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Mutual Antagonism Between Fluorescent Pseudomonads and Soil Actinomycetes

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Abstract

The inhibition of 23 strains of actinomycetes by 7 strains of fluorescent pseudomonads and *vice versa,* on 4 different media, was examined. The antagonism of pseudomonads was more frequent and more intense than that of actinomycetes. Inhibition was observed in pseudomonads and actinomycetes of both kinds of strains, antagonistic and non-antagonistic to *Fusarium oxysporum.* Some media were more favourable to the antagonism of pseudomonads and others to that of actinomycetes, hence in identical environmental conditions mutual antagonism between the two counter-partners occurred only rarely.

Keywords: fluorescent pseudomonads, actinomycetes, *Fusarium,* antibiotics, siderophores

Introduction

There are microorganisms in the soil that are antagonistic to plant pathogens. Among them are numerous strains of fluorescent pseudomonads producing siderophores which control the availability of Fe^{3+} [7, 8, 9], and antibiotics [3, 4, 11, 12], often several of them. Another important group of microorganisms that are antibiotic towards pathogens are soil actinomycetes [8].

When deliberating over the role of these two antagonists, one has to take into account the possibility of their mutual antagonism in the soil environment. There are some data indicative of such a possibility. Even in early studies of antibiosis [13], pseudomonads were found to be sensitive to many antibiotics of actinomycetes. Hence, it cannot be excluded that this sensitivity is one of the biological factors hindering the colonization of a root system by fluorescent pseudomonads introduced into the soil experimentally [2].

In turn, it was observed [5] that the proliferation of fluorescent pseudomonads following the application of fungicides was accompanied by a decrease in the number of actinomycetes, and some of their isolates were inhibited by fluorescent pseudomonads when cultured on King's B medium.

When considering antagonistic relations between the discussed microorganisms, one may ask which antagonism is stronger: that of pseudomonads towards actinomycetes, or that of actinomycetes towards pseudomonads. While this activity depends on the medium composition, it is not known whether it manifests itself in environmental conditions identical or different for the two counter-partners.

In the present work an attempt was made to arrive at a partial answer to the above questions by examining the mutual antagonism among 7 strains of Pseudomonas and 23 strains of actinomycetes on 4 different media. The sensitivity of the strains to their counter-partners was studied in connection with their effect on *Fusarium oxysporum.*

Material and Methods

Characteristics of the Strains of Microorganisms

Five strains of Pseudomonas denoted by the symbols P3, P15, P18, P23 and PD were isolated and described previously [5] in studies with loose sand treated with the fungicide methiram. One strain, denoted by PK, was obtained from the Department of Forestry Phytopathology of the Poznan Academy of Agriculture, where it had been isolated from the surfaces of acorns. The seventh strain, *P. fluorescens,* denoted by the symbol PF, came from the collection of the Department of Microbiology of Nicolaus Copernicus University in Torufi.

All actinomycetes derived from the same methiram-treated soil as the Pseudomonas strains we isolated.

The strain of *F. oxysporum* was from the collection of the Department of Agricultural Microbiology, Academy of Agriculture in Poznan.

Table 1. Ability of 7 strains of fluorescent pseudomonads to inhibit 23 isolates of actinomycetes.

Strains of pseudomonads	Number of inhibited actinomycetes				Zone of inhibition (mm)*							
					Range				Average value			
	Medium											
	PGM	KBM	BM	PDA	PGM	KBM	BM	PDA	PGM	KBM	BM	PDA
P3	21	17	2 [13]	0	5-14	5-18	10-11 [8-26]	-	7	12	11 [16]	-
P15	20	16	4 [12]	0	5-15	5-18	5-12 [5-31]	-	8	15	8 [12]	-
P18	22	17	11 [7]	0	5-15	6-21	7-20 [12-21]	-	8	15	11 [18]	-
P23	23	18	13 [5]	1	5-13	5-21	5-15 [5-25]	5	7	14	8 [17]	-
PD	23	23	23 [0]	23	16-25	7-16	5-15 [6-23]	7-16	20	11	10 [11]	11
PK	22	19	3 [17]	0	9-18	5-18	10-11 [5-26]	-	14	13	10 [15]	-
PF	22	22	0 [9]	0	5-14	5-25	- [5-25]	-	9	12	- [12]	-

Explanation: PGM - Pridham Gotllieb's medium; KBM - King's B medium; BM - Burkholder's medium; PDA - potato-dextrose medium;
* - Standard deviation = ± 1 mm
In brackets - inhibition of only aerial mycelium, not vegetative one.

Culture of Microorganisms

All strains were maintained and proliferated to obtain an inoculum on Burkholder's medium (BM) containing g/dm^3 of extract from 300 g of peeled potatoes: Na_2HPO_4 X 12 H_2O, 2; sodium citrate, 10; dl-asparagine, 1.0; bac-to-peptone, 5; dextrose, 6; agar, 15. The pH was adjusted to 7.0.

The antagonistic activity was determined by the conventional agar-streak method using the following media: (2) Pridham-Gottlieb's medium (PGM) containing g/dm^3 of distilled water: K_2HPO_4, 0.5; $MgSO_4$, 1.0; $CaSO_4$ x 7 H_2O, 0.0064; $FeSO_4$ x 7 H_2O, 0.00011; $MnCl_2$, 0.007; $ZnSO_4$ x 7 H_2O, 0.0015; dextrose, 10; meat extract, 10; agar, 15. The pH was adjusted to 7.4 [10]. (2) King's B medium (KBM) containing g/dm^3 of distilled water: proteose peptone, 20.0; glycerol, 10.0; K_2HPO_4, 1.5; $MgSO_4$ x 7 H_2O, 1.5. The pH was adjusted to 7.2 [6]. (3) Burkholder's medium (BM), its content as above. (4) Potato-dextrose agar (PDA) containing g/dm^3 of extract from 300 g of peeled potatoes: dextrose, 5.0; agar, 20.0. The pH was adjusted to 7.4.

Results and Discussion

When comparing the development of the microorganisms under study on the above media, the most luxuriant growth of pseudomonads was observed on KBM, and the poorest on PGM. Actinomycetes grew well on all the media, but during incubation they did not produce a sporulating aerial mycelium on KBM.

The data presented in Table 1 show that on suitable media actinomycetes could be strongly inhibited by fluorescent pseudomonads. The strength of the inhibition did not always depend on the medium composition, because Pseudomonas spp. PD was antagonistic to all, or almost all, actinomycetes on any kind of medium. Generally, the strongest effect was produced on PGM, on which every strain of Pseudomonas, though growing poorly, was inhibitory to at least 20 actinomycetes out of the 23 tested. The effect was less pronounced on KBM, where a dozen or so strains were inhibited. The inhibition was much weaker on BM. On this medium Pseudomonas spp. PF did not cause any clear growth-inhibition zones in any of the actinomycetes, while Pseudomonas spp. P3, PI5 and PI8 made them in only a few strains. Still, in many actinomycetes, instead of growth-inhibition zones in both the vegetative and the aerial mycelium, the inhibition of the aerial mycelium alone occured, which was not observed on media other than BM. The use of PDA was the least advantageous for the antagonistic properties of fluorescent pseudomonads to manifest themselves. Ignoring the PD strain which was active on all the media, only P23 strain was inhibitory, but only towards a single strain of actinomycetes, and very weakly, too. On BM and PDA not only a smaller number of actinomycetes were inhibited, but the inhibition zones themselves, though sometimes comparatively broad, were generally smaller than on PGM or KBM.

On the basis of the growth of fluorescent pseudomonads on the media used, the conclusion could be drawn that their production of siderophores was not the main cause of the strong inhibition of actinomycetes. The fluorescent yellow-green siderophore pigment was only visible on the KBM medium. Its enrichment with Fe^{3+} to make siderophore production impossible nullified inhibition zones in only some actinomycetes (Table 2), although the dose of FeCb employed, 320 mg/cm^3, was many times higher than the 10 mg of $FeCl_3$/cm^3 sufficient to nullify the suppression of various *Phytium* strains by *P. fluorescens.* [1].

It cannot be ruled out that pseudomonads produced other, non-fluorescent, siderophores on the remaining 3 media. However, on PGM where the inhibition of actinomycetes was the strongest, the addition of $FeCl_3$ was of no significance. On BM, Fe^{3+} had a poor effect on the inhibition by Pseudomonas of the production of an aerial mycelium by actinomycetes. It was only on PDA, a medium distinguished by the actinomycetes that grew on it exhibiting low sensitivity to pseudomonads, that the addition of Fe^{3+} nullified the inhibitory effect.

All the above data justify the opinion that the high sensitivity of actinomycetes to fluorescent pseudomonads was caused by the production by these bacteria of antibiotic substances, rather than by their control of Fe^{3+} availability. It is worth noting at this point that sometimes an addition of Fe^{3+} not only did not reduce the inhibition zone of an actinomycetes strain, but even enlarged it. It corroborates the opinion [8] that while Fe^{3+} deficiency induces sidero-

Table 2. Influence of $FeCl_3$ on inhibition of actinomycetes by fluorescent pseudomonads on various media*)

Medium	Strains of actinomycetes							
	S1		S5		S15		S44L	
	$FeCl_3$ added (µg/ml)							
Strains of pseudomonads	0	320	0	320	0	320	0	320
	Inhibition zone (mm)**)							
Pridham-Gotllieb's medium								
P3	5	5	9	10	15	15	7	9
PD	18	12	18	16	17	12	18	15
PK	12	12	12	16	11	15	10	15
PF	9	9	11	10	5	10	10	17
King's B medium								
P3	17	0	17	5	23	0	16	0
P15	8	5	11	10	19	8	0	0
P23	10	7	15	10	15	15	6	0
PD	13	0	16	5	21	10	10	0
PK	10	7	15	10	16	15	6	0
PF	4	0	6	0	5	0	0	0
Burkholder's medium								
P3	0 [17]	0 [24]	0 [10]	0 [15]	0 [18]	0 [17]	0 [11]	0 [11]
PD	6	5	8	7	10	10	9	0
PK	0 [15]	0 [10]	0	0 [7]	0	0	0	0
PF	0 [15]	0 [10]	0	0 [10]	0	0	0	0
Glucose-potato medium								
P3	0	0	20	0	19	0	0	0
PD	5	0	6	0	5	0	5	0
PK	0	0	0	0	0	0	0	0
PF	0	0	0	0	0	0	0	0

* - In brackets - inhibition zones of only aerial mycelium, not vegetative one
**) - Standard deviation = ± 1 mm

phore production in Pseudomonas, its large concentration increases their production of antibiotics.

It follows from a comparison of Table 3 and Table 1 that not only fluorescent pseudomonads were inhibitory to actinomycetes, but also actinomycetes were inhibitory to those pseudomonads, as expected. It should be emphasized, however, that actinomycetes were less active towards pseudomonads than pseudomonads towards actinomycetes. In our system of experiments (7 strains of fluorescent pseudomonads, 23 of actinomycetes, and 4 media), out of 644 theoretically possible records, an antagonistic activity was registered in pseudomonads in 354 cases, and in actinomycetes in only 82 cases. In pseudomonads the inhibition zones were narrower, too.

Table 3. Ability of 23 isolates of actinomycetes to inhibit 7 strains of fluorescent pseudomonads.

Strains of pseudomonads	Number of inhibitory actinomycetes				Zone of inhibition (mm)*							
					Range				Average value			
	Medium											
	PGM	KBM	BM	PDA	PGM	KBM	BM	PDA	PGM	KBM	BM	PDA
P3	1	1	5	13	6	5	5-9	5-14	6	5	7	7
P15	0	1	5	10	0	5	5-8	5-14	0	5	6	9
P18	0	1	0	2	0	5	0	10-14	0	5	0	11
P23	0	1	0	5	0	7	0	5-10	0	7	0	8
PD	0	0	0	2	0	0	0	5-9	0	0	0	7
PK	0	1	0	20	0	8	0	6-13	0	8	0	10
PF	2	3	3	8	7-8	4-8	14-18	5-24	7	6	16	11

Explanations: as in Table 1
* - Standard deviation = ± 1 mm

Table 4. Mutual inhibition of fluorescent pseudomonads and actinomycetes on various media.

Medium	Pseudomonads	Inhibitory organisms	Actinomycetes
Pridham-Gotllieb's medium	PF	←→	S1, S17
King's B medium	PF	←→	S18
Burkholder's medium	P3, P15 PF	←→ ←→	S13, S15, S16 S12, S42L, S44L
Potato-glucose medium	PD	←→	S17, S42L

The number of actinomycetes inhibitory to Pseudomonas was in fact smaller than the results in Table 3 might suggest. Particular strains of actinomycetes on specific media were often antagonistic to several strains of pseudomonads. As a result, the inhibitory effect on PGM, KBM, BM and PDA media was produced by not more than 2, 1, 8 and 20 actinomycetes strains, respectively.

Noteworthy is the almost total lack of response of Pseudomonas spp. PD to actinomycetes; as has been mentioned earlier, it was their especially strong antagonist. Generally, fluorescent pseudomonads were inhibited the most weakly by actinomycetes on PGM, on which their antagonistic activity was the strongest. This was rather unexpected, because in experiments involving not Pseudomonas, but *Bacillus subtilis* (unpublished), it was on this medium the antagonism of actinomycetes was the strongest. It was observed in 19 strains as compared with 12, 7 and 5 strains on PDA, KBM and BM, respectively.

The only strong response of pseudomonads to actinomycetes could be considered to be that on PDA. On this medium Pseudomonas spp. PK were inhibited by 20 strains of actinomycetes. At the same time it was a medium on which pseudomonads inhibited actinomycetes the weakest, except Pseudomonas spp. PD.

The above statements suggest that in the interrelationships between fluorescent pseudomonads and actinomycetes antagonistic properties of the particular counter-partners may often manifest themselves in different environmental conditions. This is also corroborated by the results presented in Table 4. They show that in only 12 cases out of 82 theoretically possible, a specific Pseudomonas strain was inhibitory to an actinomycetes and *vice versa* in identical environmental conditions.

It was possible to find strains inhibitory to *F. oxysporum* among both, actinomycetes and pseudomonads (Tables 5 and 6). However, actinomycetes were inhibitory on all the media, whereas pseudomonads only on KBM or BM. Also, actinomycetes caused wider inhibition zones than pseudomonads. Actinomycetes inhibitory to *F. oxysporum* were usually sensitive to pseudomonads, unless they grew on PDA, on which they only responded to Pseudomonas spp. PD. Pseudomonads, which were equally antagonistic to *F.oxysporum*, if at all sensitive to actinomycetes, responded to a minority of their strains. These observations suggest that actinomycetes can be potentially stronger antagonists to *F. oxysporum* than fluorescent pseudomonads, but actinomycetes antagonistic to *F. oxysporum* are more sensitive to pseudomonads than strains of pseudomonads antagonistic to *F. oxysporum* are sensitive to actinomycetes. However, when assessing this phenomenon we must keep in mind the well-known fact that in a natural soil environment the production of antibiotics by actinomycetes is a very rare occurrence.

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Table 5. Ability of 23 actinomycetes isolates to inhibit *Fusarium oxysporum* on various media and their sensitivity to 7 strains of fluorescence pseudomonads.

Medium	Total number of isolates inhibitory to *F. oxysporum*	Number of inhibitory isolates sensitive to:			Average inhibition zone of *F. oxysporum* growth (mm)*)
		all or majority of strains of pseudomonads	minority of strains of pseudomonads	none strain of pseudomonads	
Pridham-Gotllieb's medium	15	15	0	0	29
King's B medium	15	15	0	0	24
Burkholder's medium	19	15	4	0	42
Potato-dextrose medium	18	1	0	17	10

*) Standard deviation = ± 2 mm

Table 6. Ability of 7 strains of fluorescence pseudomonads to inhibit *Fusarium oxysporum* on various media and their sensitivity to 23 actinomycetes isolates.

Medium	Total number of strains inhibitory to *F. oxysporum*	Number of inhibitory strains sensitive to:			Average inhibition zone of *F. oxysporum* growth (mm)*)
		all or majority of actinomycetes isolates	minority of actinomycetes isolates	none of actinomycetes isolates	
Pridham-Gotllieb's medium	0	0	0	0	0
King's B medium	4	0	3	1	11
Burkholder's medium	4	0	1	3	16
Potato-dextrose medium	7	0	7	0	10

*) Standard deviation = ± 2 mm

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