



The Contribution of Microbial Origin as Herbicides

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INTRODUCTION

Microbial Herbicides

The microbial origin of herbicides is definitely a major contribution and an alternative to chemical weedicides. Some of the successes with exotic pathogens have been the use of *Puccinia chondrillina* from Southern Europe to control skeleton weed *Chondellia juncea* in Austin, while the *Cercospora riparia* to control *Ageratina riparia* in Hawaii from Jamaica and the use of introduced rust *Phragmidium violaceum* to control wild blackberry *Rubus sp.* Similarly, the use of *Cercospora rodmanii* to control water hyacinth and *Colletotrichum gloeosporioides* to control *Aeschynomene virginica*, the host specific pathotype *Phytophthora cirrospora* to control mill-weed vine *Morreria odorata* have been established. Such type of weedicides needs a close cooperation, whereas the use of endemic microbial herbicides needs cooperation from various agencies.

Bacterial Insecticides

Bacteria are often associated with plant and human diseases. However, there are certain bacteria such as *Clostridium acetobutylicum* acetone-butanol production, *Bacillus licheniformis* antibiotic-bacitracin production, *Bacillus megaterium* vitamin B12 production and *Pseudomonas fluorescens* 2-ketogluconate, are some of them which are used for industrial product formation. In addition, there are certain bacteria which are of immense importance to mankind. They are pathogenic to insects, pests and other pathogens and kill these wide range of parasitic organisms. The majority of bacteria isolated from insects can be regarded as facultative pathogens. Interests in the use of bacteria as biological control agent was stimulated by an increasing number of insects and pests.

Pseudomonads as Bacterial Insecticides

Pseudomonas aeruginosa is among the most frequently described pathogen causing disease in insects. It is still unknown that certain strains pathogenic to insects by feeding differ from those that are potentially pathogenic to man. *Pseudomonas aeruginosa* that produces toxic enzymes, has also been used as a model organism to study the mechanism of insect pathogenicity and immunity. The non-fluorescent Pseudomonads isolated from insects include *Pseudomonas alcaligenes*, *Pseudomonas cepacia*, *Pseudomonas melophilia* and *Pseudomonas acidovorans*.

Pseudomonas cepacia is known to be a versatile bacterium of soil as a plant pathogen and a human pathogen as well as a broad spectrum antagonist to plant pathogens through the production of various types of antibiotics such as pyrrolnitrin. *Pseudomonas cepacia* is a Gram-negative bacterium that has been reported to produce siderophores. This bacterium also acts as plant growth promoting rhizobacteria. Suppression of plant diseases may involve secretion of siderophores or antibiotics and aggressive root colonization by organisms that displace or exclude deleterious rhizosphere microorganisms. *Pseudomonas fluorescens* is one of the most important biological control agents of many plants' disease-causing organisms. These are also common PGPR that secrete siderophores. Seed inoculation with these organisms helps in inducing growth and suppression of diseases. It produces fluorescent siderophores called pyoverdine or pseudobactrin which is characteristic of the fluorescent Pseudomonads. The siderophores are low molecular mass, water soluble, high affinity Fe chelators. Siderophores are secreted under iron-limiting conditions as a means to secure available iron present at low concentration in soil. The ability of certain pseudomonads to utilize a wide range of ferric siderophores as a source of metabolic iron may contribute to their competitiveness and survival in the soil.

Bacterial Insecticides

Bacillus thuringiensis

This bacterium is now widely known as Bt. It is the most important bacilli reported to kill a wide range of insects like moths, beetle, mosquitoes, flies, aphids, insects, ants, termites, midges, butterflies even some pathogenic fungi such as *Pythium ultimum* and *Fusarium axysporum* depending upon the host strains of the bacterium. The Bt strains are pathogenic to cockroaches, snails and protozoans. Due to a wide range of host killing. It occupies a tremendous significance in agriculture due to exotoxin and endotoxin production and its related ease of mass production in submerged fermentation on relatively cheap media besides many chemical compounds designed for use in controlling economically and biomedically important insects.

The Bt bacterium was first discovered by a Japanese scientist Ishiwata in 1902 who isolated a bacterium from diseased or unhealthy silk larvae, and was named as *Bacillus satto*. In the year 1912, a German Microbiologist also isolated this bacterium from infected insects from a flour mill. He named the bacterium which caused the insect disease, as *Bacillus thuringiensis*. The importance of this organism was realized and development of commercial formulation of Bt as biopesticide in USA was carried out in 1960. Now hundreds of Bt strains are commercially available round the world and all the major insect pests are susceptible to these strains,

The mechanism of action of Bt endotoxins on insect is quite interesting. The endotoxin is the protein. The crystalline proteins upon ingestion by the insect larvae are solubilized under highly alkaline conditions prevalent in the midgut. The toxins are digested by the enzymes, called proteases into active fragments. These active fragments bind to receptor proteins present in the gut epithelial membrane. Upon binding, the toxin molecules form pores in the gut membrane. As

a result, the osmotic equilibrium of the cell is disturbed, the cells swell and burst. This results into the death of insect larvae.

Now, more than 50 Bt genes have been isolated, cloned and characterized. Making use of microorganisms and genetic engineering, transgenic microbes and transgenic plants are developed. Many crop plants are colonized by harmless bacteria. Such bacteria are identified and genetically transformed by vectors carrying Bt genes. These bacterial formulations are then sprayed on the crop which provide a protective cover to the crop. Certain crop plants are also being protected against insects by genetic mediation e. transgenic plants resistant to insects with Bt genes for example bollworm resistant cotton, stem borer resistant rice, corn borer resistant maize, potato beetle and tuber moth resistant potato, tomato resistant to pinworm, etc. At IARI, New Delhi, Bt transgenic cabbage and cauliflower plants have been developed. Apart from crop plants, many forest tree species are also being transformed using Bt genes. Now Bt technology is being used in expressing two different kinds of Bt genes in transgenic plants or microorganisms. This technique helps in preventing insects from developing resistance to Bt toxin proteins.

Bt formulations are being used to control *Aedes* and *Anopheles* mosquitoes, black fly which spread yellow fever, malaria and blindness respectively. The Bt genes are being expressed in aquatic bacteria and cyanobacteria so that during their propagation in lakes, the breeding of mosquito could be checked. Scientists in Anna University, Chennai are engaged in research on Bt. Bt strains are also being developed towards killing of liver flukes, tapeworms, etc. which cause disease in cattle and human.

Toxins produced by *Bacillus thuringiensis*

Bt produces several toxins, four of which will be considered here alpha exotoxin heat labile toxin, beta exotoxin fly-factor or heat-stable exotoxins, delta endotoxin crystalline toxin or just crystal or parasporal body and louse

factor. The alpha exotoxin identifies as lecithinase water soluble, heat labile and toxic to insect while, beta exotoxin defined as adenine nucleotide and ATP analogue and given the name "thuringiensin" which is water soluble heat stable toxin. The delta endotoxin in the crystal of Bt has a limited activity spectrum. It is produced during sporulation. In 1974, Ginrich et al reported that four species of mammal-biting lice were susceptible to powders containing the spore endotoxin complex of Bt-var Kurstaki (HD-1), an isolate of Bt that does not produce exotoxin, a toxin to which these lice are susceptible. It seems improbable that the endotoxin could be responsible for the action. Hence, the toxicity to lice was due to a new chemical which was called the "louse-factor"

Insect Controlling Bacteria

Among the species of *Bacillus*, there are ather obligate spore-forming bacteria such as *Bacillus papilliae* and *Bacillus lentimorbus*, facultative spore former such as *Bacillus cereus* which are pathogenic to insect. The control of Japanese beetle by inducing milky disease with *Bacillus lentimorbus* and *Bacillus papilliae* have been reported. *E. coli* is reported from flies as surface contaminant. The strains of *E. blattae* has been isolated from cockroach. Strains of *Enterobacter*, *Serratia* and *Klebsiella* are frequently reported as facultative insect pathogens.

Serratia liquefaciem and *Serratia marcescens* control lab insects in culture due to un satisfactory nutrition and physical conditions in the maintenance. *Alcaligenes odorans* has been isolated from insects where this bacterium acts as facultative pathogen.

Bacteria associated with nematodes

Relationship between the insect (host) and the nematode gut has been studied by Poiner (1979). Nematode larvae entering an insect body were reported to cause septicaemia by dissolving the insect gut wall and thus introducing bacteria to the haemolymph. An entomocidal insect-killing bacterium named *Achromobacter nematophilus* was described

initially from the nematode *Neoplectana carpocapsae* and regarded as a commensal symbiont of the nematode. The nematode genera, *Neoplectana* and *Heterorhabditis* carry in its gut bacterial species named *Xenorhabdus nematophilus* and *Xenorhabdus fluorescens* belong to family Enterobacteriaceae. Even one bacterium from each species is enough to kill insect. The advantage of using bacterial insecticides are: (i) permanency. (ii) economic feasibility due to low cost, (iii) environmental safety. (iv) absence of development of resistance.

Virus Insecticides

There are viruses or their products commercially exploited in place of chemical insecticides. Viruses of the family Baculoviridae are pathogenic to arthropods. Viruses contain lipid envelope with circular, double stranded DNA genome of 80-150 Kilo base pairs depending upon the viruses. Naked viral DNA is infectious per se. Baculoviruses are restricted in their host ranges. They do not infect vertebrates, non-arthropod invertebrates, microorganisms or plants. Indeed, baculoviruses infect only a few arthropod species. Their use dates back to the 19th century. But the commercial use of virus insecticides has been limited by their high specificity limited host range and slow action. Viruses most frequently considered for control of insects (usually saw flies and lepidoptera) are the occluded viruses, namely NPV, cytoplasmic polyhedrosis (CPV), granulosis (GV) and entomopox viruses (EPN).

Entomopathogenic Fungi

Many entomopathogenic fungi overcome their hosts only after limited growth in the haemocoel so toxins are presumed to cause host death. The importance of toxins to the virulence of an entomopathogenic fungus is difficult to evaluate. Because toxin production in the host must be preceded by following activities: (i) attachment of the infective unit on the cuticle, (ii) germination of infective unit, (iii) penetration followed by formation of germ tube and appearance of infection pegs from appressoria, (iv) multiplication of hyphal bodies in the haemocoel, (v) production of

toxic metabolites, (vi) death of the host, (vii) growth in the mycelial phase with invasion of all host organs, (viii) penetration of hyphae from the interior through the cuticle to the exterior of the insect, (ix) production of infective units on the exterior of the insects.

Metarhizium anisopliae

This fungus produces destruxin and desmethyl destruxin in silk worm larvae. These toxins are referred to as vivotoxins. In addition to low molecular weight compounds, proteases and other enzymes are produced by entomopathogenic fungi. Injection of *Entomophthora* species cultures into *Galleria mellonella* larvae caused blackening similar to that noted in infected larvae.

Metarhizium culture filtrate is toxic to coleoptera haemocytes in vitro producing changes in organelles. The extract of mycelium is toxic if it comes in the contact of adult house fly. The solvent extracts of *Metarhizium* kill silk worms if it is injected into intra haemocoelic injection. Six cyclodepsipeptides with five-member amino acids viz., B-alanine, alanine, valine, isoleucine and proline have been isolated from filtrates of *Metarhizium anisopliae* cultures.

Cytichalasin are the other fungal metabolites. Their origin is from phenylalanine or tryptophan in col to or C, polyketide chain. Their activity includes inhibition of cytoplasmic cleavage cultured mammalian cells.

Beauveria bassiana and *Beauveria brongriartii*

Beauveria bassiana and *Beauveria brongriartii* are the other fungal species that attack insects, Beauvericin is a depsipeptide. It comprises of a cyclic repeating sequence of 3 molecules of N-methyl phenylalanine alternating with three molecules of 2-hydroxy isovaleric acid. It has also been isolated from mycelium of *Paecilomyces fumosoroseus*.

Beauverolides and bassianolide are the other cyclodepsipeptides. The beauverolides H and I were isolated from *Beauveria hasziana*, while bassianolide which is composed of 4 molecules each of L-N methyl leucine and D-a-hydroxy isovaleric acid has been isolated from *Beauveria brongriart*. The sorolides A,

B, and C are also cyclodepsipeptides which were found in *Beauveria brongriartii*. Two very similar pigments, tanellin and bassianin, produced in vitro by some strains of both *Beauveria bassiana* and *Beauveria brongriartii* are concentrated in the mycelium rather than released into the medium. The dibenzoquinone pigment oosporein, produced by many isolates of *Beauveria*, probably accounts for the reddish colour of infected caterpillars. *Beauveria brongriartii* converts 20% of the original solids in a peptone medium into oxalic acid. This acid is a general poison. Oxalate crystals have been noted on the surface of insects killed by *Beauveria bassiana*.

Verticillium lecanii

Verticillium lecanii is non-fastidious and can grow on all conventional media meant for culturing of fungi. The most frequently recorded hosts are scale insects and aphids. Much less reports of hosts have been recorded in other orders: *Verticillium lecanii* sometimes hyper parasitizes phytopathogenic fungi, mostly rusts and powdery mildews.

The control of aphids and scales is possible by using *Verticillium lecanii* conidia or blastospores suspended in phosphate buffer containing 0.02% Triton X-100 as wetting agent. After evening sprays, plants were covered with polythene blackout sheets to restrict day length for flower initiation. The appraisal of *Verticillium lecanii* as a microbial insecticide is based on the choice of infectious material i.e. between conidia and blastospores. Production of conidia on agar is too expensive and also difficult to ensure culture purity. Alternatively, the conidia can be produced on a cheap granular solid substrate. *Verticillium lecanii* is reported to be a promising biological control agent against aphids of chrysanthemum in green houses.

Hirsutella thompsonii

Hirsutella thompsonii a deuteromycetous fungus, is a potential killer of citrus rust mite *Phyllocoptrula oleovora*. Most of the species are pathogenic to invertebrates. The fungal strains have also been reported from the citrus

bad mite. *Eriophyes sheldone* and coconut flower mite, *Eriophyes guerreronis*. In the USA, a commercial formulation of conidia has been introduced by Abbott Laboratories, North Chicago, Illinois. *Hirsutella thompsonii* can be cultured on agar media. The spore suspension is mixed in semisolid medium containing wheat bran (60 g) and distilled water (60 ml) to which 250 ppm per litre of chloramphenicol is added. The flasks are incubated at 25°C for 2 weeks to get bran fungal mat. It is air dried and blended, finally stored at 18-20°C.

Nomuraea rileyi

It is the Ascomycetous fungus for which first effort of mass-production was made for applying as entomopathogenic agent. This fungus usually is found to induce extensive epizootics in caterpillar pests on cabbage, clover, soybean and velvet beans and thus is a potential agent for use as a microbial insecticide. The natural epizootics were observed on *Bombys mori*, *Peridroma saucia*, *Leptinotarsa decemlineata*. The unidentified compounds were extracted from mycelium produced in submerged culture.

The other fungi

Aspergillus ochraceus, *Paecilomyces fumosaroneus*, *Fusarium solani* *Trichoderma harzianum*, *Trichoderma reesei*, *Gliocladium virens*, etc. have also been reported for other microbial pesticides.

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