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Chinese researchers develop spore-free Bt Chassis and structure-guided insecticidal protein discovery platform

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Chinese researchers combine a spore-free Bt expression chassis with AlphaFold3-based protein discovery to identify novel insecticidal proteins from diverse biological sources

Researchers at the Institute of Plant Protection, Chinese Academy of Agricultural Sciences, have developed a novel Bacillus thuringiensis (Bt) expression chassis that could help advance the next generation of protein-based biopesticides while opening a new pathway for discovering insecticidal proteins from non-traditional biological sources.

The research, published in Trends in Biotechnology under the title “A Bacillus thuringiensis AcdsR-based expression system for insecticidal proteins identified using AlphaFold3,” combines Bt chassis engineering with AlphaFold3-enabled protein structure prediction to address two longstanding challenges in microbial insecticide development: reducing the presence of live bacterial cells and spores in Bt products, and expanding the search for novel insecticidal proteins beyond conventional strain and sequence-based approaches.

Bt is among the world's most widely used microbial insecticides. Its insecticidal activity is primarily associated with Cry and other insecticidal proteins. Conventional Bt formulations typically contain mixtures of spores and insecticidal crystal proteins. While this approach has established Bt as an important biological pest-control technology, the environmental release of live bacteria and spores can add complexity to product safety assessment and application management.

The research team addressed this challenge by constructing a Bt expression chassis based on an AcdsR mutant. The engineered chassis separates insecticidal protein production from the bacterial sporulation process. It also incorporates a controllable cell-death mechanism that enables bacterial cells to be inactivated after production.

As a result, the system can generate a chassis free of live bacteria and spores while maintaining high-level accumulation of insecticidal proteins. By screening strong promoters to drive insecticidal protein expression, the researchers enabled the cells to continuously produce and accumulate target proteins while remaining in a non-sporulating state.

The resulting spore-free chassis could provide a platform for developing efficient, protein-based Bt biopesticides. Beyond improving the production architecture of microbial insecticides, the approach could potentially contribute to the development of new green crop-protection products with greater control over the biological components present in the final product.

The study also tackles a second challenge: how to discover insecticidal proteins that are substantially different from known Bt proteins.

Traditional insecticidal protein discovery has relied heavily on strain-resource screening, genome sequencing and sequence homology analysis. Although these approaches have successfully identified numerous insecticidal genes, they can be less effective when candidate proteins have substantial sequence divergence or originate from organisms outside conventional Bt resource pools.

The Chinese research team instead used protein three-dimensional structure as the starting point.

Using AlphaFold3, the researchers predicted protein structures and screened protein resources from diverse biological origins for candidates with structural characteristics resembling known insecticidal proteins. Candidate proteins were then subjected to heterologous expression and insecticidal activity testing using the AcdsR mutant Bt chassis.

The results indicated that potential insecticidal proteins are distributed across a broad range of organisms, including plants, animals, fungi and bacteria. Several candidate proteins originating outside Bt were efficiently expressed in the AcdsR chassis and demonstrated insecticidal activity against agricultural pests.

The findings establish a structure-driven discovery pathway that links structure prediction, candidate screening, heterologous expression and functional verification. In doing so, the research expands insecticidal protein mining from approaches primarily dependent on strain collections and sequence similarity toward a broader exploration of protein structural space.

This could significantly widen the pool of genes available for agricultural biotechnology. Novel insecticidal proteins identified through such approaches could ultimately support the development of new biological crop-protection products as well as provide additional genetic resources for insect-resistant crop breeding.

The Institute of Plant Protection, Chinese Academy of Agricultural Sciences, is the first affiliation on the study. Li Shiqing, a joint-training master's student at Northeast Agricultural University; Zhang Xin, a lecturer at Northeast Agricultural University; and Yan Tinglu, a doctoral student at the Institute of Plant Protection, Chinese Academy of Agricultural Sciences, are the co-first authors.

Professor Fan Dong of Northeast Agricultural University and Researcher Song Fuping of the Institute of Plant Protection, Chinese Academy of Agricultural Sciences, are the co-corresponding authors.

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The study highlights how advances in protein structure prediction and microbial chassis engineering are beginning to converge with agricultural biotechnology. By combining a spore-free Bt production platform with structure-guided discovery, the research could provide a new foundation for identifying and developing insecticidal proteins with applications in sustainable crop protection and insect-resistant crop breeding.