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B&B e-Paper No. 11:

Biofertilizers and Biopesticides (B&B) for Sustainable Agriculture No. 11

The rising star of Photobacterium: Both insecticidal and antibacterial effects

Photorhabdus luminescens, formerly *Xenorhabdus luminescens*, is an insect pathogenic gram-negative rod-shaped bacterium of the family Enterobacteriaceae, with the characteristic of forming a symbiotic relationship with *Heterorhabditis* spp. *P. luminescens* was isolated from light-emitting insects that had been infected by entomogenous nematodes of the family Heterorhabditidae. The entomopathogenic nematodes family Heterorhabditidae contains only one genus, Heterorhabditis, which is, at least currently, composed of 10 recognized species. The entomopathogenic *Photorhabdus* lives in symbiosis with nematodes that invade insects.

When the nematode is ingested by insect larvae, *P. luminescens* are carried in the gut of the infective larvae of the nematode and released directly into the haemocoel when the nematode invades an insect host. The bacterial cells start to multiply and septicaemia sets in killing the insect within 24–48 h. In addition, the bacterium can be grown as free-living organisms under standard laboratory conditions. Indigenous entomopathogenic nematodes are perhaps more suitable for inundatory release against local insect pests, because they have adapted to local climate and other population regulators. In Taiwan, information on the diversity of Heterorhabditidae and their symbiotic bacteria is currently absent; only Steinernematidae have been documented.

Started from 2004, the Taiwan Agricultural Chemicals and Toxic Substances Research Institute (TACTRI), Dr. Hsieh has been implementing the study on the secondary metabolites from symbiotic bacteria for more than 15 years. *H. brevicaudis* were successfully recovered from soil samples using the *Galleria*-bait method. Identification of the nematode was mainly based on observations under scanning electron microscopy (SEM) and nucleotide sequence of the internal transcribed spacer 1 (ITS1). The occurrence of *H. brevicaudis* and its symbiotic bacteria, *P. luminescens* subsp. *akhurstii* in Taiwan were recorded the first time. *P. luminescens* subsp. *akhurstii* was isolated from nematodes and identified by phenotypic, biochemical tests, 16S rDNA and Biolog identification system. 13 strains of symbiotic bacteria were successfully isolated from *H. brevicaudis*, among which the *P. luminescens* strain 0805-P2R has the most potential development for microbial agents.





Fig. 1. *H. brevicaudis* of entomopathogenic nematodes were successfully recovered from soil samples using the *Galleria*-bait method.





Fig. 2. P. luminescens (left) and H. brevicaudis (right) examined by electron microscopy

The metabolites and toxins from *P. luminescens* exhibited a wide spectrum of antimicrobial and insecticidal activity against native pests and plant pathogens. The fermentation broth and crude extract of *P. luminescens* could have antibacterial activities against *Bacillus cereus*, *Escherichia coli*, *Erwinia carotovora* subsp. *carotovora* and antifungal activities against *Botrytis cinerea*, *Glomerella cingulata*, *Alternaria mali*, *Phytophthora capsici*. Supernatant fluid of the *P. luminescens* culture was centrifuged, filtered and protein preparation on 18 species of fungi and 12 species of bacteria were examined by means of dual or concomitant culture methods. High antimicrobial activities against *Plutella xylostella and Glomerella mellonella* were observed. The LC50 values of protein preparations (greater than 100 kDa) against the 3rd instar larvae of the lepidopteran *P. xylostella* and *G. mellonella* were 56 and 200 ppm, respectively.

P. luminescens kills insects through the secretion of high molecular weight toxin complexes. The mortality rates of *P. xylostella* observed after diluting the *P. luminescens* strain 0805-P2R fermentation broth 50 times and diluting the supernatant 5 times were

18.89% and 91.11%, respectively. The supernatant of *P. luminescens* strain 0805-P2R had insecticidal activity, and the main insecticidal toxin complexes had a molecular weight exceeding 70 kDa. Moreover, the insecticidal activity was improved to 92.2% in the 100-fold diluted fermentation broth and to 97.8% in the 10-fold diluted supernatant in the experiments. All combinations tested showed clear indications of lethality, including swelling, vesicle formation, cytoplasm vacuolization, and brush border membrane lysis.

In addition, *P. luminescens* strain 0805-P2R suspension concentrate, a 500X dilution can effectively reduce the leaf pest incidences of papaya *Tetranychus kanzawai*. Thus, *P. luminescens* 0805-P2R as a potent microbial pesticide with bioactivity against insect pests and pathogens of agricultural importance in Taiwan. The large-scale fermentation and application technology for *P. luminescens* strain 0805-P2R have been transferred to a local company, Sinon Corporation in 2020.



Fig. 3. *P. luminescens* cultured on MacConkey and LA agar plate have red colonies (left) and white colonies (right), respectively.



Fig. 4. Moderate and severe damage to soybean leaves from *T. kanzawai* Kishida (left) and *Tetranychus urticae* Koch (right).



Fig. 5. Treatment group (A) and control group (B) show a significant difference on control effect of anthracnose in mango by using fermentation broth with *P. luminescens*.



Fig. 6. A supervised field trial of *T. kanzawai* infested papaya leaves was conducted with *P. luminescens* treatment.



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