Neth. J. Pl. Path. 88 (1982) 39-45

The use of Solacol® (validamycin) as a growth retardant in the isolation of soil fungi

W. GAMS and WILLEKE VAN LAAR

Centraalbureau voor Schimmelcultures, Baarn

Accepted 4 August 1981

Abstract

Solacol®, a formulation of the antibiotic validamycin, at 0.33% in 2% malt extract agar, reduced the spread of fungi on dilution plates drastically and allowed twice as much incubation time before subculturing; this resulted in an elevated number of species isolated. Using pure cultures of 62 common soil fungi, it was shown that all fast-growing species (except *Pythium ultimum*) were efficiently inhibited but not completely suppressed. Inhibition was comparable to that by 0.5% oxgall, though, while this substance completely suppressed several species, Solacol very strongly inhibited only *Gaeumannomyces graminis, Gerlachia nivalis, Harzia acremonioides, Verticillium biguttatum* and *Rhizoctonia solani.* In a further experiment each separate constituent of Solacol was tested against 22 fungi at equivalent concentrations. Validamycin strongly inhibited *Chaetomium globosum* and two Basidiomycetes, though hardly more than the non-ionic detergent which mainly inhibited the other fungi. A few species were, however, more inhibited by Solacol than by the detergent alone. Solacol at 0.33% is a suitable aid in dilution plating of soil fungi, by increasing the number of colonies and species observed.

Additional keywords: antibiotic, detergent, oxgall, hyperparasites, *Rhizoctonia solani, Thanatephorus, Athelia rolfsii, Sclerotium rolfsii, Verticillium biguttatum, Gliocladium roseum.*

Introduction and preliminary observations

The antibiotic validamycin, isolated from *Streptomyces hygroscopicus* (Jensen) Waksman & Henrici var. *limoneus* Iwasa et al. (1971 a, b), was reported to be effective against sheath blight of rice. It caused abnormal branching in hyphal tips of the Basidiomycetes *Thanatephorus sasakii, Th. praticola, Th. cucumeris (Rhizoctonia solani)* and *Athelia (Sclerotium) rolfsii* and further development of the hyphae was arrested (Nioh and Mizushima, 1974; Wakae and Matsuura, 1975), whereas no antimicrobial activity was observed against 2177 strains of other (not listed) fungi tested on the usual agar media (Iwasa, 1978). The first promising results of treating seed potatoes against *Rhizoctonia solani* in the Netherlands were reported by Bakkeren et al. (1977) and Bakkeren and Krul (1977), and the antibiotic was admitted in the Netherlands in 1978 for this purpose in a liquid formulation containing per litre 30 g validamycin, 4 g of a non-ionic detergent, 1 g of a preservative, 0.5 g of an anti-foam agent and 0.12 g of a blue dye, under the name

Solacol® produced by Aagrunol B.V., Groningen (Flipse, 1978). The recommended dilution is 20 times, i.e. 1.5 g a.i.·l^{-1}. In other countries the antibiotic is available in a formulation with a higher concentration of a detergent under the name Validacin® manufactured by Takeda Ltd., Tokyo, for the control of sheath blight in rice.

Besides seed potato disinfection with Solacol, another means of suppression of *Rhizoctonia solani,* viz. by hyperparasitic fungi, such as *Verticillium biguttatum* W. Gams (Gams and Van Zaayen, 1982) and *Gliocladium* species, has gained interest through the work of Jager et al. (1979). With the intention of isolating these hyperparasites quantitatively from soil, experiments were started where 0.67% Solacol was incorporated together with aureomycin (20 $\mu g \cdot ml^{-1}$) into the malt extract agar used for dilution plating of a sandy soil from Haren (prov. Groningen) and a clay soil from Sexbierum (prov. Friesland). The surprising result was that all fungi which developed on these plates grew much more slowly, though the colonies appeared normal, so that the plates could be left for eleven days instead of the usual five days at about 20°C, before subcultures were made. This allowed the development and isolation of several slow-growing fungi which had been missed on other dilution plates. *V. biguttatum* and *Gliocladium* species, however, were not isolated. Hence a selective effect on the microflora could be expected. In a parallel experiment the radial growth of *G. roseum* was inhibited by 30% with 0.33% Solacol and by 50% with 0.67%, while *V. biguttatum* did not show any growth after 9 days with 0,67% Solacol, and only a trace with 0.33% at about 20 °C.

In a simultaneous dilution plate series, *Mortierella* species were hardly inhibited by quintozene or iprodione (Rovral) (both at 50 mg·l^{-1}); *Fusarium* species tended to overgrow the Petri dishes with quintozene after a few days, while *V. biguttatum* and *Gliocladium* species again were absent. Subsequently Solacol was also successfully used in Warcup soil plates with a Columbian páramo soil sample to suppress *Mortierella* and *Zygorrhynchus* species which would otherwise rapidly overgrow the other fungi.

In order to find out whether any fungi would still be unsatisfactorily inhibited or others would be suppressed completely, 62 potentially soil-borne species representing 55 genera were taken from the CBS collection and tested against Solacol and its constituents.

Materials and methods

Colonies were grown from 8 mm diam mycelial discs and radial growth was measured after 3, 5 and 7 days of growth at 20°C in darkness. One or 0.5 ml of a 10% Solacol solution in sterile water were pipetted into the Petri dishes before pouring out 15 ml of 2% malt extract agar, resulting in 0.67 and 0.33% Solacol, respectively (200 and 100 mg validamycin·l^{-1}). A parallel series with the higher antibiotic concentration was incubated under continuous light of four fluorescent tubes giving 2500 lux, so as to detect any additional toxic effects of the antibiotic under light, similar to those known to occur with Rose Bengal (Pady et al., 1960; Ottow, 1972).

Another series was grown on the same medium to which 5 g oxgall·l^{-1} had been added before sterilization, as this compound, amongst other detergents, is now

often used with success to retard fungal growth (Miller et al., 1951; Papavizas and Davey, 1959; Steiner and Watson, 1965; Tuite, 1969).

Results and discussion

None of the fungi showed a significant change of the inhibition when grown under light and therefore details on light effects need not be given below.

The fastest growing fungi tested (with the exception of *Pythium ultimum*) all showed satisfactory inhibition on both concentrations tested, whereas oxgall had more divergent effects. In Table 1 the maximal radial increment measured over a 2-day interval is shown.

The behaviour of the remaining, less fast-growing fungi is shown in Table 2; inhibition ranged from 50 to 90% of radial growth with little difference between the two concentrations of Solacol used. Almost complete inhibition by 0.33% Solacol occurred in *Gaeumannomyces graminis, Harzia acremonioides* and *Rhizoctonia*

Table 1. Radial extension[1] of fast-growing fungi on malt extract agar containing Solacol and oxgall.

Fungal species	Control	Solacol (0.33%)	Solacol (0.67%)	Oxgall (0.5%)
Apiosordaria verruculosa	20	2.5	1.5	4
Athelia rolfsii	15	3.5	0.5	0
Botrytis cinerea	18	3	2.5	8
Cunninghamella elegans	26	3.5	3.5	8
Epicoccum purpurascens	11	2.5	1.5	3
Fusarium culmorum	13	4.5	4	8
Gaeumannomyces graminis	15	2.5	1.5	0.5
Gerlachia nivalis	>15	3.5	2.0	2.5
Harzia acremonioides	7.5	1.5	0.5	2.5
Mortierella hyalina	19	2	1.5	5
Mucor hiemalis	>20	4.5	4.5	9.5
Peziza ostracoderma	8	3.5	2.5	1
Phytophthora cactorum	9.5	5.5	5.5	0.5
Phytophthora cinnamomi	9.5	4.5	3.5	(1)
Pythium dissotocum	>25	4.5	3	0.5
Pythium ultimum	>25	>20	>20	0
Rhizoctonia solani	15	0.5	0.5	10.5
Rhizopus oryzae	>25	5.5	4	5.5
Sclerotinia sclerotiorum	>20	3	1.5	1.5
Thamnidium elegans	13	3	3	9.5
Trichoderma viride	>18	5	5	9
Zygorrhynchus moelleri	>20	7	8	3.5

[1] Maximal radial extension (mm) measured in 2-day intervals at 20 °C.

Tabel 1. Radiale groei van enkele snelgroeiende schimmels op moutextract agar met Solacol en ossegal.

Table 2. Comparison between inhibition by Solacol and oxgall of less fast-growing fungi.

Stronger inhibition by Solacol than by oxgall	About equal inhibition by Solacol and oxgall	Less inhibition by Solacol than by oxgall
Alternaria alternata	*Aspergillus niger*	*Cylindrocarpon destructans*
Arthrobotrys oligospora	*Aureobasidium pullulans*	*Exophiala jeanselmei*
Broomella acuta	*Cladosporium cladosporioides*	*Geotrichum candidum*
Chaetomium globosum	*Cladosporium herbarum*	*Mortierella ramanniana* var. *ramanniana*
Colletotrichum gloeosporioides	*Coniothyrium fuckelii*	*Periconia macrospinosa*
Doratomyces stemonitis	*Curvularia geniculata*	*Phialophora malorum*
Emericella nidulans	*Humicola grisea*	*Verticillium nigrescens*
Eurotium herbariorum	*Microdochium bolleyi*	
Gliocladium roseum	*Myrothecium roridum*	
Metarrhizium anisopliae	*Paecilomyces carneus*	
Microsporum gypseum	*Paecilomyces marquandii*	
Phoma eupyrena	*Penicillium verrucosum* var. *cyclopium*	
Plectosphaerella cucumerina	*Petriellidium boydii*	
Scopulariopsis brevicaulis	*Stachybotrys chartarum*	
Sesquicillium candelabrum	*Talaromyces wortmannii*	
Verticillium tenerum	*Thelebolus polysporus*	
	Verticillium lecanii	

Tabel 2. Vergelijking tussen de remeffecten van Solacol en ossegal bij minder snel groeiende soorten.

solani (Table 1). Complete or almost complete inhibition by oxgall occurred in *Athelia rolfsii,* the Oomycetes (Table 1) and *Exophiala jeanselmei.*

To discover which component of Solacol was responsible for the growth retardation, the experiment was repeated with 22 fungi and adding either 0.33% Solacol or the equivalant concentrations of each separate constituent to the agar: validamycin (available in powder form, technical grade with 49.8% a.i. of mainly validamycin A) (100 $mg \cdot l^{-1}$), detergent (13 $mg \cdot l^{-1}$), preservative (3.3 $mg \cdot l^{-1}$), anti-foam agent (1.6 $mg \cdot l^{-1}$), and dye (0.4 $mg \cdot l^{-1}$). The results for the first two compounds are shown in Table 3, whilst the other three had negligible effects. The preservative is apparently not efficient at considerably lower concentrations than that present in undiluted Solacol.

Validamycin alone had no inhibitory effect except on *Chaetomium globosum* and the Basidiomycetes, *Athelia rolfsii* and *Rhizoctonia solani.* But in these fungi the inhibition due to the detergent was in the same order of magnitude as that due to validamycin alone. The apparent discrepancies between the figures in Tables 1 and 3 reflect the variation occurring in this kind of experiment, but do not affect the general tendency.

The action of Solacol has been shown to be due to several components, the detergent being generally more active than the antibiotic validamycin. However, the sum of the components sometimes has a much stronger action than the single constituents, as shown for *Epicoccum purpurascens, Gliocladium roseum, Mucor hiemalis, Pythium dissotocum* and *Zygorrhynchus moelleri* in Table 3. For these fungi the experiment was repeated with similar results. As non-ionic detergents are

Table 3. Radial extension[1] of 22 fungi on malt extract agar containing 0.33% Solacol and equivalent concentrations of its main constituents.

Fungal species	Control	Solacol (0.33%)	Validamycin (100 mg·l^{-1})	Detergent (13 mg·l^{-1})
Alternaria alternata	13	3	12	4.5
Athelia rolfsii	14	1	7	7
Botrytis cinerea	19	3	20	4
Broomella acuta	5.5	2	6	2.5
Chaetomium globosum	10	1	3.5	6
Cunninghamella elegans	19	8.5	19	11
Epicoccum purpurascens	5.5	2.5	5	4.5
Fusarium culmorum	17	8	13	7
Gaeumannomyces graminis	13	0	14	3.5
Geotrichum candidum	8	4	8	5
Gerlachia nivalis	15	1.5	15	3
Gliocladium roseum	5	3	5	5
Mortierella hyalina	16	4	18	7
Mucor hiemalis	26	4	26	20
Penicillium verrucosum var. *cyclopium*	5	2.5	5	3.5
Petriellidium boydii	4.5	2	4.5	3.5
Pythium dissotocum	33	2.5	30	32
Rhizoctonia solani	28	2	8	9
Scopulariopsis brevicaulis	5	2.5	5	2.5
Thelebolus polysporus	4	2	4	3.5
Trichoderma viride	22	6	20	12
Zygorrhynchus moelleri	32	8	32	17

[1] Maximal radial extension (mm) measured in 2-day intervals at 20°C.

Tabel 3. Radiale groei van fungi op moutextract agar met 0.33% Solacol en de hoofdcomponenten daarvan in vergelijkbare concentraties.

relatively persistent in soil (Lee, 1970), whilst validamycin is very rapidly decomposed (Bakkeren et al., 1977), it is likely that the detergent contained in Solacol will also contribute to its effect on seed potatoes.

In conclusion, the addition of 0.33% Solacol to, for example, 2% malt extract agar is a convenient method by which the spread of moulds can be inhibited when using dilution plates and similar techniques. Almost all broadly spreading species are efficiently slowed down, whilst the slowest-growing fungi are inhibited the least. Because of its general and rather uniform growth-retarding action with little alteration of colonial characters, Solacol compares favourably with other inhibitors used for the same purpose. In contrast with oxgall, it allows the isolation of *Pythium* and *Phytophthora* species, whilst the spread of other fast-growing fungi, particularly Mucorales, is generally (with the exception of *Zygorrhynchus moelleri*) more efficiently reduced. *Gaeumannomyces graminis, Gerlachia nivalis, Verticillium biguttatum* and a few other fungi showed very little growth on Solacol, and for assessing these fungi the method may be less suitable.

Acknowledgements

We wish to acknowledge the assistance of Drs O. Constantinescu and Th. Limonard with the documentation. Mr J. A. Ebbers and Mr M. Bakkeren from Aagrunol B.V., Haren, kindly supplied validamycin and the other constituents of Solacol as well as information about its manufacture.

Samenvatting

Het gebruik van Solacol® (validamycin) als groeiremmer bij het isoleren van grondschimmels

Solacol®, een formulering van het antibioticum validamycine, remde de groei van schimmels in verdunningsplaten met een concentratie van 0.33% in 2% moutagar en maakte het mogelijk de periode tot afenten met een factor 2 te verlengen; daardoor was het aantal geïsoleerde soorten duidelijk toegenomen. Met reincultures van 62 algemene grondschimmelsoorten werd aangetoond, dat alle snelgroeiende soorten (met uitzondering van *Pythium ultimum*) voldoende geremd, maar niet volkomen onderdrukt werden. Het remmingspercentage was vergelijkbaar met dat van 0.5% ossegal, hoewel dit laatste sommige soorten volkomen onderdrukte; Solacol remde alleen *Gaeumannomyces graminis, Gerlachia nivalis, Harzia acremonioides, Verticillium biguttatum* en *Rhizoctonia solani* zeer sterk. In een volgend experiment werden de componenten van Solacol t.o.v. 22 fungi apart getoetst in concentraties equivalent aan 0.33% Solacol. Validamycine remde alleen *Chaetomium globosum* en twee basidiomyceten behoorlijk, maar nauwelijks meer dan de niet-ionische uitvloeier, die in hoofdzaak de overige remeffecten veroorzaakte. Enkele soorten werden echter door het complete Solacol veel sterker geremd dan door de uitvloeier alleen. Solacol in een verdunning van 0,33% wordt aanbevolen bij verdunningsplaten voor het isoleren van grondschimmels ten einde het aantal kolonies en soorten te verhogen.

References

Bakkeren, M., Jong, S. de & Krul, J., 1977. Validamycin, a new fungicidal antibiotic. Proc. 9th Br. Crop Prot. Conf., Brighton 2: 541-546.

Bakkeren, M. & Krul, J., 1977. Validamycine, een nieuw antibioticum met fungicide eigenschappen. Gewasbescherming 8: 14.

Flipse, L.P., 1978. Nieuwe en gewijzigde toelatingen van bestrijdingsmiddelen. Gewasbescherming 9: 23-25.

Gams, W. & Zaayen, A. van, 1982. Contribution to the taxonomy and pathogenicity of fungicolous *Verticillium* species. I. Taxonomy. Neth. J. Pl. Path. 88: 57-78.

Iwasa, T., 1978. Antifungal characteristics and bioassay methods of validamycin, an antibiotic effective against the sheath blight of rice plants. J. Takeda Res. Lab. 37: 307-352 (in Japanese, with English summary).

Iwasa, T., Higashide, E., Yamamoto, H. & Shibata, M., 1971a. Studies on validamycins, new antibiotics. II. Production and biological properties of validamycins A and B. J. Antibiot., Tokyo 24: 107-113.

Iwasa, T., Kameda, Y., Asai, M., Horii, S. & Mizuno, K., 1971b. Studies on validamycins, new antibiotics. IV. Isolation and characterization of validamycins A and B. J. Antibiot., Tokyo 24: 119-123.
Jager, G., Hoopen, A. ten & Velvis, H., 1979. Hyperparasites of *Rhizoctonia solani* in Dutch potato fields. Neth. J. Pl. Path. 85: 253-268.
Lee, B.K.H., 1970. The effect of anionic and nonionic detergents on soil microfungi. Can. J. Bot. 48: 583-589.
Miller, J.J., Peers, D.J. & Neal, R.W., 1951. A comparison of the effects of several concentrations of oxgall in platings of soil fungi. Can. J. Bot. 29: 26-31.
Nioh, T. & Mizushima, D., 1974. Effect of validamycin on the growth and morphology of *Pellicularia sasakii*. J. gen. appl. Microbiol. Tokyo 20: 373-383.
Ottow, J.C.G., 1972. Rose Bengal as a selective aid in the isolation of fungi and actinomycetes from natural sources. Mycologia 64: 304-315.
Pady, S.M., Kramer, C.L. & Pathak, V.K., 1960. Supression of fungi by light on media containing Rose Bengal. Mycologia 52: 347-350.
Papavizas, G.C. & Davey, C.B., 1959. Evaluation of various media and antimicrobial agents for isolation of fungi from soil. Soil Sci. 88: 112-117.
Steiner, G.W. & Watson, R.D., 1965. Use of surfactants in the soil dilution and plate count method. Phytopathology 55: 728-730.
Tuite, J., 1969. Plant pathological methods. Fungi and bacteria. Burgess Publ. Co., Minneapolis, 239 pp.
Wakae, O. & Matsuura, K., 1975. Characteristics of validamycin as a fungicide for *Rhizoctonia* disease control. Rev. Pl. Prot. Res. 8: 81-92.

Address

Centraalbureau voor Schimmelcultures, P.O. Box 273, 3740AG Baarn, the Netherlands.