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Biotechnological Aspects of Microorganisms Used in Plant Biological Control

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Abstract: The future of sustainable agriculture will increasingly rely on the integration of biotechnology with traditional agricultural practices. Most sustainable and environmentally acceptable control may be achieved using biocontrol agents due to the effort to reduce the use of agrochemicals and their residues in the environment and in food. Identifying, understanding and utilizing microorganisms or microbial products to control of plant diseases and to enhance crop production are integral parts of sustainable agriculture. Biological control has the potential to control crop diseases while causing no or minimal detrimental environmental impact. This review article states the role of antibiosis in biological control of plant diseases mediated by specific metabolites of microbial origin, as antibiotics, enzymes, volatile compounds or other toxic substances. Also, new evidence obtained with the modern tools of microbial genetics or molecular techniques, which can use to study the biocontrol mechanisms and improve the biocontrol activity, metabolites and products that could have important environmental benefits.

Key words:

INTRODUCTION

Plant pathogens include fungi are the most visible threats to sustainable food production. Plant The decreasing efficacy of the fungicides as well as risks associated with fungicide residues on the leaves and fruit, have highlighted the need for a more effective and safer alternative control measures. In recent years, biological control of plant pathogens has received increasing attention as a promising supplement or alternative to chemical control. Biological control of plant disease is defined as " The involvement of the use of beneficial microorganisms, such as specialized fungi or yeast or bacteria to attack and control the plant pathogens (i.e., fungi, bacteria, nematodes or weeds) and the diseases they are causing [1, 2].

Biological control is a potent means of reducing the damage caused by plant pathogens [3, 4]. Biological control of plant disease can occur through different mechanisms, which are generally classified as; antibiosis, competition, suppression, direct parasitism, induced resistance, hypovirulence and predation. The antagonistic activity has often been associated with production of secondary metabolites [5]. At the same time, molecular markers provide gigantic sources of data that can assist scientists in developing tools to monitor the genetic and environmental fate of these agents. Selection of appropriate molecular techniques should be based on the specific characteristics of the organism and on the desired type of information necessary to evaluate a particular step in the developmental process of a biofungicides. The identification and the biological and molecular characterization of microorganisms, useful as biocontrol agents or as producers of bioactive compounds, are of great relevance for the modern and echo-compatible agriculture [6].

Biotechnological aspects of antibiosis in biocontrol: Antibiosis is considered the most common mechanisms of action deployed by the microorganisms up to now investigated [7]. Antibiosis is defined as antagonism brought about the metabolites of fungal antibiotics or antibiotic-like compounds, lytic enzymes, volatile compounds, siderophores or other toxic substances. Several biocontrol agents have shown to be able to produce secondary metabolites as antibiotics, antibiotics like compounds or enzymes. It is relatively easy to demonstrate inhibition of one microorganism by another on agar and obtain evidence of strain dependence in the effect [8]. Actually, most of early studies on biological

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control of plant pathogens, being based on *in vitro* screening of potential biocontrol candidates, tended to select for microorganisms able to produce toxic metabolites, since the appearance of an inhibition zone was considered as a factor for screening [9].

Hydrolytic enzymes: The role of enzymes in biocontrol can often be assigned to mechanisms, parasitism and antibiosis. In particular cell wall degrading enzyme such as chitinases, β-1,3-glucanases, proteases and cellulases, are not only important features of mycoparasites for colonization of their host fungi, but also may exhibit considerable antifungal activity on their own [10]. One of its main mechanisms, antibiosis, relies on the recognition, binding and enzymatic disruption of the host-fungus cell wall [11].

Antibiotics: Antibiotics are generally defined as low molecular weight organic compounds that are produced as secondary metabolites by certain groups of microorganisms, mostly soil inhibitors that are inhibitory to the growth or other metabolic activities of other microorganisms at low concentrations [12]. Antibiotics may have a cidal (killing) effect or a static (inhibitory) effect on a range of microbes [13]. Different antibiotics have different modes of action on bacteria.These include: Prevention of proper cell wall formation; Inhibition or interference with protein syntheses and membrane integrity; Disruption of plasma and/or outer membrane function; Inhibition or interference with DNA synthesis and Inhibition of synthesis of essential small molecules miscellaneous effects [14]. Bacteriocins are antibiotic-like compounds with bactericidal specificity restricted to bacterial strains closely related to the producer [15].

Siderophores: Siderophores are low-molecular-weight (0.5 to 1.5 KDa), high specificity Fe^{3+} chelating agents [16]. They are produced under iron-limiting conditions by almost all aerobic and facultative anaerobic microorganisms examined. Systems such as siderophores, involved in the acquisition of iron under iron limited conditions, may play a major role in microbial interactions. Siderophores have been demonstrated to play a major role in plant disease suppression by some bacterial biocontrol agents which inhibit the growth or the metabolic activity of plant pathogens by sequestering iron [17].

Volatile compounds: Volatile compounds from the biological control agents has an important part of the inhibitory mechanism, especially under closed storage conditions. Production of volatile ammonia has been implicated as a possible mechanism to control soil borne pathogens [18].

Alkaloids: The basic unit in the biogenesis of the true alkaloids are Amino Acids. Alkaloids are highly reactive substances with biological activity in low doses [19]. Several strains of microorganisms produce alkaloids. Alkaloids, elimoklavine, festuklavine produce by *Trichoderma* and agroklavine, ergometrine produce by *Penicillium* have antifungal activity against, *Botrytis cinerea, Fusarium solani* and *Alternaria tenius* as well as antibacterial action which depressed the growth of several pathogenic bacteria, cause the death of living cell.

Phenols : Phenols may also be involved in protection of plants from pathogens. Antagonism of *P. fluorescens* strain 2-79 (NRRL B-15132) suppressed take-all disease of wheat can be attributed to production of the 2-acetamidophenol (0.05 g l^{-1}, in cultures) by the strain [20].

Effect of stress conditions on biocontrol agents: When planning the application of biocontrol strains, it is important to consider the environmental stresses affecting microbial activities. Besides pH, low temperature, low water potential "as a results of salt stress", antagonistic bacteria and pesticides, the presence of heavy metals in the soil is a very important stress factor affecting the growth and metabolites activities of biocontrol agents [21].

Application of molecular genetic to study biocontrol mechanisms: The molecular genetic approach to studies on biocontrol mechanisms consists of; cloning the genes encoding putative biocontrol factors, producing specifically deficient mutants of the antagonist by gene disruption, determining the loss or reduction of biocontrol ability of the mutants compared to their parental genotype, restoring ability to produce the factor by reintroducing the intact encoding sequence *via* transformation and determine restored biocontrol ability in the transformants. This approach provides the most conclusive evidence for the role of a particular factor in biocontrol [22].

Application of molecular genetic to imrove biocontrol agents

Genetical improvement of biocontrol agents: Genetically improved antagonistic hyperparasitic microorganisms tend to increase their effectiveness as biological contol

agents. Effective performance requires either improvement of the environment to favour the biocontrol agent or genetic improvement of the agent [23]. Th gols are to enhance antifungal metabolites productivity of biocontrol agents, to improve antagonistic potential of biocontrol agents, to control a broad spectrum of phytopathogens and to develop biocontrol agents tolerance of some stress conditions.

Genetic improvement can be achieved by chemical and physical mutation, sexual hybrids, homokaryons and genetic manipulation e.g, directed mutagenesis, protoplast fusion, genetic analysis of fusants; recombination, transformation or isolation of useful genes from biocontrol fungi without functional sexual stages [24, 25]. Genetic improvement of any organism is referring to development of one or more of its desired characters through genetic technique (s) (i.e. mutation and/or protoplast fusion and/or genetic manipulation) [21, 26, 27]. Random mutagenesis has been manipulated to improve production of antifungal metabolites and antagonistic potential of biocontrol agents (i.e. *Trichoderma* spp. and *Gliocladium* spp. to control a broad spectrum of phytopathogens. Transposon is a segment of DNA that is capable of transfer or jumping to another region of DNA within the genome; transposon mutagenesis: use of these jumping regions to disrupt target genes. Site-directed mutagenesis means that the mutation or alteration occurs at a specific, known site within the sequence. Protoplast fusion is a quick and easy method for combining the advantageous properties of distinct promising strains. Protoplast fusion is occur at intra-and inter-strain protoplast fusion levels. Therefore protoplast fusion has been used in successfully attempts to produce asexual hybrids of microorganisms of biocontrol strains that their sexual stages have rarely. Transformation the alteration of an organism's genome due to the uptake by the cell of exogenous DNA and the incorporation or stabilization of that genetic information within the genome. Recombinant DNA the combination of foreign DNA inserts with vector DNA (e.g., plasmid, phage, cosmid, etc.) to produce a clone within a host. Insertion or modification of genes to produce desired proteins.

Mutation as tool for the improvement: Many authors were applied the mutagensis by either the physical or the chemical mutagens to generate new biotypes with improve the potentialities of biocontrol agents and/or antifungal metabolites producers. Induced mutation is one of the most commonly used routine to restrain the genetic construction of microorganisms [28].

Use of protoplast fusion for genetic improvement or manipulation: Protoplast fusion is a quick and easy method for combining the advantageous properties of distinct promising strains [29]. Protoplast fusion techniques was used to combine genetic traits desirable for improving biocontrol activity by *T. harzianum* and increased amounts of specific proteins. Fusion of protoplast derived from two efficient biocontrol strains of *T. harzianum* resulted in the recovery of a progeny strain with greatly improved biocontrol ability. After that, directed transfer of fungal genetic sequences encoding for factors important in biocontrol will be possible as soon as such sequences are available [30].

Improvement the bioagents *via* directed mutagenesis: Many authors were applied the mutagensis by either the physical mutagens (e.g. gamma irradiation or UV-irradiation) or the chemical mutagens to generate new biotypes with improve the potentialities of biocontrol agents and/or antifungal metabolites producers [28]. Haggag [31] used mutation technique by UV light and improved the production of important *P. fluorescens* of antibiotics, phenazine, pyrrolnitrin and phloroglucinol as well as siderophore pigment production against some tomato damping-off pathogens (*Fusarium solani, Fusarium oxysporum* f.sp. *lycopersici* and *Rhizoctonia solani*). These mutant strains increased the antibiosis and the fluorescens on King's medium comparing to the wild-type. A mitogen-activated protein kinase encoding gene, *tvk1*, from *Trichoderma virens* was cloned and its role during the mycoparasitism, conidiation and biocontrol was examined in *tvk1* null mutants. These mutants showed; a clear increase in the level of the expression of mycoparasitism-related genes during direct confrontation with the plant pathogen *Rhizoctonia solani* [32].

The null mutants displayed an increased protein secretion phenotype as measured by the production of lytic enzymes in culture supernatant compared to the wild-type [33]. Consistently, biocontrol assays demonstrated that the null mutants were considerably more effective in disease control than the wild-type strain or a chemical fungicide. In addition, *tvk1* gene disruptant strains sporulated abundantly in submerged cultures, a condition that is not conducive to sporulation in the wild type. These data suggest that Tvk1 acts as a negative modulator during host sensing and sporulation in *T. virens* [33].

Genetic modification and transformation: A genetic modification approach was used to further enhance the biocontrol ability of biocontrol agents [34]. Genetically

Modified (GM) was used in strains, *P. fluorescens* F113Rif (pCU8.3) and *P. fluorescens* F113Rif (pCUP9), were developed for enhanced phenazine-1-carboxylate (Phl) production and assessed for biocontrol efficacy and impact on sugar beet in microcosm experiments [35]. An endochitinase encoding gene was cloned from the genome DNA of *T. viride*. The length of this DNA fragment is 1672 bp and contains a promoter and coding region for mRNA. After ligating it with the promoter and terminator of Trp synthetase from *Aspergillus nidulans*, this gene was introduced into *Chaetomium globosum* CG10. Approximately one-third of the transformants increased their endochitinase activity significantly. This experiment lays some foundation and presents a potential way for improvement of the biocontrol activity of *Chaetomium globosum* with genetic engineering method. *Pseudomonas aeruginosa* PNA1, isolated from the rhizosphere of chickpeas in India. mutant (FM13) deficient in phenazine production were obtained following transposon mutagenesis of PNA1 [36]. Anthranilate, an intermediate in the tryptophan biosynthesis pathway, suppressed mycelial growth of *Pythium* spp. in culture and damping-off of *P. vulgaris* and lettuces. It is concluded that anthranilate, excreted by FM13 as a consequence of the trpC mutation, may have contributed to the suppression of *Pythium* damping-off by the mutant.

Future application: Antibiosis often acts in concert with competition and/or parasitism. Understanding the mechanisms of biocontrol of plant diseases to develop rational models for the exploitation of antagonists in agroecosystems. In addition, this information is necessary for the manipulation of parameters affecting biocontrol agents and for the genetic improvement of biocontrol agents. Once the role of inhibitory compound has been established, several possibilities exist. For example, biocontrol organisms may be developed that possess enhanced production capabilities. Eventually, it may be possible to produce the inhibitory compound by chemical processes or by super-producer organisms and then apply the compound or its analog as a pesticide. Use of inhibitory metabolite compounds in place of hazardous chemicals at different stages of leather processing namely soaking, degreasing and bating at present is being developed. As against traditional chemical methods, enzymatic processes yield products of improved quality and reduce the use of hazardous and polluting chemicals. Recent advances in the study of molecular genetics of the biological control agent strains have provided a powerful tool that will aid in unravelling these basic mechanisms and for evaluating the impact of the biological control agents on the environment. Molecular biology has helped identify the modes of action of many biocontrol agents. Also can be used to increase the amount of a protein or metabolite produced by an organism. These depending on: Regulation of Gene expression, Components involved in regulation of gene expression, Regulation at initiation of transcription, Regulation at the level of transcription, Post-transcriptional control, Factors affecting the expression of a gene. Nevertheless, all these approaches are dependent on the metabolic load associated with overproduction or expression of novel genes. Eventually, it may be possible to produce the inhibitory compound by chemical processes or by super-producer organisms and then apply the compound or its analog as a pesticide.

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