Effects of Validamycin A on the Morphology, Growth and Sporulation of *Rhizoctonia cerealis*, *Fusarium culmorum* and Other Fungi

By GEOFFREY D. ROBSON,¹* PAUL J. KUHN² AND ANTHONY P. J. TRINCI¹

¹ Microbiology Group, Department of Cell and Structural Biology, School of Biological Sciences, Stopford Building, University of Manchester, Manchester M13 9PL, UK

² Shell Research Limited, Sittingbourne Research Centre, Sittingbourne, Kent ME9 8AG, UK

(Received 14 June 1988)

All Basidiomycotina screened were sensitive to validamycin A, whereas most Ascomycotina and all Mucorales and Oomycetes were insensitive. Studies with *Rhizoctonia cerealis* and *Fusarium culmorum* showed that, in semi-solid culture, the antibiotic caused a decrease in colony radial growth rate and that this was associated with a decrease in mean hyphal extension rate and an increase in hyphal branching. However, the antibiotic did not alter the morphology of *R. cerealis* grown in liquid culture (shaken or stationary). Validamycin A caused a reduction in the number and viability of conidia produced by *F. culmorum*.

INTRODUCTION

Validamycin A is an antibiotic used in Japan and China to control rice sheath blight (Wakae & Matsuura, 1975). Unlike most antifungal agents, validamycin A is not fungicidal or fungistatic but controls the spread of the pathogen, *Pellicularia sasakii* (synonym of *Rhizoctonia solani*), on the plant by reducing the maximum rate of hyphal extension without affecting the organism's specific growth rate (Trinci, 1984); the decrease in hyphal extension caused by the antibiotic is associated with an increase in branch frequency (Nioh & Mizushima, 1974; Trinci, 1985). Compounds of this type are known as paramorphogens (Tatum *et al.*, 1949) and include L-sorbose (Trinci & Collinge, 1973; Jejelowo & Trinci, 1988), 3-O-methyl glucose (Galpin *et al.*, 1977; Jejelowo & Trinci, 1988), glucosamine (Jejelowo & Trinci, 1988), sclareol (Bailey *et al.*, 1974) and α -aminoisobutyric acid (S. C. Watkinson, personal communication).

In this study we investigated the spectrum of activity of validamycin A and determined its effect on the growth and morphology of *Rhizoctonia cerealis* and *Fusarium culmorum*.

METHODS

Antibiotic, organisms, media and incubation. Validamycin A (stock no. 3, purity 93.5%) was a gift from Takeda Chemical Industries Ltd, Tokyo, Japan. *Rhizoctonia cerealis* Van der Hoeven (strain R80/101) was obtained from the Plant Breeding Institute, Cambridge, UK. *Phanerochaete chrysosporium* was obtained from Dr J. M. MacDonald of the University of Manchester Institute of Science and Technology, UK. *Bjerkandera adusta*, *Heterobasidion annosum*, *Phlebia radiata*, *Phlebia rufa*, *Phlebia gigantea* and *Pseudotrametes gibbosa* were obtained from Dr A. D. N. Rayner, School of Biological Sciences, University of Bath, UK. *Agaricus silvicola*, *Cytoderma pereniciosa*, *Daedalia quercina*, *Entoloma arborivus*, *Fistulina hepatica*, *Flammulina velutipes*, *Hericium corraloides*, *Lentinus edodes*, *Lepiota procera* and *Pleurotus ostreatus* were obtained from Dr J. N. Hedger, Department of Botany and Microbiology, University College of Wales, Aberystwyth, UK. All other fungi were obtained from the Culture Collection of the Department of Cell and Structural Biology, University of Manchester, UK. The fungi were cultured on malt extract agar (Lab M) slopes and stored at 4 °C under paraffin oil.

Fungi were grown on Vogel's medium (Vogel, 1956) containing 5 or 50 mM-glucose (instead of sucrose) as the carbon source; the medium was prepared as described by Robson *et al.* (1987). Except for *R. cerealis* and *R. solani* A 79, modified Vogel's medium was used to cultivate Basidiomycotina (and Deuteromycotina with basidiomycete

perfect stages). This was prepared by amending Vogel's medium with 10 ml filter sterilized (0.22 µm) yeast extract 'growth factor' solution per litre of medium; 100 ml yeast extract 'growth factor' solution was prepared from 5 g yeast extract (Lab M) according to Northam & Norris (1952) and stored at -20 °C until required. When necessary, medium was solidified with 15 g agar (Taiyo, Davis Gelatine) per litre of medium. All cultures were incubated at 25 °C.

Semi-solid cultures. Fungi were grown on 20 ml volumes of agar medium in 9 cm Petri dishes. Plates were inoculated with 4.5 mm diameter mycelial plugs taken from the margin of colonies which were in the linear phase of growth. The agar medium in plates was sometimes overlaid with circles (8.5 cm diam.) of Cellophane (Rayophane, British Cellophane) prior to inoculation; Cellophane was boiled (5 min) twice in distilled water to remove the plasticizers before being sterilized in water at 121 °C for 15 min. Cellophane overlaid medium was either inoculated with 4.5 mm diameter plugs taken from the margin of colonies of *R. cerealis* in the linear phase of growth or with a conidial suspension of *F. culmorum* to give not more than 200 conidia per plate (the conidia were spread over the surface of the medium using a glass spreader).

Liquid cultures. *R. cerealis* was grown in 50 ml volumes of Vogel's medium in 250 ml flasks, which were inoculated with 0.5 ml volumes of a mycelial blend prepared by homogenizing (using an MSE homogenizer at high speed) liquid grown mycelia for 2 min in 20 ml sterile distilled water, filtering the suspension through a layer of muslin, and then adjusting the concentration of the blend with sterile distilled water until it gave an optical density of about 1.0 at 540 nm. *F. culmorum* was grown in 20 ml volumes of Vogel's medium in 250 ml nephlos flasks (conical flasks with a test-tube side arm) inoculated with 0.2 ml volumes of a suspension containing 1×10^6 conidia ml⁻¹. Shake flask cultures were shaken at 200 r.p.m. on a rotary shaker with a 2.5 cm stroke. *R. cerealis* was sometimes grown in stationary liquid cultures in 9 cm Petri dishes as described by Cohen (1973).

Measurements of morphology and growth. Internode lengths (the distance between adjacent primary branches) were measured (using an Olympus travelling micrometer eyepiece) on the first three internodes of leading hyphae at the margin of colonies of *R. cerealis* in the linear phase of growth. Alternatively, internode lengths were measured on mycelia grown in 10 ml volumes of liquid medium in 9 cm Petri dishes (Cohen, 1973). Hyphal growth unit (*G*, total hyphal length of a mycelium divided by the number of hyphal tips) measurements were made on *F. culmorum* mycelia which had at least 10 hyphal tips; these mycelia were grown on Cellophane overlaid agar medium (Trinci, 1974). Colony diameters were measured with a Shadowmaster (Baty & Co) using a $\times 10$ objective. Colony dry weights of *R. cerealis* were obtained by washing mycelia off Cellophane overlaid plates onto pre-dried, weighed filter papers (Whatman no. 1); the biomass was washed with 100 ml distilled water before being dried to constant weight.

Specific growth rates of *F. culmorum* germ tubes were measured using time lapse photomicroscopy (Trinci, 1969); growth of only the first-formed germ tube was followed. Measurements of the mean rate of extension (*E*) of hyphae of young mycelia were made as described by Trinci (1974) and were calculated from,

$$E = \frac{2(H_1 - H_0)}{B_0 + B_1} \quad (1)$$

where H_0 = total hyphal length at zero time, H_1 = total hyphal length 1 h later, B_0 = no. of tips at zero time, and B_1 = no. of tips 1 h later.

The specific growth rate of *F. culmorum* in shake flask culture was determined by measuring culture optical density (Trinci, 1972) with an EEL colorimeter using a green filter (540–560 nm). Percentage germination of conidia was measured using plates of Cellophane overlaid agar medium, which were inoculated with 0.2 ml volumes of a suspension containing 1×10^4 conidia ml⁻¹. A conidium was regarded as having 'germinated' if a germ tube of any length could be detected; at least 100 conidia were counted from five fields of view under the microscope.

Measurement of spore production. Cellophane overlaid plates were inoculated and 'spread' with 0.2 ml volumes of a conidial suspension (about 1×10^6 conidia ml⁻¹). At various intervals after the onset of incubation, conidia were harvested in 10 ml distilled water, using a glass spreader to agitate the surface of the mycelium. The spore suspension was filtered through a single layer of muslin and conidia were counted using a haemocytometer.

RESULTS

Sensitivity of fungi to validamycin A

Various fungi were screened for sensitivity to validamycin A and those sensitive to the antibiotic are shown in Table 1. The following fungi were insensitive to 25 µM-validamycin A in media containing 5 mM-glucose (same conditions as in Table 1). Deuteromycotina – *Alternaria brassicicola*, *Alternaria alternata*, *Aspergillus terreus*, *Aspergillus niger*, *Aspergillus giganteus*, *Aspergillus oryzae*, *Aspergillus nidulans*, *Botrytis cinerea*, *Botrytis fabae*, *Cladosporium cucumeris*,

Table 1. *Fungi sensitive to validamycin A*

Fungi were grown at 25 °C on Vogel's (fungi other than Basidiomycotina) or modified Vogel's (Basidiomycotina) media containing 5 mM-glucose in the presence and absence of 25 µM validamycin A. The values are expressed as a percentage of the diameter of colonies grown in the absence of validamycin. All diameters were measured whilst colonies were in the linear phase of growth and two measurements were made at right angles on three replicate plates to give six colony diameters which were treated as independent variables. The diameters of all validamycin A treated colonies were significantly different (using the *t*-test, $P < 0.05$) from control colonies.

Organism*	Colony diameter of treated fungi (% of control)	Organism*	Colony diameter of treated fungi (% of control)
<i>Bjerkandera adusta</i>	23	<i>Lentinus edodes</i>	48
<i>Pleurotus ostreatus</i>	24	<i>Stereum subtomentosum</i>	62
<i>Rhizoctonia cerealis</i> ^a	28	<i>Chaetomium globosum</i> ^b	63
<i>Coprinus cinereus</i>	28	<i>Agaricus silvicola</i>	63
<i>Flammulina velutipes</i>	28	<i>Hericius coralloides</i>	66
<i>Rhizoctonia solani</i> A79 ^a	28	<i>Phlebia gigantea</i>	70
<i>Lepiota procera</i>	31	<i>Phanerochaete chrysosporium</i>	72
<i>Chaetomium bostrychoides</i> ^b	38	<i>Stereum hirsutum</i>	77
<i>Fusarium culmorum</i> ^a	39	<i>Entoloma arborivius</i>	78
<i>Fistulina hepatica</i>	40	<i>Phlebia radiata</i>	84
<i>Schizophyllum commune</i>	42	<i>Heterobasidion annosum</i>	86
<i>Daedalia quercina</i>	47	<i>Phlebia rufa</i>	88
<i>Pseudotrametes gibbosa</i>	48		

* All fungi are Basidiomycotina except for *a*, Deuteromycotina and *b*, Ascomycotina.

Table 2. *Effect of validamycin A on the radial growth rate of various Basidiomycotina and Deuteromycotina*

Fungi were grown at 25 °C on Vogel's (*R. cerealis* and *F. culmorum*) or modified Vogel's (other fungi) media containing 50 mM-glucose. All radial growth rates (mean ± SE) were measured whilst colonies (six replicates) were in the linear phase of growth. For any particular species, colonial radial growth rates with the same letter (*a-e*) are not significantly ($P < 0.05$) different.

Organism	Radial growth rate (µm h ⁻¹)					
	0	10	Validamycin A concn (µM):			
			100	500	1000	5000
<i>Rhizoctonia cerealis</i> *	259 ± 6 ^a	101 ± 2 ^b	94 ± 1 ^b	77 ± 3 ^c	60 ± <1 ^d	45 ± <1 ^e
<i>Pseudotrametes gibbosa</i> †	336 ± 7 ^a	316 ± 4 ^a	325 ± 6 ^a	213 ± 2 ^b	126 ± 2 ^c	108 ± 5 ^d
<i>Phanerochaete chrysosporium</i> †	742 ± 16 ^a	737 ± 12 ^a	739 ± 21 ^a	691 ± 8 ^b	689 ± 11 ^b	488 ± 8 ^c
<i>Phlebia gigantea</i> †	146 ± 1 ^a	144 ± 1 ^a	144 ± 2 ^a	135 ± <1 ^b	136 ± 1 ^b	71 ± <1 ^c
<i>Phlebia radiata</i> †	169 ± 3 ^a	169 ± 1 ^a	167 ± 2 ^a	169 ± 2 ^a	154 ± 2 ^b	128 ± 1 ^c
<i>Pleurotus ostreatus</i> †	112 ± <1 ^a	96 ± <1 ^b	97 ± 1 ^b	93 ± <1 ^b	95 ± <1 ^b	56 ± <1 ^c
<i>Heterobasidion annosum</i> †	224 ± 2 ^a	174 ± 3 ^b	177 ± 2 ^b	174 ± 1 ^b	72 ± <1 ^c	75 ± <1 ^c
<i>Fistulina hepatica</i> †	297 ± 5 ^a	300 ± 7 ^a	296 ± 2 ^a	302 ± 11 ^a	207 ± 1 ^b	100 ± 3 ^c
<i>Fusarium culmorum</i> *	447 ± 4 ^a	440 ± 5 ^a	365 ± 7 ^b	302 ± 3 ^c	280 ± 3 ^c	262 ± 41 ^e

* Deuteromycotina. † Basidiomycotina.

Didymella lycopersica, *Fusarium solani*, *Fusarium oxysporum*, *Fusarium sporotrichoides*, *Geotrichum candidum*, *Paecilomyces farinosus*, *Penicillium luteum*, *Penicillium italicum*, *Penicillium digitatum*, *Penicillium chrysogenum*, *Sporotrichum carnis* and *Verticillium agaricinum*; Ascomycotina – *Cochliobolus sativus*, *Gaeumannomyces graminis*, *Neurospora crassa* and *Sordaria fimicola*; Zygomycotina – *Absidia glauca*, *Basidiobolus ranarum*, *Cokeromyces poitrassii*, *Cunninghamella elegans*, *Mucor racemosus*, *Mycotypha africana*, *Syncephalastrum racemosum* and *Zygorhynchus moelleri*; Mastigomycotina – *Pythium ultimum*, *Pythium middletonii* and *Saprolegnia ferax*.

Table 3. *Effects of validamycin A on the morphology and growth of R. cerealis*

R. cerealis was grown at 25 °C in Vogel's medium containing 5 mM-glucose in the presence and absence of 1 µM-validamycin A in semi-solid, shake flask and stationary liquid culture.

Cultural conditions	Parameter measured	No added validamycin A	Plus 1 µM-validamycin A
Semi-solid culture	Colony radial growth rate (µm h ⁻¹)*	278 ± 5	96 ± <1¶
	Internode length (µm)†	188 ± 11	94 ± 6¶
	Colony surface area (cm ²)‡	26.4 ± 0.4	2.3 ± 0.1¶
	Colony density (mg cm ⁻²)‡	0.22 ± 0.02	0.78 ± 0.08¶
Shake flask liquid culture	Specific growth rate (h ⁻¹)§	0.059 ± 0.005	0.062 ± 0.004
	Doubling time (h)§	11.8 ± 0.9	11.2 ± 0.8
	Internode length (µm)	129 ± 14	131 ± 10
Stationary liquid culture	Internode length (µm)	139 ± 10	120 ± 12

* All radial growth rates (mean ± SE) of colonies (six replicates) were measured during the linear phase of growth.

† Mean ± SE of 45 internode lengths taken from the first three consecutive internodes of each of three leading hyphae from the margins of five replicate colonies in the linear growth phase.

‡ Measurements were made on colonies 120 h after inoculation and represent the means ± SE of five replicate colonies.

§ Growth followed by measuring dry weight, values are the mean ± SE of three independent experiments each with three replicates.

|| Mean ± SE of at least 21 internode lengths measured randomly in cultures 48 h after inoculation.

¶ Significantly different (using the *t*-test, *P* < 0.05) from the control.

Table 4. *Effects of validamycin A on the morphology and growth of F. culmorum*

F. culmorum was grown at 25 °C on Vogel's medium containing 50 mM-glucose in the presence and absence of 100 µM-validamycin A in semi-solid and shake flask culture.

Cultural conditions	Parameter measured	No added validamycin A	Plus 1 µM-validamycin A
Semi-solid culture	Colony radial growth rate (µm h ⁻¹)*	492 ± 22	355 ± 9¶
	Germ tube specific growth rate (h ⁻¹)†	0.284 ± 0.015	0.295 ± 0.007
	Mean hyphal extension rate (µm h ⁻¹)‡	70 ± 4	55 ± 3¶
	Hyphal growth unit length (µm)§	254 ± 8	184 ± 9¶
Liquid culture	Specific growth rate (h ⁻¹)	0.278 ± 0.009	0.278 ± 0.012

* Radial growth rates of colonies (mean of six replicates ± SE) were measured in the linear phase of growth.

† Determined by time lapse photomicroscopy for a period up to 15 h after germination. Results represent the mean of five germlings ± SE.

‡ Calculated using Equation (1). Results represent the mean (± SE) of five determinations.

§ Measurements were made on germlings with between 12 and 30 hyphal tips. Results are the mean (± SE) of at least 15 replicates.

|| Determined using culture optical density as a measure of growth. Results are the means (± SE) of five independent experiments, each with three replicates.

¶ Significantly different (using the *t*-test, *P* < 0.05) from the control.

All the Basidiomycotina (and Deuteromycotina with a basidiomycete perfect stage) were sensitive to the antibiotic, although the percentage reduction in colony diameter varied from only 12% for *Phlebia rufa* to 77% for *Bjerkandera adusta*. With the exception of *Fusarium culmorum*, *Chaetomium globosum* and *Chaetomium bostrychoides*, all Ascomycotina, (and Deuteromycotina with a perfect ascomycete stage), Oomycetes and Mucorales tested were insensitive to validamycin A.

A number of validamycin A sensitive fungi from the initial screen on media containing 5 mM-glucose were tested over a range of antibiotic concentrations on media containing 50 mM-glucose (Table 2). The antibiotic concentration at which a significant inhibition in colony radial

Fig. 1. Effect of validamycin A on the production of conidia by *F. culmorum*. Vogel's agar medium containing 50 mM-glucose in the presence (●) and absence (O) of 100 µM-validamycin A, was inoculated evenly with a conidial suspension of *F. culmorum* and incubated at 25 °C. At intervals, plates were harvested and the total number of conidia present counted. Each point represents the mean of three determinations made on each of five replicate plates; the error bars indicate the SE values.

growth rate was first observed varied from 10 µM for *Pleurotus ostreatus* and *Heterobasidion annosum* to 1 mM for *Phlebia radiata* and *Fistulina hepatica*. Increasing validamycin A concentration above that at which inhibition was first observed resulted in a further decrease in colony radial growth rate.

Effect of validamycin A on the growth and morphology of R. cerealis

Validamycin A reduced the radial growth rate of *R. cerealis* colonies by 66% and this decrease was associated with a 50% reduction in the internode length and a 355% increase in colony density (mg biomass dry wt cm⁻²) (Table 3). However, the antibiotic had no effect on the specific growth rate of the organism in shake flask culture, nor did it have a significant effect on branch frequency (internode length) of mycelia grown in liquid culture (shake flask or stationary).

Effect of validamycin A on the morphology, growth and sporulation of F. culmorum

The effect of validamycin A on the germination and growth of conidia of *F. culmorum* was also studied. Such studies could not be made with *R. cerealis*, which does not form spores. On solid medium containing 50 mM-glucose, 100 µM-validamycin A caused a 28% reduction in the radial growth rate of colonies of *F. culmorum* and this was associated with a 21% reduction in mean hyphal extension rate and a 28% reduction in the hyphal growth unit length (Table 4). However, specific growth rate of the organism on solid medium or in shake flask liquid culture was unaffected by the antibiotic. Production of conidia in both the presence and absence of validamycin A reached a maximum 100 h after inoculation; however, the maximum number of conidia produced was reduced by about 50% when cultures were grown in the presence of the antibiotic (Fig. 1). When conidia harvested from cultures grown in the absence of the antibiotic were used to inoculate medium containing validamycin A, the rate of spore germination and the final percentage of spores which germinated were unaffected by the antibiotic (Fig. 2). However, only 50% of the conidia harvested from cultures grown in the presence of the antibiotic germinated on Vogel's medium and they did so at a slower rate than conidia harvested from control cultures (Fig. 3).

Fig. 2

Fig. 3

Fig. 2. Effect of validamycin A on the germination of conidia of *F. culmorum*. Vogel's agar medium overlaid with Cellophane and containing 50 mM-glucose in the presence (●) and absence (○) of 100 µM-validamycin A, was inoculated evenly with a conidial suspension of *F. culmorum* and incubated at 25 °C. Each result is the mean of at least 100 conidia taken from five fields of view; the error bars indicate the SE values.

Fig. 3. Germination of conidia harvested from cultures of *F. culmorum* grown in the presence and absence of validamycin A. Plates containing Vogel's agar medium overlaid with Cellophane and containing 50 mM-glucose, were inoculated evenly with a spore suspension of *F. culmorum* harvested from 100 h mycelium which had been grown in the presence (●) or absence (○) of 100 µM-validamycin A. Plates were incubated at 25 °C and spore germination followed by microscopy with time. Each result is the mean of at least 100 spores taken from five fields of view; the error bars indicate the SE values.

DISCUSSION

All the Basidiomycotina (and Deuteromycotina with a basidiomycete perfect stage) tested were sensitive to validamycin A (Table 1). However, neither Oomycetes nor Mucorales and only a few Ascomycotina were affected by the antibiotic, even when tested at concentrations as high as 5 mM (results not shown). These observations confirm results obtained previously using a small number of fungi (Trinci, 1984) and suggest that sensitivity to validamycin A is due to a common factor shared by Basidiomycotina. The nature of this factor is not known but it may be a transport system which is necessary for the uptake of the antibiotic, or a metabolic pathway affected by validamycin A which is present in Basidiomycotina but not in other fungi. Asano *et al.* (1987) recovered validamycin A from the mycelium of *Rhizoctonia solani* grown in the presence of the antibiotic and showed that it was degraded intracellularly to validoxylamine A and glucose; validoxylamine A and validamycin D (containing an α -(1→4)-linked glucose residue in the place of the β -(1→4)-linked glucose) were found to be taken up only slowly by the mycelium, results which suggest that *R. solani* has an uptake system capable of transporting validamycin A. Such a 'trisaccharide' type transport system in fungi has not previously been reported.

Although validamycin A has no effect on the specific growth rate of fungi (Tables 3 and 4), it causes a decrease in the radial growth rate of colonies of sensitive fungi and this can be correlated with a decrease in branch frequency (a decrease in *G*). Other workers (Morrison & Righelato, 1974; Jejelowo & Trinci, 1988) have shown that there is a positive relationship between hyphal growth unit length and colony radial growth rate. Thus, validamycin A is a true paramorphogen, affecting only the spatial distribution of the mycelium and not its rate of production (i.e. specific growth rate).

Although validamycin A alters the morphology of mycelia and colonies of *R. cerealis* grown on solid medium, no similar effects were observed in stationary liquid culture (Table 3). However, this difference between results obtained in semi-solid and liquid cultures is not unique; Trinci & Collinge (1973) found that L-sorbose had an effect on the morphology of *Neurospora crassa* in semi-solid, but not in liquid culture. As nutrient gradients will be formed in both solid (Robson *et al.*, 1987) and stationary liquid cultures, such gradients probably play no role in the morphological effects induced by validamycin A, although gradient formation has been suggested to explain the absence of a paramorphogenic effect caused by L-sorbose in liquid culture (Moore, 1981). Restriction of the morphological effects of validamycin A and L-sorbose to cultures grown on solid medium may provide an important clue to the mode of action of these paramorphogens. The altered morphology of highly branched (colonial) mutants of *N. crassa* has been correlated with their altered cell wall compositions (Mahadevan & Tatum, 1965). Changes in wall biosynthesis may reduce hyphal extension and, if the specific growth rate of the organism is unchanged, this will inevitably result in an increase in branch frequency (Trinci, 1984). Similarly, the paramorphogen L-sorbose has been shown to inhibit the activity of β -(1 $\rightarrow$ 3)-glucan synthetase and reduce the amount of β -(1 $\rightarrow$ 3)-glucan in the cell wall of *N. crassa* (Mishra & Tatum, 1972). Validamycin A also affects the composition of walls of *R. solani* (Kido *et al.*, 1986) and we believe this may be a secondary effect of the antibiotic resulting from an altered inositol metabolism (G. D. Robson, P. J. Kuhn & A. P. J. Trinci, unpublished observations). A study of the effect of validamycin A on the inositol metabolism of *R. cerealis* will form the basis of a future paper.

Trinci (1985) demonstrated that, for *R. cerealis*, the colony radial growth rate of validamycin-A-inhibited colonies was independent of the concentration of the antibiotic in the medium, i.e. validamycin A had an 'all or nothing effect' on hyphal extension. However, this conclusion was based on concentrations of antibiotic of 1 μ M and less. In the present study we have shown that in the concentration range from 10 to 5000 μ M, the colony radial growth rate of *R. cerealis* and other sensitive fungi decreased with increase in antibiotic concentration (Table 2). With the exception of *R. cerealis*, *Heterobasidion annosum* and *F. culmorum* the fungi tested on media containing 50 mM-glucose first became 'inhibited' at an antibiotic concentration of 100 μ M or above (Table 2), despite exhibiting sensitivity to 25 μ M-validamycin A when grown on media containing 5 mM-glucose (Table 1). Glucose is able to antagonize the 'inhibitory' effect which validamycin A has on *R. cerealis* (Trinci, 1984, 1985), and the present results indicate that glucose also antagonizes the effect of the antibiotic on other fungi.

Validamycin A causes a marked reduction in both spore production and the viability of spores produced by *F. culmorum* (Figs. 1 and 2). The inhibition of conidia formation may be partly or solely due to the altered morphology of the mycelium. However, the observed decrease in conidial viability suggests that validamycin A may have a specific effect on sporulation. Conidiation is a complex process, often occurring in older regions of a colony when exogenous nutrients are exhausted. Synthesis of the reserve disaccharide trehalose usually precedes conidiation and is important in spore germination, accumulated trehalose being rapidly metabolized during the early stages of germination (Thevelein, 1984). Recently, validamycin A and validoxylamine A [formed within the mycelium by hydrolysis of the β -(1 $\rightarrow$ 4)-glucose residue of validamycin A] have been found to be potent inhibitors of trehalase activity, both *in vitro* and *in vivo* (Asano *et al.*, 1987; Kameda *et al.*, 1987). Thus, the effects of validamycin A on conidiation and conidial germination of *F. culmorum* may result from the antibiotic inhibiting trehalose metabolism. The decreased viability of conidia produced on media containing validamycin A may be due to the antibiotic accumulating in the spores, leading to the inhibition of trehalase activity and hence trehalose metabolism and germination. The inability of validamycin A to affect the germination of control conidia (produced in the absence of validamycin A) (Fig. 2) may be because they germinate before sufficient validamycin A has accumulated to affect trehalose metabolism.

We thank the Science and Engineering Research Council for a CASE studentship to the University of Manchester and Shell Research Limited, Sittingbourne.

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