The Ability of *Sordaria fimicola* to Take up and Metabolize Glucose and Sucrose

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Glucose, fructose and trehalose, but not sucrose, supported the growth of *Sordaria fimicola*. Mycelial suspensions rapidly metabolized [14C]glucose, which was shown to be taken up by a mechanism saturated at 1 mM. Comparable experiments with [14C]sucrose were complicated by contaminating [14C]hexose but provided no conclusive evidence for sucrose uptake. Mycelial extracts hydrolysed sucrose at 7% of the rate found with trehalose. It is suggested that the fungus cannot grow on sucrose because it lacks a mechanism that permits uptake of sucrose carbon.

**INTRODUCTION**

There is considerable evidence that members of the ascomycete genus *Sordaria* grow poorly on sucrose (Hawker & Chaudhuri, 1946; Hawker, 1947; Lilly & Barnett, 1951; Bretzloff, 1954). However, there has been no report of a quantitative study of the growth of *Sordaria* species on sucrose in a medium that was both chemically defined, and sterilized in a way that would have avoided hydrolysis of sucrose. Consequently, it is not known whether *Sordaria* species merely make poor growth on sucrose or whether they are completely incapable of using this sugar. The aims of the work described here were to discover if *Sordaria fimicola* can use sucrose as a carbon source, and if not, to investigate whether this is due to inability to take up sucrose or inability to metabolize it once it is taken up.

**METHODS**

**Organism and culture conditions.** *Sordaria fimicola* Ces. & de Not. was obtained from the culture collection of the Botany School, Cambridge, UK, and came originally from the Commonwealth Mycological Institute, Kew, UK. It was grown in the liquid medium described by Bretzloff (1954) except that the pH was 5.2 and the sugar was 100 mM-glucose, 50 mM-trehalose or 50 mM-sucrose. Growth was at 25 °C in 2 litre Erlenmeyer flasks that contained 200 ml constantly stirred medium. Inoculations were made with 10 ml of 8- to 10-d-old cultures and the experiments were done with 8-d-old cultures. To measure growth rate, replicate cultures were harvested at 2 d intervals. The mycelium in each culture was separated from the medium by rapid filtration through glass-fibre paper, washed five times with successive 100 ml volumes of culture medium lacking sugar, sucked dry, blotted between filter paper and weighed to give the fresh weight. Sub-samples (0.4-1.7 g fresh wt) of the weighed mycelium were made: some were dried at 85 °C to a constant weight to give the dry weight; others were added to 50, (w/v) trichloroacetic acid at 4 °C, and assayed for protein by the Lowry method using bovine serum albumin as the standard. The protein content of the mycelium was 3.8 mg (g fresh wt)-1.

**Metabolism of 14C-labelled sugars.** Isotopes were from Amersham. Cultures were harvested and washed as described above, and divided into samples that contained 2-4.5 mg protein. For measurement of uptake of glucose, these samples were added to growth medium that contained [U-14C]glucose at the following concentrations, volumes and specific activities: 100 mM, 5 ml (4.17 μCi mmol-1); 10 mM, 5 ml (16.6 μCi mmol-1); 1 mM, 25 ml (25.8 μCi mmol-1); 0.1 mM, 50 ml (410 μCi mmol-1) [1 μCi = 37 kBq]. The samples were incubated at 25 °C with gentle reciprocal shaking in Erlenmeyer flasks (volumes of 125, 125, 250 and 500 ml, respectively, for 100, 10, 1, 0.1 mM[U-14C]glucose) fitted with centre-wells that contained 0.5 ml 10% (w/v) KOH to absorb respired 14CO2. At the end of the incubation, the alkali was removed and the radioactivity counted. The mycelium was washed free of the labelled medium as described above except that the sugar-free medium was at 3 °C. The washed mycelium was immediately frozen in liquid nitrogen and kept at −26 °C until homogenized at 2 °C; 14C in the

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homogenate was determined after combustion to $^{14}$CO$_2$ (Fowler & ap Rees, 1970). For each sample the $^{14}$C recovered as respired $^{14}$CO$_2$ and the $^{14}$C in the homogenate were summed to give a measure of the uptake of $[^{14}$C]glucose. Analysis of the mycelium and of the washings by ion-exchange and paper chromatography showed that our method for measuring uptake was satisfactory. Less than 3% of the $^{14}$C in the mycelium was recovered as $[^{14}$C]glucose; charged compounds accounted for less than 5% of the $^{14}$C in the washings. For each measurement of the rate of glucose uptake, six replicate samples were harvested at intervals of 60, 30, 15 and 20 min, respectively, for samples in 100, 10, 1 and 0.1 mM-$[^{14}$C]glucose. At each concentration, uptake was linear with respect to time throughout the period of measurement. Initial attempts to measure sucrose uptake were made as described for glucose except that the sugar was $[U-^{15}$C]sucrose at the following concentrations and specific activities: 50 mM (4.5 pCi mmol$^{-1}$), 5 mM (29.6 pCi mmol$^{-1}$), 0.5 mM (190 μCi mmol$^{-1}$), 0.05 mM (4.5 mCi mmol$^{-1}$). In subsequent studies the volumes of labelled media were 5.0 ml for all concentrations of $[^{14}$C]sucrose. For determination of the distribution of $^{14}$C taken up by the mycelium, the washed samples were killed and extracted with ethanol, and analysed as described by Stitt & ap Rees (1978).

Measurement of enzyme activities. Freshly harvested mycelium was homogenized in a pestle and mortar with a little sand in 10 vols 0.187 M-Na$_2$HPO$_4$/0.007 M-citric acid buffer, pH 7.6. Then the homogenate was dialysed against 5 litres 0.012 M-Na$_2$HPO$_4$/0.004 M-citric acid buffer, pH 5.6, at 4°C for 24 h. Samples (1·0 ml) of the dialysed homogenate were added to 4 ml 0.116 M-Na$_2$HPO$_4$/0.041 M-citric acid buffer, pH 5.6, that contained either 50 mg sucrose or 50 mg trehalose, and were then incubated, with shaking, for 2 h at 25°C. The incubation was stopped by the addition of zinc sulphate, and the production of reducing sugars was determined as described by Ricardo & ap Rees (1970).

Sugars were sterilized by filtration. Each culture, and each sample at the end of the incubation in $^{14}$C-labelled sugar, was checked for contaminants by showing that incubation of portions on sucrose and on glucose media for 7 d at 25°C led to no growth of any organism on the former and growth of only S. fimbria on the latter.

RESULTS AND DISCUSSION

Growth

Mycelium grown on glucose or trehalose was used depending upon whether glucose or sucrose metabolism was studied. Fig. 1 illustrates the growth on these sugars. Fructose also supported growth but no growth could be detected on sucrose. Glucose-grown mycelium was incubated for 7 months in media containing 5 and 50 mM-sucrose. Trehalose-grown mycelium was incubated for 10 months on media that contained 50 mM-sucrose at pH 5.2, 6.5 and 7.5. No increase in fresh or dry weight or protein content was observed. No growth was observed over 7 d on the 200 occasions when sucrose medium solidified with agar (1%) was inoculated. We conclude that S. fimbria is incapable of significant growth when supplied with sucrose as the sole source of carbon, and attribute earlier reports of growth of this fungus on sucrose to the use of media that were not free of other sugars. Sucrose was not toxic to S. fimbria. Mycelium that had been incubated for 7 months on sucrose medium grew normally when transferred to glucose medium. Cultures that had been incubated for 10 months on sucrose medium grew normally on addition of trehalose.

Uptake and metabolism of $^{14}$C-labelled sugars

Glucose-grown S. fimbria readily took up glucose. Uptake [nmol min$^{-1}$ (mg protein)$^{-1}$] at 100, 10, 1, 0.1 mM was 20.8 ± 1.7, 20.9 ± 6.0, 27.2 ± 5.1, 4.5 ± 1.7, respectively (values are means ± SD of three estimates). The fact that less than 3% of the $^{14}$C found in the mycelium was present as $[^{14}$C]glucose shows that the glucose taken up was very rapidly metabolized. The extent of this metabolism is exemplified by the distribution of $^{14}$C in mycelium that had been incubated in medium containing 1 mM-$[U-^{14}$C]glucose (specific activity 72 mCi mmol$^{-1}$) for 140 min. The percentages of the absorbed $^{14}$C that were recovered in the different fractions were: CO$_2$, 6; insoluble material, 5; acidic, basic and neutral components of the water-soluble material, 13, 10, and 49, respectively. Over 80% of the $^{14}$C in the soluble neutral compounds was in trehalose. This distribution, expected of aerobic mycelium, shows that S. fimbria readily metabolized sugar under our experimental conditions.

When either glucose- or trehalose-grown mycelium was incubated in media in which the carbon source was $[U-^{14}$C]sucrose, there was appreciable uptake of label over a wide range (0.05 to 50 mM) of $[^{14}$C]sucrose concentrations (Table 1). The distribution of the absorbed label was
Sugar metabolism by Sordaria fimicola

Fig. 1. Growth of *S. fimicola* on 50 mM-trehalose (○, dry wt; ■, protein) and on 100 mM-glucose (●, dry wt). Each point represents the mean of at least three estimates; SD < 8% of the mean.

Table 1. Uptake of $^{14}$C by *S. fimicola* from media containing different concentrations of $[^{14}$C]sucrose

Samples (5–10 mg protein) of glucose-grown mycelium were incubated in 5 ml of media that contained $[^{14}$C]sucrose at the concentrations and specific activities shown. Uptake of $^{14}$C was determined after 5.7 and 24 h.

<table>
<thead>
<tr>
<th>$[^{14}$C]Sucrose supplied</th>
<th>Uptake of $^{14}$C [d.p.m. (mg protein)$^{-1}$]</th>
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<tbody>
<tr>
<td>Conc (mM)</td>
<td>Specific activity ($10^{-3}$ x d.p.m. μmol$^{-1}$)</td>
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<tr>
<td>0.05</td>
<td>5090</td>
</tr>
<tr>
<td>0.50</td>
<td>509</td>
</tr>
<tr>
<td>5.0</td>
<td>50.9</td>
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<tr>
<td>50.0</td>
<td>5.09</td>
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similar to that observed when $[^{14}$C]glucose was supplied. The only component of the soluble neutral compounds in which $^{14}$C could be detected was trehalose. The lack of significant amounts of $[^{14}$C]sucrose in the mycelium argues strongly against the view that sucrose entered the mycelium but could not then be metabolized. However, the labelling of the other compounds, at first sight, suggests appreciable uptake of sucrose followed by rapid metabolism. At face value the above analyses and the data in Table 1 suggest that sucrose at 50 mM was taken up at 95 nmol min$^{-1}$ (mg protein)$^{-1}$ and metabolized. Comparison with the rates of glucose uptake suggests that such metabolism of sucrose should have supported a rate of growth comparable to that observed with 100 mM-glucose.

There was a marked contradiction between the apparent metabolism of sucrose by *S. fimicola* and the total inability of sucrose to support growth. The most likely explanation was that the lability of sucrose (Pontis, 1977) had led to hydrolysis of a small fraction of the commercial supply of $[^{14}$C]sucrose so that the latter contained $^{14}$C-labelled hexoses, of high specific activity, that would have been readily metabolized by *S. fimicola*. The following is evidence that this was so. Paper chromatography showed that 1–2% of the label in the commercial preparation was present as $^{14}$C-labelled hexose. The uptake of $^{14}$C observed when we supplied *S. fimicola* with $[^{14}$C]sucrose, 1.15 ± 0.13% of that supplied (mean ± SEM of values from 23 samples), corresponded to the above degree of contamination. Further, we found that uptake of label from $[^{14}$C]sucrose in the medium was not linearly related to time for very long and was independent of the specific activity of the $[^{14}$C]sucrose (Table 1). In contrast, such uptake was affected by the presence of unlabelled glucose and fructose (Table 2). The form of this effect strongly suggests that the added hexoses decreased the specific activities of $^{14}$C-labelled hexoses.
Table 2. Effects of hexose on uptake of $^{14}$C by S. fimicola incubated in 50 mM-$[U-^{14}$C]sucrose

Samples (6-8 mg protein) of trehalose-grown mycelium were incubated in 25 ml medium that contained: (a) 50 mM-$[U-^{14}$C]sucrose; (b) 50 mM-$[U-^{14}$C]sucrose, 10 mM-glucose, 10 mM-fructose; (c) 50 mM-$[U-^{14}$C]sucrose, 1 mM-fructose, 1 mM-glucose. Each sample received $10^{-6} \times 8.5$ d.p.m. Uptake was measured at the times indicated.

<table>
<thead>
<tr>
<th>Period of incubation (min)</th>
<th>Uptake of $^{14}$C [d.p.m. (mg protein)$^{-1}$]</th>
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<tbody>
<tr>
<td></td>
<td>(a) $^{14}$C Sucrose + 10 mM-hexose</td>
</tr>
<tr>
<td>100</td>
<td>11453</td>
</tr>
<tr>
<td>200</td>
<td>12133</td>
</tr>
<tr>
<td>300</td>
<td>12685</td>
</tr>
<tr>
<td>400</td>
<td>14529</td>
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</table>

in the media. We argue that the experiments with $[^{14}$C]sucrose provide no convincing evidence that S. fimicola can take up this sugar, and emphasize the need for caution in using $[^{14}$C]sucrose to study sucrose uptake.

**Ability of mycelial extracts to hydrolyse sucrose**

We investigated whether S. fimicola could metabolize sucrose both by observing whether extracts of trehalose-grown mycelium showed invertase activity, and by comparing any such activity to that of trehalase. The latter was readily demonstrable and over the range pH 5.0–8.0 was optimum at pH 6-0. At this pH trehalase activity was $209 \pm 20$ nmol min$^{-1}$ (g fresh wt)$^{-1}$ (mean ± SEM of four estimates). The same extracts hydrolysed sucrose at pH 6-0 at the rate of $15.6 \pm 4.8$ nmol min$^{-1}$ (g fresh wt)$^{-1}$ (mean ± SEM of five estimates). Although the invertase activity is low relative to that of trehalase, it is a significant and measurable activity and would be expected to give some growth on sucrose provided the invertase had access to the sucrose.

**CONCLUSIONS**

We conclude that S. fimicola can not use sucrose as a carbon source. The presence of modest invertase activities in mycelial extracts suggests that this is not due to failure to metabolize sucrose. The experiments with $[^{14}$C]sucrose are consistent with the view that S. fimicola can not take up sucrose. In particular, our failure to detect significant $[^{14}$C]sucrose in mycelium supplied with this sugar argues against uptake of sucrose without metabolism. We suggest that S. fimicola can not grow on sucrose because it can neither take up sucrose nor hydrolyse it extracellularly. This apparent impermeability of the cell membrane to sucrose is comparable to that of the chloroplast envelope (Heber & Heldt, 1981) and suggests the need for a specific mechanism for the transport of sucrose across membranes.

**REFERENCES**


