Mutants of *Coprinus* selected for resistance to D-glucosamine and L-sorbose

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SUMMARY

Mutants resistant to growth inhibition have been obtained by selecting on media containing inhibitory concentrations of either D-glucosamine or L-sorbose. All of the mutants isolated were found to be sugar transport-defective and cross-resistant to growth inhibition by the three analogues 2-deoxy-D-glucose, D-glucosamine and L-sorbose. Like the mutants previously selected for resistance to 2-deoxy-D-glucose, the new mutants described are all shown to be alleles of the *ftr* cistron.

1. INTRODUCTION

The three hexose analogues 2-deoxy-D-glucose, D-glucosamine and L-sorbose all cause severe growth inhibition in *Coprinus* (Moore & Stewart, 1972). Mutants have been selected for resistance to 2-deoxy-D-glucose (Moore & Stewart, 1971). All of the mutants obtained were found to be alleles of single cistron (the *ftr* cistron) involved in sugar transport and some of them have been used in the construction of an allele map of that gene (Moore, 1972). An interesting feature of the 2-deoxy-D-glucose-resistant mutants is that they are cross-resistant to both glucosamine and sorbose. In order to see whether cross-resistance to all three analogues necessarily followed from selection for resistance to just one, attempts were made to select mutants resistant to sorbose and to glucosamine. The isolation and characterization of these mutants is described in this paper.

2. MATERIALS AND METHODS

(i) *Organism.* The strain used for the selection of mutants was the haploid wild-type number BC9/6, 6 (mating type A_6B_6). Various existing resistant mutants were used as testers and controls in the experiments described below, these all have stock numbers prefixed by the letter Z.

(ii) *Media.* The basal medium used was the carbon-free NCM (Moore, 1969), to which pre-sterilized solutions of carbon sources were added after autoclaving. Media were solidified with 1·5 % Difco Bacto-Agar.

(iii) *Selection of mutants.* (*a*) Sorbose-resistants. Suspensions of BC9/6, 6 oidia were pour-plated into NCM medium containing 5 mM sorbose. After 9 days incubation at 37 °C the plates bore a number of rapidly growing colonies which, on microscopic examination, were found to be surrounded by large numbers of swollen and distorted oidia and oidial germlings. These rapidly growing colonies were inoculated to fresh plates of NCM+5 mM sorbose as presumed resistants, and subsequently 34 were stocked as confirmed resistants and given the stock numbers AZ1/6, 6 to AZ34/6, 6.

(*b*) Glucosamine-resistants were selected in exactly the same way except that the

selection and test medium was NCM + 1·0 mM glucosamine. The 36 mutants successfully isolated were given the stock numbers BZ1/6, 6 to BZ36/6, 6.

No mutagenic treatments were used and about 2×10^8 oidia were plated in the selection media in each case.

(iv) *Characterization of the mutants.* Linear growth rates of the mutants on various media were determined as detailed previously (Moore & Stewart, 1972), and complementation tests against existing mutants were done as described by Moore & Stewart (1971). Tests for sugar accumulation were carried out by exposing either washed mycelium (dikaryons) or oidiospores (monokaryons) to liquid basal medium containing isotopically labelled sugar. After treatment the tissue was harvested and washed on a Whatman GF/A disk and the disk + harvest added directly to a vial of scintillation fluid.
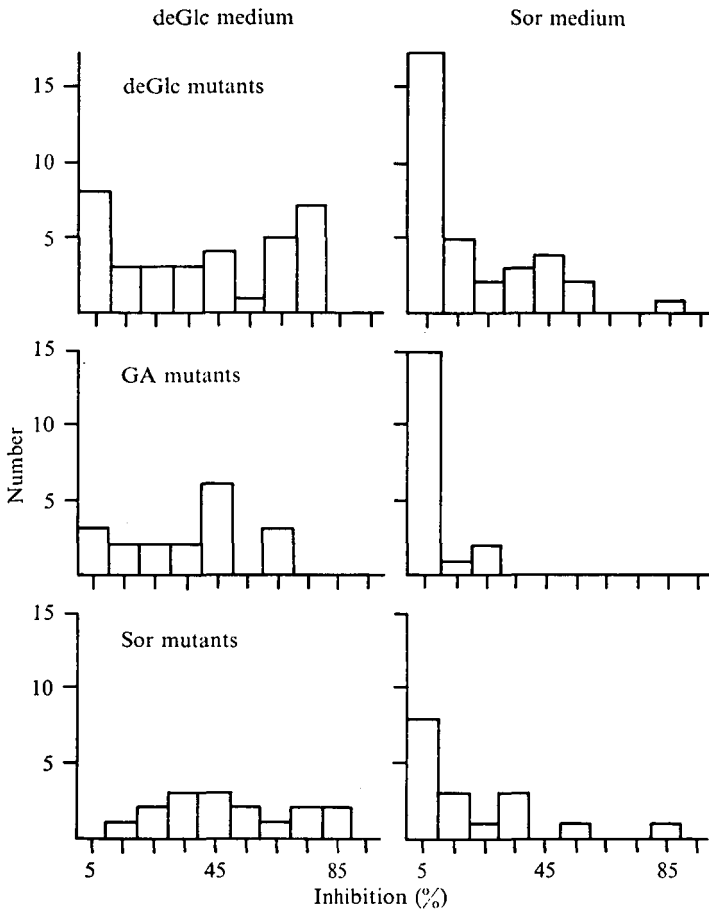

Fig. 1. Frequency histograms showing the pattern of resistance (expressed as % inhibition) to media containing either 2-deoxy-D-glucose (deGlc) or sorbose (sor) of (a) a set of mutants selected for resistance to 2-deoxy-D-glucose (deGlc mutants); (b) a set of mutants selected for resistance to D-glucosamine (GA- mutants); (c) a set of mutants selected for resistance to L-sorbose (sor- mutants).

3. RESULTS

(i) All of the mutants (both sorbose- and glucosamine-resistants) proved to be cross-resistant to their two non-selected inhibitors when growth tests were made on media

which completely inhibited growth of the wild-type. In subsequent experiments growth rates were determined for a randomly chosen set of each type of mutant. Fig. 1 shows frequency histograms in which the patterns of growth rate inhibition on deoxyglucose and sorbose media of both sets of mutants are compared with a randomly chosen set of the existing mutants (referred to as deoxyglucose-resistants). The histograms show that though the degree of resistance varied considerably from strain to strain it was generally within the range already known to encompass the majority of analogue-resistant strains. The differences which are evident in this figure will be discussed below.

(ii) All of the mutants were dikaryotized with the wild type ZBw601/40, 40 (which, like BC9/6, 6, is sensitive to inhibition by all three analogues) and with at least two established *ftr* alleles. All of the mutant × wild-type dikaryons failed to grow on medium containing 5 mM Sorbose showing that the mutations are recessive. In contrast, all of the mutant × *ftr* dikaryons grew well on sorbose medium. Since growth on sorbose is a mutant character these latter tests show that neither sorbose-selected nor glucosamine-selected analogue-resistant mutants are able to complement the *ftr* mutation. They are therefore alleles of the *ftr* cistron.

(iii) The biochemical characteristic of *ftr* alleles is that they have a lesion in sugar transport. The ability of a few selected sorbose-resistant and glucosamine-resistant strains to accumulate a variety of sugars was tested in comparison with wild-types and existing *ftr* alleles. The results are shown in Table 1.

Table 1. *Intracellular sugar accumulated by monokaryons and dikaryons of* ftr *strains*

	Sugar accumulated (nmoles/mg dry weight/15 min)					
	Initial concentration 0·12 mM				Initial concentration (3 mM)	
Culture ...	Fru*	Glc	Sor	deGlc	Fru	Glc
(a) Monokaryons						
BC9/6, 6	15·58	63·82	4·64	21·95	36·72	115·63
AZ 13	2·31	6·69	4·39	2·47	6·43	22·11
AZ 14	2·74	22·90	2·25	7·68	12·63	46·51
BZ 18	0·26	6·23	4·26	3·38	N.D.	N.D.
BZ 22	2·09	3·36	2·37	2·52	15·55	30·52
BZ 29	20·20	44·01	4·27	14·38	13·51	26·97
Z 15	4·86	5·13	4·33	3·06	N.D.	N.D.
Z 154	4·78	5·87	2·99	4·39	16·71	89·91
Z 197	4·88	5·12	4·31	3·40	35·55	18·07

	Initial concentrations 0·12 mM			
	Fru	Glc	Sor	deGlc
(b) Dikaryons				
BC9/6, 6 × ZBw601/40, 40	1·82	24·78	2·87	10·61
Z15/6, 6 × ZR15/40, 40	0·97	2·30	0·75	0·78
Z154/6, 6 × ZR154/40, 40	0·40	1·38	0·36	0·48
Z197/6, 6 × ZR197/40, 40	0·81	2·58	0·70	0·51
Z15/6, 6 × ZR154/40, 40	0·30	2·66	0·27	1·30
Z15/6, 6 × ZR197/40, 40	1·15	3·35	0·48	0·65
Z197/6, 6 × ZR154/40, 40	0·62	2·85	0·30	0·56

* Abbreviations: Fru = fructose; Glc = glucose; Sor = sorbose; deGlc = 2-deoxy-D-glucose; N.D. = not done.

4. DISCUSSION

The sorbose and glucosamine resistant strains obtained are clearly alleles of the *ftr* cistron. They have the physiological and biochemical characteristics of *ftr* mutants and they fail to complement the latter. Some differences are suggested though, in particular the sorbose-resistants seem on the whole to be rather less resistant than are mutants selected on either glucosamine or 2-deoxy-D-glucose (Fig. 1). An interesting feature of Fig. 1 is that the displays for deoxyglucose-resistants look very much like the combinations of the individual sorbose- and glucosamine-resistant displays. This may be purely fortuitous, though it could also indicate that selection for resistance to either sorbose or glucosamine leads to the recognition of distinctly different types of *ftr* alleles while exposure to deoxyglucose is a much less specific selection procedure. Indeed, a degree of functional differentiation in the cistron has been indirectly recognized (Moore, 1972) and this could be expressed, as a difference in selection pressure, during mutant selection if the functional interaction with the polypeptide differs significantly between the analogues. It should be noted that all mutants were selected on media which completely inhibited wild-type growth, so any differences between the alleles obtained must be analogue-specific. Certainly the inhibitory activity against the wild-type differs greatly, 2-deoxy-D-glucose being more inhibitory than glucosamine and the latter more so than sorbose (Moore & Stewart, 1972), also the wild-type accumulates about 5 times more deoxyglucose than sorbose (Table 1).

The data of Table 1 confirm at the biochemical level the previously obtained physiological data derived from growth rate measurements, but they are unfortunately not very much more informative. It is evident that data such as these form such a confusing pattern that a reliable picture of the comparative abilities of different alleles to transport different sugars will only be obtained from a study of the detailed kinetics of uptake. This presupposes knowledge of the kinetic characteristics of normal (wild-type) uptake and investigations of this aspect are underway. In the meantime the data of Table 1 are valuable in that (*a*) they demonstrate clearly that the mutants are transport defective; (*b*) they show for the first time that heteroallelic dikaryons are also transport defective and thus vindicate the present type of complementation test which consists of a test for growth on inhibitor media; (*c*) they show for the first time that the accumulation of all four sugars is affected (although for some reason the defect in sorbose transport is more clearly expressed in the experiments involving dikaryon mycelia than in those in which monokaryotic spores were used). With regard to the last point it is notable that at 3 mM substrate concentration the amount of glucose accumulated by the mutants is not very different from the amount of fructose accumulated by the wild-type. Since the wild-type grows equally well (in terms of specific growth rate) on glucose and fructose despite the fact that these sugars are accumulated to very different levels this feature of the mutants presumably accounts for the fact that there was 'no appreciable difference...between the growth of the mutants and that of their parent wild-type strain on media which contained either 5 mM glucose or 5 mM acetate' (Moore & Stewart, 1971).

It is thus demonstrated that selection for resistance to any one of the analogues, 2-deoxy-D-glucose, glucosamine or sorbose, results in the isolation of alleles of just one gene. This uniformity in response provides an even greater contrast to the results which have been obtained in similar work with other organisms (discussed in Moore & Stewart, 1971). In view of the profound influence that glucose has on the degree of inhibition caused by the analogues (Moore & Stewart, 1972) it does not seem illogical to suggest that the common feature in these different selection experiments which gives rise to the uniform response is the absence of glucose from the selection media. Very recent experiments in which selection has been made on a medium containing a mixture of glucose and 2-deoxy-D-glucose have yielded, in addition to *ftr*-allelic resistants,

a class of resistants which are not alleles of the *ftr* cistron and are not cross-resistant to sorbose. These new mutants are currently being examined.

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