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Susceptibility of *Saccharomyces* spp. and *Schwanniomyces* spp. to the Aminoglycoside Antibiotic G418

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Industrially useful polyploid yeasts such as the brewing yeasts do not possess any auxotrophic genetic markers and hence are not easily amenable to plasmid-mediated DNA transformations. In an attempt to obtain genetic markers, a number of useful *Saccharomyces* sp. strains and some amylolytic *Schwanniomyces* sp. strains were tested for their susceptibility to the antibiotic Geneticin G418, a 2-deoxystreptamine reported to be active against bacteria, yeasts, and plant and animal cells. All of the *Saccharomyces* sp. strains, including the brewing strains, were found to be susceptible to G418 in the concentration range of 150 to 500 μg/ml. Of the three *Schwanniomyces* species investigated, only *Schwanniomyces castellii* (strain 1402) was found to be resistant to G418 at concentrations up to 1 mg/ml. Resistance was exhibited both in liquid media and on glycerol-peptone-yeast extract agar plates. This finding is interesting in view of the possibility of using this strain as a DNA donor for transformations aimed at introducing the amylolytic capability into brewing yeasts.

In recent years there has developed a great deal of interest in the genetic manipulation of industrial yeast strains. The purpose of improving these already proven microbes has been to broaden their substrate-utilizing capability or to use them for producing foreign proteins of interest, such as insulin and interferon. Industrial yeast strains such as the brewing yeasts are polyploid/aneuploid and are genetically very stable (7). As a result of this, it is very difficult to obtain auxotrophic markers in such strains; consequently, plasmid-mediated transformation presents problems. The amylolytic yeast genus *Schwanniomyces* has not been genetically characterized. However, members of this genus have been found to produce extracellular α-amylase and glucoamylase with debranching activity (6). They are, thus, potential donors of genetic material for transforming a brewing strain of *Saccharomyces* sp. to enable it to utilize starch and dextrins which form a significant part of the nonfermented carbohydrates remaining in the final beer. Introduction of the genes for α-amylase and glucoamylase into a stable brewing yeast would also be of great value for producing industrial fermentation ethanol from starch substrates.

With a view to developing such a transformation system, strains of *Saccharomyces* spp. and *Schwanniomyces* spp. were tested for their susceptibility to various antibiotics, including gentamycin, lincomycin, and neomycin, and the response to the aminoglycoside 2-deoxystreptamine Geneticin G418 (2) was found to be most interesting. The antibiotic G418 has been found to have a wide spectrum of activity against bacteria, yeasts, fungi, algae, and plant and animal cells (2, 4, 9, 10). In eucaryotic cells G418 interferes with the function of 80S ribosomes and blocks protein synthesis. Davies and Jimenez (2) have found that bacteria producing the 3′-*O*-aminoglycoside phosphotransferase enzymes are resistant to G418. Two of these transferases, types I and II, were found to be encoded by the transposable elements Tn*5* and Tn*601*, respectively. Plasmid vectors carrying these transposons have been prepared, and *Saccharomyces cerevisiae* cells have been transformed to G418 resistance with such vectors (3, 4). The present study describes an investigation carried out to determine the susceptibility of *Saccharomyces* sp. strains and *Schwanniomyces* sp. strains to G418.

The following yeast strains were investigated: *Saccharomyces cerevisiae* strain 1434, obtained from the Berkeley Yeast Culture Collection as RH218 (a *trp1 gal2 mal SUC2 CUP1*); *Saccharomyces uvarum* (*carlsbergensis*) strain 21, a polyploid brewing lager strain; *Schwanniomyces occidentalis* strain 1401 (-ATCC 26074); *Schwanniomyces castellii* strain 1402 (-ATCC #26077); and *Schwanniomyces alluvius* strain 1442, obtained from W. M. Ingledew, University of Saskatchewan, Saskatoon, Canada. *Schwanniomyces castellii* strain 1436 was a 2-deoxyglucose-resistant mutant of strain 1402 found to be derepressed for α-amylase and glucoamylase production (8). The cultures were grown in a synthetic medium (PYN) consisting of 0.35% Bacto-Peptone (Difco Laboratories), 0.3% yeast extract (Difco), 15 mM KH_2PO_4, 4 mM $MgSO_4 \cdot 7H_2O$, 8 mM $(NH_4)_2SO_4$, and 2% (wt/vol) glucose, adjusted pH 5.0. Cells were pregrown in PYN medium and transferred to a fresh 200 ml of PYN medium in 500-ml shake flasks with or without an appropriate concentration of filter-sterilized G418. The flasks were agitated in a rotary shaker at 30°C. One- to 3-ml samples were periodically taken, and the optical density at 600 nm (OD_{600}) was immediately determined. For determining cell viability, an aliquot of the sample was mixed with a 0.01% solution of methylene blue and viable cells (no dye uptake) were counted, using a hemacytometer placed under a microscope as described previously (1).

Saccharomyces cerevisiae 1434 (RH218) has been used extensively for transformations involving complementation of the *TRP1* gene (5). When examined for resistance to G418, the yeast strain was found to be very susceptible at G418 concentrations of 150 to 500 μg/ml. Similarly (Fig. 1), cells of the brewing yeast *Saccharomyces uvarum* (*carlsbergensis*) 21 showed reduced growth as monitored by OD_{600} after addition of 300 μg of G418 per ml when compared with a control (without G418). The addition of G418, however,

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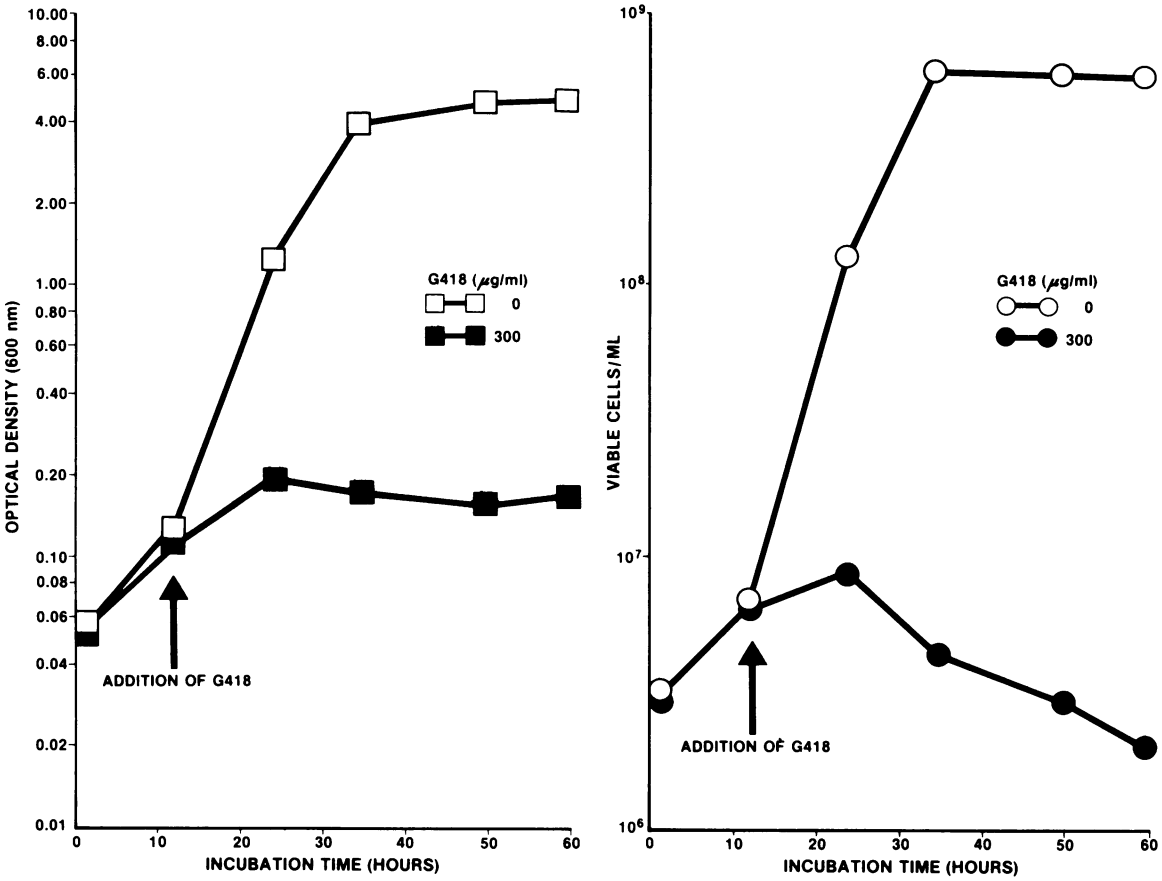

FIG. 1. Effect of addition of Geneticin G418 on growth and cell viability of *Saccharomyces uvarum* (*carlsbergensis*) 21.

brought about a significant decrease in the number of viable cells as determined by methylene blue assay (1). It is seen that both OD_{600} and the viable cell count were affected almost immediately upon the addition of G418. Cells of *Schwanniomyces occidentalis* 1401 also showed similar results with the addition of G418, i.e., a leveling off (or slight decline, possibly due to cell lysis) of OD_{600} and a significant decline in the number of viable cells from 10^7 to 1.5×10^6 per ml, whereas in the absence of G418 the concentration increased to 10^9 cells per ml. Cells of *Schwanniomyces castellii* 1402, however, demonstrated a resistance to G418 (Fig. 2). Thus, with the addition of 300 μg of G418 per ml after 12 h of growth, *Schwanniomyces castellii* 1402 cells were not affected by the addition of G418. Both OD_{600} and viable cell counts remained similar to those for the control cells (no G418 added).

For G418 to be a useful marker for spheroplast fusion or transformation, resistance or susceptibility by yeast cells would have to be demonstrated on G418-supplemented agar plates. Table 1 demonstrates cell counts obtained when cells from various yeast species were plated onto 2% glycerol–peptone-yeast extract agar plates with or without 300 μg of G418 per ml. As shown above, both the polyploid *Saccharomyces uvarum* (*carlsbergensis*) 21 and the haploid *Saccharomyces cerevisiae* 1434 were found to be very susceptible to G418. In both cases, no colonies (revertants) were found on plates inoculated with 100-fold-diluted culture. Of the *Schwanniomyces* sp. strains investigated, only *Schwanniomyces castellii* 1402 and *Schwanniomyces castellii* 1436 (a 2-deoxy-glucose-resistant mutant of strain 1402 [8]) were found to be resistant to G418 (even at concentrations of 1 mg/ml). In both cases, there was no significant difference in the CFU obtained with or without G418. Both *Schwanniomyces occidentalis* (strain 1401) and *Schwanniomyces alluvius* (strain 1442) were found to be susceptible to 300 μg of G418 per ml.

Previous reports on G418 have shown that the antibiotic has a broad spectrum of activity against bacteria, yeasts, fungi, and plant and animal cells. Apart from certain bacterial strains and yeasts transformed with bacterial plasmids carrying Tn*5* and Tn*601* transposons, no other organisms have been reported to show any resistance to G418 (3). It is, therefore, of considerable interest to find a strain of the yeast *Schwanniomyces* that can resist G418 at high concentrations. This is particularly significant in light of the fact that *Schwanniomyces castellii* is an amylolytic yeast and thus potentially useful as a donor for DNA for transformation of amylolytic genes into industrial yeasts such as the brewing polyploid strains. The mechanism of resistance to G418 by *Schwanniomyces castellii* is not quite clear. It is conceivable that the antibiotic is not taken up by the cells. Preliminary experiments have shown that the presence or absence of phosphate in the defined medium has no significant effect upon the resistance of *Schwanniomyces castellii* to G418. Further investigations are under way to determine whether exclusion of the antibiotic is the reason for resistance or

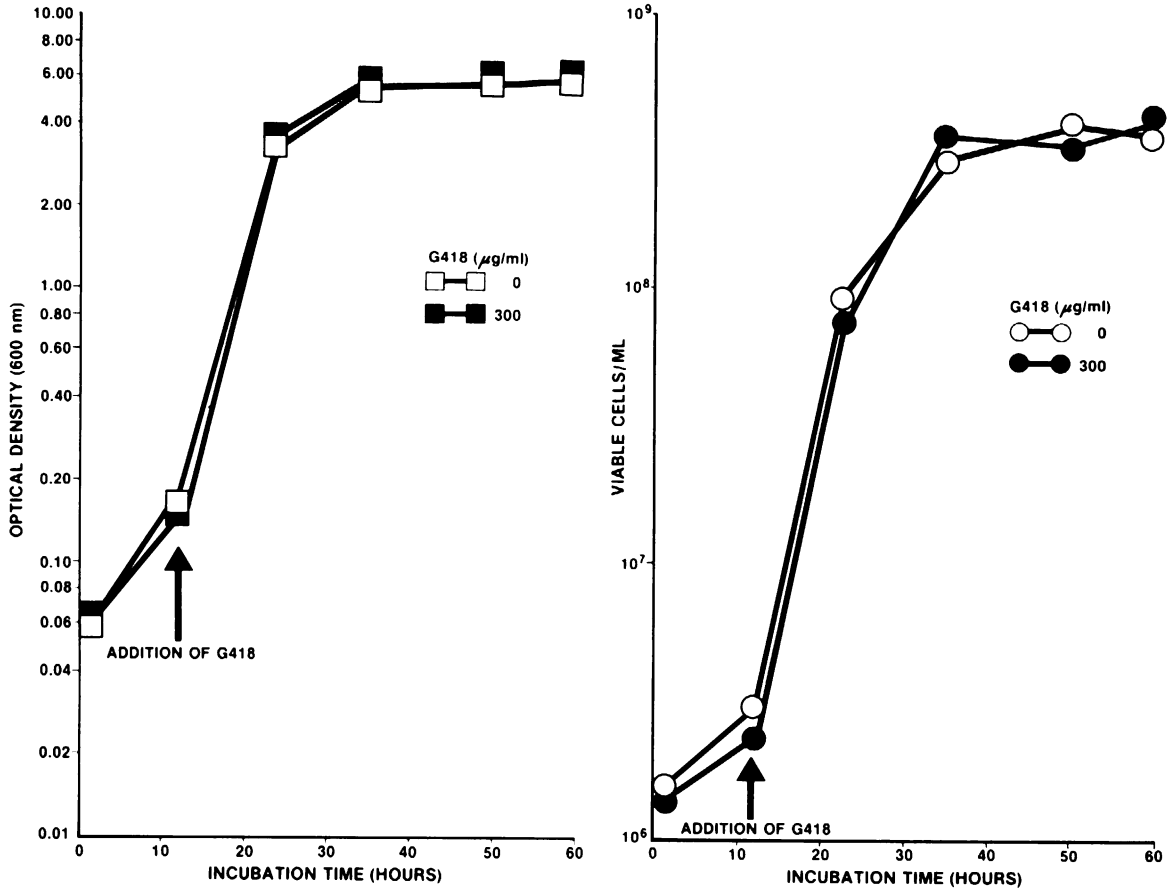

FIG. 2. Effect of addition of Geneticin G418 on growth and cell viability of *Schwanniomyces castellii* 1402.

TABLE 1. Viability of *Saccharomyces* spp. and *Schwanniomyces* spp. cells on agar plates with or without Genticin G418[a]

Yeast strain	CFU/ml	
	Without G418 ($\times 10^8$)	With G418
Saccharomyces uvarum (*carlsbergensis*) 21, polyploid	1.73 ± 0.20	$<10^2$
Saccharomyces cerevisiae 1434 (RH218), haploid	1.52 ± 0.18	$<10^2$
Schwanniomyces occidentalis 1401	1.00 ± 0.20	$<10^2$
Schwanniomyces castellii 1402	6.40 ± 0.20	$7.20 \pm 0.20 \times 10^8$
Schwanniomyces castellii 1436 (derepressed mutant of strain 1402)	1.94 ± 0.20	$1.53 \pm 0.20 \times 10^8$
Schwanniomyces alluvius 1442	2.20 ± 0.22	$<10^2$

[a] Colonies were plated on 2% glycerol-peptone-yeast extract agar plates ± 300 μg of G418 per ml and incubated at 25°C. Results are the average of three plate counts.

whether G418-modifying enzymes are present in *Schwanniomyces castellii*.

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