JOURNAL OF BACTERIOLOGY, Apr. 1979, p. 146-154
0021-9193/79/04-0146/09$02.00/0

Vol. 138, No. 1

Mating Pheromones of *Saccharomyces kluyveri*: Pheromone Interactions Between *Saccharomyces kluyveri* and *Saccharomyces cerevisiae*

JOHN McCULLOUGH AND IRA HERSKOWITZ*

Institute of Molecular Biology and Department of Biology, University of Oregon, Eugene, Oregon 97403

Received for publication 11 September 1978

Saccharomyces kluyveri is a heterothallic yeast with two allelic mating types denoted as **a**-k and α-k by analogy with *Saccharomyces cerevisiae* and from the work described here. *S. kluyveri* produces mating pheromones analogous to those of *S. cerevisiae*, but which appear to have different specificity. *S. kluyveri* thus differs from *S. cerevisiae*, *Hansenula wingei*, and *Schizosaccharomyces pombe* in that it exhibits both strong constitutive agglutination and mating pheromones. α-k cells produce a pheromone ("α-k-factor") which causes **a**-k cells to arrest in the G1 phase of the cell cycle and to undergo a morphological change. After a period of time dependent on the concentration of α-k-factor, cells exposed to the factor resume cell division. α-k-factor has no effect on **a**-k/α-k diploids or on α-k cells, but at high concentration does induce G1 arrest of *S. cerevisiae* **a** cells (**a**-c). **a**-k cells produce a pheromone ("**a**-k-factor") which causes α-k cells to exhibit a morphological change. In addition, **a**-k cells exhibit the Bar phenotype with respect to α-k-factor. Partially purified preparations of *S. cerevisiae* α-factor are more active in inducing G1 arrest of **a**-k cells than of **a**-c cells. A more purified preparation of α-c-factor is less active against **a**-k cells than **a**-c cells, suggesting that an additional factor (KRE, *kluyveri* response enhancer) may be lost during purification. Attempts to mate *S. kluyveri* and *S. cerevisiae* cells by prototroph selection and by cell-to-cell mating have been unsuccessful with all combinations of mating types. Thus, *S. cerevisiae* and *S. kluyveri* are incompatible for mating even though their pheromones exhibit some physiological cross-reaction.

The mating process of ascomycetes typically involves interactions between physiologically distinct types of cells. In yeasts such as *Hansenula wingei*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Saccharomyces kluyveri*, cells of opposite mating type mate efficiently with each other but not with cells of the same mating type. Cells of the two mating types are morphologically identical but differ physiologically in a number of respects (reviewed in reference 7). For example, cells of opposite mating type agglutinate strongly with each other (4, 22, 25).

S. cerevisiae, unlike *S. pombe* and *H. wingei*, produces diffusible mating type-specific pheromones which act on cells of opposite mating type (2, 7, 8, 15, 16, 26). The pheromone produced by α cells, α-factor, is an oligopeptide which exists in two forms, one with 12 L-amino acids and the other with 13 (24; see also reference 6). The factor produced by **a** cells, **a**-factor, has not been characterized as extensively, but appears to have a polypeptide component since its activity is lost upon treatment with trypsin (2, 26). These factors cause cells of opposite mating type to arrest as unbudded cells, in the G1 phase of the cell cycle, just before the initiation of DNA synthesis (2, 3, 12, 26). **a**/α cells, which mate with neither mating type, do not produce or respond to the factors. Because pheromone production and response is correlated with mating ability, it has been suggested that the mating factors are involved in the mating process (8). This correlation is extended by the observations that some nonmating mutants do not produce the mating factors (17) and that mutants selected as resistant to α-factor are defective in mating (19; L. Hartwell, personal communication). Since mating occurs between unbudded cells (11) and not when cells are arrested at other stages of the cell cycle (21), and since **a**- and α-factors induce G1 arrest, these factors may facilitate mating by synchronizing cell cycles prior to cell and nuclear fusion. Although the above observations are consistent with the factors being essential for mating, critical evidence—for example, existence of

nonmating mutants whose defect is correctable by addition of factors—is lacking (18).

To assess the generality of the existence of mating pheromones and to learn more about the role of mating pheromones in the mating process, we have begun a survey of ascomycetes other than *S. cerevisiae* for those which produce mating pheromones. In this paper we report our analysis of the heterothallic yeast, *S. kluyveri*, isolated from nature by Phaff et al. (20). Mating type specificity of this yeast is similar to that of *S. cerevisiae* in that hybrids formed between cells of complementary mating type mate with neither haploid type and readily sporulate when placed on acetate medium to produce four-spored asci with two spores of each mating type (1, 25).

Here we present evidence that *S. kluyveri* produces factors analogous to those of *S. cerevisiae* and that the α-factors from *S. kluyveri* and *S. cerevisiae* act on a cells of both yeasts. In addition, we present evidence for the existence of another factor secreted by *S. cerevisiae* α cells which may cause G1 arrest of *S. kluyveri* a cells or potentiate α-factor activity specifically against *S. kluyveri* a cells. Finally, we describe our unsuccessful attempts to produce hybrids between *S. kluyveri* and *S. cerevisiae* and discuss the use of pheromone response to assess relatedness of different yeasts.

MATERIALS AND METHODS

Strains. Strains are described in Table 1. X2180-G2c (a mating type) and X2180-1b (α mating type) are isogenic strains obtained in separate meioses from X2180, a diploid which arose spontaneously from a haploid strain (2; V. MacKay, personal communication).

Media. YEPD, SD (minimal medium), and nutritional supplements have been described previously (13). Other recipes are from Sherman et al. (23): Presporulation medium: 0.8% yeast extract, 0.3% peptone, 2% agar (Difco), and 10% dextrose. Sporulation medium: 1% potassium acetate, 0.1% yeast extract, 0.05% dextrose, and 2% agar. Citrate-buffered YEPD: YEPD medium in 0.1 M citrate buffer, pH 4.5 (10).

Isolation of *S. kluyveri* auxotrophs. Initial attempts to isolate auxotrophic mutants from strains 287 and 288 were unsuccessful. In addition to this failure, the observation that strains 287 and 288 were less sensitive than haploid *S. cerevisiae* strains to UV irradiation indicated that strains 287 and 288 might be homozygous diploids. The diploid nature of these strains was confirmed, and haploid strains were isolated by two successive cycles of mating and sporulation as follows: strains 287 and 288 were mated, and the resulting strain was induced to sporulate, producing meiotic products of two types—those which mated but did not sporulate and those which sporulated but did not mate. Sporulation of the latter type yielded only segregants with mating ability. One segregant from this second sporulation (strain 2a-2) was used for isolation of auxotrophic mutants by the following procedure: cells were grown in YEPD and UV irradiated to a survival of 10%. Auxotrophic mutants were identified by replica plating colonies from YEPD onto minimal medium and were obtained at a frequency of approximately 0.4%.

***S. kluyveri* genetic methods.** *S. kluyveri* diploids (or tetraploids) were formed by cell-to-cell mating or by prototroph selection. Sporulation was induced by growth first on presporulation medium at 30°C for 2 days, followed by growth on sporulation medium at 30°C for 2 to 4 days. Meiotic products were obtained after digestion with Glusulase (Endo Laboratories, New York). Since the spores adhered very strongly to each other in tetrads, separation of the spores was achieved only after allowing the spores to germinate on a YEPD agar slab for 2 h at 30°C. At this time individual spores which had not yet mated with other spores could be separated from other spores.

Purification of α-factor. The first step in the purification procedure is a modification (suggested by V. MacKay) of the first step used by Duntze et al. (9). α-c-Factor from supernatant fluids of cultures of α-c

TABLE 1. *S. cerevisiae and S. kluyveri strains*

Strain	Genotype	Source
S. cerevisiae		
X2180-G2c	a-c	V. MacKay
X2180-1b	α-c	V. MacKay
XT1172-S245c	α-c *ade6 his6 leu1 met1 trp5-1 gal2 can1*	V. MacKay
XT1177-S247	a-c *ade2-1 his2 lys1-1 trp5-18 gal2 can1*	V. MacKay
S. kluyveri		
287	a-k/a-k	Strain NRRL Y-4288-3 from C. L. Kurtzman
288	α-k/α-k	Strain NRRL Y-4288-26 from C. L. Kurtzman
289	a-k/a-k/α-k/α-k	287 mated with 288
2a-1	α-k	This work
2a-2	a-k	This work
XM12-4	α-k *thr⁻ arg⁻*	This work
XM12-17	a-k *thr⁻ arg⁻*	This work

strain X2180-1b or α-k strain 288 was bound to the weakly acidic cation exchange resin Bio Rex 70 (Bio-Rad Laboratories, Richmond, Calif.). The column was washed with 1:1 ethanol-water, followed by a wash with 0.5 M pyridine acetate buffer (pH 4.0). α-Factor was eluted with 3.5 M pyridine acetate buffer (pH 5.5). This eluate is preparation 1. α-c-Factor was further purified as follows: preparation 1 was lyophilized and redissolved in PA buffer (0.05 M pyridine acetate buffer, pH 2.4), and put on a 1- by 30-cm column of S.P. Sephadex C-25 (Sigma) which had previously been equilibrated with PA buffer. The column was sequentially washed with 100 ml of PA buffer and 100 ml of 0.28 M pyridine acetate buffer (pH 3.65). α-Factor was eluted with 100 ml of 0.5 M pyridine acetate buffer (pH 3.75). This material was lyophilized and suspended in 9:1 methanol-water, and insoluble material was removed by centrifugation. The supernatant was filtered through a Sephadex-LH20 (Sigma) column which had been equilibrated with 90% methanol. Ninety-two percent of the total activity was recovered. In agreement with the results of Duntze et al. (9), two peaks of α-factor activity eluted from the column. Only α-factor from the second peak (approximately 88% of total activity) was used in these studies and is referred to as preparation 2.

Assay of α-factor activity: (i) agar assay. A semiquantitative determination of α-factor activity was made using the serial dilution well assay on 4% YEPD agar slabs as described by Duntze et al. (9).

(ii) Liquid culture assay. Cells of **a** mating type were grown in citrate-buffered YEPD medium to exponential phase at 30°C. α-Factor was then added to the exponentially growing cells, and aliquots were removed from the cultures at various times. These aliquots were stored in 10% formaldehyde at 4°C until counted. Cell number was determined using a Petroff-Hauser counter after brief sonic disruption of the aliquots.

RESULTS

***S. kluyveri* mating factor analogous to *S. cerevisiae* α-factor.** The presence of an activity analogous to *S. cerevisiae* α-factor activity was detected in the cell-free supernatant from a culture of *S. kluyveri* strain 288 grown to stationary phase. A partially purified preparation of this activity was obtained from culture fluid by ion-exchange chromatography on Bio Rex 70 as described in Materials and Methods. This partially purified preparation caused transient arrest of exponentially growing *S. kluyveri* strain 287 cells as unbudded cells (Fig. 1 and Fig. 2B). The arrested cells apparently continued to synthesize protein, since they became enlarged and elongated into the "shmoo" form characteristic of *S. cerevisiae* **a** cells ("**a**-c cells") treated with *S. cerevisiae* α-factor ("α-c-factor") (Fig. 2A). The morphology of strain 287 cells in the absence of the strain 288 culture fluid preparation is shown in Fig. 2D. (The following abbreviations are used throughout this work: *S. cerevisiae* and *S. kluyveri* **a** and α mating types—**a**-c, α-c, **a**-k, and α-k, respectively; *S. cerevisiae* and *S. kluyveri* **a**-factor and α-factor—**a**-c-factor, α-c-factor, **a**-k-factor, α-k-factor, respectively.)

The length of the arrest period is proportional to the logarithm of the concentration of *S. kluyveri* material added (Fig. 1, inset). At the end of the arrest period, the strain 287 cells resumed budding at the same rate as the untreated controls. This response is identical to that obtained when **a**-c cells are treated with α-c factor (3, 5). The activity in the partially purified preparation acted specifically on cells of strain 287; strain 288 and hybrids formed between strains 287 and 288 were unaffected (Fig. 3). Because of these similarities to the α-factor of *S. cerevisiae*, we propose that the activity observed in culture fluids of strain 288 is due to production of a *S. kluyveri* α-factor (α-k-factor). By analogy with *S. cerevisiae*, we denote strain 288 as having α mating type (α-k) and strain 287 as having **a** mating type (**a**-k). Additional justification for these assignments is provided below.

Species specificity of α-k-factor. *S. cerevisiae* **a** strain X2180-G2c was unaffected by a concentration of α-k-factor which was capable of arresting *S. kluyveri* **a** cells (Fig. 4). At higher concentrations of α-k factor (25 times greater than required for response by **a**-k cells), X2180-G2c cells became arrested as unbudded cells and, after 90 min, elongated into the shmoo form characteristic of response to α-c-factor. *S. cerevisiae* α cells and **a**/α diploids did not respond (data not shown). The differential response of **a**-k and **a**-c cells to α-k-factor could also be seen by placing less than 25 units of α-k-factor in a well surrounded by **a**-k and **a**-c cells, in which case only the **a**-k cells responded (Fig. 4, inset). In summary, α-k-factor affected **a** strains of both species but was more effective against **a**-k strain 287 than **a**-c strain X2180-G2c.

Species specificity of α-c-factor: (i) assay on agar. *S. kluyveri* **a** cells were arrested and exhibited morphological changes in response to α-c-factor when assayed on agar (Fig. 2C). To evoke a response from **a**-k cells, the α-c-factor had to be concentrated fourfold over the most dilute preparation eliciting a response from X2180-G2c. Thus, both **a**-k and **a**-c cells responded to α-c-factor, but, by this assay, **a**-k strain 287 cells were fourfold less sensitive than cells of **a**-c strain X2180-G2c.

(ii) Assay in liquid. Figure 5A shows the effect of partially purified α-c-factor (preparation 1, see Materials and Methods) on exponentially growing cultures of **a**-c cells (X2180-G2c) and **a**-k cells (strain 287). The cells of both strains were arrested as unbudded cells for a period of time and then resumed dividing. Thus,

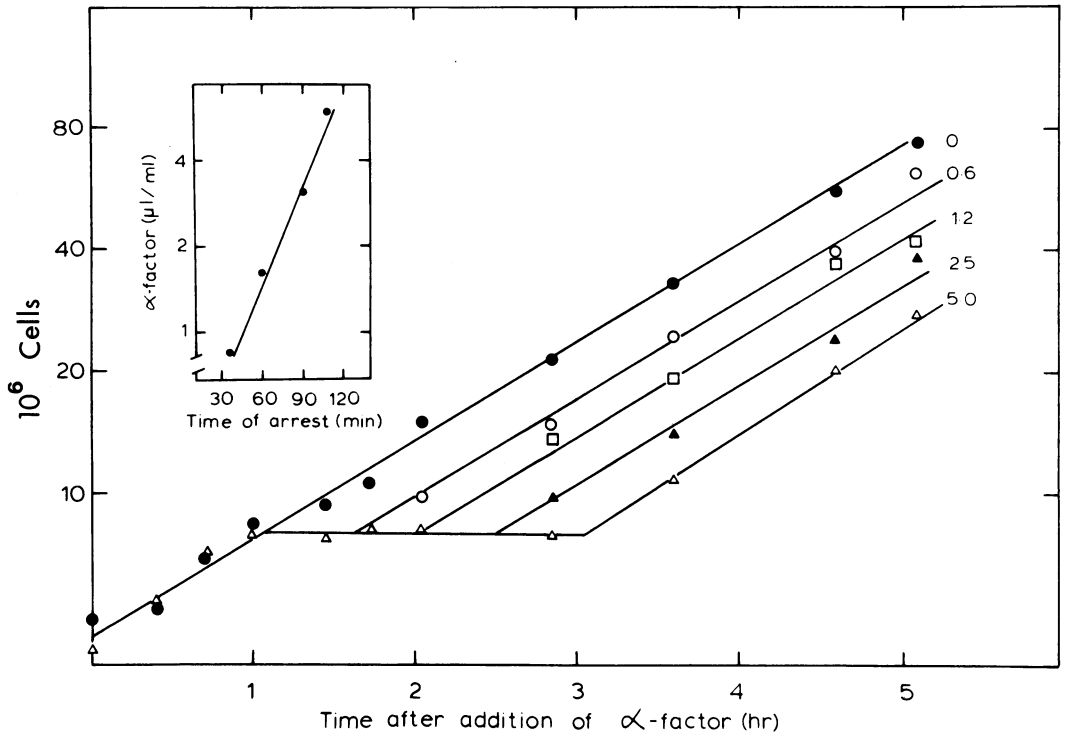

FIG. 1. *Response of S. kluyveri* **a** *cells to α-k-factor: To* **a***-k strain 287 cells growing exponentially in YEPD medium, partially purified α-k-factor (preparation 1 concentrated 500-fold by lyophilization) from a culture of strain 288 (α-k) was added at t = 0. The cultures were incubated aerobically at 30°C, and the cell number was monitored as described in Materials and Methods. 0, 0.6, 1.2, 2.5, and 5.0 indicate relative α-k-factor concentrations, in microliters of filtrate per milliliter of culture. The inset shows the length of the inhibition period as a function of the relative α-k-factor concentration.*

both strains responded to α-c-factor in a qualitatively similar fashion. Surprisingly, it was observed that more than eight times as much α-c-factor was required to arrest **a**-c cells for 2 h as was required to arrest **a**-k cells for the same length of time (see legend to Fig. 5). *S. kluyveri* **a** cells thus appear to be more sensitive to α-c-factor than *S. cerevisiae* **a** cells when assayed by this method. These results contrast with the results obtained using the well assay in which **a**-k cells appeared less sensitive to α-c-factor than **a**-c cells.

Detection of *S. cerevisiae* factor which affects *S. kluyveri*. One explanation for the disagreement between the two assay methods is that the partially purified α-c-factor preparation (preparation 1) contains an additional factor. This factor may act specifically on **a**-k cells either by itself or in concert with α-c-factor to cause G1 arrest. Alternatively, this factor may act specifically on **a**-c cells and be an inhibitor of response to α-c-factor. Such a factor would have to be too large to diffuse through 4% agar to account for failure to observe its activity in the well assay. This hypothesis was tested by further purifying α-c-factor (see Materials and Methods) and determining the relative effectiveness of this preparation (preparation 2) against **a**-k and **a**-c cells in liquid culture. The results shown in Fig. 5 support the hypothesis of an additional factor. Although **a**-k cells are about sixfold more sensitive than **a**-c cells to preparation 1 (Fig. 5A), **a**-k and **a**-c cells are equally sensitive to the more purified α-c-factor preparation 2 (Fig. 5B). Thus, the additional purification has removed something which either increases response by **a**-k cells or inhibits response of **a**-c cells to α-c-factor.

To distinguish between these possibilities, the following experiment was performed. The two preparations of α-c-factor were diluted to the same activity as determined by well assays against **a**-c cells. These preparations had a similar effect on **a**-c cells when assayed by the liquid method (Fig. 5C). In contrast, preparation 1 had much greater activity than the more purified preparation 2 when assayed against *S. kluyveri* **a** cells (Fig. 5D). These observations indicate

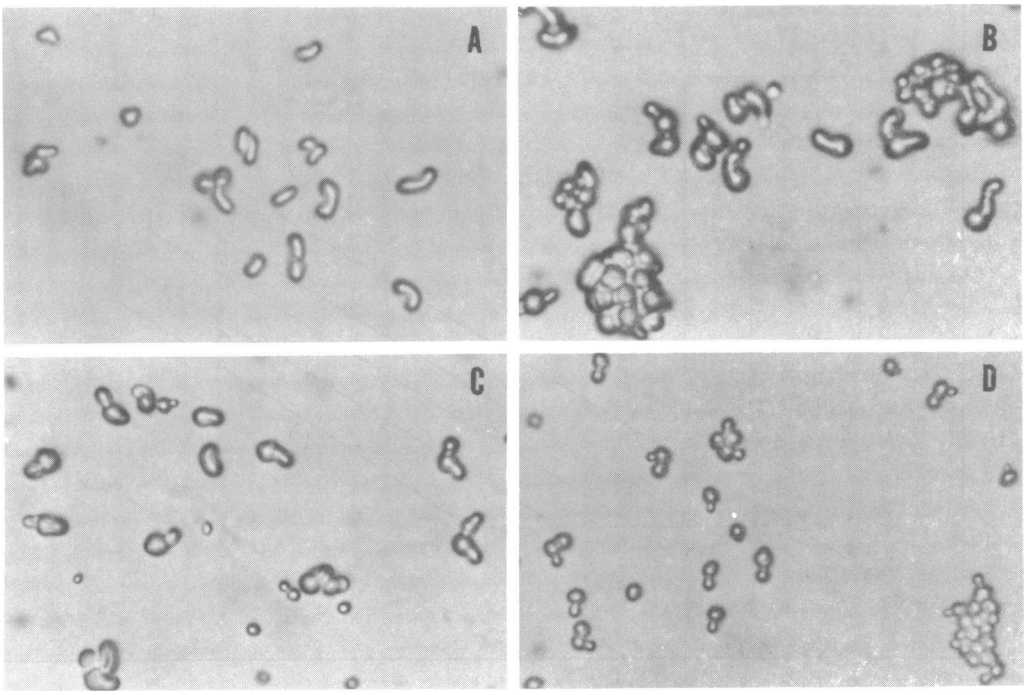

FIG. 2. *Response of S. kluyveri and S. cerevisiae* **a** *cells to α-k-factor and α-c-factor: S. kluyveri or S. cerevisiae* **a** *mating type cells were spread on an agar slab containing a well to which was added 5 μl of partially purified (preparation 1) α-c-factor or α-k-factor concentrated 100-fold from α culture fluids. The photographs were taken 4 h later. (A)* **a**-*c strain X2180-G2c treated with α-c-factor. (B)* **a**-*k strain 287 treated with α-k-factor. (C)* **a**-*k strain 287 treated with α-c-factor. (D)* **a**-*k strain 287 untreated.*

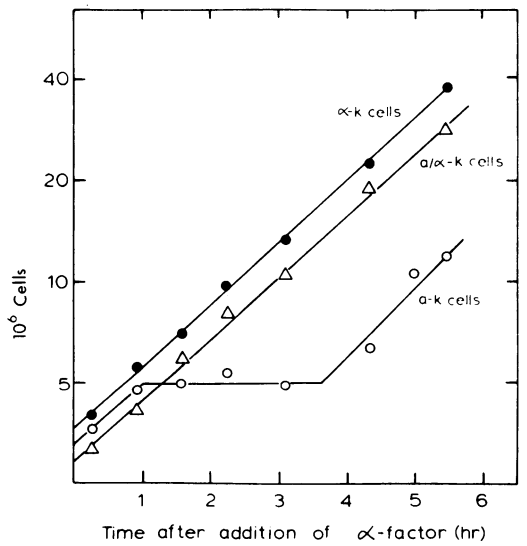

FIG. 3. *Mating type specificity of response to α-k-factor. Partially purified α-k-factor (as in Fig. 1) was added at t = 0 to cultures growing exponentially at 30°C in YEPD at a concentration of 5 μl/ml. Symbols:* ●, *288 (α-k);* △, *289 (***a**-*k/α-k);* ○, *287 (***a**-*k).*

that the factor acts specifically on **a**-k cells, perhaps as a positive effector of α-c-factor or as an independent G1 arrest factor.

Although, as shown above, further purification of the α-c-factor preparation leads to some loss of activity against **a**-k cells relative to **a**-c cells, preparation 2 is at least as active on **a**-k cells as on **a**-c cells. This result contrasts with the agar assay, in which **a**-k cells are less sensitive than **a**-c cells to α-c-factor. One explanation for this disparity is that the factor which enhances **a**-k response to α-c-factor (whose action is not seen in the agar assay) has not been completely removed from preparation 2.

***S. kluyveri* a cells have mating-specific functions analogous to *S. cerevisiae* a-factor and Bar: (i) a-factor.** An activity (**a**-k-factor) analogous to *S. cerevisiae* **a**-factor was detected using a confrontation assay. A dense streak of *S. kluyveri* **a** strain 287 was placed on an agar slab and incubated at 30°C for 24 h. α-k Cells (strain 288), placed close to the streak of **a**-k cells by micromanipulation, became arrested as unbudded cells and elongated to the form characteristic of α-c cells exposed to **a**-c factor (Fig. 6).

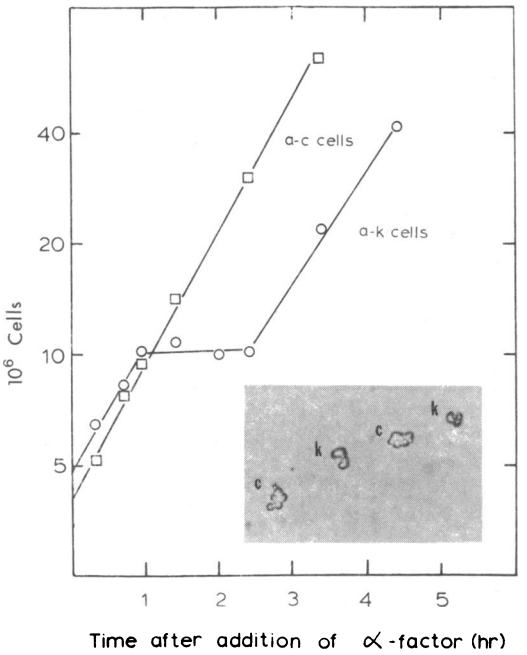

FIG. 4. *Species specificity of α-k-factor. Cultures growing exponentially in YEPD were treated at 30°C with 5 μl of partially purified α-k-factor per ml as in Fig. 1. Symbols: ○, 287 (**a**-k); □, X2180-G2c (**a**-c). The inset shows an agar slab with cells of **a**-k strain 287 (labeled k) and **a**-c strain X2180-G2c (labeled c) placed around a well containing 5 μl of partially purified α-k-factor concentrated fivefold.*

Both **a**-c-factor and **a**-k-factor are species specific: *S. cerevisiae* α cells were unaffected when placed close to an *S. kluyveri* **a** cell streak, and *S. kluyveri* α cells were unaffected when placed close to an *S. cerevisiae* **a** cell streak (data not shown). Whether cross-reactions similar to those described for the α-factors are observed at higher concentrations of the **a**-factors has not been tested.

(ii) Bar. Studies with *S. cerevisiae* have shown that when a dense streak of **a** cells is placed between an α cell streak and **a** indicator cells in a confrontation assay, the **a** indicator cells do not exhibit a response to α-factor (14). The ability of interposed cells to block α-factor activity, the Bar^+ phenotype, is controlled by the mating type locus—**a**/α cells are Bar^-, **a** cells are Bar^+. Bar^+ behavior may be due to binding of α-factor by **a** cells and/or secretion of a diffusible inhibitor of α-factor by the **a** cells (5, 14). In a confrontation assay with α-k cells providing α-factor and **a**-k cells as indicator cells, interposed **a**-k cells but not **a**-k/α-k cells blocked α-factor response. *S. kluyveri* **a** cells thus are Bar^+. Interposed *S. cerevisiae* **a** cells (strain X2180-G2c) did not inhibit the response of **a**-k cells to α-k-factor in a confrontation test, and thus are Bar^- with respect to α-k-factor. In a confrontation test between α-c and **a**-c cells, interposed **a**-k cells (strain 287) at least partially inhibited the action of α-c-factor (data not shown).

***S. cerevisiae* and *S. kluyveri* are noninterbreeding species.** We have utilized two methods, cell-to-cell mating and prototroph selection, in attempting to form hybrids between *S. cerevisiae* and *S. kluyveri*.

S. cerevisiae and *S. kluyveri* cells were placed together on an agar slab by micromanipulation and examined periodically for formation of zygotes. Of 35 cell pairs for each case, efficient zygote formation was observed between **a**-k and α-k cells (28/35) and between **a**-c and α-c cells (31/35), but not between **a**-c and α-k or **a**-k cells or between α-c and **a**-k or α-k cells.

To utilize the prototroph selection method, haploid *S. kluyveri* strains with two auxotrophic mutations were constructed (see Materials and Methods): strains XM12-4 and XM12-17 have α-k and **a**-k mating types, respectively, and carry the same *thr* and *arg* mutations. Cells (10^7) of each *S. kluyveri* strain were mixed with 10^7 cells of multiply-auxotrophic *S. cerevisiae* α and **a** strains (XT1172-S245c and XT1177-S247) and allowed to grow on rich medium (YEPD) for 2 days at 30°C. Approximately 10^{10} cells were then transferred to minimal medium agar (SD), and the plates were incubated for several days at 30°C. In none of the mixtures, **a**-k with α-c, **a**-k with **a**-c, α-k with **a**-c, or α-k with α-c, were prototrophic diploids observed. To test the possibility that mating did not occur only because of differences between these yeasts' mating pheromones, all four strains were allowed to grow on the same rich plate or in the same rich liquid culture before selection on minimal medium. In neither case were prototrophic diploids obtained.

DISCUSSION

Genetic control of mating in *S. kluyveri* is similar to that in *S. cerevisiae* as follows. (i) Mating type is determined by a pair of alleles at a single locus. (ii) Cells of opposite mating type agglutinate with each other. (iii) Diploids or tetraploids formed between cells of opposite mating type are capable of sporulation but not mating. Our results show that similarities extend also to the mating pheromone systems.

(i) Cells of each mating type secrete diffusible agents which act specifically on cells of the opposite mating type. Diploids and tetraploids formed between cells of opposite mating type

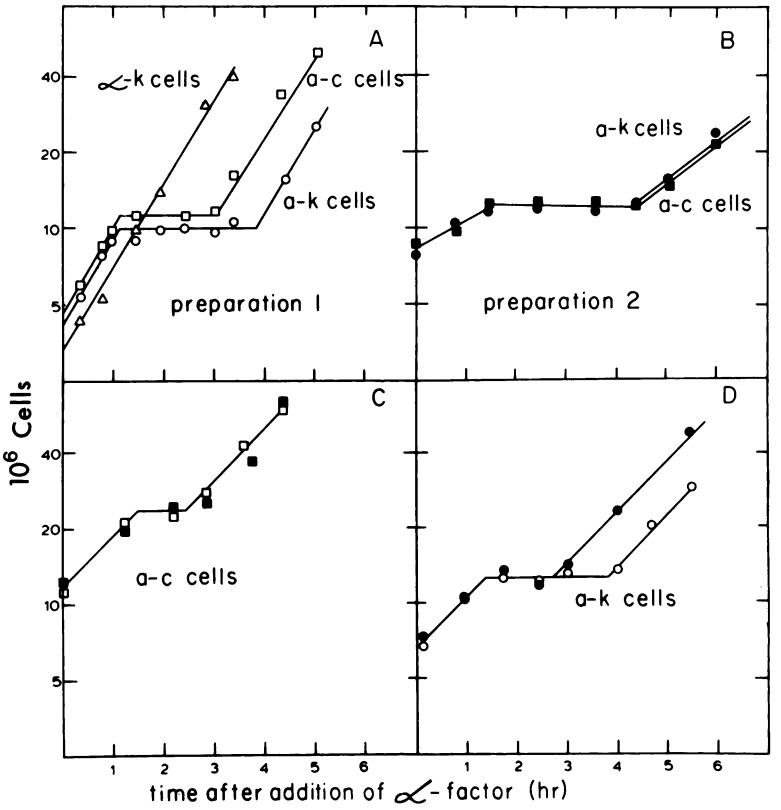

FIG. 5. *Species specificity of α-c-factor. Cells growing exponentially in YEPD (A) or citrate-buffered YEPD (B, C, and D) were treated with α-c-factor preparation 1 or 2 as in Fig. 1. Open symbols: cultures treated with α-c-factor preparation 1; closed symbols: cultures treated with α-c-factor preparation 2. Symbols: △, 288 (α-k); □, ■, X2180-G2c (**a**-c); ○, ●, 287 (**a**-k). (A) Cultures of 288 (α-k) and X2180-G2c (**a**-c) contained 10 μl of α-c-factor (100-fold-concentrated preparation 1) per ml of culture. The 287 (**a**-k) culture contained 1.25 μl of the same α-c-factor preparation per ml of culture. (B, C, and D) Cultures contained 7.5 μl of α-c-factor (200-fold-concentrated preparation 1 or preparation 2) per ml of culture.*

neither produce nor respond to these pheromones.

(ii) The pheromones cause sensitive cells to arrest as unbudded cells in the G1 phase of the cell cycle and to undergo a characteristic morphological change.

(iii) Arrest by α-k-factor is reversible. After a period of arrest dependent on the concentration of α-k-factor added, **a**-k cells resume growth at the same rate as in the absence of α-k-factor. It is not yet known whether α-k-factor activity is absent from the medium of these cultures, as is the case for cultures of **a**-c cells which have recovered from arrest by α-c-factor (5).

(iv) The response by cells of one mating type to the pheromone produced by cells of the opposite mating type is not symmetrical; in confrontation assays, **a** cells exhibit a greater response (a higher fraction of cells which remain arrested for a longer period of time) to α cells than α cells exhibit when confronted with a dense streak of **a** cells. Whether this difference is due to differences in the pheromones per se (e.g., in the diffusibility, specific activity, stability, or amount of the pheromone) or in response to the pheromones is not known.

(v) α-Factor is produced constitutively by α-k and α-c cells: pure cultures of α cells contain α-factor activity.

(vi) **a**-k Cells but not **a**-k/α-k cells have the Bar^+ phenotype, i.e., they act as a barrier to α-k-factor in confrontation tests.

The α-factors of *S. kluyveri* and *S. cerevisiae* elicit a response in the **a** cells of both species. The response to α-factor appears to be species specific, however, since higher concentrations of α-k-factor are required to observe a response by **a**-c cells than by **a**-k cells. Similarly, higher concentrations of α-c-factor are required for response by **a**-k cells than by **a**-c cells. The obser-

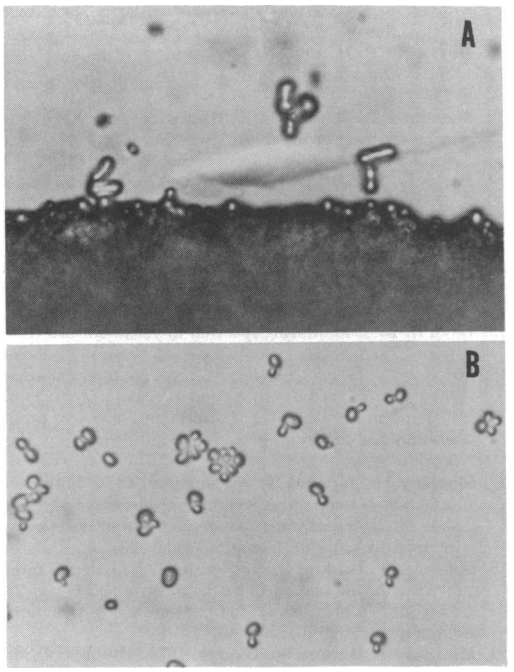

FIG. 6. *Response of α-k cells to* **a***-k-factor. (A) A dense streak of* **a***-k strain 287 cells was placed on a YEPD agar slab incubated at 30°C for 18 h. α-k strain 288 cells were then placed close to the streak by micromanipulation. The agar slab was incubated at 30°C for another 4 h at which time the photograph was taken. (B) α-k strain 288 cells in the absence of* **a***-k cells.*

vation that α-k-factor is less soluble in methanol than α-c-factor (unpublished observations) indicates that the two factors differ from each other. The species specificity of α-factor response thus may be due at least in part to a difference in α-factor itself.

No cross-reactivity of **a**-factor activities has been observed, but higher concentrations of **a**-factor have not yet been tested. The observation that **a**-k cells act as a partial barrier to α-c-factor provides a further indication that α-c-factor can act on **a**-k cells. The inability of **a**-c cells to act as a barrier to α-k-factor may be another reflection of the relatively greater response by **a**-k cells to α-c-factor than by **a**-c cells to α-k-factor.

The response of *S. kluyveri* **a** cells to α-c-factor depends on the method used to assay response and the source of α-c-factor. Using a partially purified preparation of α-c-factor, **a**-k cells were fourfold less sensitive than **a**-c cells when assayed on agar, but at least eightfold more sensitive than **a**-c cells when assayed in liquid. Further purification resulted in an α-c-factor preparation to which **a**-k cells were less sensitive than **a**-c cells by both methods of assay.

We propose that the differences in assay methods and α-c-factor preparations are due to a factor present in crude α-c-factor preparations which diffuses poorly in 4% agar. This factor, which we call KRE ("*kluyveri* response enhancer"), is mating type and species specific; that is, it acts only on *S. kluyveri* **a** cells, and not on *S. kluyveri* α or **a**/α cells or on *S. cerevisiae* **a** or α cells. The observation that KRE action is mating type specific is evidence that it is not a growth inhibitor acting specifically on *S. kluyveri* cells. KRE does not appear to be an inhibitor of α-c-factor activity against **a**-c cells because removal of KRE from α-c-factor preparations reduces α-factor activity specifically against **a**-k cells and not **a**-c cells. The action and specificity of KRE may be explained by the following hypotheses. (i) KRE may act on both *S. kluyveri* and *S. cerevisiae* cells but is rapidly degraded by *S. cerevisiae* cells. (ii) KRE may be an altered form of α-c-factor, for example, a precursor, to which only *S. kluyveri* cells are sensitive. (iii) KRE may itself cause G1 arrest of **a**-k cells or may potentiate α-c-factor activity against **a**-k cells. These possibilities are under investigation.

We have been unable to isolate hybrids between *S. kluyveri* and *S. cerevisiae*, even though selective methods were used which should have detected hybridization at a frequency of 10^{-10}. This result indicates that the level of response between the pheromone systems is not sufficient for mating. Whether failure to observe mating is due to differences in the pheromone systems or, for example, in the cell surfaces (as indicated by the failure to observe agglutination) is not known. The observations of Barker and Miller (1) on hybridization between isolates of an *S. kluyveri*-like species from the Pacific Coast and *S. cerevisiae* or *S. kluyveri* are interesting in light of our results. The Pacific Coast isolates, which differ from *S. kluyveri* in carrying a dominant gene for cellobiose utilization, agglutinate and mate readily with *S. kluyveri* to produce diploids capable of sporulation. The Pacific Coast isolates also mate with *S. cerevisiae* strains, although agglutination was not evident in any combination of mating types of the two yeasts. These hybrids sporulate inefficiently, giving rise to "a few unhealthy appearing asci and spores". The fact that the Pacific Coast *S. kluyveri*-like isolates mate with both *S. cerevisiae* and *S. kluyveri*, but that *S. cerevisiae* and *S. kluyveri* do not mate, indicates that the Pacific Coast isolate is more closely related to *S. cerevisiae* than is the *S. kluyveri* strain used in our work. It shall be interesting to determine the reactions of these three yeasts to each other's pheromones.

In conclusion, we have observed that *S. kluyveri* has a pheromone system analogous to that of *S. cerevisiae*. Although the α-factors of both yeasts act on a cells of both types of yeast, hybridization does not occur. We suggest that assaying response of one yeast to the pheromones of another may be generally useful in determining the relatedness of yeasts which do not hybridize.

ACKNOWLEDGMENTS

We thank C. P. Kurtzman for generously supplying us with *S. kluyveri* and other yeast strains; Steve Reed, Russell Chan, and Vivian MacKay for suggestions on α-factor purification; and members of our laboratory for discussion.

This work was supported by an American Cancer Society Postdoctoral Fellowship (PF1322) to J. M. and by a Public Health Service Research Grant (AI-13462) and Research Career Development Award (AI-00163) to I. H. from the National Institutes of Health.

LITERATURE CITED

1. **Barker, E. R., and M. W. Miller.** 1969. Some properties of *Saccharomyces kluyveri*. Antonie van Leewenhoek J. Microbiol. Serol. **35**:159–171.
2. **Betz, R., V. L. MacKay, and W. Duntze.** 1977. a-Factor from *Saccharomyces cerevisiae*: partial characterization of a mating hormone produced by cells of mating type a. J. Bacteriol. **132**:462–472.
3. **Bücking-Throm, E., W. Duntze, L. H. Hartwell, and T. R. Manney.** 1973. Reversible arrest of haploid cells at the initiation of DNA synthesis by a diffusible sex factor. Exp. Cell Res. **76**:99–110.
4. **Calleja, G. B., and B. F. Johnson.** 1971. Flocculation in a fission yeast: an initial step in the conjugation process. Can. J. Microbiol. **17**:1175–1177.
5. **Chan, R. K.** 1977. Recovery of *Saccharomyces cerevisiae* mating-type a cells from G1 arrest by α factor. J. Bacteriol. **130**:766–774.
6. **Ciejek, E., J. Thorner, and M. Geier.** 1977. Solid phase peptide synthesis of α-factor, a yeast mating pheromone. Biochem. Biophys. Res. Commun. **78**:952–961.
7. **Crandall, M., R. Egel, and V. L. MacKay.** 1977. Physiology of mating in three yeasts. Adv. Microbial Physiol. **15**:307–398.
8. **Duntze, W., V. L. MacKay, and T. R. Manney.** 1970. *Saccharomyces cerevisiae*: a diffusible sex factor. Science **168**:1472–1473.
9. **Duntze, W., D. Stötzler, E. Bücking-Throm, and S. Kalbitzer.** 1973. Purification and partial characterization of α-factor, a mating-type-specific inhibitor of cell reproduction from *Saccharomyces cerevisiae*. Eur. J. Biochem. **35**:357–365.
10. **Fink, G. R., and C. A. Styles.** 1972. Curing of a killer factor in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. U.S.A. **69**:2846–2849.
11. **Hartwell, L. H.** 1973. Synchronization of haploid yeast cell cycles, a prelude to conjugation. Exp. Cell Res. **76**: 111–117.
12. **Hereford, L. M., and L. H. Hartwell.** 1974. Sequential gene function in the initiation of *Saccharomyces cerevisiae* DNA synthesis. J. Mol. Biol. **84**:445–461.
13. **Hicks, J. B., and I. Herskowitz.** 1976. Interconversion of yeast mating types. I. Direct observations of the action of the homothallism (*HO*) gene. Genetics **83**: 245–258.
14. **Hicks, J. B., and I. Herskowitz.** 1976. Evidence for a new diffusible element of mating pheromones in yeast. Nature (London) **260**:246–248.
15. **Levi, J. D.** 1956. Mating reaction in yeast. Nature (London) **177**:753–754.
16. **MacKay, V. L.** 1978. Mating-type specific pheromones as mediators of sexual conjugation in yeast, p. 243–259. *In* J. Papaconstantinou (ed.), Molecular control of proliferation and differentiation. Academic Press Inc., New York.
17. **MacKay, V. L., and T. R. Manney.** 1974. Mutations affecting sexual conjugation in *Saccharomyces cerevisiae*. I. Isolation and phenotypic characterization of nonmating mutants. Genetics **76**:255–271.
18. **Manney, T. R., and J. H. Meade.** 1977. Cell-cell interactions during mating in *Saccharomyces cerevisiae*, p. 281–321. *In* J. Reissig (ed.), Microbial interactions. Chapman and Hall, London.
19. **Manney, T. R., and V. Woods.** 1976. Mutants of *Saccharomyces cerevisiae* resistant to the α mating-type factor. Genetics **82**:639–644.
20. **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 Leewenhoek J. Microbiol. Serol. **22**:145–161.
21. **Reid, B. J., and L. H. Hartwell.** 1977. Regulation of mating in the cell cycle of *Saccharomyces cerevisiae*. J. Cell Biol. **75**:355–365.
22. **Sakai, K., and N. Yanagishima.** 1972. Mating reaction in *Saccharomyces cerevisiae*. II. Hormonal regulation of agglutinability of a type cells. Arch. Mikrobiol. **84**: 191–198.
23. **Sherman, F., G. R. Fink, H. B. Lukins.** 1970. Methods in yeast genetics. A laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
24. **Stötzler, D., and W. Duntze.** 1976. Isolation and characterization of four related peptides exhibiting α-factor activity from *Saccharomyces cerevisiae*. Eur. J. Biochem. **65**:257–262.
25. **Wickerham, L. J.** 1958. Sexual agglutination of heterothallic yeasts in diverse taxonomic areas. Science **128**: 1504–1505.
26. **Wilkinson, L. E., and J. R. Pringle.** 1974. Transient G1 arrest of *S. cerevisiae* cells of mating type alpha by a factor produced by cells of mating type a. Exp. Cell Res. **89**:175–187.