SHORT COMMUNICATION

Water Stress Plating Hypersensitivity of Yeasts

By KYLIE F. MACKENZIE, ANDERS BLOMBERG† AND A. D. BROWN*
Department of Biology, University of Wollongong, Wollongong, NSW 2500, Australia

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Saccharomyces cerevisiae, when growing exponentially in batch culture, passed through a phase in which, on average, one cell in $10^4$ survived plating onto a low water activity ($a_w$) agar medium. Stationary phase cultures were resistant as were all other species tested, with the exception of Candida krusei. In continuous culture, S. cerevisiae was more resistant at low than at high dilution rates. Plating at low $a_w$ was lethal to those cells that were not protected by an adequate content of compatible solute. In naturally resistant yeasts and in S. cerevisiae that had been exposed to an adaptation process, the compatible solute was one or more types of polyhydric alcohol. Resistance in stationary phase was attributable to a different cause.

INTRODUCTION

Edgley & Brown (1983) reported major differences in the process of adaptation from a conventional broth medium to one containing NaCl (10%, w/v) between Saccharomyces cerevisiae and the xerotolerant species Saccharomyces rouxii. The differences included a dramatic drop in apparent viability by the former yeast but not by the latter. The apparent loss in viability by S. cerevisiae complicated interpretation of the adaptation sequence, in particular whether adaptation was achieved essentially by the whole population or a small residue that survived during the transition. The present paper describes a peculiarity of population dynamics that is relevant to that question. We have called this phenomenon 'water stress plating hypersensitivity'.
a 'synthetic honey agar' plate (SHA, containing 48%, w/v, glucose, \(a_w 0.924\)). Plates were seeded with 0.1 ml suspension and poured with 10 ml agar. They were incubated at 30 °C inside plastic bags to restrict evaporation. There were no qualitative differences of consequence between results obtained with a plating medium containing NaCl (8 or 10%, w/v) and one with 48% glucose (SHA; see Fig. 1). SHA was used routinely since colonies could be counted after 6 d compared with about three weeks for NaCl agar.

**Polyol chromatography.** Freeze-dried yeast was extracted with ethanol (Edgley & Brown, 1983). Polyols were analysed by thin-layer chromatography on silica gel with propan-1-ol/concentrated \(\text{NH}_4\text{OH}/\text{water} (6:2:1, \text{by vol})\) and with butan-1-ol/acetic acid/water (6:1:2, \text{by vol}) as developing solvents. They were detected with a periodate-Schiff base reagent (Baddiley et al., 1956) and with alkaline silver nitrate. Glycerol was estimated as described by Edgley & Brown (1983).

**RESULTS AND DISCUSSION**

**General characteristics**

During the course of a growth cycle in BYM, *S. cerevisiae* gave plate counts on malt agar (MA) and various low \(a_w\) stressing agars as shown in Fig. 1. The essential characteristic of the phenomenon was a huge drop in colony-forming ability on a stressing agar during the period of exponential growth. The population developed resistance as it entered stationary phase. Since stationary phase inocula were always used, the culture was also resistant at the beginning of the growth cycle.

At its maximum, the plating discrepancy (log MA count — log stressing agar count) was, for Fig. 1, about 2.54 but, under some conditions, discrepancies up to 6 were obtained. In other words, 1 c.f.u. in \(10^6\) is viable on the stressing agar under such extreme conditions. Plate counts on NaCl agar were not increased by inclusion in the agar of glycine betaine (0.25–0.5 mM) or glycerol (0.5–1.0 M), agents capable of relieving solute stress under some conditions (cf. Le Rudulier et al., 1984). Since early results indicated a close correlation between plating discrepancy and budding, we considered a mechanism in which a specific step in the cell division cycle was critically sensitive to the solute stress. The correlation was subsequently found to be superficial and strongly dependent on experimental conditions. When *S. cerevisiae* was grown in BYM in continuous culture, the plating discrepancy was about 1 for dilution rates in the range 0.02–0.05 h\(^{-1}\) and about 3 in the range 0.1–0.13 h\(^{-1}\) with a transition between the ranges.

**Distribution**

Fourteen species and strains (see Methods) were screened. Plating hypersensitivity was found only in strains of *S. cerevisiae*, including var. *ellipsoideus*, and in *Candida krusei*. None of the sensitive yeasts, when harvested from mid-exponential phase in BYM, had accumulated polyols that were detected on chromatograms \([\geq 0.02 \text{mmol (g dry yeast)}^{-1}, \text{for glycerol}]\). Every resistant yeast accumulated one or more of the polyols glycerol, erythritol, arabitol or mannitol in readily detectable quantities. Accumulation of arabitol by *D. hansenii* and *S. rouxii* had already been established (Adler & Gustafsson, 1980; Brown, 1978). Both haploid and diploid strains were included in the comparison; ploidy did not correlate with sensitivity.

**Physiological characteristics**

The heterogeneity of the population responsible for the results in Fig. 1 was physiological, not genetic, inasmuch as resistant colonies isolated from mid-exponential phase SHA plates gave rise to cultures with identical characteristics. Moreover, failure to grow on SHA was attributable to death, not dormancy. This was demonstrated by two versions of an experiment in which either excised areas or whole plates of SHA were incubated in quarter-strength Ringer’s solution at various time intervals after the plate was poured. Comparisons with appropriate controls showed clearly that, by this criterion, those cells that did not form colonies had died and were not merely suppressed.

The relation between plating discrepancy and \(a_w\) (or, in Fig. 2, glucose concentration) was complex. There was no discrepancy below 30% (w/v) glucose but there was approximate proportionality between the two variables at higher glucose concentrations (Fig. 2).
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Fig. 1. Partial growth cycle of *Saccharomyces cerevisiae*. (a) Proportion of c.f.u. with buds. (b) Population counts: ■, total c.f.u.; ○, MA colony count; □, SHA colony count; ●, colony count on NaCl agar (8%, w/v, NaCl); △, colony count on NaCl agar + glycine betaine (0.5 mM); Δ, colony count on NaCl agar + glycerol (0.5 M).

Fig. 2. Plating discrepancy as a function of glucose concentration in the stressing agar. The various symbols denote different experimental cultures. Inocula were taken from mid-exponential phase cultures in BYM.

Growth of *S. cerevisiae* in BYM containing NaCl eliminated plating hypersensitivity but there was a pronounced effect of NaCl concentration. There was no plating discrepancy with cultures grown in media containing 2% (w/v) or more NaCl; below 2%, plating discrepancy increased sharply with decreasing NaCl concentration. Furthermore, washing (three times by centrifugation in 0.01 M-potassium phosphate, pH 6.5) substantially reversed the adaptation. Adaptation was accompanied by accumulation of glycerol (from <0.05 mmol g⁻¹ for yeast grown in BYM to 4 mmol g⁻¹ when grown in 4% NaCl). Washing removed 80% of the glycerol from yeast grown in 2% NaCl. The yeast also adapted within about 1 h when incubated in a simple NaCl solution (i.e. no nutrient) at concentrations of 1–4% (w/v). Here, too, adaptation was complete in 2% NaCl. Adaptation was thus a bulk phenomenon, not selection of an inherently resistant variant.

Moreover, the amount of intracellular glycerol that accumulated in response to growth in 2% NaCl (about 0.99 aₒ) was substantially less than that needed for complete osmotic adjustment to SHA (0.924 aₒ). This suggests that the protection conferred by the accumulated glycerol was achieved primarily through its role as a compatible solute rather than by the osmoregulatory process of promoting complete turgor adjustment.

The inherent resistance of the insensitive strains and the acquired resistance of *S. cerevisiae* on adaptation are each attributable to polyol accumulation and the function of such compounds as compatible solutes. The resistance developed by *S. cerevisiae* in stationary phase is largely, but probably not wholly, attributable to the accumulation of the storage polysaccharide trehalose (Brown *et al.*, 1986); this is not an osmoregulatory process.

Water stress plating hypersensitivity is strikingly similar in several respects to the adaptation of *Escherichia coli* to NaCl as described by Doudoroff (1940).

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