Accumulation of Intracellular Solutes by Two Filamentous Fungi in Response to Growth at Low Steady State Osmotic Potential

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Glycerol and the solutes used to control the steady state osmotic potential of the medium, either glucose, glucose and fructose, or KCl, accumulated in the hyphae of *Penicillium chrysogenum* and *Chrysosporium fastidium* as the external potential was lowered. When the osmoticum was KCl, nearly all the observed hyphal osmotic potential of *P. chrysogenum* could be accounted for from the measured solute concentrations, but the proportion decreased when a glucose osmoticum was used, to 56% in the case of *P. chrysogenum* and to 65% for *C. fastidium*, when grown at an external potential of -10 MPa. Higher polyols were present in considerable quantity in both species but did not appear to be involved in osmotic adjustment.

Potassium was the predominant cation in both species when grown on glucose, but chloride was insufficient to maintain electroneutrality. The potassium to sodium ratios were considerably higher in *C. fastidium* than *P. chrysogenum*, and inversely related to the external potential in the former species only.

Glycerol and glucose were present in greater amounts at the margin than in older parts of large colonies of *P. chrysogenum*. High turgor potentials were apparently maintained throughout these large colonies which meant that the observed osmotic potential could not be accounted for by the solutes measured in older parts of the colony.

INTRODUCTION

Filamentous fungi are able to survive and grow at extremely low water potentials, yet they appear to maintain constant and high internal turgor potential under these conditions (Luard & Griffin, 1981). The generation of such turgor probably requires the accumulation, by uptake or synthesis, of one or more solutes of low molecular weight, since water loss alone is unlikely to be sufficient to produce such low osmotic potentials. Osmoregulation (osmotic adjustment) has been identified in several micro-organisms and higher plants, and a number of recent reviews indicate the variety of solutes found to fulfil this function, e.g. Hellebust, 1976; Brown, 1978; Gutknecht *et al.*, 1978; Greenway & Munns, 1980. There is some information on the physiological responses of yeasts to low water potential but little is known concerning the filamentous fungi.

Penicillium chrysogenum Thom a facultative xerophile, and *Chrysosporium fastidium* Pitt, an obligately xerophilic species which is not able to grow at high salt concentrations, have been analysed in order to identify and quantify the solutes acting as osmoregulators. The steady state conditions used by Luard & Griffin (1981) were employed, and the zonal distribution of solutes within the colony of *P. chrysogenum* has also been examined.

METHODS

Fungi and media. *Penicillium chrysogenum* (FRR = NRRL 1951) and *C. fastidium* (FRR 77) were kindly supplied by J. I. Pitt, CSIRO Division of Food Research, North Ryde. *Penicillium chrysogenum* was maintained on Czapek-Dox agar to which 200 g sucrose l^{-1} had been added and *C. fastidium* was maintained on Czapek-Dox containing 400 g glucose l^{-1} . The growth medium used throughout was Czapek-Dox agar to which 5 g Difco yeast extract l^{-1} had been added (CYA). Water potential was controlled osmotically by the addition of either glucose,

glucose and fructose (1:1, w/w) below -15 MPa, or KCl to the medium (Luard & Griffin, 1981). The osmotic potential of unsupplemented CYA was -0.56 MPa. For convenience the theoretical potential of the added solute has been referred to throughout and designated by the symbol ψ_s . The true potential of the media would be close to 0.56 MPa less than ψ_s .

Plastic Petri dishes (9 cm diam.) containing 30 ml medium were used in the steady state experiments while glass dishes (14 cm diam.) containing 150 ml medium were used to grow large colonies for analysis of different zones. All plates were overlaid with cellophane and inoculated centrally with a 4 mm diameter plug cut, in the case of *C. fastidium*, from the margin of a colony whose growth rate was linear, and in *P. chrysogenum*, from a spore suspension in 2% water agar. Plates were stored in plastic bags and incubated at 25°C .

Growth and dry weight. Radial growth rates were determined from the mean of three radii from duplicate plates. To obtain the fresh weight of a colony, the inoculum plug was removed and the colony lifted off the cellophane, blotted and weighed immediately in a tared aluminium foil boat. Dry weight was measured after drying at 85°C for at least a week.

Samples for the analyses specified below were taken from different colonies (usually three replicates) when the radius of the colony was at least 20 mm under steady state conditions or 65 mm in the zonal experiment. If less than an entire small colony was needed for analysis, sectors of the appropriate weight were cut. Rings (2 cm wide) were cut in the large colonies to give zones designated margin (M), -2 cm and -4 cm from the margin and samples were taken from each of the three zones.

Hyphal osmotic potential. Osmotic potentials of hyphae were determined by thermocouple psychrometry (Luard & Griffin, 1981).

Cations. Samples of 100 to 150 mg fresh weight were dry-ashed overnight at 420°C , extracted in 3 M-HCl and analysed with a Varian Techtron AA5 atomic absorption spectrophotometer; Na^+ and K^+ by flame atomic emission, Ca^{2+} and Mg^{2+} by flame atomic absorption.

Chloride. Samples of 150 to 250 mg fresh weight were frozen on dry ice, thawed, extracted in distilled water and titrated using a Radiometer CMT 10 chloride titrator.

Extraction. Approximately 400 mg fresh weight of colony was rapidly killed with hot absolute ethanol and extracted in 80% (v/v) ethanol in a Soxhlet apparatus for 24 h. The extract was dried on a rotary evaporator at 40°C under reduced pressure. The residue was taken up in 80% ethanol and divided equally for carbohydrate and amino acid analyses. The portions were again dried on a rotary evaporator and stored overnight over P_2O_5 . Single samples were taken from each treatment.

Amino acids. Dried samples from the 80% ethanol extracts were taken up in sodium citrate buffer pH 2.2. Samples were analysed on a model 119 CL Beckman amino acid autoanalyser.

Sugars and polyols. Ethanol soluble sugars and polyols were analysed by GLC using the procedure of Holligan & Drew (1971). Trimethylsilyl derivatives (TMS) of the dried extracts were prepared, the pyridine evaporated under reduced pressure and the derivatives redissolved in *n*-hexane. Separation of the derivatives was carried out with a Perkin Elmer F11 flame ionization gas chromatograph; the copper columns (1.8 m long, 1.5 mm internal diam.) contained 2% SE52 stationary phase on acid-washed Chromosorb W solid support. The carrier gas was high purity nitrogen supplied at 40 ml min^{-1} ; air and hydrogen were supplied at 300 ml min^{-1} and 30 ml min^{-1} , respectively. The detectors were at the same temperature as the oven and the injection ports were at 250°C . Glycerol was chromatographed isothermally at 100°C and erythritol, arabitol, fructose, glucose and mannitol with a temperature programme of 100°C to 250°C increasing by 4°C min^{-1} . The instrument was calibrated externally with these compounds. Amounts were calculated from peak areas which were determined as peak height times width at half height. Identification of peaks was by coincidence with authentic standards. SE52 will not separate the TMS derivatives of the isomeric pentitols or hexitols so that the identity of mannitol and arabitol was confirmed by paper chromatography using the method of Robyt (1975), except that the chromatogram was run for 24 h at 17°C .

RESULTS

Hyphal osmotic potential and water content

Hyphal osmotic potentials decreased as the external potential was lowered in both species under steady state conditions (Fig. 1) so that positive turgor was maintained, even at the lowest potentials, in agreement with the findings of Luard & Griffin (1981). However, contrary to those earlier results, there was no difference in the osmotic potentials of the different zones of the large *P. chrysogenum* colonies, and hence no apparent loss of turgor in the older zones (Table 1).

The water content as indicated by the fresh weight to dry weight ratios, decreased as the osmotic potential fell in *P. chrysogenum* but over a range of 15 MPa there appeared to be little, if any, change in water content in *C. fastidium* (Fig. 1). The fresh weight to dry weight ratios of *P.*

Fig. 1. Effect of osmotic potential on the fresh weight to dry weight ratios ($\circ$, $\bullet$) and hyphal osmotic potentials ($\triangle$, $\blacktriangle$) of colonies (20 mm radius) of *Penicillium chrysogenum* (a) and *Chrysosporium fastidium* (b) grown on glucose osmoticum (open symbols) and KCl osmoticum (closed symbols). The asterisks denote colonies with a radius < 20 mm.

Table 1. Effect of position in the colony on various features of the hyphae of *Penicillium chrysogenum* grown at different osmotic potentials produced by the addition of glucose

ψ_s (-MPa)	Zone	Ion content [μ equiv. (g dry wt) $^{-1}$]					Ratio $K^+ : Na^+$	Ratio fresh wt : dry wt	ψ_s (hyphae) (-MPa)
		K^+	Na^+	Mg^{2+}	Ca^{2+}	Cl^-			
0	M	487	124	27	10	63	3.9	3.87	1.06
	-2	545	167	32	17	152	3.3	4.84	1.21
	-4	345	189	36	41	87	1.8	4.69	1.00
2	M	524	66	19	3	90	7.9	3.20	3.03
	-2	339	62	20	4	80	5.5	2.51	4.53
	-4	290	66	16	4	80	4.4	2.61	3.31
10	M	403	68	15	2	57	5.9	2.91	10.33
	-2	211	48	16	3	10	4.4	1.96	10.32
	-4	197	44	17	5	22	4.5	1.94	10.25

chrysogenum differed little between colonies grown on the two types of osmotica. The water content of the large colonies, which were grown on a glucose osmoticum, increased with distance from the margin at 0 MPa, but decreased in the -2 MPa and -10 MPa treatments (Table 1).

Ions

The amount of potassium on a dry weight basis in *P. chrysogenum* decreased at lower external potentials, but the reverse was true in *C. fastidium* and the values were all lower, when these

Table 2. *Ion content of Penicillium chrysogenum grown at different osmotic potentials produced by the addition of glucose*

Values are means and standard deviations of three samples.

ψ_s (-MPa)	Ion content [$\mu\text{equiv. (g dry wt)}^{-1}$]				
	K ⁺	Na ⁺	Mg ²⁺	Ca ²⁺	Cl ⁻
0	510 $\pm$ 92	101 $\pm$ 32	29 $\pm$ 5	16 $\pm$ 6	41 $\pm$ 6
2.0	413 $\pm$ 18	60 $\pm$ 3	21 $\pm$ 3	9 $\pm$ 4	114 $\pm$ 8
4.0	444 $\pm$ 9	68 $\pm$ 4	19 $\pm$ 1	7 $\pm$ 2	90 $\pm$ 8
6.0	400 $\pm$ 17	72 $\pm$ 4	18 $\pm$ 1	8 $\pm$ 2	80 $\pm$ 5
8.0	392 $\pm$ 20	64 $\pm$ 5	18 $\pm$ 1	6 $\pm$ 3	59 $\pm$ 3
10.0	389 $\pm$ 17	65 $\pm$ 16	18 $\pm$ 2	4 $\pm$ 2	53 $\pm$ 2

Table 3. *Ion content of Chrysosporium fastidium grown at different osmotic potentials produced by the addition of glucose*

Values are means and standard deviations of three samples.

ψ_s (-MPa)	Ion content [$\mu\text{equiv. (g dry wt)}^{-1}$]				
	K ⁺	Na ⁺	Mg ²⁺	Ca ²⁺	Cl ⁻
5.0	278 $\pm$ 10	35 $\pm$ 1	12 $\pm$ 0	2 $\pm$ 1	46 $\pm$ 2
7.5	296 $\pm$ 25	38 $\pm$ 1	12 $\pm$ 0	1 $\pm$ 1	51 $\pm$ 1
10.0	302 $\pm$ 51	32 $\pm$ 2	10 $\pm$ 1	1 $\pm$ 1	58 $\pm$ 6
12.5	306 $\pm$ 2	28 $\pm$ 0	10 $\pm$ 1	1 $\pm$ 0	55 $\pm$ 6
15.0	308 $\pm$ 5	27 $\pm$ 0	10 $\pm$ 0	1 $\pm$ 1	62 $\pm$ 2
17.5	330 $\pm$ 9	25 $\pm$ 3	6 $\pm$ 0	1 $\pm$ 1	65 $\pm$ 2
20.0	345 $\pm$ 9	23 $\pm$ 0	6 $\pm$ 0	0 $\pm$ 0	88

species were grown with a glucose osmoticum (Tables 2 and 3). Sodium decreased as the external potential fell in *C. fastidium* and the amount present was approximately half that in *P. chrysogenum*. In *P. chrysogenum* the Na⁺ content was highest in the basal medium and approximately constant at lower potentials.

The K⁺:Na⁺ ratio of CYA was close to unity so that potassium was evidently selectively accumulated in the hyphae. The K⁺:Na⁺ ratios and linear growth rates of the colonies grown on a glucose osmoticum are shown in Fig. 2. In *P. chrysogenum* both follow similar patterns in response to lowered osmotic potential, with maxima at potentials below the highest potential tested. However, in *C. fastidium* there was a linear increase in the K⁺:Na⁺ ratio as the water potential fell. This appeared to be due both to increased K⁺ accumulation and Na⁺ exclusion. All the values exceeded those for *P. chrysogenum*.

Magnesium and calcium contents were low in both species (Tables 2 and 3) and decreased with low water potential. The levels of magnesium in *C. fastidium* were about half those of *P. chrysogenum* and even less for calcium.

The chloride measured was obviously insufficient to balance the cation content (Tables 2 and 3). Carbohydrate analyses suggested the presence of malic acid in ethanol extracts of *P. chrysogenum*, but organic acids were not estimated quantitatively.

The K⁺, Na⁺ and Cl⁻ contents of *P. chrysogenum* when grown on KCl are shown in Fig. 3. Potassium accumulated in proportion to the concentration in the medium and also Cl⁻ but to a lesser extent. The difference between K⁺ and Cl⁻ was approximately constant. Na⁺ remained at a slightly lower level than when grown on glucose (Table 2). No consistent differences were noticed in Mg²⁺ or Ca²⁺ levels between treatments (values not shown).

The ion contents of the three zones of *P. chrysogenum* on a dry weight basis are shown in Table 1. The K⁺ content of the hyphae was lower in the centre of the colony. This was most noticeable in the colonies from the -10 MPa treatment where the hyphae near the centre contained half the K⁺ present in the margin. The Na⁺ content showed a different pattern in each of the three

Fig. 2. Effect of osmotic potential on the potassium to sodium ratios (○) and the radial growth rates (□) of *Penicillium chrysogenum* (a) and *Chrysosporium fastidium* (b) grown on glucose osmotica.

Fig. 3. Effect of osmotic potential on the potassium (●), sodium (▲) and chloride (■) ion contents of *Penicillium chrysogenum* grown on KCl.

treatments; the level was higher in the centre of the colony in the 0 MPa colonies, approximately constant at -2 MPa and lower in the -10 MPa treatment. However, the $K^+ : Na^+$ ratio decreased with distance away from the margin at all potentials.

The chloride content was variable in the different zones, although the values were appreciably lower in the centre of the -10 MPa colonies. As in the small colonies, the Cl^- content was insufficient to balance the cations.

Fig. 4. Effect of osmotic potential on the principal organic solutes in *Penicillium chrysogenum* (a) and *Chrysosporium fastidium* (b). Glycerol (○, ●), glucose (□, ■) and fructose (△). Glucose osmoticum (open symbols), KCl osmoticum (closed symbols).

The Ca^{2+} content was again noticeably higher in the mycelium from the 0 MPa treatment compared with the colonies from -2 and -10 MPa. Mg^{2+} and Ca^{2+} remained relatively constant in the different parts of the colony at the two lower potentials.

Sugars and polyols

Polyols were present in considerable quantity in the hyphae of both species, and the compounds present in *P. chrysogenum* appear to be identical to those identified in the conidia of this species by Ballio *et al.* (1964). The changes in sugar and polyol content of the two species on a dry weight basis in response to external osmotic potential are shown in Figs 4 and 5. When glucose was used as the osmoticum, it is clear that the only compounds which accumulated proportionally as the potential decreased were the sugar in the medium and also glycerol. Amounts of glycerol were similar in the two species at equivalent potentials. The glycerol content of *P. chrysogenum* also increased when the potential was lowered with KCl but amounts

Fig. 5. Effect of osmotic potential on the content of polyols in *Penicillium chrysogenum* grown on glucose (a) and on KCl (b) and in *Chrysosporium fastidium* grown on glucose (c). Mannitol (○, ●), arabitol (△, ▲) and erythritol (□, ■).

were about half those induced by the glucose osmoticum. Neither glucose nor fructose was detected when the osmoticum was KCl. Low levels of fructose were difficult to measure accurately because at least three peaks were present in the TMS derivatives, but small amounts were present in both species grown on glucose (data not shown).

Mannitol, arabitol and erythritol showed different patterns in response to lowered osmotic potential. In the three experiments shown in Fig. 5, the amount of mannitol was highest at the highest potential tested and then declined. Although the initial level was lower in *C. fastidium*, if equivalent potentials are compared the values are similar. The erythritol present in *P. chrysogenum* was maximal at a potential below the highest tested and then decreased, although the pattern depended on the external solute. The content was also twice as high when the osmoticum was KCl as when it was glucose. Traces only of erythritol were found in *C. fastidium*. Amounts of arabitol were fairly low in both species and there appeared to be a slight increase as the external potential was lowered. The presence of equi-molar fructose and glucose that was

Table 4. *Ethanol soluble sugar, polyol and total amino acid content of hyphae from different positions in colonies of Penicillium chrysogenum grown at three osmotic potentials produced with glucose*

ψ_s (-MPa)	Zone	Solute content [$\mu\text{mol (g dry wt)}^{-1}$]						
		Glycerol	Glucose	Fructose	Mannitol	Erythritol	Arabitol	Total amino acids
0	M	47	0	tr	427	114	29	21
	-2	tr	0	tr	224	tr	tr	18
	-4	tr	tr	tr	163	tr	tr	13
2	M	813	361	tr	322	376	tr	22
	-2	116	239	tr	226	289	0	11
	-4	99	197	tr	233	353	0	8
10	M	1859	786	tr	103	87	tr	15
	-2	646	467	0	135	71	13	8
	-4	647	530	0	152	126	23	6

tr, Trace.

Table 5. *Total amino acid pools extracted from Penicillium chrysogenum and Chrysosporium fastidium grown at different osmotic potentials*

Total amino acid content [$\mu\text{mol (g dry wt)}^{-1}$]				
<i>P. chrysogenum</i>			<i>C. fastidium</i>	
ψ_s (-MPa)	Glucose osmoticum	KCl osmoticum	ψ_s (-MPa)	Glucose osmoticum
0	55	93	5.0	20
2.0	60	78	7.5	16
4.0	63	66	10.0	14
6.0	65	89	12.5	11
8.0	69	61	15.0	12
10.0	113	82	17.5	28
			20.0	26

necessary to produce potentials below -15 MPa stimulated the production of both mannitol and arabitol but not glycerol in *C. fastidium*.

Glucose and glycerol were present in larger amounts at the margin of *P. chrysogenum* colonies than the inner zone (Table 4). Mannitol was also highest at the margin of colonies from 0 MPa and -2 MPa, while erythritol and arabitol did not appear to be correlated with position in the colony.

Amino acids

There was no accumulation of amino acids by either species at low osmotic potential, nor did the total amino acid pools show any relationship to steady state osmotic potential (Table 5) although the extraction procedure may not have been complete for the less ethanol-soluble compounds. The pool size in *P. chrysogenum* was at least four times greater than in *C. fastidium* even when equivalent potentials are compared. Glutamate and aspartate were the principal components of the pools of both species (data not shown). The addition of fructose to the medium at -17.5 and -20 MPa increased the amino acid pool as it did the polyols (Fig. 5). The total amino acid pool decreased with distance from the margin of large colonies at all three potentials (Table 4).

DISCUSSION

It appears that glycerol and the solute in the medium, either KCl or glucose, are acting as osmoregulatory solutes in both *P. chrysogenum* and *C. fastidium*. An attempt has been made to determine whether these solutes are present in sufficient quantity to account for the measured osmotic potential of the two species grown at a potential of -10 MPa (Table 6). Estimates of solute concentrations in hyphae require a number of assumptions and must be applied with caution; the subject has been discussed in some detail by Griffin (1981). The volume of the cell occupied by osmotically active solutes must be known. It is assumed that the plasma membrane is the barrier which is impermeable in fungi, and that water in the wall is not therefore a solvent for cell osmotica. In liquid cultures of micro-organisms, the osmotic volume has usually been determined experimentally from concentrations of supposedly impermeable substances. This has not been attempted in these experiments but the possibility that the culture conditions (solid or liquid) or low water potential *per se* might affect wall thickness or wall to cell ratios has been tested using electronmicrographs of thin sections. The results which have been reported elsewhere (Luard, 1980) have been used to calculate a correction factor for non-solvent water in

Table 6. *Estimated concentrations and osmotic potentials of some hyphal solutes in P. chrysogenum and C. fastidium*

Internal solute	Measured content [$\mu\text{mol (g dry wt)}^{-1}$ or $\mu\text{equiv. (g dry wt)}^{-1}$]	Estimated concentration* (molal)	Osmotic coefficient (ϕ)†	ψ_s (hyphae)‡ ($-\text{MPa}$)
<i>P. chrysogenum</i> : external solute -10 MPa glucose.				
Na ⁺	65	0.065§	0.93	0.30
K ⁺	389	0.387	0.90	1.728
Glycerol	1279	1.273	1.02	3.157
Glucose	702	0.699		1.720
Mannitol	243	0.242		0.627
Erythritol	118	0.117	1.0	0.290
			calculated	7.822
			observed	13.36
<i>P. chrysogenum</i> : external solute -10 MPa KCl				
Na ⁺	43	0.040	0.93	0.185
K ⁺	2210	2.049	0.91	9.248
Glycerol	780	0.723	1.01	1.793
Mannitol	169	0.157		0.423
Erythritol	134	0.124	1.0	0.308
			calculated	11.957
			observed	12.74
<i>C. fastidium</i> : external solute -10 MPa glucose				
Na ⁺	32	0.028¶	0.93	0.129
K ⁺	302	0.261	0.91	1.178
Glycerol	1340	1.159	1.01	2.912
Glucose	1386	1.199		2.910
Mannitol	185	0.160		0.431
Arabitol	42	0.036	1.0	0.089
			calculated	7.649
			observed	11.85

* For the purpose of the calculation cations were assumed to be totally balanced by Cl^- , as insufficient anions were measured.

† NaCl and KCl from Robinson & Stokes (1955); glucose from Norrish (1966); mannitol by psychrometry; glycerol from Scatchard *et al.* (1938); arabitol and erythritol were assumed to be equal to 1.0.

‡ Calculated from the relationship $\psi_s = -RTmv\phi\rho$ where R is the gas constant, T the temperature (K), v the number of ions per molecule, m the molality, and ρ the density of water.

§ Calculated from fresh wt:dry wt 2.50, wall water 8% and non-solvent cytoplasmic water 25%.

|| Calculated from fresh wt:dry wt 2.61, wall water 8% and non-solvent cytoplasmic water 25%.

¶ Calculated from fresh wt:dry wt 2.72, wall water 8% and non-solvent cytoplasmic water 25%.

the walls. In addition, it appears that 20 to 30% of the water within the cytoplasm forms a monolayer on the surface of macromolecules such that its properties are altered and it is unable to act as solvent (Cooke & Kuntz, 1974; Griffin, 1981). A value of 25% non-solvent cytoplasmic water has been assumed.

It is apparent that although the identified solutes probably account for the measured osmotic potential when *P. chrysogenum* was grown on an osmoticum of KCl, when grown on glucose, only 56% of the observed hyphal osmotic potential could be accounted for. In the case of *C. fastidium* the figure was 65%. The discrepancies may have been a result of underestimation of ethanol soluble sugars and polyols, since an internal standard was not used, or possibly the assumptions concerning osmotic volume and non-solvent water may have been incorrect. A third possibility is that additional osmotica were not identified by the experimental procedure used here. Free organic acids seem the most likely candidates.

The presence of large amounts of polyols in these two species is in agreement with the widespread distribution of these compounds in fungi (Lewis & Smith, 1967; Curtis *et al.*, 1980; Pfyffer & Rast, 1980). However, of the polyols identified, only glycerol is known to act as an osmoregulator in fungi although other polyols are also good compatible solutes (Brown, 1978; Adler & Gustafsson, 1980). Arabitol may play a secondary role in this respect in *P. chrysogenum* and *C. fastidium* although the amounts involved are insignificant. Although the steady state experiments suggested that the pattern of mannitol was the inverse of glycerol and glucose or KCl content, the osmotic shock experiments did not implicate either mannitol or erythritol in osmoregulation (Luard, 1982). The case of arabitol and mannitol accumulation in the marine fungus *Dendryphiella salina* is not strictly comparable. Holligan & Jennings (1972*a b, c*) used starved cultures of stationary phase cells; the shock experiments of Allaway & Jennings (1970*a, b*, 1971) were followed for only 5 h and the lowest potential in both cases was greater than -1 MPa. Glycerol was in fact detected in some of their experiments but not in significant quantities. Higher polyols and their derivatives do appear to have an osmoregulatory function in some algae; for instance cyclohexane tetrol increased in *Monochrysis lutheri* (Craigie, 1969), isofloridoside (galactosyl glycerol) in *Ochromonas malhamensis* (Kauss, 1973) and mannitol in *Platymonas subcordiformis* (Kirst, 1977).

The contribution of both ions and organic solutes to the osmotic potential of most plant species is now generally accepted. For instance Gimmler & Schirling (1978) have demonstrated that Na⁺ taken up from the saline medium as well as glycerol synthesized by *Dunaliella parva* both contribute significantly. The technique used here of growing fungal colonies on solid media separated by cellophane has meant that intracellular salts can easily be measured without excessive contamination or the necessity for washing. K⁺ salts clearly contributed to some extent to the internal potential of both experimental species when grown with glucose, and very largely when the osmoticum was KCl (Table 6). Interestingly, work on isocitrate dehydrogenase has indicated that an internal K⁺ concentration sufficient to produce an osmotic potential of -9 MPa in *P. chrysogenum* would have inhibited the *in vitro* activity of this enzyme to about 6% of the maximum (Luard, 1980).

Slayman & Tatum (1964) working on *Neurospora crassa* attributed the high K⁺ content of these cells to the selective permeability of the membrane and not to binding by macromolecules. Although it has sometimes been argued that the fixed charges of the walls may be balanced by some of the cellular cations, Galpin *et al.* (1978) did not consider this to be a large proportion in *D. salina* and it has been assumed for the purpose of the calculations that the cations are located in the cytoplasm of the experimental species.

The chloride contents of both species were generally low when grown on sugar media (Tables 2 and 3), and the permeability of other fungi to the chloride ion also appears to be low (Shere & Jacobson, 1970). Kirst (1977) summarized the literature on the ion content of algae and concluded that the Cl⁻ content of non-vacuolate microalgae was very low compared with the vacuolate giant and thalloid algae, although the anions in the former were not identified. This appears to agree with the situation in fungi which are also largely non-vacuolate, at least at the growing apices. Fungi are well known for their ability to accumulate organic acids (Cochrane, 1958), and it is probable that these together with the negatively charged amino acids aspartate and glutamate contributed the remainder of the balancing charge within the cytoplasm.

The $K^+ : Na^+$ ratio may serve as an indicator of tolerance to low water potential. *Chrysosporium fastidium* had a higher $K^+ : Na^+$ ratio than *P. chrysogenum*, and was able to exclude sodium. Norkrans & Kylin (1969) compared the $K^+ : Na^+$ ratios of a salt tolerant and a less tolerant yeast, and found a correlation with halotolerance which was attributed to the ability to accumulate K^+ and exclude Na^+ by the tolerant species. However, in the case of the osmophilic or sugar tolerant yeasts, inhibition by NaCl could not be interpreted simply as an inability to maintain an adequate $K^+ : Na^+$ ratio (Rodriguez-Navarro, 1971). The case of *P. chrysogenum* growing in a saline environment has not been examined but neither *Lulworthia medusa*, a marine fungus, nor *Ophiobolus cariceti*, a freshwater isolate, were able to exclude sodium when growing in sea water, but both accumulated potassium (Davidson, 1971). The $K^+ : Na^+$ ratio of the former was about 0.6; Galpin *et al.* (1978) report a maximum value of 4.3 in *D. salina* and Jennings (1979) has calculated values as high as 16.5 for *N. crassa* from the data of Slayman & Tatum (1964). The maximum $K^+ : Na^+$ values observed for *P. chrysogenum* and *C. fastidium* were 6.9 and 15.0, respectively.

Solute contents of mycelium have been presented on a dry weight basis, but clearly there will be a considerable increase in dry weight with decreasing potential due to accumulation of osmoregulatory solutes. It is possible that the pattern of minor solutes with respect to osmotic potential may have been obscured by this treatment. Possibly a protein or nucleic acid basis might have been more useful. Since a colony grows on solid media as a relatively flat disc, a unit area basis is also an alternative, assuming that osmotic potential does not affect hyphal branching (colony density).

In summary it can be said that the xerotolerance of two filamentous fungi has to some extent been explained. Further work is needed on direct determinations of the osmotic volume of hyphae so that exact concentrations of solutes can be calculated. This is particularly true for *C. fastidium* where there was some uncertainty in interpretation of water contents. Possibly glycerol is acting to replace water molecules and maintain hydration of proteins at low water potential as proposed by Schobert (1977).

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