

Fighting caterpillar pests and managing agricultural insecticide resistance with Lepidoptera-associated *Enterococcus casseliflavus*

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Received: October 19, 2023; Accepted: November 22, 2023; Published Online: December 1, 2023; <https://doi.org/10.59717/j.xinn-life.2023.100042>

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Citation: Zhang Y. and Ju F. (2023). Fighting caterpillar pests and managing agricultural insecticide resistance with Lepidoptera-associated *Enterococcus casseliflavus*. The Innovation Life 1(3), 100042.

Probiotics are live microorganisms that are intended to benefit the health of metazoan hosts. Mutualism in insects (both pests and beneficial insects) provides a vital contribution to host fitness. With the development and demand for healthy modern agriculture, bacterial symbionts are considered to be an important toolbox for pest control and beneficial insect protection. *Enterococcus casseliflavus* is a widespread symbiont in most Lepidoptera insect pests. Recent studies indicated that *E. casseliflavus* provides beneficial phenotypes to the host, including supplying a diet lacking amino acids and enhancing insecticide resistance. We propose that *E. casseliflavus* should have enormous biotechnological potential awaiting further exploitation for Lepidoptera insect pest control or beneficial insect protection through symbiont-targeted insecticide resistance management, because this symbiont is widespread in natural caterpillar hosts, easily isolatable and cultured, and rapidly spreadable within the caterpillar population. We outline the rationales for why *E. casseliflavus* is suitable for application in pest management and envision possible plans of symbiont-targeted therapeutics via four potential approaches: genome modification, phage engineering, predatory biocontrol, and synthetic microbiome. This perspective opens up a grand blueprint for the future of symbiont-targeted

insect pest suppression or economic insect promotion.

Lepidoptera insects, including butterflies and moths, are the most significant radiation of plant-feeding insects, which also function ecologically as pollinators and predators. Many have been notably known as crop pests due to their larvae stage (called caterpillar) overconsuming in plant leaves.¹ Microbiota- and natural enemy-based insect pest control avoid insecticide resistance and environmental pollution problems from a natural perspective. Still, due to the lack of exploitable resources for insect pest control, there is an urgent need to investigate further available symbiotic bacteria and their interaction and transmission mechanisms within the host to enrich the resource pool of pest control targets and contribute to food security.

In recent years, numerous studies indicated that the microbiome is involved in caterpillar adaptation to adversity conditions, including toxin exposure, extreme environment, and natural enemies.^{2,3} *Enterococcus* species (e.g., *casseliflavus*, *mundtii*, *faecalis*, and *durans*) are arguably the dominant bacterial symbionts in most caterpillars.² Especially *E. casseliflavus* has been experimentally confirmed quite recently as both an amino acid provider and a probiotic enhancer related to evaluated insecticide resistance of *Spodoptera*

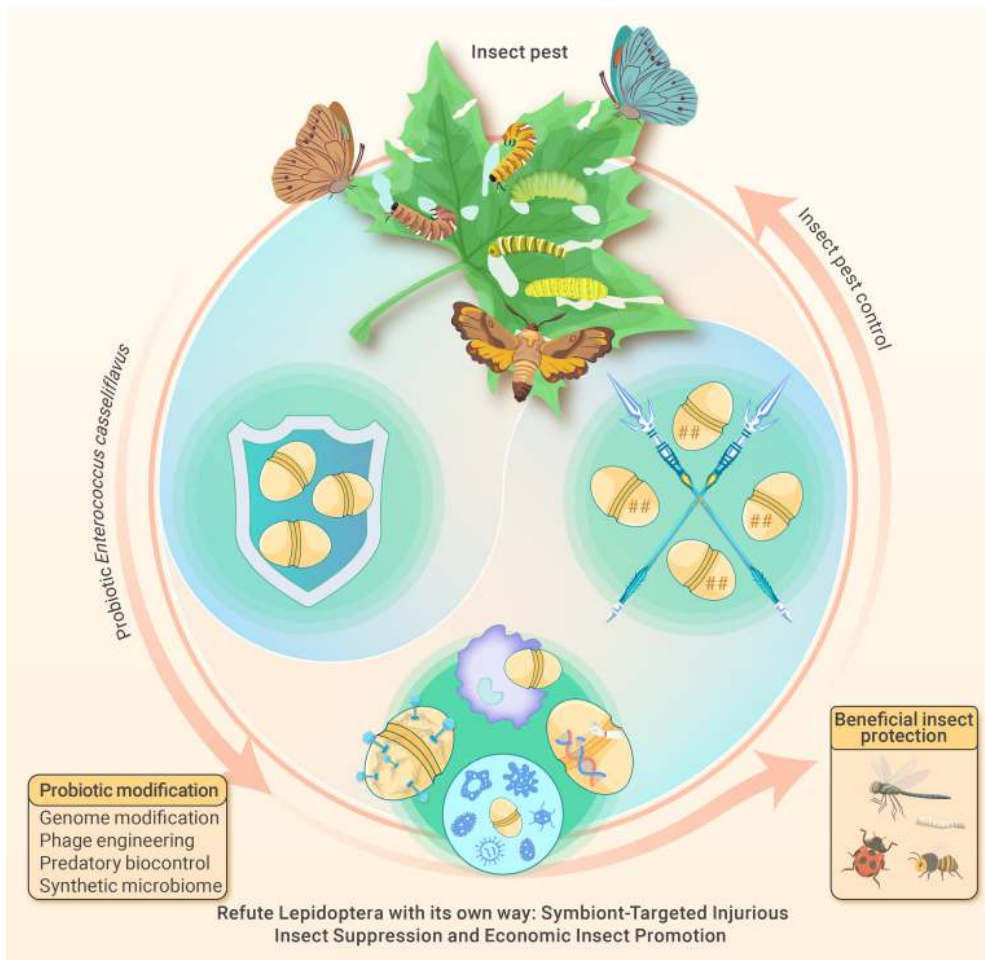


Figure 1. A schematic diagram of *Enterococcus casseliflavus* applied in caterpillar insecticide resistance management (e.g., via biodegradation) and Symbiont-Targeted Insect Control (STIC) including Injurious Insect Suppression and Economic Insect Promotion based on traditional Chinese culture "Tai Chi" and the old Chinese saying that "refute somebody with his own way" *E. casseliflavus* is a promising "shield" for the caterpillar hosts because it can mediate beneficial phenotypes for the host. We propose genome modification, phage engineering, predatory biocontrol, and synthetic microbiome to modify the "shield" to a "sword" that fights caterpillar pests, while also as "shield" for protecting and promoting useful insects (bee and silkworm) promotion.

litura.⁴ Coincidentally, we recently discovered that a new bacterial strain EMBL-3 isolated from *Spodoptera frugiperda* can also mediate host insecticide resistance of this agricultural invasive insect which suggested *E. casseliflavus* as a "popular symbiont star" widespread in the caterpillar community, providing a stable, beneficial phenotype to the host.⁵ Thus, in this perspective, we present a Lepidopteran bacterial symbiont that has received little attention as a novel target for insecticide resistance management of insect pest, and also for economic insect promotion under the background of widely used insecticide globally. In combination with the old Chinese saying "refute somebody with his own way" and the idea of Tai Chi, the aim of green pest control is achieved by combining biological control with synthetic biology (Figure 1).

JUSTIFICATION OF *E. CASSELLIFLAVUS* AS SUITABLE NOVEL TARGETS OF INSECT PEST CONTROL

To our knowledge, *E. casseliflavus* widely infects Lepidoptera insects, including Noctuidae, Bombycidae, Pyralidae, Sphingidae and Nymphalidae etc. These family species were identified in most Lepidoptera insects not only by DNA sequencing but also by isolating. Pure strains of *E. casseliflavus* can be easily isolated and cultured using only ordinary LB medium, which indicates that they are easily culturable bacteria. They can horizontally spread rapidly within the caterpillar population through cross-diet or cannibalism, as is the case for *S. frugiperda*, an agricultural migratory pest to China in early 2019,⁶ which implies that genetically modified strains e.g., symbiotic or pathogenic bacterium modified to be harmful to insect pests, can quickly spread among the caterpillar population in the field.⁵ In recent years, the ability of *E. casseliflavus* to enhance host insecticide resistance has been reported, suggesting its potential as a target for resistance management.⁶ In conclusion of the above reasons, *E. casseliflavus* is a potentially suitable novel target for insect pest control.

POTENTIAL METHODS FOR APPLICATION *E. CASSELLIFLAVUS* APPLICATION IN THE AGRICULTURAL FIELD

Four potential methods are proposed for exploiting *E. casseliflavus* in agricultural insect pest control and insecticide resistance management. First of all, genome modification of *E. casseliflavus* is easy due to the pure strains and genome maps being conveniently obtained from different Lepidoptera insects. However, the interaction between *E. casseliflavus* and their host insect should be clarified to meet the need of finding host phenotype-related functional genes as the targets for modification or knockout. A recent study showed that *E. casseliflavus* isolated from *S. frugiperda* of different insecticide resistance levels has a genomic variation that may contribute to host insecticide resistance via biodegradation.⁷ Identifying microbial genomic variation related to host insecticide resistance is vital for genome modification-based insecticide management. The first step of genetically modifying with a new insect-associated symbiont is to determine effective tools and approaches, typical workflows like homologous recombination and CRISPR system as the tools for Genetically modification have been developed in many insect symbionts. However, attention should also be paid to the field fitness of these genome-modified strains, which determines the practical effect of genome modification-based *E. casseliflavus* application in the agricultural field.

Moreover, phage biocontrol of *E. casseliflavus* is another potentially important application method that guarantees the effective treatment of antibiotic-resistant bacterial infections. Phages attach to bacterial host cells through receptor-binding proteins (RBPs) on the virion particles, which have a high affinity for a bacterial surface receptor and contribute to host specificity. Based on host specificity and bacterial treatment, the primary bacteriophage of *E. casseliflavus* can be identified, modified and induced to become a valuable tool for targeted therapy of *E. casseliflavus*. Engineered phages of insect symbionts via RBPs can also be used to deliver molecule vehicles, including antibiotics or insecticides, to help manage insecticide resistance. In addition, it is also one of the green, healthy, and friendly application measures to explore suitable methods to induce temperate phages in the symbionts' genome into virulent phages and kill beneficial symbionts from inside the cells.⁸

Symbiotic or pathogenic prey bacteria are constantly threatened by bacterivorous predators such as nematodes, protozoa, and predatory bacteria (e.g., Myxobacteria and *Bdellovibrio*-and-like-organisms). Predatory biocontrol can be applied in insect pest control when symbiont targets are known, provided that *E. casseliflavus*-targeting predators are available. Predators can potentially consume beneficial symbionts of caterpillars, especially *E. casseliflavus*, while making caterpillars more sensitive to insecticides. Exploring the agriculture application potentials of bacterial predation relies on integrating the power of emerging culture-independent technologies with the classic culture-based methodology that can obtain specific predatory bacteria that target insect pest-associated probiotics or beneficial insect pathogens in high throughput. The central question is how these predators can be cocktailed in specific hosts without destroying the agricultural microbial community, including plants and soil microbiome, which is the focus of future research.⁹

The other complex ecological interactions between symbiotic, pathogenic

and other microbes also help us to identify target bacteria and further understand their nature to inform insect control. Based on the concept of synthetic microbiome, i.e., microbial communities of desired function, such as those harmful to pests but beneficial to crops, are rationally designed and artificially synthesized to replace the original indigenous microbiome of caterpillar pests to reduce their harmfulness and adaptability.¹⁰ To this end, reformed *E. casseliflavus* is likely to be one of the keystone members of such synthetic microbial communities, and of course, to fulfill this endeavor, it is imperative to develop more versatile resources of available caterpillar microbial strains.

CONCLUSION

In recent decades, high levels of insecticide resistance have developed in insect pests and the pesticides overused caused economic insects' death have been noticed. Therefore, it is important to explore and develop green and safe pesticides or novel methods to control or protect harmful and useful insects. This perspective presents a new insect symbiont target, *E. casseliflavus*, for fighting caterpillar pests and for protecting beneficial insects with four approaches, including genetically modified symbionts, phage biocontrol, predatory biocontrol and synthetic microbiome. Considering *E. casseliflavus* as a widespread, easily isolated and widely infected symbiont, using advanced methods to develop it to control more insect pests or promote the growth of economic insects will have broad social-economic prospects. Nevertheless, it is still a long way off for applications of these approaches in most agricultural injurious and useful insects. This perspective inspires the development of insect symbiont-related engineering to solve societal challenges, ranging from insect pests control to economic insect health promotion.

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FUNDING AND ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China under Grant No. 32302393 (to ZYH) and Grant No. 22241603 (to JF), the China Postdoctoral Science Foundation Certificate No: 2023M733188 (to ZYH) and the HRHI program 202309010 of Westlake Laboratory of Life Sciences and Biomedicine (to JF). The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.