Progress in Synthetic Biology Research

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Abstract: Synthetic biology is a frontier field of life sciences in the 21st century. In recent years, the global development of synthetic biology has been rapid, and it has become a key engine driving the bio-economy and achieving the "dual carbon" goals. Synthetic biology has made many important research progresses in recent years, with breakthroughs in gene mining, strain construction, genome synthesis, etc. For example, scientists have constructed *Vibrio natriegens* engineering strains using synthetic biology methods, which can degrade multiple organic pollutants simultaneously in high-salt industrial wastewater and high-salt soil. In the field of agricultural synthetic biology, scientists have successfully deciphered the biosynthetic pathway of the locust aggregation pheromone 4-vinylanisole (4VA), realized precise regulation of the biosynthesis and release process of locust pheromones, and completed artificial intervention in their swarming behavior. In addition, scientists have used core synthetic biology tools such as gene editing, circuit design, and metabolic engineering transformation to deeply analyze the complex physiological mechanisms of insects, construct engineered insect cell factories, and develop new biomaterials and high-value active molecules, providing revolutionary ideas for sustainable agricultural development. RNA pesticides are leading the third revolution in the history of pesticide development. The biosynthesis and green clean production of RNA pesticides are the priority development directions of global biomanufacturing layout, and the empowerment of synthetic biology is accelerating the rapid commercialization of RNA pesticides.
Shanxi Province has made a forward-looking layout, listing synthetic biology as one of the key industrial chains. It took the lead in unveiling and establishing the School of Synthetic Biology of Shanxi University in 2021. In 2023, it completed the construction of an integrated talent training system covering undergraduate, master's and doctoral programs, focusing on frontier exploration and innovation in directions such as gene editing and chassis organism construction, biocatalysis and green synthesis, and environmental microorganism development and pollution control. Shanxi University has jointly established the Modern Industrial College of Synthetic Biology with leading synthetic biology enterprises in Shanxi Province, namely Kaisai Biology and Jinbo Biology. It adopts a dual-track teaching model of "theory + practice" to promote the construction of an ecological closed loop of "teaching - research - industry" and help cultivate high-quality interdisciplinary talents. This report will explore how to combine Shanxi's characteristics with international frontiers, discuss the training of synthetic biology talents, promote agricultural synthetic biology research, and contribute core scientific and technological strength to the bio-economic transformation and green agricultural development in Shanxi and even the whole country.
Keywords: Agricultural Synthetic Biology;Cathay Biotech Inc.;Jinbo Biotech Inc.;School of Synthetic Biology of Shanxi University

A novel dsRNA virus parasitic in symbiotic fungus to enhance fungal virulence by suppressing biotin biosynthesis in honeybees

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Abstract: Mycoviruses pose a significant threat to biodiversity and natural ecosystems, yet their role in enhancing the virulence of entomopathogenic fungi-particularly in key pollinator insects-remains poorly understood. Here, using high-throughput sequencing we identified a novel mycovirus, Aspergillus tubingensis alternavirus 1 (AtAV1), isolated from the entomopathogenic symbiotic fungus Aspergillus tubingensis, a symbiont of the honeybee pathogen Ascosphaera apis. While transmission electron microscopy and co-inoculation assays revealed that AtAV1 infection alone had no obvious effect on the development of Asp. tubingensis and honeybees survival, metabolomic analyses demonstrated that AtAV1 disrupts biotin biosynthesis in A. tubingensis. Combined histopathological and metabolomic analyses revealed that this disruption cascaded into reduced biotin and fatty acid production in honeybees, triggering gut damage and elevated mortality. Transcriptomic profiling further indicated that AtAV1-induced biotin shifts contributed to the suppression of fatty acid metabolism and immune response disorder, revealing a mechanism for fungal virulence enhancement. Strikingly, field surveys detected AtAV1 in over 80% of sampled honeybee colonies across China, underscoring its potential role in widespread pollinator decline. Our findings elucidate the critical role of mycoviruses in modulating fungal pathogenicity and highlight their potential risks to pollinator health and ecosystem biodiversity.
Keywords: mycovirus; entomopathogenic fungus; hypervirulence; Apis mellifera; biotin biosynthesis

Bioconversion of Agricultural Organic Waste by Black Soldier Fly (*Hermetia illucens*)

Ling Tian

Genome-Scale CRISPR-Cas9 Editing and Synthetic Breeding in Silkworm

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Abstract: This study presents a genome-wide CRISPR-Cas9 editing platform combined with synthetic biology strategies to advance functional genomics and biological breeding in Bombyx mori (silkworm). We developed a piggyBac-mediated transposon system to stably deliver pooled sgRNA libraries into silkworm cells and embryos, enabling large-scale genetic screens. A library of 94,000 sgRNAs targeting 16,571 protein-coding genes was constructed for cell-based screens, identifying essential genes for cell viability under normal conditions. Context-specific positive screens further revealed key genes and pathways governing resistance to biotic (baculovirus) and abiotic (temperature, environmental pH, and environmental pollutants) stresses, as well as hormone signaling. In parallel, we generated the first large-scale CRISPR-induced mutant library in a non-model multicellular organism via microinjection of 66,650 embryos, establishing 1,726 transgenic lines and 300 phenotypically distinct mutants. Phenomic analysis uncovered genes critical for visual and economically valuable traits, such as silk production, survival, and cadmium tolerance. Our integrated approach merges high-throughput genome editing with synthetic biology principles, offering a versatile resource for annotating silkworm functional genomics and accelerating the design of genetically optimized strains for agricultural and industrial applications.
Keywords: CRISPR library;Synthetic Biology;SIlkworm

Nanodelivery system and RNA pesticide development

Shuo Yan

Development of Insect Gene Editing Tools and Their Applications in Synthetic Biology

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Abstract: Synthetic biological agriculture applies synthetic biology to innovate agricultural systems, supporting emission reduction and carbon sequestration. Insects represent sustainable protein sources and synthetic biology platforms due to their biomass production, reduced environmental footprint, and nutritional value. CRISPR/Cas9 editing systems are established for consumed species including Locusta migratoria and Gryllus bimaculatus. These insects require fewer resources and generate lower greenhouse gases per protein unit than conventional livestock, positioning them as candidates for future food and green manufacturing. Our team developed insect gene-editing tools and a standardized genetic engineering platform. We implemented in vivo and in vitro CRISPR/Cas9 activity assays to advance editing precision. Integrated microinjection, rearing, and mutant screening systems enable standardized genetic manipulation. Through promoter screening and gene-editing technologies, foreign gene expressions were achieved in insect embryos, providing tools for studying gene function and developmental and metabolic processes in non-model insects. This platform enables editing of immune genes to improve nutritional quality and growth performance of edible insects and utilizes engineered insect cells and living systems to efficiently synthesize high-value-added bioactive substances. Future work will exploit insect resources to advance insect-driven synthetic biological agriculture innovation, promoting regional green agriculture and bioeconomic development.

Keywords: Insect gene editing;CRISPR/Cas9;Synthetic biology;Sustainable protein;Standardized platform;Bioeconomic development

CRISPR-based gene drives to suppress Culex mosquito populations

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Abstract: *Culex* mosquitoes pose a significant public health threat as vectors of diseases such as West Nile and lymphatic filariasis, and also transmit pathogens threatening livestock, companion animals, and endangered bird species. Rampant insecticide resistance makes controlling these mosquitoes challenging and necessitates the development of novel control strategies. While gene drives have shown promise in other mosquito species, progress in *Culex* has lagged behind. Here, we developed a CRISPR-based genome editing toolkit and demonstrated the first successful implementation of gene drives in *Culex quinueafasciatus* by targeting two genomic *loci*. Building on this foundation, we engineered a population suppression gene drive targeting the *doublesex* gene, which efficiently converts genetic females into sterile intersexes, promoting male-biased inheritance and removal of fertile females from the population. The system achieved super-Mendelian inheritance (up to 71%) and generates partially dominant sterile resistance alleles via end-joining repair, producing intersexes with markedly reduced fertility and hatchability. This work establishes a self-limiting and scalable gene drive framework for *Culex* population suppression and highlights the potential of leveraging active genetics for sustainable control of insect vectors and agricultural pests.

Keywords: CRISPR-gene drive;Active genetic;Culex mosquitoes;Population suppression;Sustainable vector control strategies

Green biosynthesis using genetically engineered silkworms

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Abstract: The high capacity for protein biosynthesis in silk glands and efficient genetic manipulation technologies, including germ-line transformation and genome editing, makes the silkworm ideal as a bioreactor for producing exogenous proteins. Bombyx mori has been used to express exogenous proteins with potential biomedical applications, such as the human platelet-derived growth factor, epidermal growth factor, and fibroblast growth factor, as well as those with commercial applications including improving silk fiber mechanical properties through coexpression of spider silk proteins which enhance the tensile strength of silk fibers. In recent studies, we described different transformation strategies in genetically engineered silkworms to achieve custom-designed silk production. In addition, we also showed the ability to produce natural plant pigment and essential oil in transgenic silkworms by introducing multiple enzyme-mediated biosynthesis pathways.
Keywords: Bombyx mori ;germ-line transformation; genome editing; custom-designed silk production; plant pigment and essential oil biosynthesis

Development of RNA nanopesticides targeting immune genes of green peach aphid

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Abstract: Novel green technology RNA pesticide has been put forward as a promising method for plant protection, but the instability and short life disadvantage of double-stranded RNA (dsRNA) seriously constrain the commercialization process of RNA pesticide. Herein, a star polycation (SPc)-based nano system was constructed for co-delivering *hemocytin* (*hem*) dsRNA and botanical pesticide matrine to develop a novel multicomponent nano-pesticide for achieving sequential bioactivity against devastating green peach aphids. The self-assembled matrine/SPc complex could be further complexed with dsRNA through trostatic interaction and hydrogen bond to form nano-sized matrine/SPc/dsRNA complex. The screened ds*hem* fragment led to efficient gene silencing and high mortality rate through SPc-based topicaltion, and the main lethal mechanism was through down-regulating *hem* gene to result in severe bacterial infection. The ds*hem* fragment was successfully expressed in pET28-BL21 (DE3) RNaseIII-system to prepare matrine/SPc/dshem complex. Both initial acting time and persistence of matrine/SPc/ds*hem* complex were remarkably improved in field, which overcame the short life disadvantage of ds*hem* and slow-acting property of matrine simultaneously.
Keywords: RNAi;RNA pesticide;nanocarrier

CRISPR-based gene drive using engineered Cas9 variants

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Abstract: CRISPR-based gene drives have emerged as promising genetic tools for disseminating engineered traits into wild insect populations to achieve population modification and suppression. This could allow control of disease vectors, invasive species, and agricultural pests. Most utilize the SpCas9 nuclease, which recognizes guide RNA (gRNA)-complementary 20-bp genomic sequences adjacent to an NGG protospacer adjacent motif (PAM). However, the application of alternative Cas9 variants with improved editing fidelity and expanded targeting ranges remains largely unexplored. Here, we demonstrate proof-of-concept gene drives in Drosophila melanogaster using PAM-flexible Cas9 variants. We show that several of these, including eSpCas9 (1.1), LZ3, and SpG, achieve drive conversion efficiencies comparable to the original SpCas9 at NGG target loci. Furthermore, SpG and the near PAM-less SpRY demonstrate slightly reduced but still substantial drive conversion at NGA and NHN PAMs, respectively, where SpCas9 exhibits no cleavage activity. Our engineered Cas9 approach expands the CRISPR toolbox for gene drive design and broadens the range of editing. Specifically, this will allow targeting of conserved AT-rich regions and enhanced compatibility with tethered drive approaches, which can achieve confined population suppression.
Keywords: Gene drive;CRISPR nucleases;Genome editing

In Vivo Targeted Protein Degradation via an E2 Enzyme–Driven Nanobody Platform

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Abstract: Functional studies of genes and proteins often rely on knockdown using RNA interference (RNAi), which may be ineffective for proteins with long half-lives or when immediate depletion is required. Targeted protein degradation (TPD) offers a promising alternative. While most TPD methods exploit E3 ligases, E2 ubiquitin-conjugating enzyme–based strategies remain underexplored. Here, we developed degradE2-VHH05, an E2-driven TPD system where the E2 enzyme Eff is fused to the nanobody NbVHH05, which recognizes the VHH05 nanotag. This tool enables efficient protein degradation in Drosophila and mosquito cells. In vivo, degradE2-VHH05 rapidly degraded endogenous VHH05-tagged H2Av, outperforming RNAi. We show H2Av is essential for intestinal stem cell (ISC) homeostasis, as its depletion impairs proliferation and reduces response to bacterial infection. Transcriptomic analyses reveal distinct gene expression changes compared to RNAi, modulating stem cell pathways without triggering immune responses. Overall, our results suggest that, compared to RNAi, degradE2-VHH05 enables more efficient and rapid protein depletion while avoiding immune-related artifacts, highlighting the potential of E2 enzyme–based degradation as both a complementary and alternative strategy to conventional E3 ligase-dependent TPD.

Keywords: Drosophila;Protein degradation;NbVHH05;Eff;H2Av

Engineered Higiant enhances the bioconversion ability of Hermetia illucens

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Abstract: The black soldier fly (BSF), *Hermetia illucens*, has garnered attention for its proficiency in converting organic waste into valuable biomass. In the context of rapid population growth and urbanization, BSF with higher bioconversion efficiency is in urgent need. One promising approach to improve the efficiency is generating new lines by molecular breeding, which has succeeded in plants and various livestock. Here, we systematically screened and edited the BSF genes related to the growth and development. And we developed a BSF strain with an enhanced bioconversion efficiency using CRISPR/Cas9. By knocking out the gene *giant* (*gt*) in BSF, we obtained individuals with larger size and higher reproductive capacity. Notably, the mutant strain increased the bioconversion efficiency of food waste by 13.10%, and exhibited consistent performance across diverse organic waste substrates. In addition, our study elucidates multifaceted roles of *gt* in larval growth and developmental plasticity. Moreover, mutants showed reduced reproductive competitiveness against WT in mixed populations. In conclusion, our study provides a superior and safe insect chassis for biomanufacturing and underscores the potential of molecular breeding to enhance efficiency in BSF farming for sustainable organic waste recycling.

Keywords: Hermetia illucens;organic waste;molecular breeding;giant