Microbial Pesticides and Natural Products as Alternatives

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Introduction

There is a long history of hunting for new natural products for pharmaceutical purposes. For example, penicillin, cyclosporin and streptomycin are natural products from microorganisms. However, the efforts dedicated to screening of microbial natural products for pesticides is small compared to pharmaceutical natural product programs.

Approximately 400 insecticidal/miticidal, 30 herbicidal, and less than 20 nematicidal microbial natural products are known, compared to the tens of thousands of known pharmaceutically active natural products from microorganisms. The technology for discovering and commercializing microbial and other biopesticides, however, is proven and in some cases quite powerful.

Many plant and microbial natural products have become commercial pesticides (for example, pyrethrins and synthetic pyrethroids from *Pyrethrum* daisy, azadiractin from the neem tree, avermectin from the microbe *Streptomyces avermitilis*, and spinosyn from the microbe, *Saccharopolyspora spinosa*). The natural world can provide alternatives to existing chemicals both in the short and long term. We have only scratched the surface. Out of 1.5 million species of fungi, only 73,000 species have been described. A small percentage of these have been tested in pesticidal screens. In addition, other than *Bacillus thuringiensis*, only a small number of strains of known genera have been tested as pesticides.

Microbially-Derived Pesticides

Complex Fermentation Products

Avermectin (Avid®, Agrimek®) is the most successful natural product pesticide (sold by Merck to Novartis). It is a purified natural product compound with a complex structure, produced in fermentation by the microbe, *Streptomyces avermitilis* (structure shown below). It controls mites, leafminers, and cockroaches. A second generation product, emamectin, with improved caterpillar activity, is being launched by Novartis.

Dow Agrosciences has launched spinosyn (or spinosad), the common name for a purified, complex natural product compound produced in fermentation by the microbe, *Saccharopolyspora spinosa* (structure shown below), with excellent activity on caterpillars, leafminers, thrips, certain mites and some other insects. It has an excellent toxicological and environmental profile. This could become the country's largest selling fruit and vegetable insecticide within five years and may prove, in the long run if resistance can be managed, more profitable than Dow AgroSciences' major insecticide, chlorpyrifos. Dow AgroSciences also promises a series of similar naturalyte products and other companies have recently accelerated their efforts to more systematically assess the enormous diversity that exists in nature.

Both avermectin and spinosyn were discovered from pharmaceutical screening programs. Certainly there are more molecules like avermectin and spinosyn to be discovered. To find more natural product molecules like this, natural product screening programs must be sustained over several years (rather than stopping and starting with different scientists each time), dedicated specifically to pesticide discovery, not just spillovers from pharma programs. Opportunities to formulate mixtures of microbial and natural products, possibly in conjunction with pheromones, are just beginning to be seriously explored.

Bacillus thuringiensis (B.t.)

The efficacy and reliability of *Bacillus thuringiensis* has improved dramatically over the last five years. When it was first discovered and commercialized as a bioinsecticide, the first products were not optimized for stability, formulation and manufacturing. With a tiny investment compared to industrial investment in chemical pesticides, B.t. is now a mainstay of many integrated pest management programs (Starnes et al., 1993). Remarkable, there were more acre-treatments made with B.t. than any OP by vegetable growers surveyed by USDA in 1996 (pesticide use data provided by C. Benbrook, and are derived from NASS surveys). Only two insecticides were applied more often – methomyl (2 million acre-treatments) and permethrin (2.1 million). Methamidophos was the most frequently applied OP, with 890,000 acre treatments, essentially all on tomatoes and potatoes. There were 1.3 million B.t. acre treatments across a wide range of vegetable crops – three times more acre treatments than chlorpyrifos and more than twice imidacloprid's use.

On fruit crops, B.t. ranked fourth among all insecticides in 1997 in terms of acre treatments – behind azinphos methyl, chlorpyrifos and avermectin. Many people dismiss B.t.'s commercial success because so few pounds are applied. With the cost per acre treatment down to $10.00 to $20.00 and product stability and efficacy improving, B.t. clearly has a promising future, especially as broad spectrum insecticides fall by the wayside as a result of resistance, regulation, or grower concern over health and ecological impacts.

Other microbes now in development or recently commercialized (e.g., *Beauveria, Metarrhizium*) could be improved through formulation and fermentation much in the same way as B.t.

There are many examples of novel approaches to improving B.t. performance and reliability, through strain improvement, development of larger crystal proteins (multiple copies of the protein gene) and other methods. A specific example is the discovery of Zwittermicin A, a novel fungicidal compound discovered by the University of Wisconsin (He, et al., 1994) that is produced by *Bacillus cereus* and other Bacilli (Figure 2). Novo Nordisk Entotech, Inc. discovered that this compound is an enhancer of B.t., and is produced in certain B.t. strains at very low levels. (Manker et al., 1992; Manker et al. 1995). When the compound is combined at higher levels with the B.t. protein, there is significant enhancement of efficacy in the field against the hardest to kill caterpillars such as *Helicopverpa zea* and *Spodoptera exigua*. Other opportunities for natural-product based potentiation are bound to emerge.

Synthetic Leads

Agrichemical companies have successfully used natural products as sources of promising molecules to develop into new synthetic chemicals. Many old chemicals are based on plant natural products (e.g. pyrethroids, carbamates). Strobilurin compounds, a key class of new biofungicides, were discovered by a university from a mushroom fungus and found, in their natural state, to be too unstable. Several companies made synthetic derivatives to improve the stability and are now launching commercial products based on this chemistry.

BASF and Zeneca have successfully introduced products, each with slightly different spectrum on plant diseases. Zeneca's azoxystrobin was approved by US EPA as a reduced risk chemical pesticide. Strobilurin derivatives are expected to reach $500 million in revenue in the next five years and take the lead as the largest selling chemical fungicide class. American Cyanamid's pyrrole compounds are also derived from a microbial natural product and are expected to replace many older chemical insecticides.

Like the complex fermentation products, there is great potential to continue to find new reduced risk synthetic leads derived from natural products.

Figure 1. Examples of known, successful natural products produced in fermentation, engineered into plants or chemically synthesized.

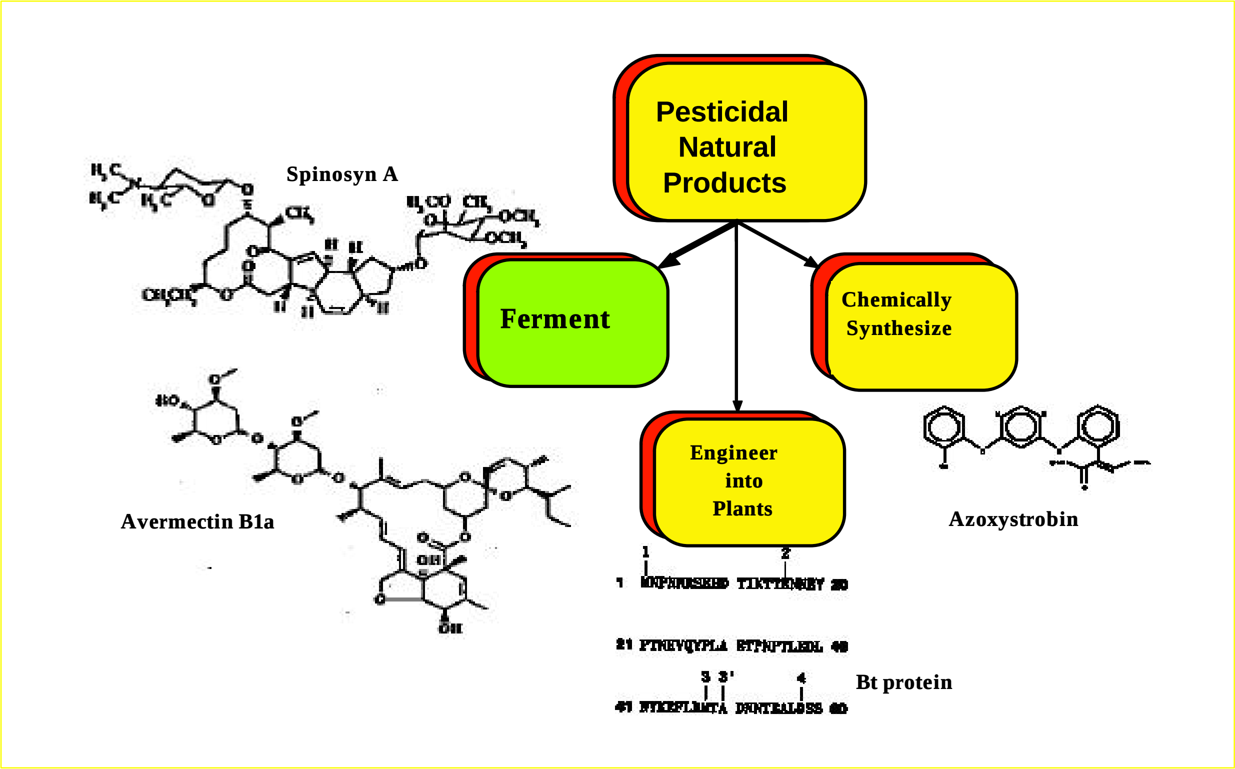
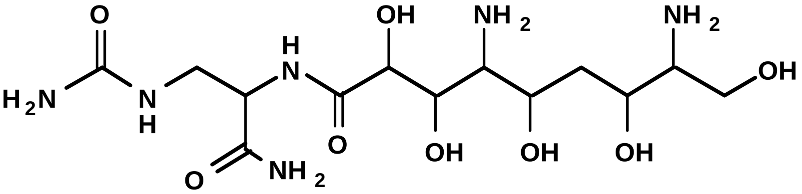


Figure 2. Structure of Zwittermicin A, which was found to enhance the efficacy of *Bacillus thuringiensis*.



Bacteria as Biofungicides

The use of *Bacillus* as a biocontrol agent for controlling plant diseases has been researched in academic laboratories for over 20 years (Blakeman and Fokkema, 1982; Rodgers, 1993), yet there are few commercial products (e.g., Gustafson's Kodiak® as seed treatments) that use Bacilli. Given the potential number of *Bacillus* strains that could be useful as fungicides, why aren't there more successful commercial fungicides based on the Bacilli?

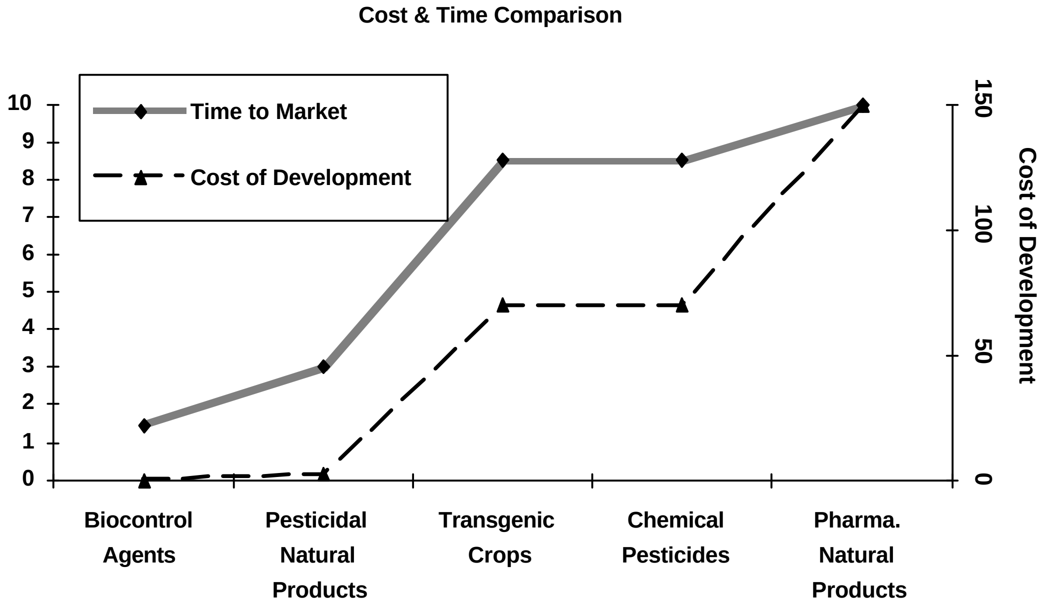
AgraQuest asked this question when we first started the company. We reasoned that despite all the university research on fungicidal Bacilli, 1) relatively few strains had been screened, 2) few strains were tested in commercial fermentation conditions, and 3) few strains were optimally formulated for best efficacy.

Our goal was to find a biofungicide strain that would be as good as a chemical pesticide in **performance, consistency and ease of use**. Within six months, we found a *Bacillus* strain, QST713 that shows excellent field activity as a preventative fungicide. In tests on more than 200 field trials, it has performed as well as the best commercial standard on many plant diseases, such as grape powdery mildew,*Botrytis*, fire blight, and apple scab. The company moved this strain from the lab bench to EPA registration submission in just 18 months, including process development, scale-up, formulation, field testing, and toxicology. The product has more than a year shelf life, does not need to be refrigerated, and is formulated and sprayed like a chemical pesticide. To summarize a successful approach to rapidly discovering and developing a biopesticide:

- Let nature do the genetic manipulation / selection (to avoid genetic engineering and complicating the regulatory process)
- Find hardy, high producing strains, with high activity on the pest / pathogen
- Optimize the fermentation process on pesticidal activity not just cell yield
- Formulate to increase activity by 25% or more
- Compare to chemical pesticides on efficacy, cost and ease of use

The cost to develop a biofungicide product like this is approximately $3 million over three years compared to $100 million over seven to ten years.

Figure 3. Cost and time of pesticide development



Conclusions

The microbial world has great potential for new pesticides as reduced risk fermented natural product compounds, lead molecules for synthetic chemistry and as microbial pesticides. There are successful examples of commercial products on the market and in development from all three categories. The relative R+D effort invested into pesticidal natural product and biopesticide discovery is small compared to the industrial dollars allocated to pharmaceutical natural products, chemical pesticides and most recently transgenic plant research. Likewise, investment in manufacturing, formulation, transportation and application technology and infrastructure has been dominated for forty years by synthetics. Little wonder biologicals and natural products are yet to capture the process and handling efficiencies farmers and IPM specialists have grown accustomed to.

There is great potential to rapidly and inexpensively develop biopesticide alternatives that have the ease of use, efficacy, reliability, and value to the customer of synthetic chemical pesticides. A number of factors are bound to converge in the next five to 10 years and will greatly accelerate the already underway transition to pest management systems grounded in prevention and treatment with very safe natural biopesticide products.

References Cited

Blakeman, J.P., Fokkema, N.J. 1982. Potential for biological control of plant disease on the phylloplane. Annu. Rev. Phytopathol. 20: 167-192.

He, H., Silo-Suh, L.A., Handelsman, J., Clardy, J. (1994) Zwittermicin A, an antifungal and plant protection agent from *Bacillus cereus.* *Tet. Letters* 35: 2499-2502.

Manker, D.C., Lidster, B.D., MacIntosh, S.C., Starnes, R.L., Potentiator of *Bacillus* pesticidal activity, US Patent Application Serial # 08/146,852, PCT # WO 9409630.

Manker, D.C., Starnes, R.L., Beccio, M.G., Lidster, W.D., MacIntosh, S.C., Swank, J.K., Jiménez, D.R., Edwards, D.R. (1995) A synergistic metabolite from *Bacillus thuringiensis* with potential for use in agricultural applications. Invited Symposia ACS Pacifichem '95 Honolulu, Hawaii, December 1995.

Starnes, Robert L., Chi-Li Liu, and Pamela G. Marrone. 1993. History, use and future of microbial insecticides. American Entomologist, July: 83-91.

Rodgers, P.B. 1993 Potential of Biopesticides in Agriculture. Pestic. Sci. 39: 117-129.