STEROLS AS COMPONENTS IN THE MATING REACTION OF *HANSENULA WINGEI*

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ABSTRACT

HUNT, D. E. (Charles V. Chapin Hospital, Providence, R.I.) AND P. L. CARPENTER. Sterols as components in the mating reaction of *Hansenula wingei*. J. Bacteriol. **86**:845–847. 1963.—Agglutinative mating strains 5 and 21 of the yeast *Hansenula wingei* did not agglutinate after the cells of these two strains were treated with nystatin. The agglutination-inhibiting action of this antibiotic could not be reversed by washing, or heating, or washing and heating the cells. When cells of the two mating types, which had not been treated with nystatin, were heated and washed three times with a chloroform-ethanol (1:3, v/v) solution, the agglutination reaction was reduced significantly. If unwashed cells of mating strain 5 were tested for agglutination with chloroform-ethanol washed cells of mating strain 21, normal agglutination occurred readily. The reciprocal of this agglutination test also resulted in normal agglutination. Evidence is presented that the factor(s) required for the mating reaction in *H. wingei* can be supplied by either strain, and sterols or related compounds have an active role in the mating reaction.

Wickerham (1956) described a new species of yeast, *Hansenula wingei*, which shows a strong mating reaction when two haploid mating types (strains 5 and 21) are brought together. Brock (1958*a*) developed a quantitative method for the evaluation of mating reactions, which is based on the reduction of turbidity when the two mating types are mixed. He later investigated this phenomenon with special emphasis on cell-surface properties and agglutination (1958*b*, 1959), and suggested that the mating reaction is due to hydrogen-bond formation between a protein on strain 21 and a polysaccharide on strain 5. Brookbank and Heisler (1963) investigated the immunology of *H. wingei*, but their work did not reveal specificity for mating type.

Brock (1958*a*) also reported the following. (i) Twice-washed cells of *H. wingei* would not agglutinate, except when various cofactors were added. (ii) The reaction was not influenced over a pH range of 3 to 9. (iii) Heating the cells for 5 min at 100 C intensified the agglutination reaction. Lampen et al. (1959) found that binding of the antibiotic nystatin by a yeast, *Saccharomyces cerevisiae*, was unaffected by pH and was intensified by heating. It therefore appeared interesting to investigate the effect of nystatin on the mating reaction of *H. wingei*.

MATERIALS AND METHODS

Organisms. *H. wingei* NRRL Y-2340 strains 5 and 21, which are agglutinative mating types, were used in this investigation. These organisms were maintained on agar slants containing 3% glucose, 0.7% yeast extract, 0.5% KH_2PO_4, and 2% agar. For experimental work, cells of *H. wingei* were grown in liquid medium of the same composition minus the agar.

Materials. The nystatin used in this work was pharmaceutical grade (E. R. Squibb & Sons, New York, N.Y.). An aqueous nystatin suspension (5.0 mg/ml) was prepared and added as indicated.

Preparation of cells. Three 250-ml Erlenmeyer flasks containing 100 ml of liquid medium were inoculated with 0.1 ml of a shaken 24-hr broth culture of *H. wingei* strain 5, and three other flasks were inoculated with strain 21. After these flasks were shaken overnight at 28 C, 5 ml of the nystatin suspension were added to two flasks of strain 5 cultures and to two flasks of strain 21 cultures. The remaining pair of cultures served as controls. Then, 2 hr later, the flasks were removed from the shaker, and the cells were col-

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TABLE 1. *Effect of nystatin on agglutination of Hansenula wingei mating strains 5 and 21*

Prepn	Cell treatment*	Optical density†	
		Nystatin	Control
1	Nonheated	1.98	0.75
2	Heated	2.00	0.36
3	Water-washed and heated	1.98	0.40
4	Nystatin-treated strain 5 plus nystatin-nontreated strain 21	0.70	—
5	Nystatin-treated strain 21 plus nystatin-nontreated strain 5	0.73	—
6	Chloroform-ethanol (1:3, v/v) washing	1.94	1.98
7	Heated and washed with chloroform-ethanol	1.98	1.80

* Except where indicated, both strains 5 and 21 were treated in the same manner in each preparation.

† In all instances, the optical density measurements were taken 5 min after the two mating types were prepared. The final nystatin concentration in nystatin-treated cells was 0.25 mg/ml. Controls were not treated with nystatin. Higher optical density indicates a lesser degree of agglutination.

lected by centrifugation and used for agglutination tests.

Quantitative determinations of mating reaction. The determinations were essentially the same as those described by Brock (1958*a*); however, in this work turbidities, which indicated the degree of agglutination, were determined at 525 mμ with a Bausch and Lomb Spectronic-20 transistorized colorimeter.

Results and Discussion

When nystatin was added to cultures of *H. wingei* mating strains 5 and 21, cells from these cultures did not agglutinate (Table 1).

Heating the cells for 5 min at 100 C, which regularly stimulates agglutination, failed to induce agglutination in the nystatin-treated cells. Washing or heating, or both, of the cells did not stop the agglutination-inhibiting action of nystatin, which has been found to be difficult to remove from yeast cell walls (Lampen et al., 1959). Whether nystatin was added immediately before or 2 hr before the agglutination tests were conducted made no apparent difference in its agglutination-inhibiting action. Apparently, it complexes quickly with the sterol (Lampen et al., 1962) of the yeast cells.

When nystatin-treated cells of either mating type were tested for agglutination with nystatin-nontreated cells of the opposite mating type, agglutination occurred readily. This suggests that either strain can furnish the factor needed for agglutination. It was found that the mating reaction could be diminished significantly by washing cells of the two mating strains three times with a chloroform-ethanol (1:3, v/v) solution. This, of course, would remove sterols. Since sterol removal or binding sterol with nystatin prevents the mating reaction, it appears that sterol(s) is involved in the mating reaction in *H. wingei.*

That the cells of the two mating types agglutinate due to hydrogen bonding seems to be apparent, since Brock (1959) reported that 8 M urea completely inhibits the mating reaction. This poses the following question. How can a sterol form hydrogen bonds and promote agglutination between the two mating strains? An examination of the structure of ergosterol, a common yeast sterol, would seem to rule out hydrogen bonding in this compound, since ring I of the cyclopentanoperhydrophenanthrene nucleus (which contains the hydroxyl group) is saturated. Perhaps another factor(s) in addition to sterol may be necessary for agglutination, especially since other species of yeast, which also contain sterols, will not agglutinate with *H. wingei.* Relatively little is known about yeast sterols. Despite exhaustive research on the sterols of *S. cerevisiae,* a new sterol was discovered after 30 years of study (Eddy, 1958). Therefore, it appears possible that an unknown sterol, peculiar to *H. wingei,* is responsible for the mating reaction.

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