Effect of 2-Deoxy-D-Glucose on Mycelial Growth of Filamentous Fungi

That 2-deoxy-D-glucose (deoxyglucose) is a potent metabolic inhibitor for a very wide range of cell types is well documented (review by Webb, 1966). The reported metabolic effects are very diverse and much work has been done in attempts to establish the site or sites of action. Investigations with deoxyglucose in fungal systems have also revealed its inhibitory properties. There is a wide variety of response ranging from the death of a very high proportion of yeast cells treated (Heredia, de la Fuente & Sols, 1964; Megnet, 1965) to full survival and a high level of resistance to inhibition of growth in *Coprinus lagopus* (Moore, 1968) and *Glomerella cingulata* (Atkin, Spencer & Wain, 1964).

Table 1. *Colony diam (mm) on glucose media after 5 days at* 25°

	5 mM deGlc*	5 mM Glc	5 mM Glc+deGlc at 5 mM	0·5 mM	0·05 mM	0·005 mM
Phycomycetes						
Cunninghamella echinulata	3†	78	48	78	82	80
Mortierella marburgensis	0	63	17	46·5	54	64
Mucor hiemalis	0	84	0	17	48	75
Rhizopus nigricans	0	38·5	0	0	18	28
Ascomycetes						
Byssochlamys fulva	0	20	0+	13	22	18
Chaetomium globosum	4†	18	12	17	13	19
Neocosmospora vasinfecta	32	31	39	37	35	31
Xylaria polymorpha	4	23	19	20·5	21	22
Basidiomycetes						
Armillaria mellea	0	7	0	3·5	5·5	6
Coprinus lagopus	0	31	25	33	40	45
Fomes annosus	0	15	12·5	17·5	19	18·5
Ganoderma applanatum	0	12	0+	7·5	14	16
Lenzites sepiaria	0	13	2·5	13·5	14·5	15·5
Pellicularia subcoronata	0	11	0	5	8·5	10
Polysticus versicolor	0	39	5	34	37	34
Schizophyllum commune	0	46	5	17	41	38
Fungi imperfecti						
Alternaria solani	0	47·5	0+	32	42	39
Aspergillus nidulans	0	42	0	16	47	46
A. niger	0	39	38·5	41·5	45	41·5
A. oryzae	19	36	26	35	36	36
Botrytis cinerea	0	48	0	10	15	35
B. fabae	0	22	0	0+	16	24
Cladosporium herbarum	0	37	40	46	44	46
Penicillium claviforme	0	22	0	11	20	21
P. italicum	0	23	0+	8	20	23
Verticillium alboatrum	0	13	0	0	11	12·5

* deGlc = 2-deoxy-D-glucose; Glc = glucose. † Signifies weak, thin growth. 0+ = trace of growth visible around the inoculum.

An interesting feature of deoxyglucose inhibition is that it can be reversed or alleviated by normal hexose, but the extent of the alleviation greatly depends on the identity of the normal hexose. *Coprinus lagopus* was

at least 100 times more sensitive to inhibition by deoxyglucose when fructose was present in the medium than when glucose was present (Moore, 1968). A similar situation was observed with human cells, though the sensitivity difference was only about five to ten-fold (Barban & Schulze, 1961); while *Saccharomyces cerevisiae* was only about twice as sensitive in the presence of fructose (Heredia *et al* 1964). This report summarizes a survey undertaken to examine the expression of this sensitivity differential in a range of filamentous fungi as an aid to the choice of suitable strains for further work on the metabolic effects of deoxyglucose.

Table 2. *Colony diam(mm) on fructose media after 5 days at* 25°

	5 mM Fru*	5 mM Fru + deGlc at: 5 mM	0·5 mM	0·05 mM	0·005 mM
Phycomycetes					
Cunninghamella echinulata	70	27	60	60	85
Mortierella marburgensis	57·5	0	5·5	7	44
Mucor hiemalis	87	0	5	15	82
Rhizopus nigricans	28	0	0	0	9·5
Ascomycetes					
Byssochlamys fulva	16	0	6	13	20
Chaetomium globosum	18	10†	10	12	20
Neocosmospora vasinfecta	31	36	35	35	37
Xylaria polymorpha	22·5	2·5	9	11	22
Basidiomycetes					
Armillaria mellea	7	0	0	4	7·5
Coprinus lagopus	25	0	0	19†	25
Fomes annosus	18	9·5	14·5	15	19
Ganoderma applanatum	13	0	0	0+	12
Lenzites sepiaria	12·5	0	3	11	11·5
Pellicularia subcoronata	12	0	0	3†	11
Polysticus versicolor	33	0	7	18	33
Schizophyllum commune	35	0+	4	11	31
Fungi imperfecti					
Alternaria solani	39	0	6	17	37
Aspergillus nidulans	30	0	4	10	40
A. niger	38·5	4	35·5	42·5	38
A. oryzae	34	23	35	35	36
Botrytis cinerea	35	0	0	0+	12
B. fabae	18·5	0	0	0	14
Cladosporium herbarum	31	29	25	23	29
Penicillium claviforme	21·5	0	0	5·5	16·5
P. italicum	15·5	0	0	0	11
Verticillium alboatrum	16	0	0	4†	14·5

* Fru = fructose; deGlc = 2-deoxy-D-glucose; † signifies weak, thin growth; 0+ = trace of growth visible around the inoculum.

The fungi used were obtained from a collection maintained in the Department of Cryptogamic Botany of this University. The standard medium contained: NH_4NO_3, $1{\cdot}2 \times 10^{-2}$M (final concentration); Na_2HPO_4, $1{\cdot}0 \times 10^{-2}$M; KH_2PO_4, $1{\cdot}0 \times 10^{-2}$M; Na_2SO_4, $2{\cdot}0 \times 10^{-3}$M; thiamin hydrochloride, $1{\cdot}5 \times 10^{-6}$M; and agar to 1·5% (w/v). For the growth of *Cunninghamella echinulata*, *Ganoderma aplanatum*, *Lenzites sepiaria*, and *Pellicularia subcoronata* it was found best to replace the ammonium nitrate of the above

medium with ammonium chloride ($3{\cdot}0 \times 10^{-3}$M final concentration). Trace elements and mixed vitamins were added to all media. Media were sterilized by autoclaving for 10 min at 121° after which the filter-sterilized hexose solutions were added.

Inocula were disks (2 mm diam) cut with a punch from established colonies growing on the standard medium + 5 mM glucose. Incubations were made at 25°, colony diameters were scored after 5 days. The colony diameters shown in Tables 1 and 2 represent the mean values of two measurements of six replicates. In all but a few cases individual measurements varied from the mean by not more than 10%.

The data show that while there is a wide variation in the extent of inhibition, the majority of the forms tested were about ten times more sensitive in the presence of fructose than in the presence of glucose (i.e. glucose was about ten times more effective than fructose in reversing the inhibition caused by deoxyglucose). *Neocosmospora vasinfecta* and *Aspergillus oryzae* were exceptional in growing vigorously with deoxyglucose as sole carbon and energy source. *Cunninghamella echinulata*, *Chaetomium globosum* and *Xylaria polymorpha* showed weak growth on deoxyglucose alone, coupled with relatively little inhibition on the other media. *Aspergillus niger* has been reported as being able to utilize deoxyglucose as sole carbon and energy source (Atkin *et al.* 1964). The absence of such a response here may result from strain or cultural differences. None of the basidiomycetes tested showed any response to deoxyglucose alone.

Fungi which did not utilize deoxyglucose all showed greater inhibition on fructose media than on glucose media, most with about a tenfold difference. Exceptions were *Cladosporium herbarum* and *Fomes annosus* which suffered only slight inhibition and showed little difference in effect of the normal hexoses, *Byssochlamys fulva*, showing a considerable amount of inhibition but little difference in effect of normal hexoses; and *Mortierella marburgensis* and *Copinus lagopus* in which the difference in effect of the normal hexoses is most highly marked, in the case of *C. lagopus* glucose being about 500 times more effective than fructose in relieving inhibition.

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