Antonie van Leeuwenhoek **37** (1971) 379–384

Sex-specific growth responses in yeasts

ALBERTA I. HERMAN

Northern Regional Research Laboratory, Peoria, Illinois 61604, U.S.A.

HERMAN, A. I. 1971. Sex-specific growth responses in yeasts. Antonie van Leeuwenhoek **37**: 379–384.

Growing cells of complementary mating types from two genera of yeasts, *Saccharomyces* and *Hansenula*, exhibited sex-specific responses. Two kinds of responses were observed. Either one of the sexes grew preferentially toward its mate which ceased to bud and increased in size, or both mating types grew preferentially toward each other. A growth response was observed between mating types of *Saccharomyces cerevisiae* and *Hansenula anomala* presumably complementary.

INTRODUCTION

Yanagishima et al. (1968), Yanagishima (1969), and Duntze, MacKay and Manney (1970) have shown that sex-specific compounds are formed by complementary mating types of the yeast *Saccharomyces cerevisiae*. The agents elicit a specific response in cells of the opposite gender. Exposure to the active constituents causes sensitive cells to increase in size. In the yeast *Kluyveromyces lactis* another kind of sex-specific growth response is expressed. In *K. lactis*, cells of opposite mating type grow preferentially toward each other (Herman, 1970*a*, *b*). The difference in the type of sex-specific response that is observed in cells of *S. cerevisiae* and *K. lactis* could be real or it could be due to dissimilar assays used by the investigators (Yanagishima et al., 1968; Yanagishima, 1969; Duntze et al., 1970; Herman, 1970*a*, *b*). To clarify this uncertainty, cells of complementary mating type of *S. cerevisiae* were grown under conditions similar to those followed in the *K. lactis* study (Herman, 1970*a*) and examined for sex-specific responses. Further, to determine whether sex-specific growth responses occur in other genera of yeasts, responses were measured between pairs of cells of complementary mating types from representatives of the genus *Hansenula*.

MATERIALS AND METHODS

Microorganisms. All the yeasts studied are prototrophic with the exception of stock NRRL Y-2047. The yeast mating pairs with their ARS Culture Collection numbers are: *Saccharomyces cerevisiae* NRRL Y-2047 and NRRL Y-2048; *Hansenula anomala* NRRL Y-2153-4 and NRRL Y-366-8; *Hansenula wingei* NRRL Y-2340 strain 5 and NRRL Y-2340 strain 21.

S. cerevisiae NRRL Y-2047 and NRRL Y-2048 were received by the ARS Culture Collection in Peoria in the early 1950's from Dr. Sol Spiegelman with the information that they were the mating type testers from the Carbondale breeding stock. *H. anomala* NRRL Y-2153-4 and NRRL Y-366-8 were isolated by Dr. L. J. Wickerham and are used as the mating type testers for this species (Wickerham and Burton, 1954). *H. wingei* NRRL Y-2340 strain 5 and NRRL Y-2340 strain 21 were isolated and described by Wickerham (1956) as sexually agglutinative stocks of opposite sex. The strains were used by Brock (1958), Taylor (1964), and Taylor and Orton (1968) in their investigations of the mating-agglutinative reactions in this species.

Culture methods. Yeasts were grown on yeast-malt (YM) medium (Wickerham, 1951).

Single cells of complementary mating types were positioned at arbitrarily chosen distances from one another with a De Fonbrune micromanipulator on a block of YM medium solidified with 3% agar and treated as described earlier (Herman, 1970*a*, *b*). At least three or four pairs of cells were tested in each observation. Two or three independent observations were performed on each species set.

RESULTS

Growth response between sexes in S. cerevisiae. Fig. 1 represents a typical pattern of budding in each of the two mating types of *S. cerevisiae* when it is

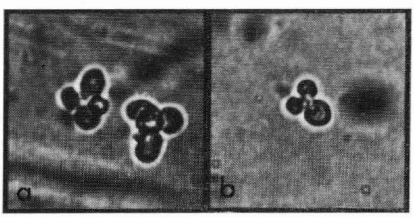

Fig. 1. Budding cells of like mating type of *Saccharomyces cerevisiae* growing in close proximity. (a) Two clones of NRRL Y-2048 mating type separated initially by 11 μm. (b) Cells of NRRL Y-2047 mating type. Bright field. 1000 ×.

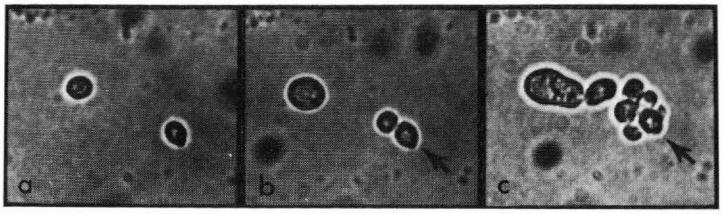

Fig. 2. Clones of complementary mating types of *S. cerevisiae* growing in close proximity. (a) A single cell of NRRL Y-2047 and of NRRL Y-2048 (right) separated by 11 μm. (b) After 3 hr. (c) After 6 hr. Arrows indicate mother cells. Bright field. 1000 ×.

grown separately. The cells bud randomly and most of the buds are similar in size. The response between growing cells of opposite sex positioned 11 μm apart is seen in Fig. 2. Initially, both cells were about equal in size (Fig. 2a). Three hours later (Fig. 2b) the cell of Y-2047 mating type enlarged about twofold and the cell of the opposite sex, Y-2048, began to bud. After 6 hr (Fig. 2c) Y-2047 continued to enlarge and formed one bud. During the same period, Y- 2048 produced several buds. Most of these were directed toward the opposite sex, Y-2047. When cells of either Y-2047 or Y-2048 were grown in close proximity to the diploid Y-2047 X Y- 2048, the clones grew away from each other.

Growth responses between *a* and α cells of *S. cerevisiae* that were derived from the Mortimer, Roman, Hawthorne breeding stocks were similar to those displayed by the Carbondale testers. The Mortimer, Roman, Hawthorne stocks were obtained from Dr. Harlyn Halvorson (University of Wisconsin, Madison) and bear his code numbers D587-4B (α mating type) and D585-11C (*a* mating type). When cells of D587-4B and D585-11C were grown in close proximity to each other, the α cells (D587-4B) formed numerous buds, most of which were oriented toward the *a* cell. At the same time, the *a* cell (D585-11C) increased in size about twofold.

Although mating stocks and method of assay differed from those employed by Yanagishima et al. (1968) and Yanagishima (1969) and Duntze et al. (1970), a sex-specific response similar to the one they reported was observed in *S. cerevisiae*. Evidently there is a real difference between the preferential budding response of *K. lactis* and the cell enlargement of *S. cerevisiae*. Reasons for this difference are not known.

Response between sexes in species of Hansenula. Two heterothallic species from the genus *Hansenula* were examined for sex-specific responses. Mating types of *H. wingei* Y-2340 strain 5 and Y-2340 strain 21 and *H. anomala* Y-2153-4 and Y-366-8 were used.

The response between *H. wingei* mating types resembled that observed in

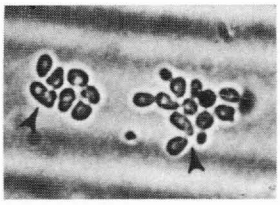

Fig. 3. Clones of complementary mating types of *Hansenula wingei* growing in close proximity. Cells of NRRL Y-2340 strain 5 and NRRL Y-2340 strain 21 (right) were initially separated by 22 μm. Arrows indicate mother cells. Bright field. 1000 ×.

K. lactis. Cells of opposite sex grew preferentially toward one another (Fig. 3). On the other hand, the response between *H. anomala* mating types (Fig. 4) resembled the pattern observed between *S. cerevisiae* mating types. Initially (Fig. 4a) cells of both sexes of *H. anomala* were about equal in size. After 4 hr (Fig. 4b) the cell of one mating type, Y-366-8, had enlarged about twofold while the other mating type, Y-2153-4, formed numerous buds; most of these were aligned toward the cell of the opposite sex. This pattern became more evident after another hour's growth (Fig. 4c). Heterothallic segregants from the diploid Y-366-8 X Y-2153-4 exhibited growth responses similar to the parent cells. Five spore isolates derived from the sporulated diploid Y-366-8 X Y-2153-4 were obtained by microdissection and their growth responses with Y-366-8 and Y-2153-4 were determined. Growth in close proximity to Y-2153-4 caused two segregants with mating type analogous to Y-366-8 to swell; three segregants with mating type similar to Y-2153-4 grew toward cells of Y-366-8 which, in turn, increased in size. The response in *H. anomala* is similar to that of *S. cere-*

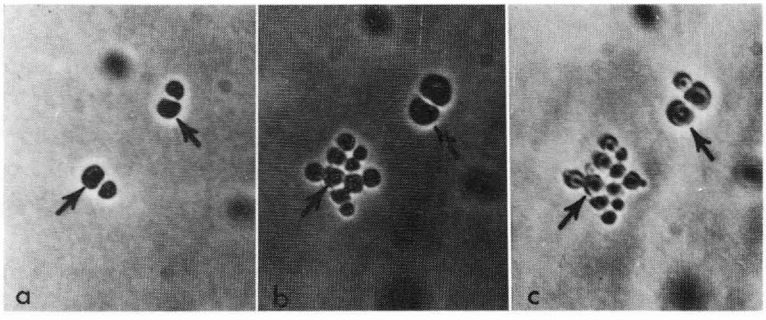

Fig. 4. Clones of complementary mating types of *Hansenula anomala* growing in close proximity. (a) Distance between cells of NRRL Y-366-8 (above) and NRRL Y-2153-4 (below) is 15 μm. (b) After 4 hr. (c) After 5 hr. Arrows indicate mother cells. Bright field. 1000 ×.

visiae. The cell of one mating type increased in size and produced few buds. The opposite sex formed numerous buds, most of which were directed toward the clone of the complementary gender.

Response between S. cerevisiae and H. anomala. Because *S. cerevisiae* and *H. anomala* showed a similarity in their sex-specific growth pattern, one wondered whether a cross-response would occur between cells of presumed complementary mating types from the two genera. Single cells of the *S. cerevisiae* stock Y-2047 that undergo enlargement were grown in proximity to single cells of the *H. anomala* stock Y-2153-4 that normally induce cell enlargement with its tester type (Fig. 5). Fig. 5d, the control, shows the pattern of growth that occurred between *S. cerevisiae* Y-2048 and *H. anomala* Y-2153-4, cells of presumably like mating type. An avoidance pattern was noted similar to that observed typically between cells of like sex. The growth response between presumed complementary cell types, Y-2047 and Y-2153-4, appears in Fig. 5a, b, c. Both cells grew preferentially toward one another. The response in *H. anomala* was more pronounced. Although *S. cerevisiae* cells enlarged somewhat, the swelling was not so marked as that elicited by the mate from its own species.

DISCUSSION

Sex-specific growth responses have been observed between opposite sexes of heterothallic yeasts from three independent genera, *Saccharomyces* (Yanagishima et al., 1968; Yanagishima, 1969; Duntze et al., 1970), *Kluyveromyces* (Herman, 1970*a*, *b*) and *Hansenula*. This kind of response may be common among heterothallic yeasts.

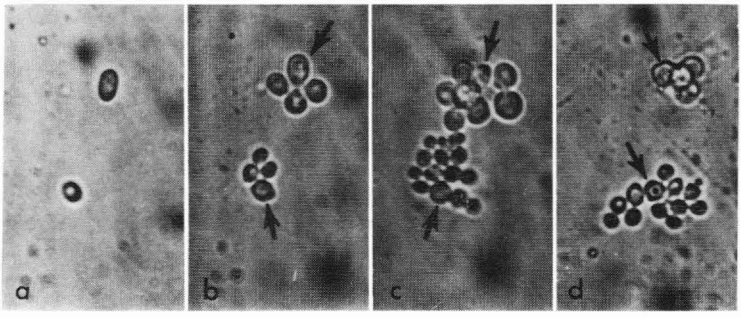

Fig. 5. Clones of *S. cerevisiae* and *H. anomala* growing in close proximity. (a) A cell of *S. cerevisiae* NRRL Y-2047 is above; *H. anomala* NRRL Y-2153-4 is below. Distance between cells is 15 μm. (b) After 5 hr. (c) After 7 hr. (d) Clones of *S. cerevisiae* NRRL Y-2048 (above) and *H. anomala* NRRL Y-2153-4 after 6 hr growth. Mother cells, indicated by arrows, were initially separated by 16 μm. Bright field. 1000×.

Two kinds of responses have been observed. The target cell may bud preferentially in a direction toward the secretor cell as in mating pairs of *K. lactis* and *H. wingei* or it may increase in size and form fewer buds as in *S. cerevisiae* and *H. anomala*. Because intergeneric responses were observed between mating types of two rather widely separated taxons, *Saccharomyces* and *Hansenula*, quite possibly the active constituents that are synthesized by yeast mating types possess broad specificity.

Testing for sex-specific growth responses could be used to identify analogous mating types in genera or species of yeast that usually do not interbreed. It might also be used as an adjunct in deciding which parental combinations would be most compatible for hybridizations between species that interbreed with a low frequency.

Received 23 *March* 1971

REFERENCES

BROCK, T. D. 1958. Mating reaction in the yeast *Hansenula wingei*. — J. Bacteriol. **75**: 697–701.

DUNTZE, W., MACKAY, V. and MANNEY, T. R. 1970. *Saccharomyces cerevisiae*: A diffusible sex factor. — Science **168**: 1472–1473.

HERMAN, A. 1970*a*. Sex-specific growth response in the yeast *Kluyveromyces lactis*. — Can. J. Genet. Cytol. **12**: 66–69.

HERMAN, A. 1970*b*. Interspecies sex-specific growth responses in *Kluyveromyces*. — Antonie van Leeuwenhoek **36**: 421–425.

TAYLOR, N. W. 1964. Inactivation of sexual agglutination in *Hansenula wingei* and *Saccharomyces kluyveri* by disulfide-cleaving agents. — J. Bacteriol. **88**: 929–936.

TAYLOR, N. W. and ORTON, W. L. 1968. Sexual agglutination in yeast. VII. Significance of the 1.7S component from reduced 5-agglutinin. — Arch. Biochem. Biophys. **126**: 912–921.

WICKERHAM, L. J. 1951. Taxonomy of yeasts. — U.S. Dep. Agr. Tech. Bull. No. 1029, Washington, D.C.

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

WICKERHAM, L. J. and BURTON, K. A. 1954. A simple technique for obtaining mating types in heterothallic diploid yeasts, with special reference to their uses in the genus *Hansenula*. — J. Bacteriol **67**: 303–308.

YANAGISHIMA, N. 1969. Sexual hormones in *Saccharomyces cerevisiae*. — Antonie van Leeuwenhoek **35**: Suppl.: Yeast Symposium 1969 C9-10.

YANAGISHIMA, N., SHIMODA, C., KUMANO, H., TAKAHASHI, T. and TAKAO, N. 1968. Hormonal regulation of mating reaction in yeast. — Proc. XII. Int. Congr. Genet. **1**: 65.