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THE EFFECT OF AN EGYPTIAN ISOLATE OF *STREPTOMYCES AFGHANENSIS* ON SOME PLANT VIRUSES

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Summary. – An Egyptian isolate of *Streptomyces afghanensis* was examined for the production of antiviral metabolites. Concentrated broth exudates of this microorganism were tested against tobacco mosaic virus (TMV), tomato mosaic virus (ToMV) and potato virus X (PVX) infecting *Nicotiana tabacum* L. cv. White Burley. Both concentrated metabolites and their acetone extract inhibited local lesion development of the tested viruses. In all cases, maximum antiviral effect was observed 2 hr after infection. The ultrafiltrate of broth culture reduced the number of local PVX lesions produced on the challenged half leaves in the case of later applications.

Key words: Streptomyces afghanensis; antiviral effect; plant viruses

Introduction

There are many screening studies for antivirals from actinomycetes, however, most of them were focused on animal and human viruses. Investigations on plant viruses included mostly the use of known manufactured antibiotics produced by actinomycetes (Bancroft and Key, 1964; Furusawa *et al.*, 1970; Hirai *et al.*, 1968; Kumoment and Samuel, 1971; Ouchi *et al.*, 1969).

In this paper the antiviral activity from exudates of *Streptomyces afghanensis* is demonstrated against three different plant viruses which produce local lesions and systemically infect many economic plants.

Materials and Methods

The isolate of Streptomyces afghanensis was obtained during a study aimed at the isolation of actinomycetes contaminating water supply system of Ismailia (Dewedar *et al.*, 1990).

Media. Starch nitrate (soluble starch, 20 g; $NaNo_3$, 2 g; K_2HPO_4, 1 g; KCl, 0.5 g; $MgSO_4.7H_2O$, 0.5 g; $FeSO_4.5H_2O$, 0.01 g; $CaCO_3$, 1 g; H_2O to 1 liter of solution). The medium was adjusted to pH 7 and used as a broth or solidified by adding agar to 2 %. Soft starch nitrate (7 g/l) amended with 10 %

glycerol was used for deep culture preservation of the stored microorganism. In addition, soft agar medium with omitted glycerol was used for the plaque assay technique to detect the presence of lysogenic phage (Adams, 1959).

Testing for lysogeny. Feshly grown subcultures and two-years-old cultures that were inoculated in glycerol-starch nitrate agar in sealed ampoules were used. These cultures were compared for the growth characteristics, the spontaneous or UV-induced phage production. For such purpose a single colony of new and old cultures was isolated and propagated. Some of these cultures were subjected to a germicidal UV lamp for 30 min followed by incubation at 30 °C for 4 days and then assayed for phage (Adams, 1959).

Production of antiviral metabolites. Spore inoculum from 7-days-old slants was prepared in a sterile saline. One ml of the spore suspension was inoculated into 250 ml Erlenmeyer flasks containing 100 ml starch-nitrate broth. Cultures were incubated at 30 °C for 7 days and then the broth was used after discarding mats. One litre broth culture was mixed with equal volume of acetone or ultrafiltered through 45 μm membrane filter. The acetone extract and the filter-sterilized broth were concentrated 50 times using rotary evaporater.

Viruses. TMV was obtained from Dr. Van Loon (Netherlands), PVX and ToMV were kindly supplied by Prof. Allam (Ain Shams University, Egypt). These viruses were propagated and maintained in our laboratory on *Nicotiana tabacum* L. cv. White Burley.

Testing for antiviral activities. Clay pots (30 cm diameter), each containing 10 plants of 60 days-old *N. tabacum* L. cv. White Burley were used at a stage of 5 foliage leaves. Inocula of TMV, ToMV and PVX were prepared in 0.01 mol/l phosphate buffer pH 7. Half of the second foliage leaf was mechanically inoculated by 100 μl of virus inoculum after dusting with carborundum (600 mesh). The opposite half leaf was challenged by the same virus inoculum 3 days after the first infection (only in the case of the ultrafiltrate broth).

S. afghanensis acetone extract or ultrafiltrate was applied in 100 μl aliquots 6, 4, 3 and 2 hr before infection and 0, 1, 2, 3, 4, 6, 12, 24 and 48 hr post infection (p. i.). Developed local lesions were counted after 10 days p. i. in at least 10 plants with each treatment. Plant pots were grown at temperature averaged 28 ± 3 °C during day time and 18 ± 2 °C during night time. The produced local lesions on the opposite half leaves were counted 10 days post challenge.

Results and Discussion

The Egyptian isolate of *S. afghanensis* is of the grey group which does not exudate pigments neither in the broth medium nor in the agar one. The characteristics of this organism is highly stable without any change of morphological or physiological properties. Moreover, the exposure of fresh or old cultures to UV radiaton did not excise any integrated phages and the plaque assay was negative. Thus, this *S. afghanensis* isolate is non-lysogenic. The absence of any integrated phage ensures a stable genetic constitution of this microorganism under controlled conditions.

In the antiviral experiments the local lesions assay of TMV, ToMV and PVX was chosen because of its feasibility and since by its use conclusive data could be obtained.

From Table 1 it appears that both the concentrated acetone extract and the ultrafiltrate of *S. afghanensis* broth produced antiviral activity on the tested viruses. The maximum antiviral activities were observed when the preparations were applied 2 hr p. i.

At later times of application of the preparations the antiviral effect decreased and finally disappeared: at 4th hr p. i. for TMV, at 6th hr for ToMV and at 12th

Table 1. The antiviral effect of preparations from cultures of S. afghanensis

Time of application	Number of local lesions								
	TMV			ToMV			PVX		
	a	b	c	a	b	c	a	b	c
Virus control	120	120	110	160	160	150	100	100	90
Before infection (hr)									
6	120	118	112	160	160	140	110	102	90
4	122	118	110	160	148	158	106	104	92
3	128	116	108	156	150	144	110	110	94
2	118	120	106	150	160	160	98	100	89
0	110	120	108	160	128	148	90	98	90
After infection (hr)									
1	108	80	110	152	98	150	92	58	94
2	0	4	11	8	24	152	6	12	88
3	6	40	112	12	48	158	14	24	70
4	40	60	108	35	96	146	36	36	60
6	110	120	106	140	122	140	52	48	40
12	118	124	100	146	140	128	68	88	24
24	112	120	90	142	150	116	83	86	18
48	112	124	80	150	150	112	94	92	16

Local lesion numbers of TMV, ToMV and PVX developed on half leaves of *N. tabacum* L. cv. White Burley treated with acetone extract (a) and ultrafiltrate (b) of *Streptomyces afghanensis* broth cultures. The opposite half leaves of (b) treatment were challenged with the correspondent virus (c). Average counts were made 10 plants 10 days after virus inoculation.

hr for PVX. Earlier applications (6–0 hr before infection) of the preparations did not had any antiviral effect. It seems that the acetone extract contains more active antiviral substances than the ultrafiltrate.

Challenge experiments with the opposite half leaves show that the ultrafiltrate induced antiviral state against PVX which increased with time.

Most of the previous work was done with pure and identified antibiotics concerning their antiviral activities against phytoviruses (Bancroft and Key, 1964; Dowson, 1978; Herai *et al.*, 1968; Kassanis *et al.*, 1975; McCarthy *et al.*, 1972; Misra and Neinhaus, 1977; Nakata and Suzuki, 1975; Phatak and Batra, 1966; Sela *et al.*, 1969; Semal, 1967; Yun and Hirai, 1968). The inhibition of virus tumour formations in tobacco by the antibiotic mithramycin was demonstrated by Misra and Neinhaus (1977).

The present data suggest that some metabolites of *S. afghanensis* possess antiviral activity against TMV, ToMV and PVX. No big differences were

obtained between the effects of the acetone extract and the ultafiltrated broth. The presence of one or more antiviral principles in the metabolites should be explored together with the factors affecting the productivity and the antiviral potentiality.

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