

Indigenous Biopesticide takes on agricultural pests

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ICGEB scientists have developed "Bioprahar", a microbe-based biopesticidal formulation effective against a range of pests of agronomic importance.

Scientists at the New Delhi-based International Centre for Genetic Engineering and Biotechnology (ICGEB) have developed a bacterial formulation for the control of a range of agricultural pests. Named as "Bioprahar", the formulation is being commercially launched by Nirmal OrganoBiotech Ltd, its industry partner, in 2005. The formulation has been found effective in controlling diamond-back moth in cabbage and cauliflower; white woolly aphids in sugarcane; mealy bugs in grapes, citrus and mango; and white ants in teak plantations.

The active ingredient of the biopesticide formulation developed by ICGEB is *Photorhabdus luminescens* sub sp. *akhurstii*, a bacterium, which resides in the intestinal tract of nematode *Heterorhabditis indica*. This bacterium secretes several extracellular macromolecules, which are lethal to pests.

Due to their mutualistic association with *Photorhabdus* bacteria, nematodes of the genus *Heterorhabditis* are well known parasites against insect pests. They are commonly used in pest management programs to control soil borne pests. The modus operandi of this symbiotic pair is that on entering the insect, *Photorhabdus* is released into the host gut where they multiply rapidly and kill the insect. They also degrade the insect upon which the nematodes feed and multiply.

Taking this lead forward, scientists (Dr Raj Bhatnagar and Dr Raman Rajagopal) of the Insect Resistance Group at ICGB, isolated and characterized the bacterium strain from a Heterorhabditis nematode species prevalent in the southern part of India and evaluated its efficacy against several pests. In parallel, the researchers purified and studied the insecticidal toxin complex produced by the bacterium. Their studies showed that this complex is lethal to pests and is responsible for the death of the insect.

After having finished this groundwork, came the tricky task of making an effective formulation. Speaking about this uphill task, Dr Bhatnagar said, "The biggest challenge was to make a viable formulation, which does not require a cold chain. Though initial studies showed good results, application in the open fields remained a challenge. We prepared a liquid formulation consisting of the bacterium and the growth medium, but its shelf life was very short. The formulation was then further fine-tuned and stabilized with emulsifiers and stabilizers so that it remains biologically active for a longer duration".

"Presently the biopesticide formulation consists of the bacterium, surfactants and adjuvants. Its shelf life is over a year with no drop in the potency or efficacy of the formulation," he added.

The field tests were conducted in agriculture fields together with Dr NGV Rao at Punjabrao Deshmukh Krishi Vidhalaya (PDKV), Akola against the diamond-back moth pest of cauliflower and cabbage; Vasant Dada Sugar Institute, Pune against the white wooly aphids of sugarcane; and the fields of commercial alliance partner Nirmal Biotech against mealy bugs of grapes, mangoes and citrus. A specific regimen of spraying on each crop was developed and the results obtained after two growing seasons revealed excellent control of the targeted pests. Overall the efficacy of the formulation was comparable to any chemical pesticide.

Significantly the application of chemical pesticides in protection of cabbage and cauliflower from pests is second to cotton. It has been shown that "Bioprahar" effectively controls these pests and will thus reduce usage of chemical pesticides in these crops to a large extent.

Similarly, sugarcane production in India has suffered immensely during the last couple of years due to the white wooly aphids. "Our field evaluation data, at six districts in Maharashtra revealed effective control of white wooly aphids. This formulation will minimize the problems faced by sugarcane farmers because of wooly aphids. Nearly 90 percent control of the white wooly aphids was seen in sugarcane fields," explained Dr Bhatnagar.

The effective control of mealy bug in the case of citrus, grape and mango is expected to promote organic farming paradigm and make the production export competent. In addition, the biopesticide displayed excellent control of termites. It was found that the whole colony of white ants collapsed in 24-48 hours.

Simultaneous evaluation of mammalian toxicity at two different independent laboratories, PDKV, Akola and National Toxicology Centre, Pune, demonstrated the safety of formulation based on standard tests. The formulation did not show any allergenicity, toxicity or any other side effects against rabbit, mice or rats.

The patent for "Bioprahar" and "Biwooly Kill", a related formulation for white wooly aphids, has been filed in India and PCT countries in 2004.

Rolly Dureha

Personal genome sequencing possible for \$1000 soon

A step toward the \$1,000 personal genome using readily available lab equipment

The theoretical price of having one's personal genome sequenced has fallen from the prohibitive \$20 million dollars to about \$2.2 million, and the goal is to reduce the amount further to about \$1,000 to make individualized prevention and treatment realistic.

The sharp drop is due to a new DNA sequencing technology developed by Harvard Medical School (HMS) researchers Jay Shendure, Gregory Porreca, George Church, and their colleagues. Their findings have been reported on August 4 in the online edition of Science. The team sequenced the E. coli bacterial genome at a fraction of the cost of conventional sequencing using off-the-shelf instruments and chemical reagents.

The Church group's technology is based on converting a widely available and relatively inexpensive microscope with a digital camera for use in a rapid automated sequencing process that does not involve the much slower electrophoresis, a mainstay

of the conventional Sanger sequencing method.

"Meeting the challenge of the \$1,000 human genome requires a significant paradigm shift in our underlying approach to the DNA polymer," write the Harvard scientists.

The new technique calls for replicating thousands of DNA fragments attached to one-micron beads, allowing for high signal density in a small area that is still large enough to be resolved through inexpensive optics. One of four fluorescent dyes corresponding to the four DNA bases binds at a specific location on the genetic sequence, depending on which DNA base is present. The fragment then shines with one of the four colors, revealing the identity of the base. Recording the color data from multiple passes over the same sequences, a camera documents the results and routes them to computers that reinterpret the data as a linear sequence of base pairs.

In their study, the researchers matched the sequence information against a reference genome, finding genetic variation in the bacterial DNA that had evolved in the lab.

"These developments give the feeling that improvements are coming very quickly," said HMS professor of genetics, Church, who also heads the Lipper Center for Computational Genetics, MIT-Harvard DOE Genomes to Life Center, and the National Institutes of Health (NIH) Center for Excellence in Genomic Science.

"The cost of \$1,000 for a human genome should allow prioritization of detailed diagnostics and therapeutics, as is already happening with cancer," Church said.

Source: Harvard Medical School

DNA buckyballs for drug delivery

Researchers at the Cornell University have made DNA buckyballs - tiny geodesic spheres that could be used for drug delivery and as containers for chemical reactions.

The researchers are making buckyballs out of a specially prepared, branched DNA-polystyrene hybrid. The hybrid molecules spontaneously self-assemble into hollow balls about 400 nanometers (nm) in diameter. The DNA/polystyrene "rods" forming the structure are each about 15 nm long.

About 70 percent of the volume of the DNA buckyball is hollow and the open spaces in the structure allow water to enter. Dan Luo, Cornell assistant professor of biological and environmental engineering in whose lab the DNA structures were made, suggested that drugs could be encapsulated in buckyballs to be carried into cells, where natural enzymes would break down the DNA, releasing the drug. "They might also be used as cages to study chemical reactions on the nanoscale," he said.

The nanoscale, hollow buckyballs are also the first structures assembled from "dendrimer like DNA." If three strands of artificial DNA are created such that portions of each strand are complementary to portions of another, the three strands will bind to each other over the complementary portions, creating a Y-shaped molecule. By joining several Ys in the same way, Luo's research group created molecules with several arms, a sort of tree shape (dendri- means tree in Greek). Then they attached polystyrene molecules to the dendrimerlike DNA forming a hybrid molecule called an amphiphile - a molecule that both likes and hates water. DNA is hydrophilic - attracted to water - while polystyrene is hydrophobic - water repels it.

The researchers expected the amphiphiles to assemble in water into some sort of solid structure arranged so that DNA would have a maximum interaction with water and polystyrene would avoid water as much as possible. Other researchers have used other amphiphiles to make spheres, rods and other solids. The hollow buckyballs were an intriguing and serendipitous surprise. A model suggests that one buckyball consists of about 19,000 amphiphiles, with their water-loving DNA mostly on the outside of the rods that form the structure. How these tens of thousands of molecules were able to self-organize to form such an intricate and complex structure is still an open question, the researchers say. They are seeking collaborators to solve the puzzle.

Source: Cornell University