Effect of Fungal Metabolites on Colonization of Fungi on Nutrient Agar from Soil Inocula

During the rhizosphere study of *Andrographis paniculata* Nees, a medicinal plant, the authors noted that a smaller number of fungi colonized the rhizosphere and rhizoplane than the non-rhizosphere. Fungi on rhizoplane were more restricted than in rhizosphere. The reason may probably be attributed to the antagonistic effect on the microbial population of the rhizosphere. DIX[1] reported that the competition among most of the rhizosphere fungi was reduced when dead bean root substrate had been precolonized by one of the potential antagonists. DWIVEDI and GARRETT[2] studied the fungal competition in agar plate colonization from mixed soil inocula and found certain penicillia and *Trichoderma viride* to have the best tolerant capacity. The present study was designed to study the effect of metabolites of certain rhizoplane fungi on colonization of fungal flora on agar plates from the rhizosphere soil inocula of the test plant.

Materials and methods. Nine dominant rhizoplane fungi viz., *Mucor luteus*, *Neocosmospora vasinfecta*, *Botryodiplodia theobromae*, *Trichoderma lignorum*, *Aspergillus niger*, *Curvularia lunata*, *Fusarium culmorum*, white sterile mycelium and dark sterile mycelium were grown in 250 ml pyrex conical flask in Czapek's liquid culture. Equal size of agar discs from the margin of 1-week-old colonies were inoculated in the respective culture flasks. The flasks were incubated for 10 and 20 days at 25 °C and thereafter liquid cultures filtered in sterile flasks through a bacteria-proof filter. 5 ml of metabolite of each fungus were added to 15 ml freshly prepared and sterilized but cooled (35 °C) Czapek's agar medium. 1 ml of the rhizosphere soil suspension of the test plant was poured into the sterilized Petri dish and 5 plates for each metabolite were prepared as replicate. The plates were incubated at 25 °C in a culture room for a week and the % occurrence was calculated on the basis of fungi appearing in control.

Table I. Effect of metabolites supplied by 10-day-old cultures on the % occurrence of rhizosphere fungi

Name of fungi	C	M	N	B	T	A	Cl	F	W	D
Group I										
Sterile phycomycete	2	1	–	–	–	–	–	–	–	–
Botryodiplodia theobromae	1	–	–	2	–	–	–	–	–	–
Pestalozzia sp.	1	–	–	–	–	–	–	–	1	–
Aspergillus candidus	1	2	–	–	–	–	–	–	–	–
Spicaria silvatica	1	–	2	–	–	–	–	–	–	–
Verticillium terrestre	1	–	–	–	1	–	–	–	–	–
Papularia sphaerosperma	1	–	–	–	–	–	–	–	–	–
Humicola grisea	1	–	–	–	–	–	–	–	–	–
Fusarium oxysporum	1	–	2	–	–	–	–	–	–	–
Myrothecium roridum	1	–	–	–	–	–	–	–	–	–
Neocosmospora vasinfecta	1	–	6	–	–	–	–	–	–	–
Group II										
Aspergillus spulhureus	1	–	3	–	–	–	–	3	–	2
Paecilomyces fusisporus	2	–	2	–	–	–	–	–	4	–
Fusarium roseum	1	–	2	–	–	–	–	3	–	3
Sclerotium sp.	3	2	–	–	2	–	–	–	4	–
Group III										
Mucor luteus	4	1	–	11	1	–	–	3	–	5
Phoma glomerata	2	–	–	2	1	–	–	3	3	6
Trichoderma lignorum	2	1	–	–	1	1	1	–	4	–
Aspergillus sydowi	1	1	–	–	–	–	–	3	1	2
A. luchuensis	10	16	–	–	13	–	11	–	20	–
Curvularia lunata	1	–	2	2	–	–	–	2	1	–
Fusarium poae	8	–	2	–	4	–	2	–	–	3
F. chlamydosporum	3	2	–	–	3	–	5	–	3	–
Group IV										
Rhizopus nigricans	3	1	3	–	2	1	1	3	1	3
Aspergillus flavus	4	6	11	–	–	1	6	1	1	2
A. terreus	3	–	9	9	2	1	1	18	1	6
A. niger	22	20	5	8	16	22	27	2	21	8
Penicillium citrinum	2	3	3	2	1	2	–	2	4	–
Fusarium culmorum	5	25	20	8	2	2	–	18	10	18
White sterile mycelium	2	1	3	–	6	2	1	–	1	–
Dark sterile mycelium	6	–	2	2	5	–	10	–	5	–
Total number of species	31	14	16	9	15	8	10	12	17	11

C, control; M, *Mucor luteus*; N, *Neocosmospora vasinfecta*; B, *Botryodiplodia theobromae*; T, *Trichoderma lignorum*; A, *Aspergillus niger*; Cl, *Curvularia lunata*; F, *Fusarium culmorum*; W, white sterile mycelium; D, dark sterile mycelium.

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Table II. Effect of metabolites supplied by 20-day-old cultures on the % occurrence of rhizosphere fungi

Name of fungi	C	M	N	B	T	A	Cl	F	W	D
Group I										
Sterile phycomycete	2	–	–	–	–	–	–	–	–	–
Botryodiplodia theobromae	1	–	–	–	–	–	–	–	–	–
Pestalozzia sp.	1	–	–	–	–	–	2	–	–	–
Cephalosporium sp.	1	–	–	–	–	–	–	–	–	–
Aspergillus candidus	1	2	–	–	–	–	–	–	–	–
Spicaria silvatica	1		–	–	–	–		2	–	–
Verticillium terrestre	2	–	–	–	3	–	–	–	–	–
Papularia sphaerosperma	1	–	–	–	–	–	–	3	–	–
Humicola grisea	1	–	–	–	–	–	–	–	–	–
Curvularia lunata	1	–	–	–	–	–	–	–	–	–
Fusarium oxysporum	1	–	–	–	–	–	–	–	–	–
Myrothecium roridum	1	–	–	–	–	–	–	–	–	–
Neocosmospora vasinfecta	1	–	–	–	–	–	2	–	–	–
Aspergillus sulphureus	1	–	–	–	–	–	–	–	–	–
Group II										
Aspergillus sydowi	1	–	2	–	–	–	–	3	–	5
Paecilomyces fusisporus	2	–	–	–	–	–	3	–	3	–
Fusarium roseum	1	–	2	–	–	–	–	2	–	2
Sclerotium sp.	3	2	–	–	3	–	–	–	4	–
Fusarium poae	8	–	–	–	9	–	7	–	–	–
F. chlamydosporum	3	–	–	–	2	–	6	–	–	–
Aspergillus flavus	4	–	11	–	–	–	–	3	–	5
Dark sterile mycelium	6	–	–	–	7	–	10	–	–	2
Group III										
Mucor luteus	4	–	–	14	3	–	–	2	–	2
Phoma glomerata	2	–	6	–	3	–	–	–	3	3
Trichoderma lignorum	2	2	–	–	4	3	–	–	2	–
Aspergillus luchuensis	10	15	–	–	7	–	–	2	18	–
A. terreus	3	–	6	12	2	–	–	23	–	3
Penicillium citrinum	2	–	–	–	3	–	5	3	3	–
White sterile mycelium	2	2	–	–	7	–	6	–	2	–
Group IV										
Rhizopus nigricans	3	2	3	–	3	–	–	2	2	1
Aspergillus niger	22	9	–	5	16	17	31	–	29	3
Fusarium culmorum	5	31	20	–	3	–	–	25	15	31
Total number of species	32	8	7	3	15	2	9	11	10	10

C, control; M, *Mucor luteus*; N, *Neocosmospora vasinfecta*; B, *Botryodiplodia theobromae*; T, *Trichoderma lignorum*; A, *Aspergillus niger*; Cl, *Curvularia lunata*; F, *Fusarium culmorum*; W, white sterile mycelium; D, dark sterile mycelium.

Results and discussion. Majority of the commonly occurring fungi in the rhizosphere of test plant appeared in control. A comparison was made between the % occurrence of rhizosphere fungi occurring in control and in presence of different metabolites supplied by the selected rhizoplane fungi.

Fungi showed different degrees of tolerance to different metabolites supplied by the selected rhizoplane fungi. It is evident from the Tables I and II that the metabolites used from 20-day-old cultures produced more toxic effect on the total % occurrence of rhizosphere fungi in comparison to metabolites taken from 10-day-old cultures. The total number of fungal species appearing in the presence of metabolites of 20-day-old cultures was also reduced considerably. The maximum toxic effect was generated by the metabolite of *Aspergillus niger* followed by *B. theobromae*, *C. lunata* and *N. vasinfecta*. *Trichoderma lignorum* was noted to have the best tolerant capacity for the metabolite of *A. niger*, and was isolated with little increased frequency in the metabolite of 20-day-old cultures. *R. nigricans*, *A. flavus*, *A. terreus*, *P. citrinum*, *F. culmorum* and white sterile mycelium showed high degree of tolerance to the 10-day-old metabolites of *A. niger*. Only 3 species viz., *M. luteus*, *A. terreus* and *A. niger* were found to have the best tolerant capacity for the metabolite of *B. theobromae* taken from 20-day-old cultures but 9 fungal species were observed to have tolerance for metabolites from 10-day-old cultures. Similar to them the metabolite of *C. lunata* was also very toxic and only 9 species could grow in the presence of 10- and 20-day-old cultural filtrates. Fungi having the best tolerant capacity were *F. poae*, *F. chlamydosporum*, *A. niger*, *P. citrinum*. White sterile mycelium and dark sterile mycelium had little effect on the % occurrence of fungi. 81% of the total fungal population could appear in the presence of 20-day-old culture filtrate.

Several fungi were stimulated by the effect of metabolites. *Fusarium culmorum*, a secondary colonizer of the rhizoplane of the test plant was stimulated in the presence of *M. luteus*, *N. vasinfecta*, white sterile mycelium and dark sterile mycelium. Many other fungi viz., *A. flavus*, *A. terreus*, *M. luteus*, white sterile mycelium, dark sterile mycelium, *T. lignorum*, *A. niger*, *F. roseum*, *P.*

glomerata, A. luchuensis, P. citrinum, Paecilomyces fusisporus, Sclerotium sp. and *A. sydowi* increased in their % occurrence in some of the metabolites of test fungi.

GARRETT[3] reported that the ability of tolerance to the toxin of metabolites by other organisms is directly related to the competitive saprophytic ability. DIX[1] reported that some frequently occurring fungi in rhizosphere succumbed to antagonism and did not appear on the root surface. Results in the present study demonstrate that the pioneer colonizers as well as secondary and late colonizers of rhizoplane, which have more tolerance capacity to the metabolites produced by the coexisting fungi, have considerable effect on the biological equilibrium of the rhizosphere. Thus the quantity of selective antagonist influences the colonization of root surface and the rhizosphere if not entirely, then to a great degree.

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Effect of Actinomycin D on the Multiplication of the Infectious Pancreatic Necrosis Virus of Trout

Actinomycin D, which blocks all DNA-directed RNA synthesis, inhibits the replication of DNA viruses. In general, RNA viruses are not effected by concentrations of actinomycin which inhibit cellular RNA synthesis. The antibiotic does, however, have an inhibitory effect on some RNA viruses. The growth of some strains of poliovirus under special culture conditions has been restricted with actinomycin D[1,2]. Also, the multiplication of some myxoviruses, including influenza virus, appears to be inhibited by relatively low concentrations of actinomycin added at early times after infection but not at later times[3-6]. The inhibition of reovirus replication by actinomycin appears to be concentration dependent. Reovirus type 3 replication in L cells is inhibited by 1–2 μg actinomycin per ml[7] and virus formation remains sensitive to inhibition throughout most of the replicative cycle[8]. However, lower concentrations of actinomycin (0.5 μg/ml), which reduce cell RNA synthesis by more than 90%, apparently have no inhibitory effect on reovirus replication[9,10].

Although initially designated a picornavirus[11-14], the infectious pancreatic necrosis (IPN) virus of trout has recently been found to resemble members of the reovirus group in size and morphology[15,16]. Preliminary evidence from studies in our laboratory indicates that the RNA of IPN virus is not double-stranded and the virus is, therefore not a reovirus; however, IPN virus was found to have an effect on macromolecule synthesis in the infected cell that is remarkably similar to that reported for the reoviruses (upublished data). Only one report of the effect of actinomycin D on IPN virus has appeared: MALSBERGER and CERINI[12] reported that actinomycin D, at a concentration of 2.0 μg/ml, resulted in a greater than 99% inhibition of IPN virus in rainbow trout gonad cell cultures. The report presented here describes studies to determine if, among other similarities, IPN virus resembles reovirus in its sensitivity to actinomycin D.

Monolayer cultures of the rainbow trout gonad (RTG-2) cell line[17], propagated at 22 °C, were employed as the host system, with the maintenance medium being Eagle's minimum essential medium with Earle's balanced salts solution and supplemented with 2% fetal calf serum. Actinomycin D (Calbiochem, Los Angeles, Calif. 90054) was stored in amber vials as an aqueous stock (200 μg/ml) from which the desired concentration was made in maintenance medium.

The same stock of RTG-2 propagated IPN virus (Dry Mills strain) was used throughout this work. Virus activity was assayed by infectivity titrations of supernatant and cell fractions sonicated for 1 min at 20 kc/sec (Sonic Dismembrator, Quigley-Rochester, Rochester, N.Y.). Infectivity titrations were made in Micro-Test II tissue culture plates (Falcon Plastics, Los Angeles, California) using a micro-titration technique. Infectivity titers are expressed in $TCID_{50}$'s as calculated by the method of REED and MUENCH[18].

The effect of various concentrations of actinomycin D on IPN virus multiplication is summarized in Table I. As shown, the production of infective IPN virus in RTG-2 cells was inhibited by more than 99% in the presence of all concentrations of actinomycin tested. Additional experiments demonstrated that the inhibition of IPN virus does not require continous exposure to actinomycin. Similar degrees of inhibition of the virus were observed in cultures which had been pretreated with actinomycin 1 or 2 h prior to infection, washed free of residual antibiotic, and incubated in normal maintenance medium.

The results presented in Table II indicate that the inhibitory action of actinomycin D involves a step very early in the replicative process of IPN virus. Pretreatment

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