Biocontrol of phytopathogens: Antibiotic production as mechanism of control

A.L. Ulloa-Ogaz, **L.N. Muñoz-Castellanos** and **G.V. Nevárez-Moorillón**

Universidad Autónoma de Chihuahua Circuito Universitario s/n. Campus Universitario II, Chihuahua, Chih., C.P. 31125, Mexico

There is great interest to discover biologically active natural products from higher plants, bacteria, fungus and yeasts that are better than synthetic agrochemicals and are much safer, from health and environmental considerations. The development of natural products as herbicides, fungicides, for biological control promises to reduce environmental hazards.

Keywords: phytopathogens; biocontrol; antibiotics

1. Biocontrol

Biological control is the use of organisms (or their metabolites or products) that are natural enemies of a pest or pathogen, to reduce or eliminate its harmful effects on plants or products [1]. The main reason why many agricultural products are not completely destroyed by pests and diseases is the naturally occurring biological control agents which are organisms capable of antagonizing with pests or pathogens, with the reduction of its harmful effects [2].

Recently, the application of genetic engineering to improve crops that are resistant to pests or pathogens has opened endless possibilities for biological control [3]. A current example is cotton crops that produce a protein (naturally produced by the soil bacteria *Bacillus thuringiensis*) which is toxic to cotton pests, but harmless to other insects and without adverse effects in humans [4]. Nevertheless, the market for biological control agents is limited by the lack of rigorous evaluation methods that could anticipate the complex interactions between plant, pest or pathogen, biological control agent, soil and the environment; all factors present in the crop field [5].

Biological control of plant pests and pathogens continues to encourage research and development in many fields [6]. Plant pathogens are just one class of targets of biological control, which is also used to limit the presence of other pests such as insects, parasitic nematodes, and weed. Biocontrol involves the elimination of pest organisms by using other organisms [7]. Moreover, the relationships of many environments can result in multiple interactions among organisms and their environment, many of which might contribute to effective biological control [8].

A wide range of soil microorganisms have demonstrated activity in the control of various soilborne plant pathogens, including *Fusarium* wilt pathogens. Several groups of biocontrol fungi, including *Laetisaria*, *Stilbella*, *Cladorrhinum*, and *Penicillium* spp., have been used to control soilborne pathogens and have also shown activity against *Fusarium* produces infections [9]. Combinations of fungi and bacteria provide protection under different conditions, and occupy different or complementary niches [10]. The combination of multiple compatible biocontrol organisms have proven to be effective in many cases; an example is the biocontrol of *Fusarium* by a combination of nonpathogenic strains of *F. oxysporum* and fluorescent strains of *Pseudomonas* [11].

Microorganisms isolated from the rhizospheric zone of an specific crop may be better adapted to that plant, and may provide better biological control than organisms isolated from other plants, since they are already adapted to the plant or plant part as well as to the particular environmental conditions in which they must function [12]. Several organisms may enhance the level and consistency of control by providing multiple mechanisms of action, a more stable rhizosphere community, and effective control over a wider range of environmental conditions [13].

2. Microorganisms as natural antagonists

Over the past one hundred years, research has demonstrated that phylogenetically diverse microorganisms can act as natural antagonists of various plant pathogens. Interactions that lead to biocontrol include antibiosis, competition, induction of host resistance, and predation [14].

From the bacterial and fungal strains isolated from a particular environment for evaluation of biocontrol activities, around 1-10% has the capacity to at least inhibit the growth of pathogenic microorganisms [15]. Some of those microorganisms that were initially isolated from rizhospheric soil have been successfully commercialized and are currently marketed as EPA-registered biopesticides in the United States. Among them are found bacteria from the genera *Agrobacterium, Bacillus, Pseudomonas*, and *Streptomyces* and fungi belonging to *Ampelomyces, Candida, Coniothyrium*, and *Trichoderma* genera[16].

The antagonistic microorganisms (bacteria, yeasts and fungi) are able to provide biologic control against plant different pathogens, and are used to control several fruit and vegetables infections [17]. To select antagonistic microorganisms, the following general characteristics should be considered:

1. Ability to colonize the surface of vegetables and persist in the site effectively.
2. Better ability than the pathogen to obtain nutrients.
3. Ability to survive under different environmental conditions [18].

There are many other specific characteristics of the antagonist organism, including its genetic stability, that can be effective at low concentrations, should not require special nutritional elements; it has to be effective for a wide range of pathogenic microorganisms in a variety of fruits and vegetables [19]. It is important also that the microorganism can be produced in large quantities in an economical growth medium, that can remain viable in the formulation for a long period of time, it needs to be easy to apply, should not produce secondary metabolites that cause damage to human health. It is also important that the microorganism is resistant to fungicides and can support commercial and non-pathogenic processes on the host plant [20].

To properly use biocontrol microorganisms, it is important to understand the mechanisms of action involved in biocontrol activity, to develop safe application processes; this is also an important background to select new and efficient strains. Basic information must be generated at both, the biochemical and the molecular level, contributing in this way, in the elucidation of effects such as antibiosis, competition for nutrients and induction of resistance [21]. It is desirable to propose the combined effect of various antagonists in order to assure and adequate disease control, with a reduction in dose and using the least amount of synthetic products; the use of additives to enhance the antagonistic effect of biocontrol microorganisms can also be considered [22].

Until now, knowledge on the mechanisms of action in biocontrol agents includes antibiosis, production of lytic enzymes, parasitism, competition for nutrients and space and induction of resistance [23]. According to studies conducted to elucidate the mechanisms of action of antagonistic microorganisms, the production of antibiotics and competition for nutrients are the most prominent [24].

3. Antibiotic production

Multiple interactions between the diverse groups of soil organisms are common, including predation and competition for resources, where chemical substances are used [25]. Some of these chemicals found in the soil and that have effect on other organisms, can be used for medicinal purposes as antibiotics, including [26]:

1. Penicillin, isolated from the penicillin fungus which is found in soils.
2. Aminoglycosides, such as streptomycin and kanamycin, as well as tetracyclines were isolated from soil dwelling actinomycetes.
3. Lipopeptides such as daptomycin have also been derived from *Steptomyces*, a type of actinomycete.

These bacterial antagonists enforce suppression of plant pathogens by the secretion of extracellular metabolites that are inhibitory at low concentration [27]. Antibiotics produced by plant growth promoting rhizobacteria (PGPR) include 2,4 Diacetyl phloroglucinol, phenazine-1-carboxyclic acid, phenazine-1-carboxamide, pyoluteorin, pyrrolnitrin, oomycinA, butyrolactones, kanosamine, zwittermycin-A, aerugine, rhamnolipids, cepaciamide A, pseudomonic acid, azomycin, antitumor antibiotics FR901463, cepafungins and antiviral antibiotic karalicin. All these antibiotics have antiviral, antimicrobial, insecticidal, antihelminthic, phytotoxic, antioxidant, and cytotoxic effects, and can also produce a plant growth promoting effect [28]. Each of these antibiotics have a different mode of action, some attack the cellular membranes; others have inhibitory effects on the ribosome or other cellular constituents [27]. This is the reason why some organisms are susceptible to some antibiotics but not others, depending on the specific form of cellular constituent the antibiotic attacks [1]. One well-known example of production of antibiotics is pyrrolnitrin, a natural product produced by some *Pseudomonas* spp.; from this compound, the fungicide fludioxonil can be derived. This fungicide is used for seed treatment, foliar spray, or soil drench. A variety of pathogenic and non-pathogenic microorganisms can induce plant defenses and may be useful as biocontrol agents [3].

Two other common antibiotics, streptomycin and oxytetracycline, are registered in the United States for control of fire blight, a disease of pear and apple caused by *Erwinia amylovora* [5]. Streptomycin was an effective chemical compound used for the management of fire blight until some pathogenic strains resistant to the antibiotic appears in several growing regions. Oxytetracycline is registered for use only on pear and is considered less effective than streptomycin for suppression of antibiotic-sensitive populations of *E. amylovora* [6]. Two of the mechanisms usually cited for this microbial biocontrol are:

Antibiosis action, through the production of specific or non-specific metabolites with antibacterial, antifungal and antinematode activity. Several antibiotic substances produced by the pseudomonads have been particularly well characterized [5]. The ones identified with biocontrol properties include the phloroglucinols, phenazine derivatives, pyoluteorin, pyrrolnitrin, cyclic lipopeptides and hydrogen cyanide. Among the other antibiotics characterized are agrocin 84 (*Agrobacterium* sp.), herbicolin A (*Erwinia* sp.), iturin A, surfactin, and zwittermicin A (*Bacillus* sp.) and xanthobacin (*Stenotrophomonas* sp. [10].

Lytic enzyme action, is a feature of several bacteria with biocontrol ability, and involves the direct degradation of the pathogen cell wall material, or the disruption of a particular developmental stage. For example, chitinase production by *Serratia plymuthica* has been reported that inhibits spore germination and germ-tube elongation in *Botrytis cinerea*, while ß- 1,3-glucanase synthesized by *Paenibacillus* sp. and *Streptomyces* sp. can lyse fungal cell walls of *Fusarium oxysporum* f. sp. *cucumerinum*. Other enzymes produced by bacteria with biocontrol activity include hydrolase, laminarinase and protease [29-30].

Antibiotics can cause intense physiological effects on organisms at subinhibitory concentrations. Quinolone and macrolide antibiotics have been reported to block cell signaling, and the production of virulence factors in *P. aeruginosa.* Subinhibitory concentration of antibiotics can suppress adherence mechanisms and the production of extracellular virulence factors in bacteria [31]. Secondary metabolites can impact soil microbial ecosystems in a variety of ways, and at a variety of levels [25]. The production of antibiotics has been demonstrated to be a widespread mechanism exerted by microorganisms to control a wide variety of phytopathogens. Because of all these properties, advanced molecular techniques are now being used to characterize the diversity, abundance, and activities of microbes that live in and around plants, including those that significantly impact plant health [1].

4. Antibiotic resistance

Some microorganisms that are not susceptible to a particular antibiotic, can be the initial source of a resistant strain over time [28]. This represents a public health problem in human pathogens, but also antibiotic resistance seen in the clinical environment can often be found in the soil environment. The reason of this phenomenon is that soil microorganisms are often exposed to a wide range of compounds in their local environment, some of which may be harmful such as antibiotics, and this causes that these organisms develops resistance or go extinct [32]. For example, antibiotic producers must contain some antibiotic resistance mechanisms, to prevent them committing suicide through production of their own toxin [16].

Soil environment is very important for research into the mechanisms of antibiotic resistance, including possible mechanisms which are not yet seen in clinical microbiology [12]. The biochemical processes occurring in antibiotic resistance is just starting to gain much interest [33]. Evolution has even taken antibiotic resistance one step further; it has been demonstrated that out of 18 antibiotics tested from 8 major classes of antibiotic of both natural and synthetic origin, 13 to 17 supported the growth of bacteria when the antibiotic was available as sole carbon source [32]. Microorganisms are highly adaptable, in ways which we are only recently coming to understand.

Antibiotic resistance occurs when antibiotics are a constant pressure on a given population; those organisms with natural resistance can survive and reproduce whereas those organisms which do not have the resistance factor die [28]. Once a resistance factor has developed, it can be spread rapidly within a population where DNA is transferred from one bacterium to another. Transfer of DNA containing antibiotic resistance genes can occur through three processes [29]:

1. *Transformation.* When a bacterium dies and lyses (splits open), other bacteria which are actively-growing in close proximity can pick up its DNA.
2. *Transfection.* Phage, which are viruses that infect bacteria and fungi, sometimes pick up extra genes from the microorganisms that they infect which are then passed on to other organisms which they infect.
3. *Conjugation.* Bacteria can fuse their cell membranes together and exchange plasmids or fragments of their chromosomes These processes can occur between distinct ‘species’ of bacteria meaning that mechanisms of antibiotic resistance may only have to evolve once and can then spread throughout an entire community [10].

There are numerous reports of the production of antifungal metabolites produced by bacteria *in vitro* that may also have activity *in vivo* [33]. These include ammonia, butyrolactones, 2,4- diacetylphloroglucinol (Ph1), HCN, kanosamine, Oligomycin A, Oomycin A, phenazine -1- carboxylic acid (PCA), pyoluterin (Plt), pyrrolnitrin (Pln), viscosinamide, xanthobaccin, and zwittermycin A [11] as well as several other uncharacterized compounds. Mutants lacking production of antibiotics or over-producing mutants have been used to demonstrate the role of antibiotics in biocontrol. Alternatively, the use of reporter genes or probes to demonstrate production of antibiotics in the rhizosphere is becoming more commonly used [32].

Antibiotic production by bacteria, particularly pseudomonads, seems to be closely regulated by a two-component system involving an environmental sensor (a membrane protein) and a cytoplasmic response factor [34]. Mutation in either gene has similar multiple effects on antibiotic production. However, the environmental signals that control the two-component system are unknown. Interestingly, the *gacA* gene is required for biocontrol activity in *P. fluorescens* CHA0 in the rhizosphere of dicotyledous plants, but not in plants of the Gramineae family, although the mechanisms are unclear [4].

5. Biocontrol mediated by allelochemicals

Nowadays, there is a great demand for compounds with selective toxicity that can be degraded by the plant or by the soil microorganisms [35]. Plant, microorganisms, soil organisms and insects can produce allelochemicals which provide new strategies for maintaining and increasing agricultural production in the future. Compounds with allelopathic activity could be used in a future for the synthesis of herbicides, insecticides, nematicides, and fungicides that are not based on the persistent compounds derived from petroleum, which are of public health concern [2].

Additionally, the production of allelochemicals depend largely upon environmental conditions: usually stressed plants are more active, and stress conditions can include extreme temperatures, drought, soil nutrient deficiency and high pest incidence. Also, the range and concentration of chemicals that a given species can produce can vary with environment conditions [11]. Anaerobic metabolites, alkaloids, phenolics, terpenoids, and steroids are bioactive chemicals which can be find in roots and rhizospheres in wetlands [35]. That bioactivity includes allelopathy, growth regulation, enzymatic activities, metal manipulation by phytosiderophores and phytochelatines, various pest-control effects, and poisoning [36]. Terpenoid and phenolic compounds are the most common compounds involved in allelopathic interactions. Terpenoids are the largest group of plant chemicals (15,000-20,000) with a common biosynthethic origin, and its pathway generates a large structural diversity and complexity of compounds, thus generating enormous potential for mediating ecological interactions [37]. Terpenoids may produce effects on seeds and soil microbiota through volatilization, leaching from plants or decomposition of plant debris.

It also has been determined that complex biological and biochemical interactions among roots, rhizosphere organisms, and the rhizosphere solution determine the overall biogeochemical processes in the wetland rhizosphere and in the vegetated wetlands [2]. Consequently, most soils possess the biological capability to inhibit or reduce their soil microflora's tendency toward disease, and could be considered disease suppressive to some extent [3].

Attempts to simplify the biological basis of disease in soils have reduced this concept to two broad mechanisms; I) a general suppression based on the activity of the total microbial biomass that is not transferable between soils [38], and II) a specific suppression that depends on the activity of specific groups of microorganisms. In general, microbial biocontrol mechanisms have been classified according to effect and have included such actions as parasitism/predation, niche competition, antibiosis and systemic induced resistance [28].

6. Conclusions

The use of microorganisms, especially bacteria with biocontrol activity as an adjunct to commercial chemical fungicides, can be a common agricultural practice in the near future. Therefore, it is necessary to understand the influence within and between microbial communities resident in the soil agro-ecosystem. There is still many facts to be discovered in this important environment.

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