaur

Pages 2044-2047 | Received 15 Mar 2014, Accepted 25 Apr 2014, Published online: 28 May 2014

Abstract

Volume 347, Issue 1
October 2013

Preparative-cum-quantitative mass-directed analysis of swainsonine and its *in situ* activity against *Sf*-21 cell line

Digar Singh, Gurvinder Kaur ✉ Author Notes

FEMS Microbiology Letters, Volume 347, Issue 1, October 2013, Pages 7–13, https://doi.org/10.1111/1574-6968.12214

Published: 01 October 2013 **Article history** ▾

Abstract

Swainsonine is a polyhydroxy indolizidine alkaloid with various research and potential therapeutic applications. In this work, swainsonine was partially purified (2.5-folds) with acetone–methanol solvent system from *Metarhizium*

Exploitation of Endogenous gene(s)

“Genes which are over expressed during the infection process”

Metarhizium anisopliae

Gene	Source	Function	Outcome
Pr1	***M. anisopliae***	Degrade insect cuticle	LT_{50} - 25 % *Manduca sexta*

Beauveria bassiana

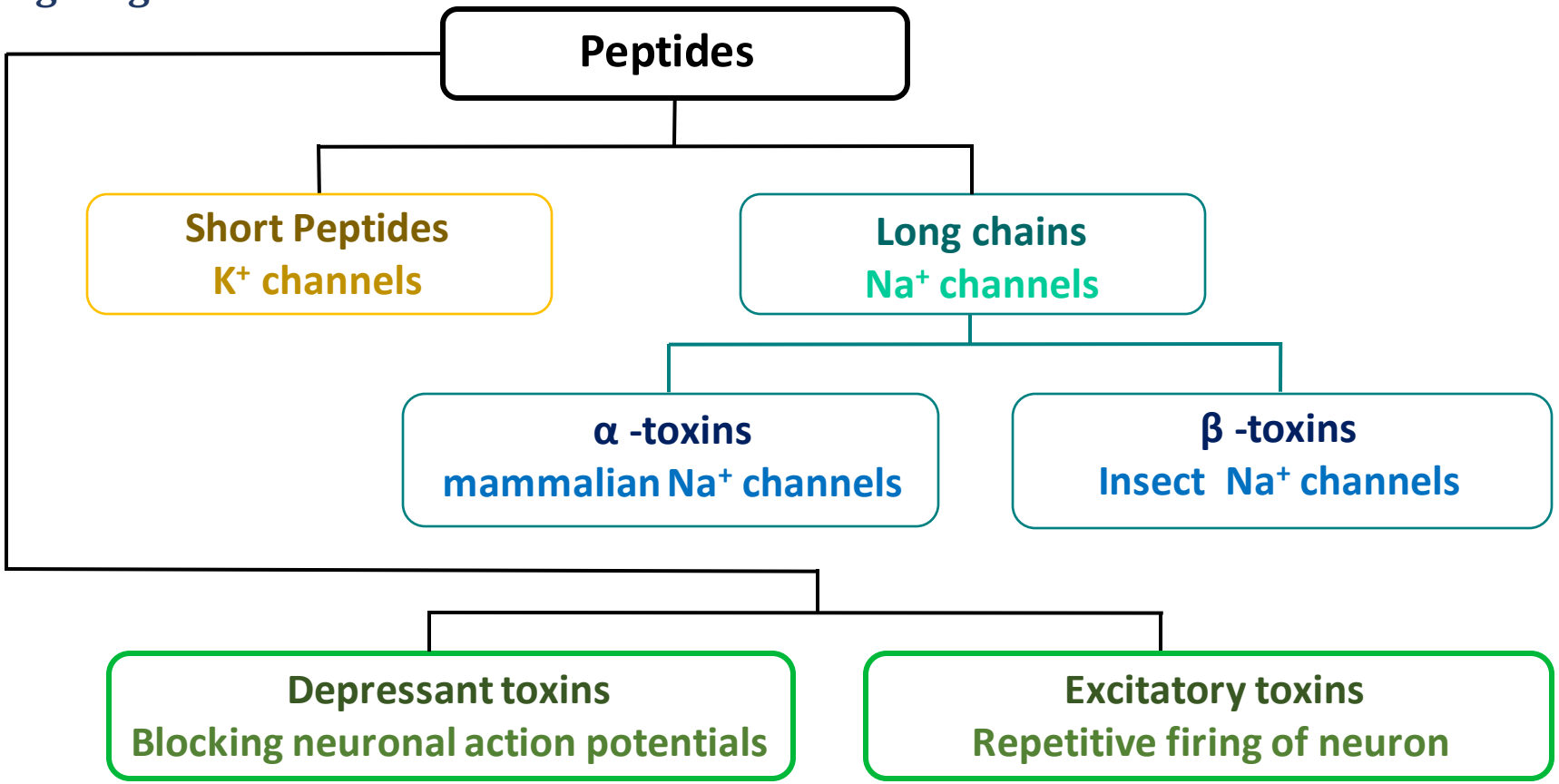
Gene	Source	Function	Outcome
CHIT1	***B. bassiana***	Degrading insect cuticle	Increased virulence by 23 %
BbCDEP-1 + BbCHIT1	***B. bassiana***	Degrading insect cuticle	Decreased spore dosage by 67 %

Exploitation of insecticidal toxins

Scorpion and spider neuropeptides

"The most important tool for scorpions and spiders to **predate** and exercise **self-defense**"

- Toxins or Peptides comprised of proteins ranging from **30-80 amino acid** residues mainly **targeting ion channels**



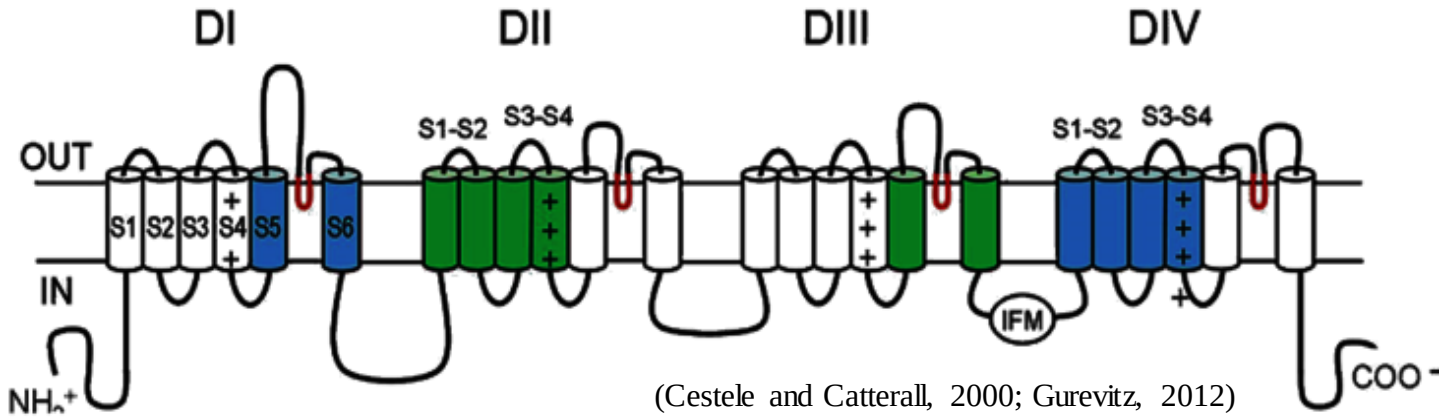
Scorpion toxins action on VGSCs

α -toxins
mammalian Na^+ channels

Scorpion α-toxins bind to receptor site 3 (extracellular loops S1-S2 and S3-S4 at DIV and S5-S6 at DI) on sodium channels which blocks the channel activation

β -toxins
Insect Na^+ channels

β-toxins bind to receptor site 4 (extracellular loops S1-S2 and S3-S4 at DII and S5-S6 at DIII) and trap inactivation channels which induce repetitive firing of action potentials



(Cestele and Catterall, 2000; Gurevitz, 2012)

Metarhizium engineered to control crop pest

AaIT1

Well characterized β-insect excitatory toxin which binds to receptor site 4 on VGSCs.

It has been proven that is not toxic to mammals even at 100 fold more concentration.

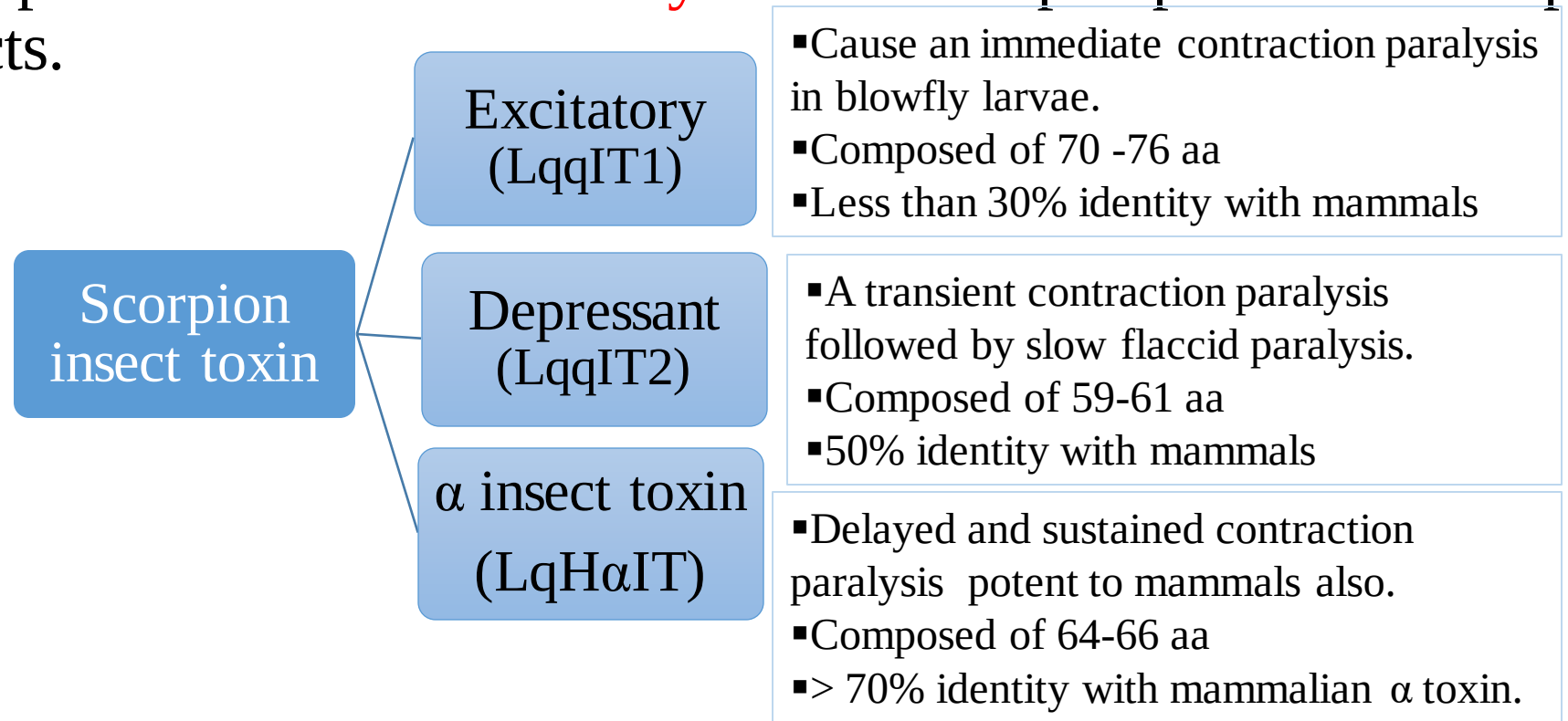
Gene	Source	Function	Outcome
AaIT1	***Androctonus australis***	Modifies the gating mechanism of sodium channel	22-fold increase in virulence
AaIT1+ Pr1	***A. australis and B. bassiana***	Dual activity with cuticle degrading and sodium channel blocker function	Reduced LT_{50} -40 %
BjαIT	***Buthotus judaicus***	Sodium channel modifier	Increased virulence

β-BUTX Lqq1a-Scorpion neurotoxin

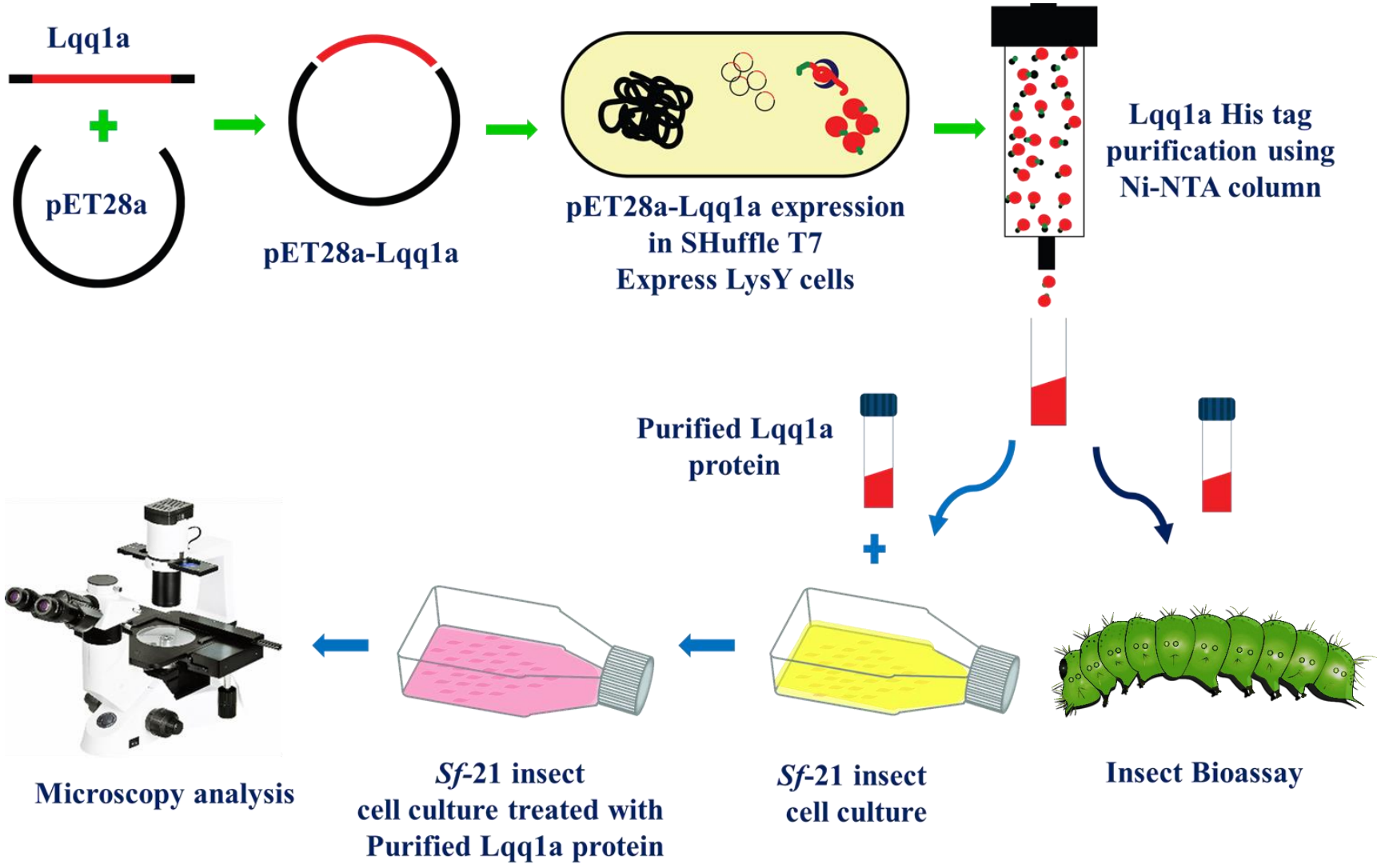
- *Leuirus quinquestriatus quinquestriatus*, **β-BUTX Lqq1a toxin**
  - 70 amino acids including 8 half cysteine residues at the positions 16-37, 22-42, 26-44, 38-64
    - Flaccid paralysis in insects which is caused by repetitive firing (Kopeyan *et al.*, 1990)
    - β toxins- bind to the receptor site 4 (Domain II and III) (Gurevitz, 2012)
    - Closely resembles insect specific β toxin Aa IT1 from *A. australis*
    - Non toxic to mammals (Ji *et al.*, 2002)

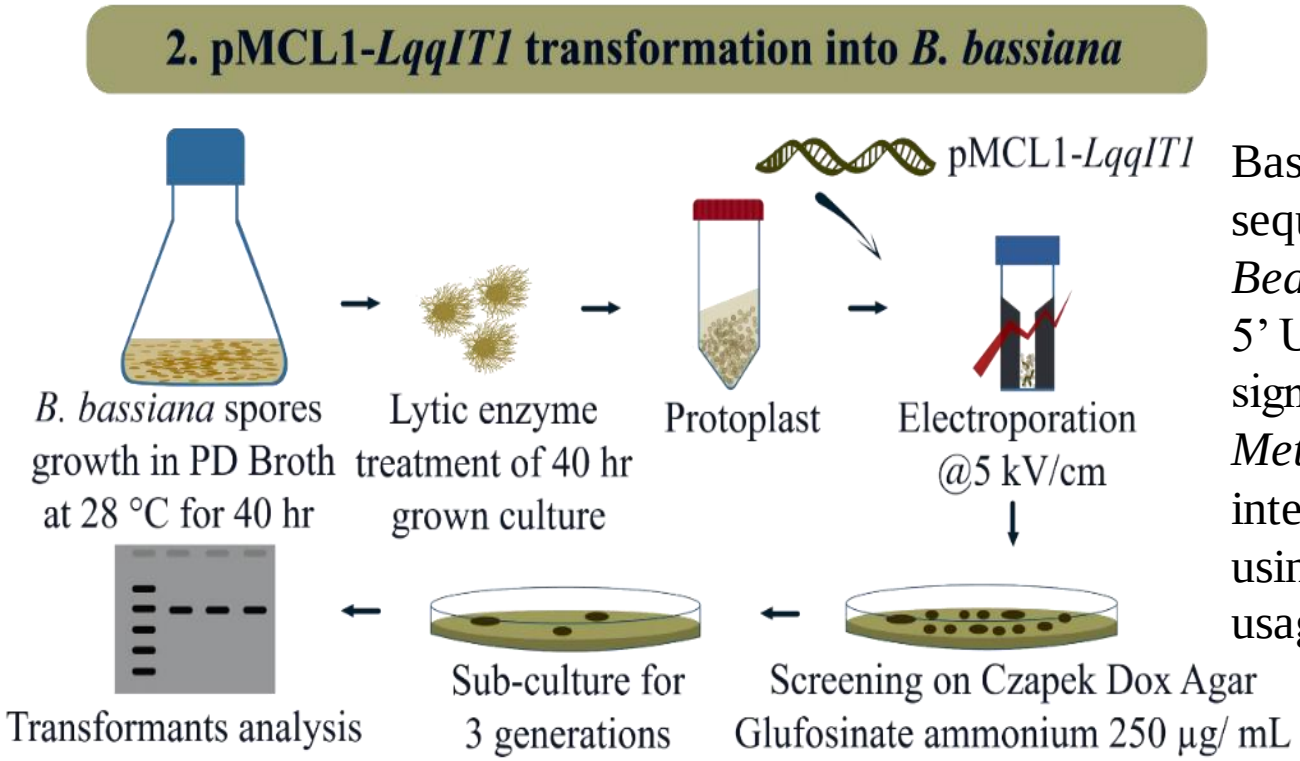
Engineering entomopathogenic fungi

- Use of Scorpion toxin Gene in fungi to enhance the pathogenicity by rDNA technology.
- Scorpion toxin are selectively active on lepidopterous and dipterous insects.



Schematic representation of bacterial expression, purification and cell culture studies of recombinant Lqq1a





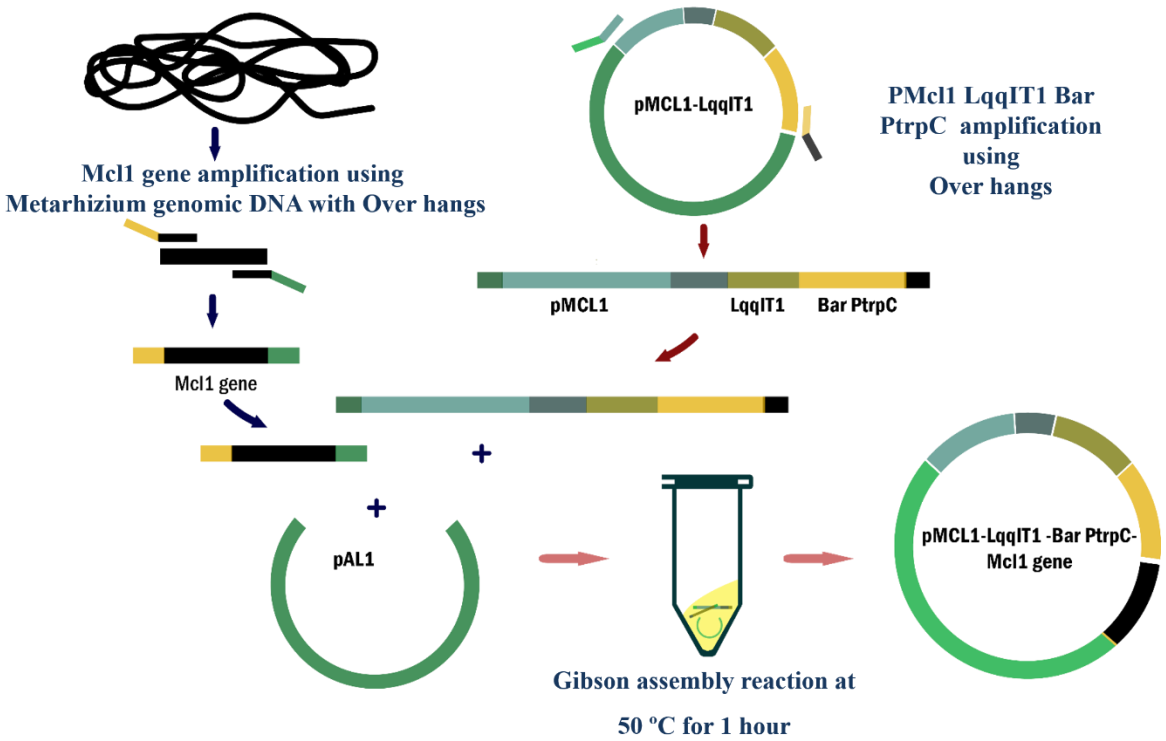
Based on the native protein sequence, corresponding gene sequence has been codon optimized for the expression in *Beauveria, Metarhizium*. In addition to the toxic gene sequence 5' Untranslated region (UTR, Kozak sequences) and Mcl1 signal peptide has been included at the 5' region from *Metarhizium* collagen like protein, to transport the protein of interest to hemolymph. The sequence was codon optimized using *Metarhizium anisopliae* and *Beauveria bassiana* codon usage

Schematic representation of PMcl1-Lqq IT1 cloning in *B. bassiana* using protoplast transformation.

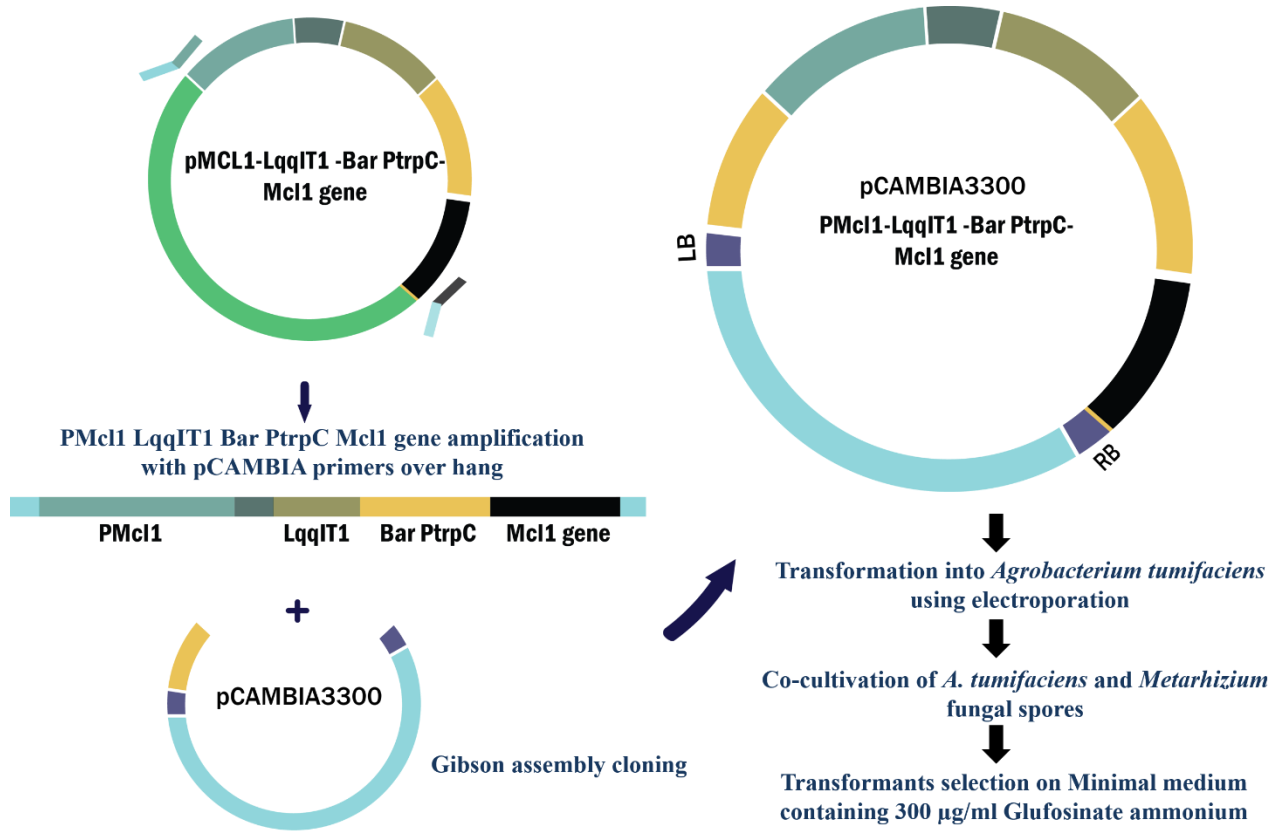
LqqIT1 protein expression and purification studies in *E. coli* and testing the efficacy of purified protein on insects (or) insect cell lines (*Sf*-9 and *Sf*-21) and Human cell line.

2a. Cloning, expression of the LqqIT1 in *Metarhizium* and *Beauveria* and insect toxicity studies

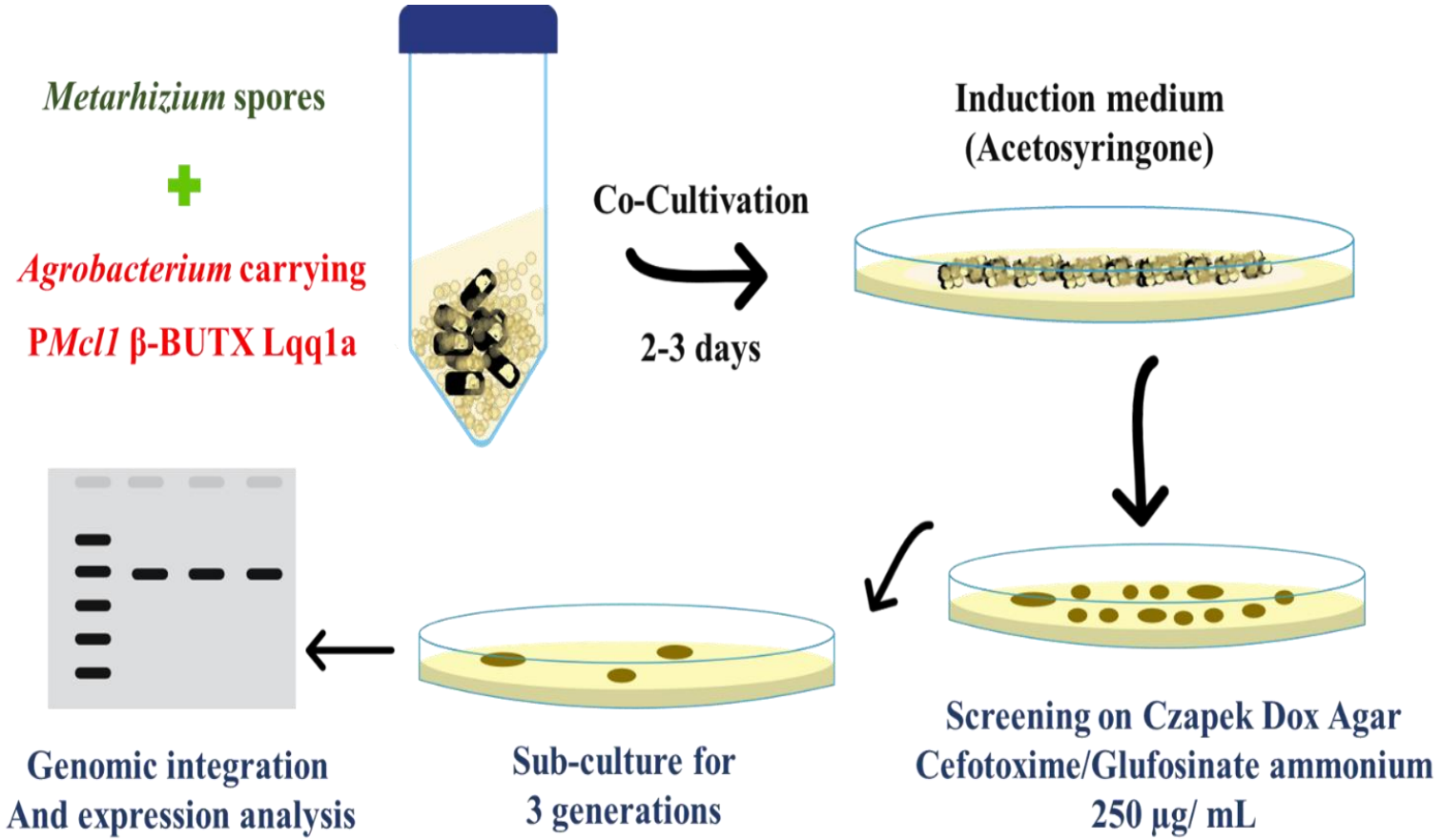
3a. Genes stacking using Lectin and *B. thuringiensis* Cry protein with LqqIT1 and insect toxicity assays



Schematic representation of LqqIT1 cloning into *Metarhizium anisopliae* pMcl1 Lqq IT1 Bar PTrpC Mcl1 gene overlap vector construction.



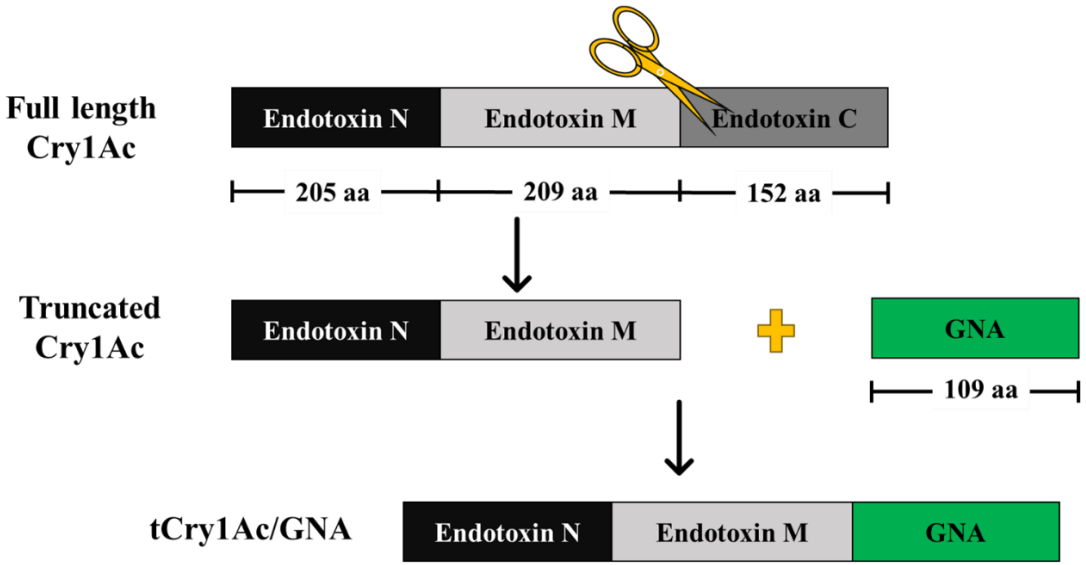
Schematic representation of LqqIT1 cloning into *Metarhizium anisopliae* pCAMBIA 3300 PMcl1LqqIT1 overlap vector construction.



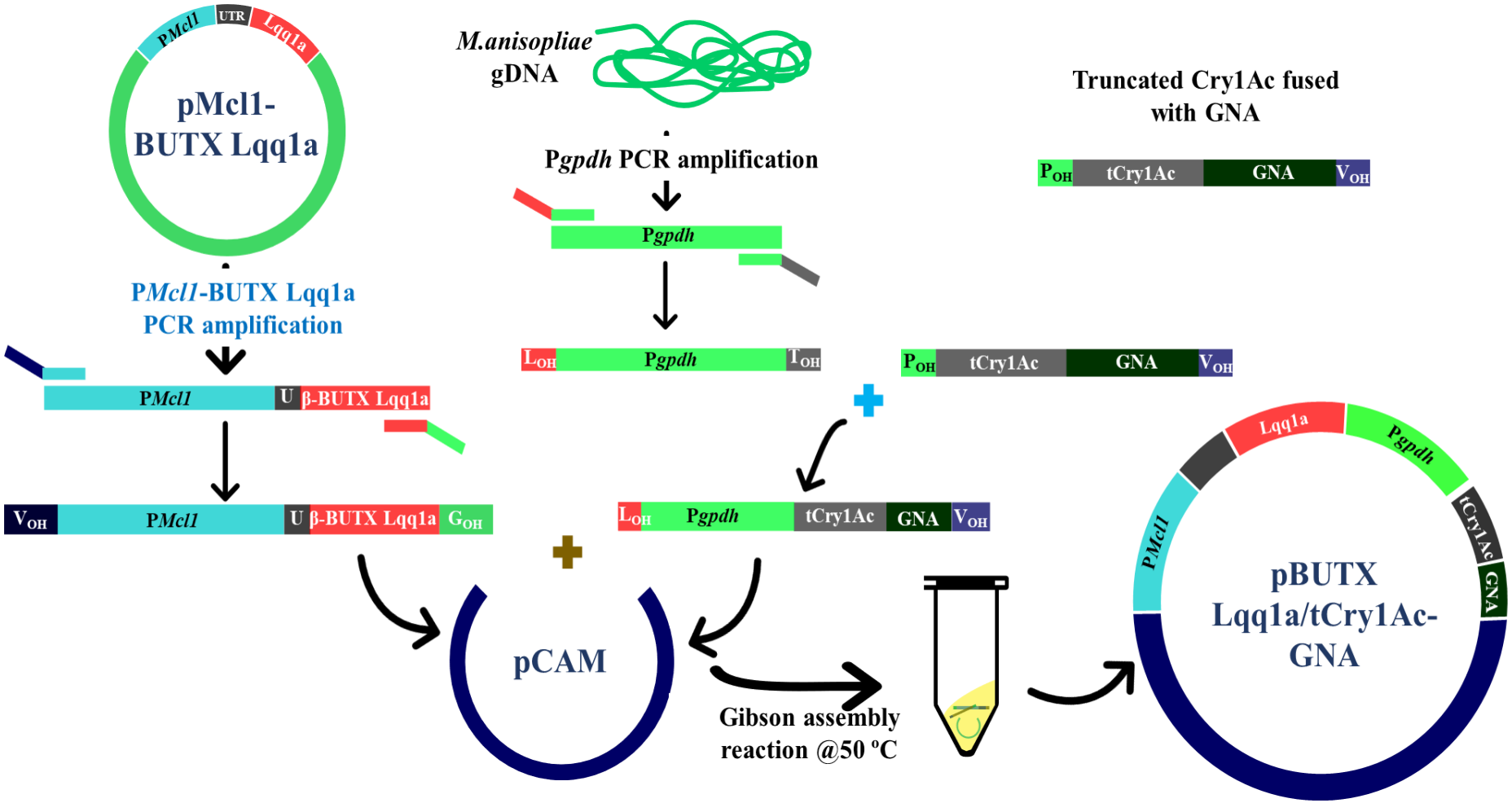
Schematic representation of *Agrobacterium* mediated transformation of *M. anisopliae*

Genes stacking using Lectin and B. thuringiensis Cry protein with LqqIT1 and insect toxicity assays

Plant lectins are heterogeneous group of defense protein molecule that recognize and **bind reversibly to the carbohydrate moieties**



Schematic representation of strategies followed for the fusion of Cry1Ac and GNA lectin



Flow chart for the cloning of β-BUTX-Lqq1a and Cry1Ac/GNA in *Agrobacterium* plasmid (pCAMBIA3300)

Article

Dhanasingh, M., and Gurvinder Kaur Saini. "Cytotoxic and lethal effects of recombinant β-BUTX-Lqq1a peptide against lepidopteran insects and cell lines." Toxicology in Vitro (2019).

May 2019 · Toxicology in Vitro 60(October):44-50

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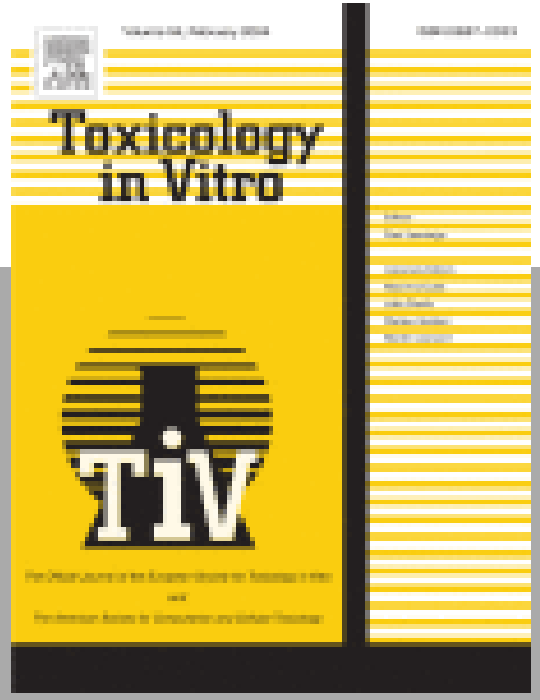
Toxicology in Vitro
Volume 94, February 2024, 105737



In vitro anticancer effects of recombinant anisoplin through activation of SAPK/JNK and downregulation of NFκB

Author links open overlay panel , , ,

https://doi.org/10.1016/j.tiv.2023.105737Get rights and content

Future of Entomopathogenic fungi

- Good mass production – formulation – application (to reach the field for use by farmers).

- Strain improvement – via genetic engineering

(over expressing extracellular enzymes to increase the rate of virulence).

- Engineering fungi with other neuro toxic genes from Scorpion, snake venom, wasps, etc.

- Continued investment in research, technology transfer and education