Co-Leaders		

Title

Identification of novel Bacillus thuringiensis effectors active against the coffee berry borer, leaf-cutter ants and burrowing nematodes through prospection of tropical agroecosystems and pangenomics.

Summary

Costa Rica and Brazil are major producers and exporters of coffee and banana worldwide. Farmers in these countries face difficulties to control the coffee berry borer, leaf-cutter ants, and burrowing nematodes, and the chemical insecticides that they regularly use to this end have undesirable environmental and public health consequences. We propose to perform a pangenomic-driven prospection of novel Bacillus thuringiensis (Bt) toxins/effectors active against these pests in agroecosystems from both countries. This effort is justified by the notion that autochtonous Bt strains kill local insect populations more efficiently than cognates from other latitudes. The proposed products have the potential to turn into at least three bioinsecticide prototypes. Moreover, the strain collection to be generated can be tested for alternative pest activities and the sequences can be placed in internet so that licensed users can develop other bioproducts and thereby reduce pesticide usage elsewhere.

Keywords

Bacillus thuringiensis (Awaiting Moderation)
Hypothenemus hampeii (Awaiting Moderation)
Atta cephalotes (Awaiting Moderation)
Radopholus similis (Awaiting Moderation)
Pangenomics (Awaiting Moderation)

Key Name	Team Position	Degree	Institution

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Duration

24 months

Thematic Area

Natural Resource Management Improvement

Country(ies) of Implementation

Brazil
Costa Rica

Problem Definition

The agriculture of Costa Rica heavily relies on coffee, bananas, shortcycle crops, cattle for beef and dairy, and forest plantations. Biological control of insect pests holds potential for increasing the sustainability of agroecosystems. The products based on Cry/Cyt proteins from Bacillus thuringiensis (Bt) largely dominate this market. Relevant to this proposal, Brazil is a leading developer of this kind of technology and integrated pest management regimes prioritizing biopesticides are improving in Costa Rica. Brazil and Costa Rica are among the main producers of coffee and banana worldwide and suffer from similar pests and control issues. In this proposal, we focus on the coffee berry borer (CBB), Hypothenemus hampei, leaf-cutter ants from the genus Atta, and Radopholus similis, on account of their impact to both economies and their relevance to producers from both countries and the tropics. The expected results have the potential to enhance the productivity due to reductions in pesticide usage. The use of pangenomics to screen large collections of Bt isolates genomically saves times and resources. This will be the first large scale strategy to create a Bt genes bank.

Objectives

General Objective: To identify novel Bacillus thuringiensis toxins and effectors active against the coffee berry borer, leaf-cutter ants, and the burrowing nematode of banana through prospection of Costa Rican and Brazilian agroecosystems and soil by pangenomics strategies. Specifics objectives: 1. To prospect coffee, and banana agroecosystems in Costa Rica and Brazil for Bt strains to expand current strain collections 2. To determine the pangenome of at least 500 Bt strains to identify novel cry genes and to make an inventory of cry genes and virulence factors with predicted activity towards the CBB, leaf-cutter ants, and burrowing nematodes of banana 3. To transform a acrystaliferous Bt strain with sequence stretches containing novel cry genes to establish a bank of clones with multiple toxin/effector combinations

Methodology

Bt isolation: We plan to recover a minimum of 600 Bt isolates from soil and death insects collected at coffee, citrus, palm, and banana agroecosystems in Costa Rica and Brazil. For Bt isolation, we will resuspend samples in LB-acetate broth, perform a thermal shock to kill vegetative cells, and plate mixtures onto PEMBA plates. White colonies over blue agar zones will checked microscopically for the production of parasporal crystals. Presumptive identifications will be confirmed via 16S rDNA sequencing. Plasmidial DNA sequencing: Plasmid DNA (pDNA) from all isolates will be obtained using commercial kits. For next generation sequencing (NGS), 30 pools of 20 plasmids each will be sequenced using PacBio long-read sequencing technology to better deal with interspersed repeats and recombination events. Sequence assembly, annotation, and analysis: Quality assessment will be done using the FASTX-Toolkit. Thereafter, reads will be assembled de novo using Velvet or SPAdes. To provide hints on fragment affiliation and to facilitate supercontig reconstruction, tetranucleotide usage patterns will be checked using Tetra. Gene annotation will be done using Prokka and custom databases containing sequences from previously described Bt plasmids and the DNA sequences of all cry/cyt genes known to date. Genes encoding Cry or Cyt proteins and other effectors will be aligned against known cognates deposited in databases using MUSCLE of MAFFT. Dendrograms will be produced with Seaview and visualized with FigTree. Sequences stretches showing similarity to known sequences will be depicted using Easyfig. Bt transformation: At least 30 sequenced toxin loci will be amplified by PCR from the pDNA pools using long-range polymerases or synthesized in silico. These DNA fragments will be cloned into a Bacillus-compatible expression vector. Recombinant plasmids will be transformed by electroporation into an acrystaliferous Bt strain. Bioassays: Coffee bean borer and leaf-cutter ant bioassays will be performed adding Bt toxins to artificial solid diets. For nematode bioassays, egg otheca will be exposed to Bt toxins on petri dishes. All bioassays will be done in insect rearing chambers to control for environmental variables. The CR node is well familiarized with these procedures and has insect colonies for the first two pests. DNA shuffling: Homologous DNA templates will be shuffled using the so-called Staggered extension process. Briefly, PCR amplified toxins will be mixed with one or more primers and subjected to very short annealing and extension steps. If the identity of the sequences to be shuffled is low, a combination of ITCHY (incremental truncation of hybrid enzymes) and sequence-homology independent recombination will be favored for hybrid toxin generation. Hybrid toxin genes will be Sanger-sequenced, cloned and expressed in the aforementioned acrystaliferous Bt strain in order to perform bioassays (as described above).

Innovation

To the best of our knowledge, this is the first pangenome-based strategy for prospection of bacterial resources for biological control of pests ever done in Latin America. This strategy allows processing of large numbers of samples, improving the odds of finding of novel toxins/effectors and thereby improve agriculture productivity.

Expected Results

• At least 400 new Bt strains from Costa Rican and Brazilian agroecosystems, incorporated along with their metadata into the databases and collections maintained by the participants of the consortium at their laboratories. • Draft sequences of at least 40 Bt plasmids • Annotated plasmid reconstructions (supercontigs) • A database of Cry/Cyt proteins and other virulence factors • A database summarizing the replication and maintenance modules of the Bt plasmids sequenced • Sequence comparisons, similarity matrixes, and dendrograms to highlight novel effectors • A collection of at least 20 Bt transformants containing novel combinations of toxins and effectors • Rearings for each of the pests targeted by this proposal • Tables comparing the LD50 of the transformants • At least 3 novel hybrid toxins exhibiting higher toxicities than the toxins from which they were derived

Potential Development Impact

Within 5 years, we expect to be engaged in the scale-up process and the formulation of one or two bioinsecticide prototypes, to have protected them intellectually and to be negotiating with investors their industrialization.

Growth Potential/Sustainability

A large of this project can be replicated in regions of high biodiversity value or in understudied locations with unique ecological features, such as African countries, provided that local groups with basic microbiological and molecular biology skills collect samples, cultivate Bt and isolate DNA. If these skills are not available, they can be transferred to technicians with a workshop. Sequencing and data analysis can be outsourced or performed in collaboration with researchers from other countries, who likely would be interested in getting the chance to tap such promissory resources. The main products of the project are

a collection of isolates whose spores can be stored at room temperature and databases that can be maintained in portable hard discs or even in the cloud, facilitating their universal access to licensed users. Since sequences and spores do not decay, bioassays to species not included in this project or alternative screening methods can be performed at any time in the future by us or others. In this regard, the University of Costa Rica and Embrapa are consolidated institutions with defined politics regarding innovation and solid financial perspectives.

Key Bibliography

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Flow Chart

FlowchartBt_20150313172549.jpg

Environmental and Social Safeguards Statement and Legal Compliance

I hereby attest to the best of my knowledge that all environment and social safeguards will be in place regarding potential negative environment and social impacts of the project including any potential impacts on indigenous people. I also attest the project will comply with the specific legislation in all implementing countries including but not limited to those related to germplasm exchange, testing involving human subjects, use of biotechnologies, and intellectual property.

Budget

Grand Total: 330000

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