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Water Relations of Sugar-tolerant Yeasts: the Role of Intracellular Polyols

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Although there is much information about the general biology of the sugar-tolerant ('osmophilic') yeasts (Scott, 1956; Onishi, 1963; Anand & Brown, 1968), there has been so far no physiological explanation of the ability of these organisms to thrive under conditions of decreased water availability. Unlike the halophilic bacteria which also thrive at low water activity, partly because of a specific requirement for high concentrations of sodium chloride (Brown, 1964; Larsen, 1967), the sugar-tolerant yeasts, with few exceptions, do not require low water activities but merely tolerate them. A variety of solutes can be used to adjust the water activity for these organisms. In natural habitats and in many experimental situations the solutes are commonly sugars. Tolerance of salts is also sometimes encountered (Onishi, 1963). The nature of the solute used to adjust water activity can affect the water relations of the yeast (Anand & Brown, 1968). The purpose of this note is to make a preliminary report of a physiological basis of the water relations of sugar-tolerant yeasts.

The sugar-tolerant yeast *Saccharomyces rouxii* (strain YA; Anand & Brown, 1968) and a non-tolerant yeast, *Saccharomyces cerevisiae* (strain Y41; Anand & Brown, 1968), were used throughout the investigation. Other strains described by Anand & Brown (1968) were used for selected comparisons. The organisms were maintained and grown as previously (Anand & Brown, 1968); the water activity of growth media was adjusted with polyethylene glycol (mol. wt 200), sucrose or glucose as required. Mass of yeast was determined on organisms which had been washed twice in the centrifuge with water at 0 °C and dried in an oven for 36 to 48 h at 85 °C. NADP-specific isocitrate dehydrogenase was prepared from strain YA in crude extracts after disrupting the organisms in a modified Hughes press (Anand, 1969). The enzyme was assayed as described by Anand (1969). This method is essentially the same as that used for the corresponding enzyme from halophilic bacteria (Aitken, Wicken & Brown, 1970) except for the concentration of salt in the reaction mixture. An extensive comparison has previously failed to demonstrate any difference between the isocitrate dehydrogenases of strains YA and Y41 (Anand, 1969; A. D. Brown, unpublished results). For chemical analyses of intracellular contents the yeasts were freeze-dried and extracted overnight with cold aqueous trichloroacetic acid (5 %, w/v). The extracted residue was washed twice with water; all washings were pooled, extracted with diethyl ether to remove the trichloroacetic acid, concentrated and freeze-dried. Polyols were identified tentatively by paper chromatography in *n*-butanol + acetic acid + water (6:1:2, by vol.), *n*-butanol + ethanol + water (6:1:2, by vol.) and methyl ethyl ketone + acetic acid + saturated aqueous boric acid (9:1:1, by vol.), their reaction with silver nitrate, their rapid reaction with the periodate and Schiff's reagent characteristic of polyols (Baddiley, Buchanan, Handschumacher & Prescott, 1956), and by their failure to react with aniline phthalate. Polyols were estimated in solution by the method of Hanahan & Olley (1958).

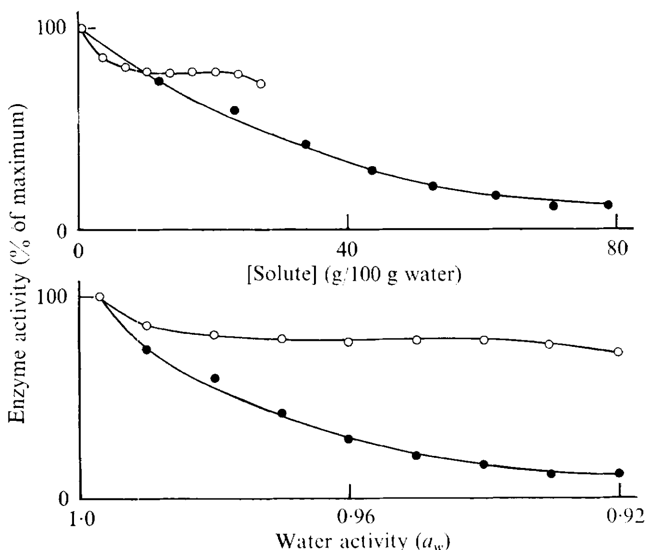

Fig. 1. Activity of a crude cell-free preparation of the NADP-specific isocitrate dehydrogenase from *Saccharomyces rouxii* (YA) measured in various concentrations of glycerol (○ --- ○) or of sucrose (● --- ●). The upper figures shows enzyme activity at a series of solute concentrations expressed as g solute/100 g water. The lower figure shows the same results plotted against the water activity of the assay solution. The curves showing enzyme activity against molal solute concentration are practically identical with those in the lower figure.

Of the cellular physiological properties which have been examined, two were found to be consistently different between sugar-tolerant yeasts on the one hand and non-tolerant yeasts on the other. One of these properties is permeability to sucrose; the other is the intracellular accumulation of polyols. The differences in permeability to sucrose are complex, being affected among other things by the presence, at least in the non-tolerant strain Y41, but not the tolerant strain (YA) of invertase. Details of permeability differences will be described elsewhere.

The other property, the accumulation of polyols, is of direct relevance to the water relations of the yeasts. All the sugar-tolerant strains described by Anand & Brown (1968) (except for strains YC, YG, YH and YK which are no longer available for study), as well as a small yeast (YO) recently isolated from a jar of slightly damp fermenting sucrose, contained one or more polyols as a major intracellular component. Polyol was not detected in any of the non-tolerant yeasts.

In strain YA and most of the other tolerant yeasts the polyol has been identified tentatively as arabitol but there were some interesting exceptions. The small yeast YO, which from preliminary observations seems to be slightly less tolerant than the others (it does not grow in 70 % (w/w) fructose (a_w 0·765); see Anand & Brown, 1968), contained a hexitol but no arabitol. Strain YE (see Anand & Brown, 1968), which has atypical water relations, contained arabitol as the major solute and had, in addition, glycerol and traces of a hexitol.

The intracellular polyols were present when the organisms were grown in basal medium (a_w 0·997) and also at lower water activities in media adjusted with polyethylene glycol (mol. wt 200), with sucrose, or with glucose. The concentration of arabitol within strain YA when grown at a_w 0·997 was approximately equivalent to 18·5 % of the dry mass of the organism (about 1217 μmol/g); the effect of water activity of the growth medium on intracellular polyol concentration will be described elsewhere. It is noteworthy that the

polyols, including glycerol, were retained within organisms washed several times with water. Retention under these conditions is unlikely to be a membrane phenomenon but is, instead, suggestive of some kind of binding, possibly of the type proposed by Ling (1962).

The function of the intracellular polyol in determining the water relations of a yeast cannot be fully appreciated without a detailed account of effects of the solute on enzyme function. The simple plot of enzyme activity against a_w (or solute concentration) shown in Fig. 1, however, gives an indication of the widely different effects of the two non-electrolytes in determining the apparent water relations of an enzyme. The action of arabitol is basically similar to that of glycerol as will be shown elsewhere.

'Osmophilic' yeasts have long been known in polyol fermentations (Spencer, 1968). To our knowledge, the polyols they produce have not previously been suspected to be a determinant of the water relations of these organisms. There is no evidence that the primary function, in this sense, of the intracellular polyol is an osmotic one. Instead the polyol functions as a 'compatible solute', analogous to K^+ in halophilic bacteria (cf. Aitken *et al.* 1970). A compatible solute may be loosely defined as one which, at high concentration, allows an enzyme to function effectively. A comparison of polyols with K^+ is possible at a physiological level as well as an enzymological one; the halophilic bacteria accumulate massive concentrations of the ion from a low extracellular concentration (Christian & Waltho, 1962) whereas the sugar-tolerant yeasts synthesize the polyol but retain it against a high concentration gradient. This type of comparison can be extended to sucrose and Na^+, both of which are very inhibitory at high concentrations but are not actively accumulated by the relevant microorganisms. The kinetics of inhibition by sucrose are also remarkably similar in some respects to the analogous inhibition by Na^+ and will be described elsewhere.

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