ABSTRACT

Microbes are genetically highly diverse and have evolved a variety of strategies in order to compete for nutrients, including pathogens of insects. Insecticidal bacteria and their toxins genes have been explored for agricultural use for decades. Traditional methods to discover insecticidal bacteria and toxin genes are based on isolating bacteria on selective media, focusing primarily on the spore-forming species Bacillus thuringiensis. Thus, the diversity and novelty of isolated bacteria is limited by what can form colonies on the selective media. An alternative approach to identifying toxin genes is based on nucleotide homology searches in all available sequenced genomes. However, most of the bacteria from soil microbial communities have not been cultured, and their genomes have not been sequenced. Our approach to identify novel insect toxins is based on mining metagenomes, in which the genetic potential of the whole microbial community includes uncultured species. In addition, we use enrichment strategies on natural microbial communities to increase the abundance of insecticidal bacteria. The metagenomes from the enriched communities are then assessed using the Second Genome (SG) discovery platform which includes statistical models trained on known insect toxins. The output from this analysis is a set of potentially novel insecticidal proteins that can be tested in vivo.

OBJECTIVES

• Enrich soil microbial communities for insecticidal bacteria
• Obtain metagenomes of the enriched communities and also public genomic and metagenomic data
• Identify novel insect toxins in metagenomes based on statistical models

I. SG discovery platform from metagenomes is based on SG knowledge bases, ‘omics pipelines and toxin-specific statistical modeling

II. The manually curated ontology system in the SG KnowledgeBase facilitates cross-study comparisons

III. APPROACH

- High sequencing depth (~150K reads per sample)
- Yet, more OTUs remain to be discovered
- 13,800 OTUs identified (at 97% nucleotide identity to GreenGenes)
- 10% of OTUs had >97% nucleotide identity to sequenced genomes

IV. RESULTS

1. High microbial diversity with most of identified OTUs not in culture

2. Changes in alpha microbial diversity varied between carbon sources

3. Strong shifts in soil microbial communities within 72 hours after addition of complex organic carbon compounds

4. Individual carbon compounds stimulated different phyla containing insecticidal-like taxa

5. Metagenomes contained homologues to insecticidal toxins with variability within and among treatments

CONCLUSIONS

- Combination of microbiology, bioinformatics and data science allows to mine the genetic potential of the total microbial community for novel insecticidal toxins and gain access to uncultured OTUs.
- The SG Discovery platform is applicable to other fields for discovery of microbial proteins beneficial for agriculture and human health.

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