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SPECIFIC, SOLUBLE FACTOR INVOLVED IN SEXUAL AGGLUTINATION OF THE YEAST *HANSENULA WINGEI*

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ABSTRACT

TAYLOR, NEIL W. (Northern Regional Research Laboratory, Peoria, Ill.). Specific, soluble factor involved in sexual agglutination of the yeast *Hansenula wingei*. J. Bacteriol. **87**:863-866. 1964.—A factor was liberated by snail enzymes from unisexual mating type 5 of *Hansenula wingei* NRRL Y-2340. This factor is the (or a) factor on type 5 involved in sexual agglutination of *H. wingei* because it agglutinates only the active opposite mating type; it is absorbed appreciably only by the active opposite mating type; and it is inactivated by a mercaptan, the same agent which inactivates sexual agglutination in type 5. The sedimentation rate of the factor, 31 Svedbergs, indicates it to be soluble.

In some species of yeasts, unisexual mating types agglutinate strongly and specifically with opposite mating types of the same species (Wickerham, 1956, 1958, 1960). As part of a study of the mechanism of this reaction, an agglutinating factor was found in snail enzyme digests of *Hansenula wingei* (Wickerham, 1956) mating type 5. This factor (5-factor) behaves similarly to the cells of type 5 in its effect on the opposite mating type. Preparation and some properties of the factor are described.

Opposite mating types of two species were examined, *H. wingei* NRRL Y-2340, types 5 and 21 (Wickerham, 1956), and *Saccharomyces kluyveri* (Phaff, Miller, and Shifrine, 1956) NRRL Y-4288, types 3 and 26 (Wickerham, 1960). These types were also used to test specificity of the 5-factor. Brock (1959) showed that, with respect to ability to agglutinate with the opposite type, the two mating types of *H. wingei* are quite different in sensitivity to chemical and enzymatic treatments. On this basis, he suggested that the surface structure of type 21 involved in agglutination was protein, whereas that of type 5 was carbohydrate. From sensitivity to chemicals such as disulfide bond cleaving agents, acid, and dinitrofluorobenzene, we found that types 3 and 5 are similar and that types 21 and 26 are similar (*unpublished data*). The bisexual cultures from which the mating types are derived do not agglutinate with either mating type, and thus may serve as partial controls for the active mating types.

MATERIALS AND METHODS

Cultures obtained from L. J. Wickerham of the Northern Regional Research Laboratory were grown at 25 C in YM medium (30 g of yeast extract, 3.0 g of malt extract, 5.0 g of peptone, and 30 g of glucose per liter of water), except that 3% glucose was used (Haynes, Wickerham, and Hesseltine, 1955). After 2 days, cells were harvested, extracted with 8 M LiBr, washed with 0.1% NaCl solution, and kept in water in the cold. All work was done with cell preparations (termed cells below) pretreated with 8 M LiBr. All tests and preparations were made up in sodium acetate buffer of pH 5.6, 0.1 ionic strength, unless otherwise indicated. Absorbancies (A) of cell preparations were measured at 590 mμ in round tubes (10-mm) in a Spectronic-20 colorimeter (Bausch & Lomb, Inc., Rochester, N.Y.). At A = 0.5, the preparations contained about 0.2 mg (dry weight) of cells per ml. For agglutination tests of cells, equal volumes of cell suspensions made up each to A = 0.5 were mixed and shaken for 10 min on a reciprocating shaker; for tests of 5-factor, type 21 was made up to A = 0.5, including the 5-factor to be analyzed, and shaken for 2 hr. Extent of agglutination was judged visually as complete, partial, or not detectable. The above procedures were developed in other work for which future publication is planned. Snail digestive enzyme was the preparation, Suc d'*Helix pomatia* [Industrie Biologique

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TABLE 1. *Agglutination factors in yeast extracts**

Extracted type	Inactivation of extracted cells	Agglutination with type			
		H. wingei		*S. kluyveri*	
		5	21	3	26
Hansenula wingei 5.	Partial	−	+	−	−
H. wingei 21.....	Total	−	−	−	−
Saccharomyces kluyveri 3.....	Total	−	−	−	−
S. kluyveri 26.....	Partial	−	−	−	−

* The snail juice extract of each type was tested for ability to agglutinate cells of each type.

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Sedimentation rates were determined in a Spinco SW 39 (Division of Beckman Instruments, Inc., Palo Alto, Calif.) swinging-bucket rotor at speeds from 30 to 35,000 rev/min in gradients containing 2 to 10% or 3 to 20% glucose. Each gradient was dispensed in about 20 successive fractions, which were tested for agglutination activity. The sedimentation rate of the factor was calculated as the mean of the values corresponding to the edges of the agglutinating band in the centrifuge. Sedimentation rates $S_{20,w}$ were corrected for glucose gradient viscosity (5 to 10% correction), temperature of the run, and density of solvent (4% correction).

RESULTS AND DISCUSSION

Preparation of 5-factor. When cells of type 5 were treated with snail digestive juice, the supernatant fluid, after the cell residue was centrifuged off, agglutinated cells of type 21. A convenient preparation, similar to the method of Eddy and Williamson (1957) for preparing protoplasts, was to add 0.1 to 0.3 ml of juice to 10 ml containing about 0.5 g (dry weight) of type 5 cells; reaction time was 1 to 3 days at 25 C. Prolonged treatment time with the higher enzyme concentration seemed to have little effect on 5-factor. The 5-factor was recovered from the reaction medium, after cells were removed, by adding type 21 and shaking for 30 to 60 min. About three times as many type 21 cells as type 5 cells were required to recover all the 5-factor in the digest. The absorbed 5-factor was eluted from type 21 cells with 0.003 M K_2CO_3; the pH of the eluate was 10, and was adjusted to pH 5.6.

Of the four mating types from the two species treated with enzymes, only *H. wingei* type 5 yielded an agglutinating factor, which in turn agglutinated only type 21 (Table 1). Cell residues of types 21 and 3 were completely inactivated by the enzymes, when tested for agglutination with the opposite mating type. Those of types 5 and 26 were partially inactivated. Further treatment of type 5 with fresh enzyme did not completely inactivate its agglutinating ability; there is evidently some heterogeneity in the factor or in the way it is attached to the cell surface.

Agglutination of type 21 suspended in a solution of 5-factor indicates the factor is multivalent. Unless the absorbed 5-factor disturbs the general repulsive force between cells, each particle of 5-factor combines with two type 21 cells and forms a many-celled agglutinating clump. Digests from the other mating types may have contained univalent agglutinating factors, which would inhibit agglutination of cells of the opposite type. No such inhibition was observed in a digest of type 21.

Chemical analyses for carbohydrate and protein indicated very little of these materials to be present in the eluates containing 5-factor. From these analyses and the minimal volume of 5-factor eluate necessary to agglutinate 1 ml of 21 cells, only a few tenths of 1% of material, compared to dry cell weight, appeared to be required to agglutinate type 21 cells. However, large variation in the carbohydrate-protein ratio in different eluates indicated further purification to be necessary.

Specificity. The 5-factor is specific in that it agglutinates only the opposite mating type of the same species. Further information on specificity of action was obtained by measuring absorption of 5-factor by various cells in suspension. Three preparations of type 21 were made inactive for mating agglutination by treatment with 0.3% 2,4-dinitrofluorobenzene at pH 8.0, 5 M HCl, and 0.1 M NaOH; reaction times were 16 hr at 25 C. These preparations, as well as the active mating types and the bisexual types, were each diluted to A = 0.5, and a sample of 5-factor was added to each. After the suspensions were shaken 1 hr, the supernatant solutions from centrifugation were recovered and titered for the 5-factor by adding type 21 at A = 0.5 to tubes containing amounts of the supernatant solution decreased serially by a factor of 0.6. Agglutination titer duplicated within one tube or better. Table 2 gives the concentration of 5-factor in the solutions

after absorbent removal, relative to the concentration of 5-factor in an identical solution without absorbent. Extensive absorption of 5-factor is shown only by active cells of type 21. The results with inactivated type 21 indicate that certain features of the type 21 cell surface are necessary for absorption, and that these features disappear with application of the three inactivation procedures. For the 5-factor, specificity of action seems quite well established.

None of the absorbents agglutinated with type 21 after treatment with 5-factor followed by washing with buffer except agglutinable type 21. These were already agglutinated, but, when diluted by the appropriate amount, they further agglutinated an equal volume of type 21 cells. The type 3 absorbent was not tested because type 3 and type 21 mutually agglutinate. The experiments indicate that, where testable, the cells which absorbed little 5-factor did not absorb enough on the surface to agglutinate type 21.

Whether the 5-factor is specifically the agglutination factor on the surface of type 5 cells can be tested further by comparing chemical inactivation of 5-factor and type 5 cells. One of the most distinct differences between cells of types 5 and 21 is the inactivation of type 5 by mercaptoethanol (and other disulfide-breaking reagents) as compared with resistance of type 21 to this reagent (*unpublished data*). When 5-factor was treated with 1.2% mercaptoethanol in pH 6.6 buffer, agglutinating ability was destroyed in 15 min. After the reaction period, 2 moles of acrylonitrile per mole of mercaptoethanol were added to stop the reaction, and the preparation was tested for ability to agglutinate type 21. Short reaction times, up to 4 min, as well as suitable controls without mercaptoethanol, acrylonitrile, or both, allowed good agglutination of type 21 cells by 5-factor. These reaction conditions are milder (i.e., of lower pH) than those used previously in inactivating type 5 cells. However, the 5-factor behaves qualitatively as do the type 5 cells in this property also.

The lower pH was used above to inactivate 5-factor because, under the conditions used to inactivate 5 cells, 5-factor appeared to be much more sensitive to mercaptoethanol. In 0.14% mercaptoethanol at pH 8, 5-factor activity disappeared within a few minutes, before it could be assayed. Under similar conditions, except that 4 M urea was also present, type 5 cells are inactivated after somewhat more than 1 hr. Urea tends to increase extent and rate of reaction of type 5 cells with mercaptoethanol; thus, despite this difference in reaction conditions, the much more rapid inactivation of 5-factor as compared to type 5 cells is apparent.

TABLE 2. *Absorption of 5-factor by cell preparations*

Type as absorbent	Concn of 5-factor*	Recovered absorbents' ability to agglutinate type 21
Hansenula wingei 21; inactivated by FDNB	1.0	No
H. wingei 21; inactivated by HCl	0.8	No
H. wingei 21; inactivated by NaOH	1.0	No
H. wingei 21	0.08	Yes
Saccharomyces kluyveri 3.	0.8	—
S. kluyveri 26	0.6	No
H. wingei; bisexual	1.0	No
S. kluyveri; bisexual	1.0	No

* Concentration of 5-factor after exposure to absorbents is given as ratio to initial concentration.

Sedimentation rate. To obtain an estimate of the size of the 5-factor, sedimentation rate was determined in glucose gradients. The average $S_{20,w}$ of seven runs was 31 Svedbergs with a standard deviation of 4. A few runs were discarded in which mixing had evidently occurred. Width of the agglutinating band averaged 0.5 of the mean sedimentation rate.

A sedimentation rate of 31 corresponds to a molecular-weight range from about 400,000 for a solid sphere of carbohydrate to about 5 million for a B-512 dextran (Senti et al., 1955); one to several million is thus a reasonable estimate of particle molecular weight. This value is small enough to indicate that the 5-factor is a soluble entity, considerably richer than cell walls in the structures involved in mating agglutination.

LITERATURE CITED

BROCK, T. D. 1959. Mating reaction in *Hansenula wingei*. Relation of cell surface properties to agglutination. J. Bacteriol. **78**:59–68.

EDDY, A. A., AND D. H. WILLIAMSON. 1957. A method of isolating protoplasts from yeast. Nature **179**:1252–1253.

HAYNES, W. C., L. J. WICKERHAM, AND C. W. HESSELTINE. 1955. Maintenance of cultures of industrially important microorganisms. Appl. Microbiol. **3**:361–368.

PHAFF, H. J., M. W. MILLER, AND M. SHIFRINE. 1956. The taxonomy of yeasts isolated from *Drosophila* in the Yosemite region of California. Antonie van Leeuwenhoek J. Microbiol. Serol. **22**:145–161.

SENTI, F. R., N. N. HELLMAN, N. H. LUDWIG, G. E. BABCOCK, R. TOBIN, C. A. GLASS, AND B. L. LAMBERTS. 1955. Viscosity, sedimentation, and light scattering properties of fractions of an acid-hydrolyzed dextran. J. Polymer Sci. **17**:527–546.

WICKERHAM, L. J. 1956. Influence of agglutination on zygote formation in *Hansenula wingei*, a new species of yeast. Compt. Rend. Trav. Lab. Carlsberg Ser. Physiol. **26**:423–443.

WICKERHAM, L. J. 1958. Sexual agglutination of heterothallic yeasts in diverse taxonomic areas. Science **128**:1504–1505.

WICKERHAM, L. J. 1960. Extracellular invertase production by sexually agglutinative mating types of *Saccharomyces kluyveri*. Science **131**: 985–986.