Genetic Transformation in Yeasts

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SUMMARY

The transformability of 25 strains of yeasts belonging to 23 species in three genera – *Saccharomyces*, *Hansenula* and *Candida* – was investigated with respect to ten characters. Eighteen hour cultures at about the end of the log phase of growth, addition of 10 µg DNA/ml, and an incubation time of 30 min at 30 to 37 °C usually yielded best results. Of the 109 pairs of strains investigated 11 yielded positive results, which included intraspecific, interspecific and intergeneric transformation. Three adenine-requiring mutants of *S. cerevisiae* were transformed to prototrophy with the DNA of *S. cerevisiae* var. *ellipsoideus*. DNA of the latter strain also transformed three other species of *Saccharomyces*. In *Candida*, reciprocal transformation was observed between *C. utilis* and *C. lipolytica*. *Hansenula petersonii* transformed *H. jadinii* to rhamnose utilization. In intergeneric experiments, *H. mrakii* was transformed with *S. cerevisiae* var. *ellipsoideus* DNA and *C. utilis* with the DNA from *H. petersonii*. Attempts at transformation between *Saccharomyces* and *Candida* were unsuccessful. The GC content of DNA from the strains which yielded positive results varied from 36.0 to 37.3 mol %. The implications of these findings for the inter-relationships and taxonomy of yeasts are discussed.

INTRODUCTION

The yeasts, which include about 160 species belonging to three distinct classes of fungi, are not considered to be a well-defined taxonomic group. The controversy regarding the classification of yeasts is largely due to the inconsistency and imprecision of the characters used for taxonomic purposes (Santa Maria, 1960). Although data are available regarding DNA base composition and DNA/DNA hybridization (Nakase & Komagata, 1971 *a, b, c*; Bicknell & Douglas, 1970; Bak & Stenderup, 1969), very little work has been done on genetic transformation of yeasts. The transformability of yeasts has not been unequivocally demonstrated. Oppenoorth's (1959) observation that the capacity for fermentation of sucrose and raffinose can be transferred from one strain of *Saccharomyces chevalieri* to another, could not be confirmed by Harris & Thompson (1960) or Laskowski & Lochmann (1961). Oppenoorth (1962) demonstrated transformation to prototrophy of two adenine- and thymineless mutants of *S. cerevisiae*. Tuppy & Wildner (1965) reported the transformation of spheroplasts of respiratory-deficient *S. cerevisiae*. In most of these experiments adequate controls were lacking (Mortimer & Hawthorne, 1966). Further, the information that we have so far relates to only two species of *Saccharomyces*, and it is thus of little significance for taxonomic purposes.

Table 1. *Strains of yeast used*

Strain	Phenotype*												Source†
	Suc	Mal	Lac	Sta	Inu	Man	Gal	Raf	Rha	Gel	Ade		
<i>Saccharomyces cerevisiae</i> var. <i>ellipsoideus</i> ITCC-Y 57	+	+	—	—	—	—	ND	ND	ND	—	+	<i>a</i>	
<i>S. cerevisiae</i> α ad ₁ S733A	ND	+	—	—	—	—	ND	ND	ND	ND	—	<i>b</i>	
<i>S. cerevisiae</i> α ad ₄ HR	ND	+	—	—	—	—	ND	ND	ND	ND	—	<i>b</i>	
<i>S. cerevisiae</i> α ad ₂ DV147	ND	+	—	—	—	—	ND	ND	ND	ND	—	<i>b</i>	
<i>S. acetii</i> VT19-1	—	—	—	ND	ND	ND	—	ND	ND	—	ND	<i>c</i>	
<i>S. hienipiensis</i> AC132	—	ND	—	ND	—	ND	ND	—	—	—	ND	<i>c</i>	
<i>S. nilssonii</i> var. <i>malacitensis</i> AC11	+	—	—	—	—	+	ND	+	—	ND	ND	<i>c</i>	
<i>S. norbensis</i> AC645	—	—	—	—	—	—	—	—	—	ND	ND	<i>c</i>	
<i>S. oleaceus</i> AC4	—	—	—	—	—	—	ND	—	—	—	ND	<i>c</i>	
<i>S. onubensis</i> VB31	+	—	—	—	—	+	ND	+	—	ND	ND	<i>c</i>	
<i>S. oxidans</i> VB22	+	—	—	—	—	—	—	+	—	—	ND	<i>c</i>	
<i>S. lactis</i> NCYC416	+	+	+	ND	ND	ND	—	ND	ND	—	ND	<i>c</i>	
<i>S. mellis</i> NCYC173	—	—	—	ND	ND	ND	ND	ND	ND	—	ND	<i>c</i>	
<i>Candida lipolytica</i> NCYC153	—	—	ND	ND	ND	ND	ND	ND	ND	+	ND	<i>d</i>	
<i>C. utilis</i> NCYC322	+	+	—	—	+	+	—	ND	—	—	ND	<i>d</i>	
<i>C. albicans</i> IGC3436	—	+	—	+	—	+	ND	—	—	—	ND	<i>e</i>	
<i>C. krusei</i> IGC2455	—	—	—	—	—	—	ND	ND	ND	—	ND	<i>e</i>	
<i>C. norvegensis</i> IGC2604	—	+	—	ND	ND	ND	ND	—	—	—	ND	<i>e</i>	
<i>C. tenuis</i> IGC3145	—	+	ND	ND	ND	ND	ND	ND	ND	—	ND	<i>e</i>	
<i>C. tropicalis</i> IGC3097	+	+	—	+	—	+	ND	—	—	—	ND	<i>e</i>	
<i>Hansenula californica</i> NRRL-Y1680	—	—	—	—	—	+	ND	—	+	+	ND	<i>f</i>	
<i>H. capsulata</i> NRRL-Y1842	—	+	—	+	—	+	ND	—	+	ND	ND	<i>f</i>	
<i>H. jadinii</i> NRRL-Y1542	+	+	—	—	+	+	—	+	—	—	ND	<i>f</i>	
<i>H. mrakii</i> NRRL-Y1364	—	—	—	—	—	+	ND	—	+	+	ND	<i>f</i>	
<i>H. petersonii</i> NRRL-Y3807	+	+	—	—	+	+	ND	+	+	ND	ND	<i>f</i>	

* Suc⁺, Mal⁺, Lac⁺, Sta⁺, Inu⁺, Man⁺, Gal⁺, Raf⁺, Rha⁺, indicate capacity to utilize sucrose, maltose, lactose, starch, inulin, manitol, galactose, raffinose and rhamnose, respectively, as the sole source of carbon; Gel⁺, ability to liquify gelatin; Ade⁺, adenine independent; ND, not determined.

† *a*, Indian Type Culture Collection, New Delhi, India; *b*, Dr R. K. Mortimer, Berkeley, California, U.S.A.; *c*, Dr J. Santa Maria, Madrid, Spain; *d*, National Collection of Yeast Cultures, Nutfield, Surrey; *e*, Dr N. van Uden, Oeiras, Portugal; *f*, Dr L. J. Wickerham, Peoria, Illinois, U.S.A.

METHODS

Strains. Twenty-five strains were used in the transformation experiments. These included 13 strains of *Saccharomyces*, 7 strains of *Candida*, and 5 strains of *Hansenula* (Table 1).

Media. The yeast cultures were usually grown and maintained in MYGP medium, which consisted of: yeast extract, 3 g; malt extract, 3 g; peptone, 5 g; glucose, 10 g; and water to 1 l. Wickerham's (1951) synthetic medium was used for testing biochemical characteristics of the strains used. MYGP medium containing 10 to 15 % gelatin was used for determining gelatin liquefying capacity and scoring gelatin liquefying transformants.

Transformation. Transformation experiments were carried out following the procedure described earlier (Khan & Sen, 1967). The experiments were terminated by the addition of DNase to a final concentration of 10 μ g/ml; the mixture contained 5 μ mol MgCl₂/ml and was incubated at 37 °C for 5 min. Serial tenfold dilutions were plated on appropriate media for scoring the transformants.

Each transformation experiment included two controls as follows: (i) In one control, the same batch of recipient cells was exposed to donor DNA treated with DNase, all other conditions of transformation remaining unchanged. DNase was added at a concentration

of 10 µg/ml, together with 5 µmol MgCl₂/ml for 30 min at 37 °C. (ii) In the other control, the same batch of recipient cells was subjected to the same experimental conditions except that no donor DNA was added. Before each experiment the sterility of the DNA was checked to ensure that the DNA was not contaminated with donor cells.

To check for contamination during the experimental period, a control plate was exposed for 5 min at the same time as dilutions of the transformation mixture were plated out. The contaminants, if any, were carefully examined for any resemblance to the transformants isolated.

In those cases where positive results were obtained in the transformation experiments an additional control was included in which the recipient cells were treated with both their own DNA and also the DNA of unrelated organisms such as *Penicillium chrysogenum* 1640, *Streptomyces rimosus* NCIB8229 and *Mycobacterium phlei*, and the protocol used for the isolation of transformants was followed under identical conditions.

In no control experiments were colonies detected which resembled the donor with respect to the marker used.

Isolation and purification of transforming DNA. As the thick cell walls of yeasts make extraction of DNA difficult, the wall was first digested with the gut juice of the African giant snail, *Acatina fulica*. The large-sized snails were collected and allowed to starve at least for 48 h. The reddish-brown fluid of the gut was collected and centrifuged in the cold; the supernatant was dialysed against distilled water for 8 h at 0 °C. The dialysis bag was changed at 2 h intervals because the gut-fluid attacked the dialysis membrane after a prolonged period of contact. The product after dialysis was a viscous fluid and no further attempt was made to remove the pigments or other impurities. This crude 'enzyme' preparation was stored at 0 °C. Organisms harvested from an exponentially growing culture (1 g wet wt) were incubated with 2.0 ml of a solution containing 1.3 M-mannitol, 0.0001 M-EDTA in 0.01 M-tris-maleate buffer (pH 5.7) and 2.0 ml of the 'enzyme' preparation at 30 °C with occasional shaking. Samples (0.1 ml) of the suspension were withdrawn from time to time and diluted to 10 ml with water before recording the extinction in a Spectronic 20 photoelectric colorimeter at 700 nm. When the extinction of the solution at 700 nm decreased by about 50 %, the incubation mixture was centrifuged in the cold at 10000 g for 5 min. The pellet, containing mostly spheroplasts, was washed with buffered mannitol. The washed spheroplasts were suspended in saline EDTA (0.15 M-NaCl+0.1 M-EDTA) and treated with sodium lauryl sulphate at a final concentration of 2 %. The mixture was then heated at 50 to 60 °C for about 15 min when it became transparent and viscous, indicating lysis of spheroplasts. Although Marmur's (1961) method has certain defects, it was used for DNA extraction since no other method yielded better results.

For extraction of labelled DNA, Wickerham's (1951) medium containing 0.1 mg KH₂PO₄/ml and 5 µCi orthophosphate [³²P]/ml obtained from the Isotopes Division, Bhabha Atomic Research Centre, Bombay, India, were used. The culture was grown for 18 h. The cells were collected by centrifugation, washed with phosphate buffer (0.01 M, pH 7.0) and used for DNA extraction.

Genetic markers. Except for adenine-requiring mutants it was necessary to choose appropriate markers. As most of the strains were resistant to antibiotics including nystatin and griseofulvin, antibiotic resistance could not be used as a marker. One of the important characters used for the classification of yeasts is carbon assimilation. Using the improved and standardized procedure of Wickerham (1951), which is highly reproducible, we tested the capacity of each strain to utilize mannitol and the following carbohydrates as the sole sources of carbon – glucose, galactose, rhamnose, maltose, sucrose, lactose, raffinose,

inulin and starch. The failure of a particular strain to utilize a substrate was checked repeatedly, and when necessary the incubation time was prolonged to 30 to 50 days. Strains which exhibited no growth whatsoever on a particular substrate were used as recipient strains in transformation experiments, and strains able to utilize the same substrate were used as donors. The strains were also checked for their capacity to liquefy gelatin; those able to liquefy gelatin were used as donors and those unable were used as recipients. The characters which were useful for transformation purposes and the sources of the strains are listed in Table 1.

Transformation assay procedure. Wickerham's synthetic medium, containing the appropriate substrate as the sole carbon source, was used to detect transformants acquiring carbohydrate- or mannitol-utilizing capacity. The same medium, containing glucose as the carbon source, was used when adenine requirement was the marker character. The procedure for scoring transformants capable of gelatin liquefaction has been described earlier (Khan & Sen, 1967).

From the selection plates 3 to 10 transformant colonies were selected at random and each transformant isolate was checked through several transfers for the acquisition and retention of the markers. The following morphological and physiological characters of the donor, recipient and the isolated transformant cultures were compared carefully to find out whether any character other than the marker was also integrated: colour, texture, smoothness of surface, shape, cross-section and margin of colonies; occurrence, shape and size of vegetative cells; formation of hyphae; nature of asci, ascospores and blastospores, if any; sediment or pellicle formation; assimilation of carbohydrates as sole carbon source relevant to the characters of the recipient strains shown in Table 1; assimilation of nitrate and liquefaction of gelatin.

Determination of guanine and cytosine (GC) content of DNA. DNA was dissolved in EDTA-phosphate buffer of low ionic strength (0.001 M-EDTA + 0.01 M-phosphate) and ionic contaminants removed by dialysis against the same buffer at 5 °C for 24 h. Thermal denaturation (T_m) values were obtained following the method of Marmur & Doty (1962). From the T_m values obtained, the GC content was calculated from the equation

$$GC = 2.43(T_m - 49.3),$$

a relationship derived from Marmur & Doty's (1962) original equation.

RESULTS

Eleven pairs of donor and recipient organisms yielded transformants in experiments involving 109 pairs. No transformant was detected in any experiment when the transforming DNA was treated with DNase before incubation with recipient cells or when the recipient cells were treated with their own DNA or DNA of unrelated organisms. Transformants detected in experiments in which the control plates showed signs of contamination were discarded.

Conditions of transformation

The strains which yielded positive results in preliminary experiments were chosen to determine the optimum conditions for transformation. Since one object of the work was to investigate intraspecific, interspecific and intergeneric transformation, optimum conditions were worked out with respect to each type of transformation.

Age of recipient. To determine the age when the cultures are most competent, the recipient cultures were grown on MYGP broth in shake culture; samples were removed

Fig. 1. Relationship between age of recipient cells and attainment of competence for transformation. Samples of recipient were withdrawn periodically from the different phases of growth and treated with the donor DNA for 30 min at 30 °C. The reaction was stopped with DNase, the mixture diluted, plated and transformation frequency calculated. Growth was measured turbidimetrically at 540 nm with a Spectronic 20 photoelectric colorimeter. Numbers of cells treated for transformation are given in the text. (a) *Saccharomyces cerevisiae* α ad₁S733A. ●, Growth curve; ○, transformation with *S. cerevisiae* var. *ellipsoideus* ITCC-Y57 DNA (10 µg/ml), using adenine requirement as the marker. (b) *S. aceti* vt19-1. ●, Growth curve; ○, transformation with *S. cerevisiae* var. *ellipsoideus* ITCC-Y57 DNA (20 µg/ml) using sucrose utilization as the marker. (c) *S. mellis* NCYC173. ●, Growth curve; ○, transformation with *S. cerevisiae* var. *ellipsoideus* ITCC-Y57 DNA (10 µg/ml) using sucrose utilization as the marker. (d) *Candida utilis* NCYC322. ●, Growth curve; ○, transformation with *Hansenula petersonii* NRRL-Y3807 DNA (10 µg/ml) using rhamnose utilization as the marker.

periodically and treated with DNA (10 µg/ml) from the appropriate donor strain, for 30 min at 30 °C. The transformation mixture was shaken on a rotary shaker at 200 rev./min. At the end of the incubation period the reaction was stopped with DNase (10 µg/ml). Transformation was noted in all the cases examined but the frequency varied considerably depending on the age of the recipient cells.

When a 4 h culture (2×10^7 organisms/ml) of the adenine-requiring mutant of *S. cerevisiae* α ad₁S733A was transformed with DNA from *S. cerevisiae* var. *ellipsoideus* ITCC-Y57, 0.21 % of the organisms became adenine-independent; the frequency of transformation increased gradually with age, reaching a maximum of 0.92 % at 18 h. Incubation beyond 18 h did not enhance transformation frequency further (Fig. 1a). When *S. aceti* vt19-1 in early exponential phase (2.5×10^7 organisms/ml) was treated with DNA from *S. cerevisiae* var. *ellipsoideus* ITCC-Y57, 4 % of the recipient organisms were transformed; with 18 h cultures this increased to 13.7 % (Fig. 1b). *S. mellis* NCYC173 was transformed (7.5 %) with respect to sucrose utilization using 6 h cultures (1.9×10^7 organisms/ml); during the following 12 h, the organisms retained their competence and the frequency almost doubled (Fig. 1c). *Candida utilis*

Fig. 2. Dependence of transformation frequency on DNA concentration. ○, Transformation of *S. cerevisiae* α ad₁s733A (1.95×10^7 to 2.41×10^7 organisms/ml) with the DNA of *S. cerevisiae* var. *ellipsoideus* ITCC-Y57; ●, transformation of *S. aceti* VT19-1 (1.30×10^6 to 1.61×10^6 organisms/ml) with *S. cerevisiae* var. *ellipsoideus* ITCC-Y57 DNA; △, transformation of *Candida utilis* NCYC322 (2.28×10^7 to 2.34×10^7 organisms/ml) with the DNA of *Hansenula petersonii* NRRL-Y3807. Age of recipient 18 h, temperature of incubation with transforming DNA 30 °C. Markers as in Fig. 1.

NCYC322, on the other hand, was poorly transformed during the early phase of growth, though thereafter the frequency of transformation rose rapidly to 1 % of the 2.1×10^7 organisms/ml of the recipient culture (Fig. 1d).

Our observations indicate that these strains of yeasts are highly competent for transformation during the exponential phase of growth. Since in most cases the highest transformation frequency was observed with late log or early stationary phase cultures, in subsequent experiments 18-h-old recipient cultures were used.

Concentration of transforming DNA. When the effect of different concentrations of DNA on transformation frequency was studied, it was observed that transformation frequency increased linearly with DNA concentration, reaching a maximum at about 10 to 18 µg/ml. Higher concentrations of DNA failed to elicit further increases (Fig. 2).

Time of exposure to DNA. To determine the optimum time of exposure to DNA, *S. cerevisiae* α ad₁s733A and *Candida utilis* NCYC322 were incubated with transforming DNA from *S. cerevisiae* var. *ellipsoideus* ITCC-Y57 and *Hansenula petersonii* NRRL-Y3807, respectively, for 5, 10, 20, 30 and 40 min; for *Candida*, samples were withdrawn after 1 and 3 min incubations. In each case, the reaction was stopped with DNase, and transformations with respect to adenine requirement (first cross) and utilization of rhamnose as the sole carbon source (second cross) for *S. cerevisiae* α ad₁s733A and *C. utilis* NCYC322 were scored. It is evident from Fig. 3 that the number of transformants increased with increase in the time of exposure to DNA; the maximum frequency was observed in both cases after 30 min exposure.

Optimum temperature of transformation. The optimum temperature of transformation was determined by keeping the concentration of DNA and the time of incubation fixed at 10 µg/ml and 30 min respectively, but varying the temperature of incubation.

Saccharomyces cerevisiae α ad₁s733A could not be transformed to prototrophy when the transformation experiment was done at 1 °C (Table 2). When the incubation temperature was increased to 30 °C, the frequency of transformation was maximum. When the tempera-

Fig. 3. Transformation frequency as affected by time of exposure to transforming DNA. ○, Transformation of *S. cerevisiae* α ad₁s733A (1.30×10^6 to 1.61×10^6 organisms/ml) with the DNA of *S. cerevisiae* var. *ellipsoideus* DNA; ●, transformation of *Candida utilis* NCYC322 (2.28×10^7 to 2.34×10^7 organisms/ml) with the DNA of *Hansenula petersonii* NRRL-Y3807. Age of recipient cells 18 h, DNA concentration 10 μ g/ml, temperature 30 °C. Markers as in Fig. 1. Samples were withdrawn at periodic intervals and reaction was stopped with DNase.

Table 2. Effect of temperature on transformation

Incubation temperature (°C)	Transformation frequency (%)		
	<i>S. cerevisiae</i> α ad ₁ s733A	<i>S. aceti</i> vt19-1	<i>C. utilis</i> NCYC322
	$\times$ <i>S. cerevisiae</i> var. <i>ellipsoideus</i> ITCC-Y57 DNA	$\times$ <i>S. cerevisiae</i> var. <i>ellipsoideus</i> ITCC-Y57 DNA	$\times$ <i>H. petersonii</i> NRRL-Y3807 DNA
1 $\pm$ 1	0	ND	ND
6 $\pm$ 1	ND	ND	0.1
18 $\pm$ 2	ND	1.7	0.5
30 $\pm$ 1	0.93	2.7	0.8
37 $\pm$ 1	0.46	0.5	1.0
42 $\pm$ 0.5	ND	ND	0.4
45 $\pm$ 1	ND	0	ND

ND, not determined.

ture was raised to 37 °C, however, fewer cells were transformed. Experiments with *S. aceti* vt19-1, using the same donor strain (*S. cerevisiae* var. *ellipsoideus* ITCC-Y57) and sucrose utilization as the marker, revealed similar trends. The highest frequency was noted at 30 °C; at 44 to 46 °C no transformant was detected. In the intergeneric transformation of *Candida utilis* NCYC322 with DNA from *Hansenula petersonii* NRRL-Y3807 with respect to rhamnose utilization, a similar pattern was evident. These results indicate that enzyme-catalysed reactions may be involved in the transformation of yeasts.

Effect of agitation of the incubation mixture. Since agitation of the transformation mixture increases the chances of contact between DNA molecules and the appropriate sites on the surface of the recipient cells, transformation frequencies observed under stationary and shake culture conditions were compared. For agitation of the transformation mixture, the tubes were placed on a rotary shaker and shaken at 200 rev./min; control tubes were kept

Table 3. *Effect of agitation of the incubation mixture on transformation*

Donor	Recipient	Phenotype selected	Condition	$10^{-6} \times$ viable colonies/ ml	$10^{-4} \times$ trans- formants/ ml	Trans- formation frequency (%)
<i>S. cerevisiae</i> var. <i>ellipsoi-</i> <i>deus</i> ITCC-Y57	<i>S. cerevisiae</i> <i>xad</i> ₁ S733A	Adenine requirement	Stationary	10.7	7.4	0.68
			Agitated	10.7	10.0	0.93
<i>H. petersonii</i> NRRL-Y3807	<i>C. utilis</i> NCYC322	Rhamnose utilization	Stationary	22.3	15.0	0.70
			Agitated	22.3	25.0	1.00

Table 4. *Effect of anaerobic condition on transformation**

Donor	Recipient	Phenotype selected	Condition	$10^{-6} \times$ viable colonies/ ml	$10^{-4} \times$ trans- formants/ ml	Trans- formation frequency (%)
<i>S. cerevisiae</i> var. <i>ellipsoi-</i> <i>deus</i> ITCC-Y57	<i>S. aceti</i> VT19-1	Sucrose utilization	Aerobic	2.4	6.5	2.6
			Anaerobic	13.5	9.5	0.7
<i>H. petersonii</i> NRRL-Y3807	<i>Candida utilis</i> NCYC322	Rhamnose utilization	Aerobic	22.3	22.5	1.0
			Anaerobic	22.3	22.5	1.0

* To obtain anaerobic condition the experiment was carried out in tubes provided with side arms fitted with stopcocks for evacuation of air from inside. After addition of DNA (10 µg/ml in the case of *H. petersonii* and 9 µg/ml in the case of *S. cerevisiae*), the tubes were evacuated and shaken. After 30 min incubation the reaction was stopped with DNase, the mixture diluted, plated and transformation frequency calculated.

under stationary conditions and incubation in both cases was at 30 °C for 30 min, the concentration of DNA being 10 µg/ml.

In the transformation to prototrophy of *S. cerevisiae xad*₁S733A with DNA from *S. cerevisiae* var. *ellipsoideus* ITCC-Y57, transformation frequency under agitated conditions was about 40 % higher than that under stationary conditions. Essentially similar results were obtained in the transformation experiments with *C. utilis* NCYC322 with the DNA of *H. petersonii* NRRL-Y3807 (Table 3).

Aerobic conditions. It was found that the requirement for aerobic conditions depends on the nature of the recipient cells. Thus, in the transformation of *S. aceti* VT19-1 to sucrose utilization with DNA from *S. cerevisiae* var. *ellipsoideus* ITCC-Y57, the frequency was improved by about 300 % under aerobic conditions. In the transformation of *C. utilis* NCYC322 with DNA from *H. petersonii* NRRL-Y3807, however, the percentage of rhamnose-utilizing transformants did not increase (Table 4).

Conditions used. These observations clearly indicate that no set of experimental conditions can be formulated which would be optimum for the production of transformants in all cases. Hence, the conditions which had yielded the highest frequency in the majority of the cases were used in subsequent experiments concerned with the assessment of transformability of the yeast strains studied.

Intraspecific, interspecific and intergeneric transformation

To investigate the transformability of the yeast strains belonging to different genera and species, the recipient cells were exposed to 10 µg DNA/ml for 30 min at 30 °C under agitated and aerobic conditions.

Table 5. *Intraspecific transformation in Saccharomyces cerevisiae*

Donor	Recipient	Phenotype selected	$10^{-6} \times$ viable colonies/ml	$10^{-4} \times$ transformants/ml	Average transformation frequency (%)
<i>S. cerevisiae</i> var. <i>ellipsoideus</i> ITCC-Y57	<i>S. cerevisiae</i> α ad ₁ S733A	Ade ⁺	11.0	10.0	0.9
<i>S. cerevisiae</i> var. <i>ellipsoideus</i> ITCC-Y57	<i>S. cerevisiae</i> α ad ₄ HR	Ade ⁺	3.9	5.0	1.3
<i>S. cerevisiae</i> var. <i>ellipsoideus</i> ITCC-Y57	<i>S. cerevisiae</i> α ad ₂ DV147	Ade ⁺	34.5	15.0	0.4

Intraspecific transformation. Three adenine-requiring mutants of *S. cerevisiae* α ad₁S733A, α ad₂DV147 and α ad₄HR were used as recipient strains. *S. cerevisiae* var. *ellipsoideus* ITCC-Y57 was chosen as the donor strain. The results (Table 5) reveal that all three adenine-requiring strains of *S. cerevisiae* could be transformed to prototrophy. The transformation frequencies, however, varied considerably. The frequency was lowest (0.4 %) with α ad₂DV147 as the recipient strain; α ad₄HR yielded the highest frequency (1.3 %).

Interspecific transformation. Experiments were carried out with ten species of *Saccharomyces*, seven species of *Candida* and five species of *Hansenula*. Of the 76 possible pairs of species which could be examined for interspecific transformation, 36 pairs of species were investigated using at least one marker (Table 6).

Of the 45 possible combinations of ten species of *Saccharomyces* included in this study, taken two at a time, 18 pairs were investigated. DNA from *S. cerevisiae* var. *ellipsoideus* ITCC-Y57 could transform *S. aceti* VT19-1, *S. mellis* NCYC173 and *S. oleaceus* AC4 to the utilization of sucrose. The frequency of transformation was quite high for *S. aceti* VT19-1 (10 %) and *S. mellis* NCYC173 (13.5 %); *S. oleaceus* AC4 gave a frequency of 1.3 %. The capacity to metabolize maltose, however, could not be conferred on any of the recipient strains.

Seven species of *Candida* were investigated and of the 21 possible combinations of the seven species, 11 pairs of strains were tested. *Candida utilis* NCYC322 was found to be transformable (3.6 %) with the DNA from *C. lipolytica* NCYC153 for gelatinase production. DNA from *C. utilis* NCYC322 in turn could transform *C. lipolytica* NCYC153 to the utilization of sucrose with a frequency of 0.55 % (Table 6). Both *C. lipolytica* NCYC153 and *C. utilis* NCYC322 produced cream coloured, pasty, smooth, circular and convex colonies with entire margins; in the latter, the vegetative cells were in short chains with pseudohyphae, but in the former there were true hyphae and long chains of vegetative cells. The transformants obtained by treatment of *C. utilis* NCYC322 with DNA from *C. lipolytica* NCYC153 resembled the recipient *C. utilis* NCYC322 in all respects except that in addition to acquiring the capacity to liquefy gelatin, the vegetative cells occurred sometimes in long chains and true hyphae appeared in addition to pseudohyphae. The transformant obtained by treatment of *C. lipolytica* NCYC153 with the DNA of *C. utilis* NCYC322 was identical with the recipient *C. lipolytica* NCYC153 in all morphological characters. Both the transformant types could liquefy gelatin and assimilate sucrose.

Of the ten possible combinations of five *Hansenulae*, transformation in five pairs was investigated. The property of rhamnose utilization was transferred from *H. petersonii* NRRL-Y3807 to *H. jadinii* NRRL-Y1542. The frequency of transformation was 1.4 % (Table 6).

Table 6. *Interspecific transformation in Saccharomyces, Candida and Hansenula*

Donor	Recipient	Phenotype selected	$10^{-4} \times$ transformants/ ml	$10^{-6} \times$ viable colonies/ ml	Trans- formation frequency (%)
<i>Saccharomyces</i>					
<i>S. cerevisiae</i>	<i>S. aceti</i>	Suc	250	24.8	10.0*
	<i>S. aceti</i>	Mal	0	24.8	Nil
	<i>S. hienipiensis</i>	Suc	0	19.0	Nil
	<i>S. mellis</i>	Suc	250	18.5	13.5
	<i>S. mellis</i>	Mal	0	19.0	Nil
	<i>S. nilssonii</i>	Mal	0	11.8	Nil
	<i>S. norbensis</i>	Suc, Mal	0	11.2	Nil
	<i>S. oleaceus</i>	Suc	30.0	22.4	1.3
	<i>S. oleaceus</i>	Mal	0	22.4	Nil
	<i>S. onubensis</i>	Mal	0	20.0	Nil
	<i>S. oxidans</i>	Mal	0	18.0	Nil
<i>S. lactis</i>	<i>S. aceti</i>	Suc, Mal, Lac, Gal	0	24.0	Nil
	<i>S. cerevisiae</i>	Lac	0	20.0	Nil
	<i>S. hienipiensis</i>	Suc, Lac	0	19.0	Nil
	<i>S. mellis</i>	Suc, Mal, Lac	0	19.0	Nil
	<i>S. nilssonii</i>	Mal, Lac	0	11.8	Nil
	<i>S. norbensis</i>	Suc, Mal, Lac, Gal	0	11.2	Nil
	<i>S. oleaceus</i>	Suc, Mal, Lac	0	22.4	Nil
	<i>S. oxidans</i>	Mal, Lac, Gal	0	18.0	Nil
<i>Candida</i>					
<i>C. lipolytica</i>	<i>C. albicans</i>	Gel	0	3.8	Nil
	<i>C. krusei</i>	Gel	0	1.7	Nil
	<i>C. norvegensis</i>	Gel	0	1.5	Nil
	<i>C. tenuis</i>	Gel	0	8.4	Nil
	<i>C. tropicalis</i>	Gel	0	8.4	Nil
	<i>C. utilis</i>	Gel	76.0	21.0	3.6
<i>C. utilis</i>	<i>C. lipolytica</i>	Suc	11.0	20.0	0.55
	<i>C. lipolytica</i>	Mal	0	20.0	Nil
	<i>C. albicans</i>	Suc, Inu	0	3.8	Nil
	<i>C. krusei</i>	Suc, Mal, Inu, Man	0	1.7	Nil
	<i>C. norvegensis</i>	Suc	0	1.5	Nil
	<i>C. tenuis</i>	Suc	0	8.4	Nil
	<i>C. tropicalis</i>	Inu	0	8.4	Nil
<i>Hansenula</i>					
<i>H. californica</i>	<i>H. jadinii</i>	Gel	0	6.6	Nil
<i>H. jadinii</i>	<i>H. californica</i>	Inu	0	22.0	Nil
	<i>H. capsulata</i>	Suc, Inu	0	22.4	Nil
	<i>H. mrakii</i>	Suc, Mal, Inu, Raf	0	11.0	Nil
<i>H. mrakii</i>	<i>H. jadinii</i>	Gel	0	6.6	Nil
<i>H. petersonii</i>	<i>H. jadinii</i>	Rha	29.0	20.5	1.4
	<i>H. mrakii</i>	Suc, Mal, Inu, Raf	0	11.0	Nil
	<i>H. californica</i>	Inu	0	22.0	Nil
	<i>H. capsulata</i>	Suc, Inu	0	22.4	Nil

* DNA concentration 18 µg/ml.

Table 7. Relationship between uptake of ^{32}P -labelled DNA and transformation

Duration of incubation with transforming DNA (min)	Uptake of [^{32}P]DNA (c.p.m./mg dry wt of cells)	Specific activity of DNA supplied (c.p.m./mg)	Specific activity of re-isolated DNA (c.p.m./mg)	Dilution of [^{32}P]DNA	$10^{-6} \times$ viable colonies/ml	$10^{-4} \times$ trans-formants/ml	Transformation frequency (%)
1	18	1.46×10^6	3×10^3	$\times 487$	450	50	0.1
5	165	1.46×10^6	8×10^3	$\times 182$	—	—	—
10	230	1.46×10^6	20×10^3	$\times 73$	—	—	—
30	350	1.46×10^6	40×10^3	$\times 36$	450	450	1.0

Table 8. Helix coil transition temperature (T_m) and DNA base composition of yeasts

Strain	T_m ($^{\circ}\text{C}$)	GC content (mol %)
<i>Saccharomyces cerevisiae</i> var. <i>ellipsoideus</i> ITCC-Y57	64.0	36.0
<i>S. lactis</i> NCYC 416	64.0	36.0
<i>S. oleaceus</i> AC4	64.1	36.3
<i>S. acetii</i> V119-1	64.4	37.0
<i>Hansenula mrakii</i> NRRL-Y1542	64.1	36.3
<i>H. jadinii</i> NRRL-Y1542	64.2	36.5
<i>H. petersonii</i> NRRL-Y3807	64.5	37.3
<i>Candida utilis</i> NCYC322	64.5	37.3

Intergeneric transformation. The possibility of intergeneric transformation within 23 species of yeasts was also studied. Of the 150 possible intergeneric pairs, 70 pairs were examined for this purpose, but only two yielded positive results. DNA from none of the *Saccharomyces* strains could transform the strains of *Candida* to sucrose or maltose utilization. The gelatin liquefying capacity of *C. lipolytica* NCYC153 and the capacity of *C. utilis* NCYC322 to metabolize sucrose, maltose, inulin or mannitol could not be transferred to any *Saccharomyces* strain. Attempts at intergeneric transformation between *Saccharomyces* and *Hansenula* and between *Hansenula* and *Candida*, however, met with some success. *Hansenula mrakii* NRRL-Y1364 DNA transformed *S. cerevisiae* var. *ellipsoideus* ITCC-Y57 to sucrose utilization with a frequency of 6.6 %. DNA from only one strain of *Hansenula* transformed a strain of *Candida*: *H. petersonii* NRRL-Y3807 DNA conferred the property of rhamnose utilization on *C. utilis* NCYC322 at a transformation frequency of 1.8 %. Intergeneric transformation in yeasts thus appears to be infrequent.

Correlation of transformation with DNA uptake

Since the yeasts possess relatively tough cell walls, it was necessary to demonstrate that transforming DNA actually entered the recipient cells and that the uptake of DNA was correlated with the frequency of transformation. ^{32}P -labelled DNA from *H. petersonii* NRRL-Y3807 was purified and recipient cells of *C. utilis* NCYC322 were incubated with this DNA with agitation at 30 $^{\circ}\text{C}$. The reaction was stopped with DNase as described earlier. The contents of one set of tubes were centrifuged, the cells washed by centrifugation until the supernatant became free from radioactivity, and dried cells were counted for determination of ^{32}P uptake. The other set of tubes was used for extraction of DNA and determination of the specific activity of re-isolated DNA. Before the DNA was extracted from the

1 min and 30 min tubes, samples were removed for determination of transformation frequency.

The results (Table 7) show that the recipient cells took up [32 P]DNA and that uptake increased with time. The increase in DNA uptake roughly correlated with the increase in transformation frequency as a function of time of exposure to DNA. After 1 min exposure, the frequency was 0.1 %; after 30 min, DNA uptake increased 20-fold and the transformation frequency was enhanced tenfold. The amount of DNA integrated into the recipient genome is not known and we have no data concerning the amount of DNA which was degraded within the recipient cells. DNA isolated from the recipient cells would include both the integrated and the unintegrated DNA. If we assume that the entire amount of [32 P]DNA which was recovered from the recipient cells was integrated within the recipient genome, which is very unlikely, the extent of integration of the transforming DNA within the recipient genome would be only a small proportion of the DNA which entered the recipient cells.

DNA base composition of yeasts

The GC content of the strains which yielded transformants was determined from the T_m . The melting temperatures of the yeast strains studied, though these belonged to different genera, were found to be very close (between 64.0 and 64.5 °C). The GC content ranged between 36.0 and 37.3 mol % (Table 8). According to Nakase & Komagata (1971*a, b, c*) the GC contents of *Hansenula*, *Saccharomyces* and *Candida* lie in the ranges 31.5 to 50.2, 32.4 to 45.9, and 30 to 63.2 mol %, respectively.

The results of the transformation experiments are in agreement with the close similarity of the GC contents of the strains studied. *Saccharomyces cerevisiae* var. *ellipsoideus* ITCC-Y57 and *H. mrakii* NRRL-Y1364, which yielded transformants, have GC contents of 36.0 and 36.3 mol %, respectively. Similarly *H. petersonii* NRRL-Y3807 and *C. utilis* NCYC322, which also yielded transformants, have almost identical GC contents of the order of 37.3 mol %.

Similarity of GC content, however, does not necessarily imply that organisms are transformable. Thus, *S. lactis* NCYC416, which has a GC content of 36 mol %, failed to show transformation, when used as a donor or recipient, even when *S. cerevisiae* var. *ellipsoideus* ITCC-Y57 (36 mol % GC) was used.

DISCUSSION

The investigations described here support the earlier observations of Oppenoorth (1959, 1962) concerning transformation in yeasts. In the transformation experiments 109 pairs of strains were used, of which 11 were amenable to transformation. This compares favourably with the results concerning transformation of other groups of micro-organisms. The time-response and the DNA concentration curves are also typical of similar kinetic studies on transformation in other micro-organisms. The fact that no transformants were detected when DNA pre-treated with DNase was used, indicates DNA-mediated transformation. The possibilities of mutation – spontaneous or otherwise – are ruled out by the observation that the recipient cells did not acquire the marker character when treated with their own DNA or DNA obtained from unrelated strains. None of the recipient cells exhibited the marker character during the 5 years of study, unless transformed. Whereas in the case of bacteria relatively low concentrations of DNA are effective in transformation, for yeasts 10 to 20 µg DNA/ml appear to be the optimum concentrations.

Genetic events are apparently initiated rapidly. Thus, even in intergeneric transformation between *C. utilis* NCYC322 and *H. petersonii* NRRL-Y3807, 0.1 % of the recipient cells were

transformed within 1 min of application of DNA. In no case was an increase in transformation frequency observed after 30 min exposure to DNA. Agitation of the transformation mixture improved the transformation frequency slightly but aerobiosis was not obligatory. In *C. utilis* NCYC322, transformation remained unaffected due to anaerobic conditions, but in *S. acetii* VT19-1, the frequency improved several-fold in the presence of air. In this respect, *S. acetii* resembles *Bacillus subtilis* and *Haemophilus influenzae*, where aeration stimulates the development of competence (Horvath, 1968; Stuy, 1962).

In addition to appropriate conditions for transformation, failure to detect transformation between any two strains may depend on the lack of DNA homology. It is therefore difficult to assess the taxonomic or phylogenetic significance of the observations recorded here. It is, however, interesting to note that *S. lactis* could not transform any strain of any other species of *Saccharomyces*, although three to four marker characters were used in several experiments. On morphological grounds, Van der Walt (1965) has suggested the placement of *S. lactis* under a separate genus *Kluyveromyces*. Bicknell & Douglas (1970) found no homology between the DNA of *S. lactis* and *S. cerevisiae*. *Candida utilis* and *C. lipolytica* were amenable to reciprocal transformation, suggesting a close relationship. The observation that *H. petersonii* DNA can transform both *H. jadinii* and *C. utilis* is in agreement with the contention of Wickerham (1964) that these three organisms are probably on the same evolutionary line, representing a section of homothallic species dependent on warm blooded animals. Although some success was recorded in the intergeneric experiments between *Saccharomyces* and *Hansenula* and between *Hansenula* and *Candida*, similar experiments with *Saccharomyces* and *Candida* uniformly yielded negative results. *Saccharomyces* and *Hansenula* are treated by most taxonomists under Endomycetaceae, and *Candida* is placed in a separate family Cryptococcaceae (Lodder & Kregar van Rij, 1952). The possibility that *Hansenula* may be a connecting link between *Saccharomyces* and *Candida* deserves careful consideration.

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