Effect of Tween 80 on the Morphology of *Trigonopsis variabilis*

By NICOLLE H. PACKER AND AUDREY M. BERSTEN

Department of Biochemistry, University of Sydney, Sydney,
New South Wales 2006, Australia

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When Tween 80 (polyoxyethylene sorbitan mono-oleate) was given as a supplement in basal ammonium sulphate/glucose medium to the yeast *Trigonopsis variabilis*, it induced the development, throughout growth, of populations consisting almost entirely of triangular cells. This induction was inhibited by an increase in growth temperature and by adding specific concentrations of short-chain alcohols to the medium. Only Tween 80 among the Tween detergents and Span 80 (sorbitan mono-oleate) among the Span detergents induced the formation of triangular cells, and Brij 96 (polyoxyethylene-10-oleyl ether) and sodium oleate were ineffective. These results indicate that the ester-linked sorbitan oleate is the chemical characteristic which is responsible for the effect of Tween on the morphology of *T. variabilis*, in a system more reproducible than any other previously investigated.

INTRODUCTION

The non-fermentative yeast *Trigonopsis variabilis* CBS1040 is a unique species which forms both triangular and ellipsoidal cells. The nitrogen source (Sentheshanmuganathan & Nickerson, 1962; Picci & Verona, 1963; Sasek & Becker, 1969), carbon source (Matthewson & Barnett, 1974) and growth temperature (Rambelli, 1959; Picci & Verona, 1963) are all factors which affect the relative proportions of the two forms when *T. variabilis* is grown in minimal medium. The various reports have presented contradictory evidence for the extent and reproducibility of the dimorphism induced by any one of these different growth conditions. The general conclusion (Matthewson & Barnett, 1974) has been that the development of triangular cell shape depends on the highly transient, undefined conditions which occur during growth in batch culture.

Tween 80 (polyoxyethylene sorbitan mono-oleate) has been used routinely as a growth supplement for *Saccharomyces cerevisiae* under anaerobic conditions. Our results show that Tween 80, when given as a supplement to *T. variabilis* under aerobic conditions, induces the development of populations consisting almost entirely of triangular cells. The effect of Tween 80 can be inhibited by the addition of alcohols to the growth medium.

METHODS

Media. *Trigonopsis variabilis* strain NRRL Y1579 corresponding to the type strain CBS1040⁺ was maintained on Sabouraud agar slopes. The culture medium was a minimal glucose/salts/vitamin medium (Sentheshanmuganathan & Nickerson, 1962) containing 0.2% (w/v) ammonium sulphate as sole nitrogen source. Glucose (2%, w/v) and the alcohols were sterilized separately and then added to the sterilized medium; detergent supplements were added to the medium before sterilizing.

Tween 80, sodium lauryl sulphate and Triton X-100 were purchased from BDH. To ensure reproducibility of the effect of Tween 80, this was also purchased from a second commercial source (Beith Chemicals, Bankstown, Sydney, Australia). Tween 20, 40, 60, 85 and Brij 96 were supplied by Sigma; Span 20 and 80 were obtained from Koch-Light; and deoxycholate, cholate and oleic acid were purchased from Calbiochem.

Growth conditions. Organisms were grown in batch culture in 50 ml medium continuously shaken on a rotary shaker (150 rev. min⁻¹) at 30 °C. The inoculum (0.1 ml) was taken from exponentially growing cells grown under the same conditions. Less than 10 % of the cells in the inoculum were triangular.

Growth was determined by measuring the absorbance at 700 nm of a 10-fold dilution of the cell suspension. The number of triangular cells was estimated by counting an average total of at least 250 cells on a wet mount slide at a magnification of $\times 1200$.

RESULTS AND DISCUSSION

Induction of triangular morphology

Growth of *T. variabilis* in minimal medium at 30 °C with ammonium sulphate as the sole nitrogen source and glucose as the carbon source resulted in a culture consisting predominantly (95 %) of ellipsoidal cells (Fig. 1). This is consistent with the observations of Sentheshanmuganathan & Nickerson (1962).

Supplementing this medium with 1 % (v/v) Tween 80 produced cultures consisting of 98 % triangular cells (Fig. 1). This high proportion of triangular cells was constant throughout growth until late-stationary phase, when the cells assumed a less definite triangular shape. The growth rate and the yield were the same whether or not the medium contained Tween 80 but the extent of triangular cell formation was dependent on the concentration of Tween 80. At a concentration of 0.1 %, Tween 80 initially induced the development of triangular cells (75 %) but as growth continued there was a sharp decrease in the proportion of such cells (21 %) (Fig. 1). Related members of the Tween class of detergents, which consist of different fatty-acid side-chains esterified to the same polyoxyethylene sorbitan backbone, did not significantly increase the proportion of triangular cells at any stage compared with growth in medium without detergent supplements (Table 1).

Span 80, a detergent consisting of oleic acid esterified to a sorbitan backbone without the ethylene oxide units of Tween 80, also increased the percentage of triangular cells (67 %) in the growing culture. The lower water solubility of the Span detergents compared with that of the Tween detergents may account for the decreased induction of the triangular cell shape. Span 20 (sorbitan monolaurate) produced spherical cells similar to those grown in medium supplemented with Tween 20 (polyoxyethylene sorbitan monolaurate). Triangular cells were not formed when Brij 96, a detergent with oleic acid attached by an ether linkage to the polyoxyethylene backbone, was added to the growth medium. Similarly, neither Tween 85 (polyoxyethylene sorbitan trioleate) nor 0.1 % (w/v) ethanolic oleic acid [final ethanol concentration of 0.04 % (v/v) in the medium], supplied as the free acid or the sodium salt, induced any change in the ellipsoidal morphology (Table 1).

To determine whether Tween 80 had a direct physical effect on cellular morphology, independent of growth, a sample of ellipsoidal cells grown on ammonium sulphate/glucose minimal medium was harvested, washed and resuspended in medium with Tween 80 but without glucose and incubated for 24 h at 30 °C. No change in morphology or cell number was observed. In a similar experiment, triangular cells grown with Tween 80 were washed, resuspended in medium without Tween 80 and glucose and incubated for 24 h. There was no reversion to the ellipsoidal cell shape. These results indicate that active growth in the presence of Tween 80 was necessary for the development of the triangular cell form. Once the triangular shape had been determined, the presence of the detergent was not required to maintain the triangular morphology of cells in a static population.

Other detergents, such as 1 % (w/v) cholate, 0.1 % (w/v) deoxycholate, 0.001 % (v/v) Triton X-100 and 0.01 % (w/v) sodium lauryl sulphate, at concentrations that did not inhibit growth, did not induce formation of triangular cells.

Table 1. Effect of addition of detergents on the cellular morphology of *Trigonopsis variabilis*

Supplements were added to minimal medium containing 0.2 % ammonium sulphate and 2 % glucose before inoculation. Cultures were grown at 30 °C. Cell shape was determined as described in Methods.

Supplement		Maximum percentage of triangular cells
Common name	Chemical name	
No supplement		< 5
1 % Tween 80	Polyoxyethylene sorbitan mono-oleate	98
1 % Span 80	Sorbitan mono-oleate	67
1 % Tween 60	Polyoxyethylene sorbitan monostearate	< 5
1 % Tween 40	Polyoxyethylene sorbitan monopalmitate	16
1 % Tween 20	Polyoxyethylene sorbitan monolaurate	< 5
1 % Span 20	Sorbitan monolaurate	< 5
1 % Tween 85	Polyoxyethylene sorbitan trioleate	< 5
1 % Brij 96	Polyoxyethylene-10-oleyl ether	< 5
0.1 % Sodium oleate		< 5

Fig. 1

Fig. 1. Percentage of triangular cells formed during growth at 30 °C on minimal medium containing 0.2 % ammonium sulphate and 2 % glucose (■), supplemented with 0.1 % Tween 80 (△) and 1 % Tween 80 (▲). Growth (○) was not affected by addition of the detergent (●).

Fig. 2

Fig. 2. Effect of temperature (○) and ethanol concentration (●) on the maximum percentage of triangular cells formed during growth at 30 °C on minimal medium containing 0.2 % ammonium sulphate and 2 % glucose and supplemented with 1 % Tween 80.

Inhibition of triangular cell formation

The maximum proportion of triangular cells which were formed during growth of *T. variabilis* in medium containing 1 % Tween 80 decreased from 98 % at growth temperatures of 25 °C and 30 °C to 56 % at 35 °C (Fig. 2). Picci & Verona (1963), using different nitrogen sources in the medium, also observed the inhibitory effect of high growth temperatures on triangular cell formation.

Ethanol served as a weak sole carbon source for *T. variabilis*, in which case no triangular cells were formed in the presence of Tween 80. Significantly, addition of absolute ethanol in

increasing concentrations to Tween 80-supplemented medium with glucose as the carbon source decreased the maximum number of triangular cells formed, and the cell population was totally ellipsoidal in the presence of 2 % (v/v) ethanol (Fig. 2).

Therefore, to differentiate between the effects of carbon metabolism and a direct physico-chemical action of an alcohol, alcohols of different chain lengths were added to Tween 80-supplemented growth medium. Methanol (4 %, v/v) and propanol (1 %, v/v) could not act as carbon sources for growth, but when they were added to growth medium containing glucose as carbon source and supplemented with Tween 80, triangular cell formation was completely inhibited. Ingram (1976) found that similar concentrations of alcohols of various chain lengths had a direct physico-chemical interaction with the membranes of *Escherichia coli* K12. An inhibitory effect of alcohols on the uptake of Tween 80 by *Staphylococcus aureus* has been demonstrated by Mates (1973).

In general, detergents modify protein-lipid interactions, solubilize specific proteins and lipids from membranes, lower the surface tension of liquids and alter membrane permeability (Helenius & Simons, 1974). The precise way in which Tween 80 exerts its effect on micro-organisms (Rao & Rao, 1975) is not known, although the oleic acid component of Tween 80 is incorporated into the membrane lipids of *S. cerevisiae* (Hossack & Rose, 1976). The specific effect of Tween 80 on the morphological differentiation of *T. variabilis* has been shown here to relate to a particular chemical structure. Oleic acid permeates the yeast cell wall (Medvedeva, Meissel & Volkova, 1969), but to exert its morphological effect on *T. variabilis* a basic requirement of oleate esterified to sorbitan has been indicated (Table 1), even though the Tween 80 detergent may represent a structurally heterogeneous mixture of polyoxyethylene sorbitan mono-oleate molecules.

The significant effect of Tween 80 on the morphology of *T. variabilis* and the simple means of its inhibition by alcohols offers a convenient and more reproducible system, than has previously been available, for further investigation of the morphogenesis of this yeast.

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