IMPORTANT BIOCONTROL TRAITS OF MICROBIAL ANTAGONISTS OF LEAF ROT DISEASE OF COCONUT

By
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ABSTRACT

Leaf rot disease of coconut, which occurs superimposed on root (wilt) disease of coconut in about 65 % of the cases, is an important disease prevalent in Kerala State of India. Colletotrichum gloeosporioides and Exserohilum rostratum are the main fungal pathogens of the disease, which disfigure the leaves and reduce yield substantially. Several biocontrol agents have been identified which reduce the disease development by these fungal pathogens. Pseudomonas *spp. exhibit a host of mechanisms, which act in concert to bring about biocontrol activity. Bacillus spp. work mainly through production of antibiotics and other non-volatile metabolites. Mycoparasitism was the main contributing factor for actinomycetes group.*

INTRODUCTION

Micro-organisms are central to our understanding of biological control of plant diseases and their potential in reducing disease incidence and severity is well demonstrated in many crops worldwide, leading to their practical applications in agriculture. The surface of aerial plant parts provides a habitat for epiphytic micro-organisms, many of them are capable of influencing the growth of pathogens, thus, paving the way for biological control on foliar surfaces (Blakeman and Brodie, 1976; Blakeman and Fokkema, 1982). These saprophytic organisms play an important part in control of foliar pathogens thus reducing the incidence of foliar diseases on crops in the field.

In recent times, bacterial antagonists have received considerable attention mainly because of their ability to reduce the inoculum's density of the pathogen, which is effected through several means. An efficient bio-control agent may affect pathogens by a combination of mechanisms (Chet, 1987). Hence, to devise ways of enhancing the antagonistic effects of foliar micro-organisms against pathogens, it is necessary to understand the mechanisms responsible for biological control.

The production of volatile and non-volatile secondary metabolites, siderophores, hydrogen cyanide (HCN), antibiotics, etc. have been implicated in the control of plant pathogens (McKeen *et.al*., 1986; Fravel, 1988; Droby *et al*., 1989; Chet *et. al*., 1990; Thomashow and Weller, 1990; Ferriera *et.al*., 1991). Mycoparasitism / hyperparasitism by microbial antagonists can also be distinguished, which involve hyphal interference, penetration of host mycelium and *hyphal lysis* (Amer *et. al*., 1997; Gupta *et al*., 2001).

Leaf rot disease, which occurs in the Southernmost District (Kerala) of India, is an economically important disease of coconut plantation crop. The disease is caused mainly by fungi *Colletotrichum gloeosporioides* and *Exserohilum rostratum*, which produce lesions on innermost tender leaves. These lesions coalesce rotting a large leaf area, which falls off after drying. Each successively emerging leaf gets infected, thus, reducing the photosynthetic area to a great extent and the yield of the palm is adversely affected.

Several microbial antagonists have been identified against the fungal pathogens of this disease. Out of this, seven bacterial / actinomycetal anta-gonists were selected for further studies.

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This paper presents investigations into some of the mechanisms employed by these bio-control agents, which may explain the basis of their bio-control activity.

MATERIALS AND METHODS

The biological control agents of leaf rot disease of coconut used in the study were:

Pseudomonas aeruginosa

Pseudomonas sp.

Bacillus sp. LP-8

Bacillus sp. CR-17

Thermomonospora mesophila

Streptomyces sp.

An unidentified bacterium

These bio-control agents were tested for the following traits:

1. Production of volatile secondary metabolites : by paired petri plate method (Johri *et al*., 1975)
2. Production of non-volatile meta-bolites: by agar-dilution technique
3. Production of hydrogen cyanide (HCN): detection through biochemical reaction
4. Production of siderophores : by spectrophotometric method (Mayer and Abdallah, 1978) and Chrome Azurol S (CAS) agar plate method (Schwyn and Neilands, 1987)
5. Antibiosis : by agar – well method
6. Study of mycoparasitism : by dual culture method (Lundborg and Unestam, 1980)

RESULTS AND DISCUSSION

Naturally occurring resident micro-organisms on aerial plant surfaces have the ability to survive and grow in this extremely difficult habitat, and if these micro-organisms possess an effective antagonistic action against a plant pathogen, then they can become ideal bio-control agents. This guiding principle had formed the basis for the work on biological control of leaf rot, a foliar disease of coconut.

As a result, seven bio-control agents were identified as promising candidates for biological control of leaf rot disease of coconut (Gupta *et.al*., 2000). However, for determining the mode, form and optimum timing of application of antagonist inoculums, it is necessary to understand the mechanisms by which the antagonistic interactions between the phylloplane microflora and pathogens are brought about. Hence, the bio-control agents of leaf rot disease were screened for different traits which may be involved in the antagonistic reactions on foliar surfaces.

All the seven bio-control agents produced antibiotics (Table.1). Production of antifungal antibiotics by phylloplane bacteria - *Pseudomonas* spp. (Teliz-Ortiz and Burkholder, 1960), *Bacillus* (Swinburne *et.al*., 1975) and actinomycetes – *Streptomyces spp*. (Fawcett and Spencer, 1970) is a widely reported phenomenon and has been proposed to be a probable cause of pathogen inhibition (Johnson, 1931).

Siderophores have been implicated in the control of plant pathogens particularly by fluorescent pseudomonads (Thomashow and Weller, 1990). In our studies also, the two *Pseudomonas* species and one unidentified bacterium were found to produce siderophores, volatile metabolites and also hydrogen cyanide (details in Table.2). Cyanide production by certain

fluorescent pseudomonades has been found to influence plant pathogens (Voisard *et. al*., 1989) and may contribute to biological control due to induction of plant resistance (O'Sullivan and O'Gara, 1992).

All seven bio-control agents were found to produce non- volatile secondary metabolites which were inhibitory towards fungal pathogens. Mycoparasitism which is the most vital mechanism of antagonism of fungal antagonists (Elad *et. al.*, 1981; Howell, 1982) was evident in the case of actinomycetes, *Thermomonospora mesophila* and *Streptomyces* sp. Coiling around host hyphae was observed in both the antagonists (Table.3). Some kind of hyphal interference was also observed in case of pseudomonas which was probably due to the production of antifungal secondary metabolites.

Antibiosis-mediated hyphal perforations, lysis and fragmentation by Pseudomonas GRC_2 against fungal pathogens had earlier been reported by Gupta *et al.* (2001).

From the results presented here, it is evident that the antagonists of leaf rot disease especially *Pseudomonas spp*. possess a combination of bio-control traits, which can play an important role in plant disease control.

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Table 1 : Plant disease suppression mechanisms operated by biocontrol agents of leaf rot disease of coconut

Biocontrol agent	Antibiosis	Mycoparasitism	Production of			
			HCN	Siderophores	Volatile metabolites	Non- volatile metabolites
Pseudomonas aeruginosa	+	+	+	+	+	+
Pseudomonas sp.	+	+	+	+	+	+
Bacillus sp. LP-8	+	--	--	--	--	+
Bacillus sp. CR-17	+	--	--	--	--	+
Thermomonospora mesophila	+	+	--	--	--	+
Streptomyces sp.	+	+	--	--	--	+
Unidentified bacterium	+	--	+	+	+	+

Table 2 : A summary of the biocontrol traits possesed by bacterial antagonists of leaf rot disease

Pseudomonas aeruginosa	***Pseudomonas* sp.**	***Bacillus* sp. LP-8**	***Bacillus* sp. CR-17**	**Unidentified bacterium**
Produces fluorescent siderophores with very high affinity for ferric iron under low- iron conditions.	--	--	--	--
Also produces blue- green, water soluble siderophores. Initial studies suggest that these possess both hydroxamate and phenolate groups and hence, fall under the category of pyoverdins.	Produces water soluble and diffusible siderophores.	--	--	Siderophores are produced under certain growth conditions.
Produces more than one type of antibiotics including pyocyanin.	Produces more than one type of antibiotic compounds.	Antibiotics (water soluble) are secreted into the growth medium.	Antibiotics appear to be the main mode of inhibitory action.	A mixture of antibiotic compounds are produced.
Produces HCN which may work through plant- induced resistance to certain pathogens.	Also produces hydrogen cyanide.	--	--	Hydrogen cyanide is produced.
Produces both volatile and non-volatile inhibitory secondary metabolites.	Produces volatile and non-volatile metabolites during the stationary phase of the growth.	Non- volatile, antifungal in character, secondary metabolites are produced.	Non- volatile secondary metabolites are produced.	Both volatile and non- volatile metabolites are synthesized.
Some kind of antifungal secondary metabolite – mediated hyphal interference is evident.	Hyphal interference in the zone of antibiotics elaboration and diffusion is seen.	--	--	--
Diversified nutrient utilization capability may confer competitive advantage for colonization on foliar surfaces.	Ability to use diverse substrates for its growth.	--	Able to utilize an array of growth substrates.	--

Table 3: A summary of the biocontrol traits possessed by antagonistic actinomycetes

Thermomonospora mesophila	***Streptomyces* sp.**
Produces red- coloured, water soluble, diffusible antifungal compound	Produces diffusible and water soluble antifungal compound, also produces pinkish-red coloured non- diffusible compound
Shows mycoparasitism/ hyperparasitism	Mycoparasitizes the plant pathogen (hyphal interactions)
Produces some non- volatile secondary metabolites which are inhibitory to fungal pathogens	Non- volatile secondary metabolites are produced

Legends of the photographs

1. A coconut palm affected by leaf rot disease.
2. The fungal pathogens attack the innermost tender leaves of the palm.
3. Antibiotics elaborated into the medium by *Bacillus* spp. (LP-8 and CR-17) inhibit the growth of the fungal pathogen (in the centre).
4. *Thermomonospora mesophila* secretes a red-coloured, water soluble, diffusible anti-fungal compound
5. *Thermomonospora mesophila* mycoparasitizing the main fungal pathogens of leaf rot disease. Zones of mycoparasitism are shown by arrows.