# THE YEAST ASSOCIATED WITH SOME *DROSOPHILA* BREEDING SUBSTRATES IN THE TUCSON, ARIZONA, AREA

by

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ABSTRACT

Little overlap (13%) of the 38 yeast species isolated was observed among five different Drosophila breeding substrates and four species of Drosophila. Pichia membranaefaciens was the only species present in high concentrations in more than one substrate.

The most common of the six yeast species in the necrotic oranges and associated D. arizonensis, D. pseudoobscura, and D. melanogaster were Kloeckera apiculata, P. fermentans, and P. kluyveri. The oak and sycamore slime fluxes contained 14 species of yeast all having low frequencies of isolation (less than 33%) which resulted in this substrate having the highest yeast diversity of all sources. The most common of the 14 yeast species in the mesquite slime fluxes and associated D. carbonaria were Cryptococcus albidus var. diffluens, P. membranaefaciens, Torulopsis sp., and Candida sp. The necrotic Opuntia contained four species of yeast, P. membranaefaciens, Cr. cereanus, Pichia sp., and T. sonorensis, which although present in high concentrations had low frequencies of isolation (less than 38%).

Although the yeast flora of the flies did not differ significantly from the yeast flora of their substrates, those yeast species exhibiting pseudomycelium and pellicle formation were found to be in a higher relative frequency in the fly than in the substrate.

The physiological factors affecting yeast distribution are discussed.

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# INTRODUCTION

The importance of yeast in the nutrition of Drosophila was first noted by Delcourt and Guyenot (1910) and later by Lobe and Northrop (1916); they observed that Drosophila, when raised under aseptic conditions, grows more normally on fruit or potato when yeast is present. Northrop (1917) found that yeast alone was a sufficient diet for complete development of D. melanogaster.

Baumberger (1919) noted that phytophagous insects that are amply supplied with carbohydrates have difficulties in obtaining protein and that many insects, e.g., Drosophila, that feed in decaying or fermenting vegetable matter of low protein content have an unusually short period of growth. Baumberger (1919, p. 43) concluded from his laboratory experiments that "the function of yeast in the ecology of the insect is to concentrate on the surface of the medium and to synthesize into nucleoprotein, the urates, ammonia, or amino acids of the substratum."

In 1944 Dobzhansky and Epling in search of the natural food of D. pseudoobscura and D. persimilis found similar bacterial and yeast forms in crops of adult Drosophila and also in moist decaying areas on and within oaks. Wagner (1944) also suspected that the requirement for yeast may be important in nature as well as in the laboratory and demonstrated that yeast from Opuntia fruits have different nutritional qualities for D. mulleri and D. aldrichi which breed in Opuntia. Wagner

suggested that nutrition may be an isolating mechanism among populations of Drosophila in one part of a common range. In 1949 Wagner showed that these yeasts have different nutritional qualities for three other members of the mulleri group, D. mojavensis, D. arizonensis, and D. buzzatii.

As a result of the limited amount of information pertaining to the taxonomy of yeast occurring in Drosophila, Shehata, Mrak and Phaff (1955) surveyed yeast found in Drosophila and suspected feeding places in south and central California, and Phaff et al. (1956) isolated the yeast found in the alimentary canal of Drosophila in the Yosemite region of California. In 1956, Carson, Knapp and Phaff surveyed the yeast flora of some natural breeding sites of Drosophila in the same areas and concluded that the known breeding sites of temperate Drosophila are not important as sources of food for adult flies. Because certain yeasts are common in several species of flies (Phaff et al. 1956), Carson et al. (1956) suggested that most species of temperate Drosophila share common feeding sites that are probably small and ephemeral. In 1957, da Cunha, Shehata and de Oliveira studied the diets and nutritional preferences of tropical Drosophila and demonstrated that the yeast in the adults are very similar to the yeasts in the feeding substrates although the differentiation of food preferences among species of tropical Drosophila is greater than that found among temperate Drosophila. Their explanation was that in temperate regions the variety of breeding places are few and, therefore, divergence of food preference may not be advantageous.

Cooper (1959) reviewed all studies on food preferences of Drosophila and concluded that different yeast species are unequal in

attractiveness to different species of Drosophila and that different species of yeast are not equivalent in supporting the growth of Drosophila larvae. In the same paper he showed that, although larvae and adults of every species studied prefer certain yeast to others, the preferences which the adults exhibit are not identical with those of the larvae of the same species.

Recently, Starmer et al. (in press) have reported the results of a survey of yeasts associated with cactiphilic Drosophila (Fellows and Heed 1972) and their feeding sites in the Sonoran Desert, and Heed et al. (1976) have compared these results with those of the temperate and tropical regions. The predominent yeast species in all cactiphilic Drosophila and their breeding sites is Pichia membranaefaciens (68 percent of all isolates), and Heed et al. (1976, p. 158) presume, therefore, "that this yeast is not a causal factor leading to strict host plant selection" by the species of cactiphilic Drosophila, but may be "one of the causal factors that has led to spatial isolation of the larvae among the species to avoid competition for a common resource." Also, the prevalence of closely related species in the genus Pichia, with P. membranaefaciens being most frequent, associated with Drosophila from the desert, temperate, and tropical regions suggests that the genus Pichia may have co-evolved with Drosophila.

The present study was undertaken to survey the yeast flora of four potential Drosophila breeding substrates and adult Drosophila that feed upon these substrates. These substrates include slime fluxes of Prosopis juliflora, mesquite, on which Drosophila carbonaria breeds

exclusively (Patterson and Stone 1952); necrotic stems of Opuntia; slime fluxes of Quercus emoryi, emory oak; and necrotic fruits of Citrus sinensis, oranges, in which D. pseudoobscura, D. arizonensis, and D. melanogaster breed.

## MATERIALS AND METHODS

The yeast flora of five different Drosophila breeding substrates and of the adult Drosophila that were found on each sampled substrate were studied. Samples of eight rotting platyopuntias, Opuntia phaeacantha var. discata (Griff.) Benson and Walkington, were collected at Ironwood Picnic Grounds (approx. elev. 2,500 feet) in the Tucson Mountains west of Tucson, Arizona, on February 28, 1975. Samples of eight rotting oranges, Citrus sinensis (L.) Osbeck, and of 37 D. pseudoobscura Frolowa 1929, 62 D. arizonensis Patterson and Wheeler 1942, and 11 D. melanogaster Meigen 1830 were collected at a citrus grove, Tucson, Arizona, on March 1, 1975. Samples of slime fluxes from 18 oaks, Quercus emoryi Torr. 1848, one sycamore, Platanus wrightii S. Wats. 1875, and 11 mesquite trees, Prosopis juliflora var. velutina (Woot.) Sarg. 1902, and of 17 D. carboneria Patterson and Wheeler 1942 were collected on two trips, May 4 and May 20, 1975, to Happy Valley (approx. elev. 4,000 feet) located east of the Rincon Mountains which lie east of Tucson, Arizona. The general appearance, pH, and temperature of the substrates were recorded in the field at the time of sampling. The platyopuntias and oranges sampled were brought into the lab and the number of each species of Drosophila that eclosed was recorded.

All necrotic tissues were sampled by placing approximately 1 g of tissue into a screw cap tube containing 10 ml of sterile distilled

water and then serially diluting and plating the $10^2$, $10^4$, and $10^6$X dilutions on AYM, yeast extract-malt extract agar (Difco) acidified with 7 ml of 1.0 N HCl per liter (final pH of 3.7-3.9). Some of the oak samples were also streaked directly on to AYM. Adult Drosophila were surface sterilized by washing in 70% ethanol for one minute, rinsed in sterile distilled water, placed on AYM, crushed well with sterile forceps and dissecting needles so as to rupture the crop, and streaked. All samples were plated within 1-2 hrs after collecting.

The plated samples were observed for yeast colonies three to five days after incubation at 25-27°C. For every sample, the number, dilution, and description of each different type of yeast colony were recorded, and a representative of each colony type was brought into pure culture. If two different colonies from the same sample were identified as the same species, they were considered as a single isolate.

In cases where numerous samples from the same source repeatedly gave several identical colony types, only isolates representing each colony type from a few of the samples were identified in order to decrease repetition without sacrificing a significant amount of accuracy. It was found that the physiological properties of all isolates representing a particular mature colony type, i.e., one that was two to three weeks old, were identical. Following identification of all representative isolates, the mature colonies of all other isolates were observed, assigned colony types, and thereby identified. This procedure was used for the oranges and associated flies and for the mesquite and associated flies.

In order to identify the yeast isolates, the procedures of Van der Walt (1970) were used to observe the following characteristics: morphological characteristics of vegetative reproduction and vegetative cell shape; cultural characteristics of growth on liquid and solid media; characteristics of spores produced on vegetable juice agar, Gorodkowa agar, Kleyn's acetate agar, and yeast extract glucose agar; and physiological characteristics which included the utilization of 28 carbon compounds (see Appendix A) and nitrate, growth at 37°C, and fermentation of glucose.

The method for determining the average relative frequency of major yeasts (see Tables 3 and 6 in Results section) is that for every sample the relative frequency of each common yeast species present was calculated by dividing its concentration by the total concentration of all common yeast species. Then relative frequencies of each yeast species of all samples from the particular source was averaged to arrive at the average relative frequency. Since the flies were not diluted, each colony type was enumerated by assigning it one of the following three categories: abundant, medium, and infrequent indicating approximately 1000, 100, and 10 colonies, respectively. These approximate numbers were used in place of the concentrations in the above calculations when determining the average relative frequency of yeast species in Drosophila.

The measure of yeast diversity was calculated by the method described by Pielou (1972).

## RESULTS

The yeast flora of each of the four substrates sampled is presented first and is followed by the over-all yeast distribution and diversity and the morphological and physiological characteristics of the yeast. The taxonomic designation and total number of isolates of each yeast species isolated can be found in Appendix A.

### *Citrus* and Associated *Drosophila*

Three of the four yeast species found in *Citrus* (Table 1) were isolated from at least 75% of the samples. The predominant yeast was *Kloeckera apiculata* since it was isolated from all of the citrus samples and was present in the highest concentration, approximately $10^8$ cells per cc. *Pichia kluyveri* and *P. fermentans*, the second and third most common yeast, were found in 88% and 75% of the orange samples, respectively, and were both found in concentrations of about $10^7$ cells per cc. *Torulopsis stellata* was represented by only one isolate but may be more common because after three days of incubation on AYM isolation plates *Kl. apiculata* and *T. stellata* colonies were morphologically indistinguishable. Nevertheless, only 6 out of 72 isolates of this colony type from both citrus and associated flies were *T. stellata*, so that *T. stellata* must represent only a very small part of the yeast present. The average number of species of yeast per fruit was 2.75 (Table 2).

Table 1. The number of isolates of yeast from *Citrus* and associated adult *Drosophila*, and the concentrations of isolates from *Citrus*.

| Source | *Citrus* | *D. arizonensis* | *D. pseudoobscura* | *D. melanogaster* | Total |
|---|---|---|---|---|---|
| Number of Samples: | 8 | 35 | 23 | 5 | 71 |
| *Kl. apiculata* | 8 (5-9)* | 33 | 20 | 5 | 66 |
| *P. fermentans* | 6 (5-8) | 33 | 21 | 5 | 65 |
| *P. kluyveri* | 7 (4-8) | 24 | 11 | 1 | 43 |
| *T. stellata* | 1 | 2 | 3 | | 6 |
| *C. vini* | | | 1 | | 1 |
| *P. membranaefaciens* | | 1 | | | 1 |
| Total: | 22 | 93 | 56 | 11 | 182 |

* Range of concentration (# cells/cc of sample) given as the nearest base 10 exponent.

Table 2. Average number of yeast species per source.

| Source | Mean | Variance | Standard Error |
|---|---|---|---|
| Citrus | 2.75 | .50 | .25 |
| D. pseudoobscura | 2.43 | .44 | .14 |
| D. arizonensis | 2.66 | .29 | .09 |
| D. melanogaster | 2.20 | .20 | .20 |
| Quercus | 1.78 | 2.07 | .34 |
| Prosopis* | 4.44 | 2.53 | .53 |
| D. carbonaria | 3.0 | 2.63 | .39 |
| Opuntia | .88 | .70 | .30 |

* Only the nine mesquite with feeding flies were used in the calculations.

The yeast species in the adult Drosophila feeding on the citrus fruits were nearly identical to that found in the fruits, and, in addition, the average number of yeast species per fly was not statistically different ($\alpha = .05$) from that in the fruits (Table 2). Of the 110 flies aspirated from the fruits sampled, 56% were D. arizonensis, 34% were D. pseudoobscura, and 10% were D. melanogaster (and/or D. simulans).

Of the 69 flies plated, 6 quickly became covered with mold leaving 63 functional samples which all gave yeast. The two most common yeast, Kl. apiculata and P. fermentans, each isolated from 92% of all flies, showed only minor variation in isolation frequency among species of Drosophila. However, the third most common yeast, P. kluyveri, was isolated in 68%, 58% and 20% of D. arizonensis, D. pseudoobscura, and D. melanogaster, respectively. The percent isolation of T. stellata averaged 9% for all three species of Drosophila. P. membranaefaciens and Candida vini were each represented by one isolate, and although their three day old colonies were confused with those of P. kluyveri, they can still be regarded as representing only a very minute part of the yeast flora in the flies for the same reasons as given in the case of T. stellata. One can conclude then that the order of abundance of P. fermentans and P. kluyveri in the flies has changed from what it was in the oranges. The yeast flora of the male and female flies was not different.

The method of enumeration of yeast from each fly gave only a rough estimate of the yeast concentration; nevertheless, the abundance of colonies present is noteworthy. For each yeast species the percent

of all flies that gave approximately 1000 colonies was 78% for Kl. apiculata, 42% for P. fermentans and 20% for P. kluyveri. This information exactly reflects the order of abundance of yeast in the flies determined by frequency of isolation.

The above observations led to a comparison of the average relative frequencies of the three most common yeasts in the flies with those in the oranges to determine if the flies were selecting certain yeast species during feeding. The frequencies are found in Table 3. The data suggest that the flies are selecting P. fermentans and avoiding Kl. apiculata while neither avoiding nor selecting P. kluyveri. The frequency of P. fermentans in the flies (pooled) is statistically different ($\alpha = .05$) from the frequency of that yeast species in the fruits.

Only the three species of Drosophila feeding on the Citrus were reared from the 8 sampled fruits that were observed in the laboratory. Of the 1886 flies that eclosed, 72% were D. arizonensis, 25% were D. pseudoobscura, and 3% were D. melanogaster. This roughly corresponds to the species abundance of those flies initially collected.

There was no correlation between the abundance of any particular species of yeast or number of flies reared and the physical characteristics of the oranges. The pH varied from 4 to 5, and the temperature varied from 17°C to 24°C, depending upon exposure to direct sunlight. Each orange had from 1 to 3 ovoid holes, made perhaps by birds, from which the flies were aspirated and the sample was taken.

Table 3. A comparison of the average relative frequencies of major yeast between *Citrus* and associated *Drosophila*.

| | *D. pseudoobscura* | *D. arizonensis* | *D. melanogaster* | *Drosophila*, pooled | *Citrus* |
|---|---|---|---|---|---|
| *Kl. apiculata* | | | | | |
| $\bar{X}$ | .57 | .58 | .61 | .58 | .81 |
| S | .37 | .32 | .40 | .34 | .26 |
| $S_{\bar{X}}$ | .08 | .05 | .18 | .04 | .09 |
| *P. fermentans* | | | | | |
| $\bar{X}$ | .29 | .31 | .30 | .30 | .09 |
| S | .28 | .29 | .26 | .28 | .14 |
| $S_{\bar{X}}$ | .06 | .05 | .12 | .04 | .05 |
| *P. kluyveri* | | | | | |
| $\bar{X}$ | .14 | .11 | .10 | .12 | .11 |
| S | .26 | .23 | .21 | .23 | .13 |
| $S_{\bar{X}}$ | .06 | .04 | .10 | .03 | .05 |

## *Quercus* and *Platanus*

Fourteen species of yeast (Table 4) were isolated from 18 *Quercus* samples, yielding an average of 1.78 species of yeast per sample. The most common yeast, *Cryptococcus albidus* var. *albidus*, was isolated from only 33% of the oak samples. All other yeast species were isolated from 6% to 22% of the oaks. The majority of the yeast species were present in low concentration, 1000 or fewer cells per cc of slime flux. Only *Candida ingens*, *Kluyveromyces drosophilarum*, *Saccharomyces globosus*, *Torulopsis wickerhamii*, and *T. species* #5 were present in greater concentrations. Yeast were not isolated from 3 of the samples.

There were no correlations between presence or abundance of any particular yeast and the physical characteristics of the oak fluxes. The pH values varied from 6 to 11, but most (78%) varied from 8 to 9; temperature of the fluxes varied from 18°C to 23°C. No fluxes were actively exuding but appeared as small moist holes most of which were about one-half inch in diameter and of variable depth.

However, there appeared to be a difference in the number and kinds of yeast species present in a temporal sense. The two collection trips to the same area were made only 16 days apart. On the first trip, in early May, six samples were collected, 4 of which contained yeast, yielding four yeast species; the average number of yeast species per sample was one. On the second trip, in late May, 12 samples were collected, 11 of which contained yeast, yielding 11 yeast species; the average number of yeast species per sample was 2.1. The only species collected on both trips was *K. drosophilarum*. The species collected on

Table 4. The number and concentration of isolates of yeast from *Quercus* and *Platanus*.

| Source | *Quercus* | *Platanus* |
|---|---|---|
| Number of Samples: | 18 | 1 |
| *C. ingens* | 2 (< 2-4)* | |
| *Cr. albidus* var. *albidus* | 6 (< 2-3) | 1 (3) |
| *Cr. albidus* var. *diffluens* | 3 (< 2-3) | 1 (3) |
| *Cr. laurentii* var. *magnus* | 3 (< 2) | |
| *Cr. luteolus* | 1 (< 2) | |
| *Kl. apiculata* | 1 (2) | |
| *K. drosophilarum* | 4 (< 2-6) | |
| *P. pastoris* | 2 (< 2) | |
| *Rh. glutinis* var. *glutinis* | | 1 (2) |
| *Rh. species* | 1 (<2) | |
| *S. globosus* | 2 (< 2-4) | |
| *T. ernobii* | 1 (3) | |
| *T. wickerhamii* | 3 (3-7) | |
| *T. species* #5 | 1 (5) | |
| Unknown #3 | 1 (< 2) | |
| Total: | 31 | 3 |

* Range of concentration (# cells/cc of sample) given as the nearest base 10 exponent. Single colonies are indicated by < 2.

the first trip were Cr. laurentii var. magnus, Cr. luteolus and T. species #5, whereas the remaining 10 species found in oaks were collected only on the second trip.

The single sycamore sample resembled the oak samples in most respects. It contained three yeasts, Cr. albidus var. albidus and Cr. albidus var. diffluens, which are shared commonly with the oaks, and Rhodotorula glutinis var. glutinis, which is found in Drosophila carbonaria. The concentration of the yeast and the physical characteristics of the sample compare well with the majority of the oak samples.

No Drosophila adults or larvae were observed feeding on the oaks or the sycamore.

## Prosopis and Associated Drosophila

The 11 Prosopis samples contained 14 species of yeast (Table 5), and three yeast species were found in over 70% of the samples. Both Cryptococcus albidus var. diffluens and Pichia membranaefaciens were present in 82% of the samples, and Torulopsis sp. #1 was isolated from 73% of the samples. Candida species #1 and Torulopsis species #2 were isolated from 55% of the samples, and all other yeast species were isolated from 9% to 27% of the samples. The concentrations of yeast varied from $10^3$ to $10^8$ cells per cc for the 10 mesquite slime flux samples and was $10^3$ or less for the single mesquite sap sample. Six of the yeast species (Table 5) exhibited two similar colony types on the initial plate; this resulted in the underestimation of the frequency of these species since only one representative of each colony type per plate was isolated.

Table 5. The number of isolates of yeast from Opuntia, Prosopis, and associated Drosophila and the concentration of isolates from Opuntia and Prosopis.

| Source | Prosopis* | Prosopis** | Prosopis*** | D. carbonaria | Opuntia | Total |
|---|---|---|---|---|---|---|
| Number of Samples: | 1 | 1 | 9 | 17 | 8 | 36 |
| C. species #1 | | | 6 (4-7) | 4 | | 10 |
| C. species #2+ | | | 1 (6) | | | 1 |
| Cr. albidus var. albidus+ | | | | 1 | | 1 |
| Cr. albidus var. diffluens+ | 1 (< 2)++ | 1 (5) | 7 (3-8) | 12 | | 21 |
| Cr. cereanus | | | | | 2 (6-7) | 2 |
| Cr. infirmo-miniatus | | | | 1 | | 1 |
| Cr. species | 1 (3) | | | | | 1 |
| P. membranaefaciens | | 1 (5) | 8 (4-7) | 13 | 3 (6-7) | 25 |
| P. onychis | | | | 2 | | 2 |
| P. species | | | | | 1 (7) | 1 |
| Rh. glutinis var. glutinis | | | | 1 | | 1 |

Table 5.--Continued.

| Source | Prosopis* | Prosopis** | Prosopis*** | D. carbonaria | Opuntia | Total |
|---|---|---|---|---|---|---|
| Number of Samples: | 1 | 1 | 9 | 17 | 8 | 36 |
| Rh. minuta var. minuta | | | | 1 | | 1 |
| Rh. pallida | 1 (< 2) | | | | | 1 |
| T. sonorensis | | | | | 1 (7) | 1 |
| T. species #1 | | | 8 (3-6) | 14 | | 22 |
| T. species #2+ | | 1 (6) | 5 (6-7) | 2 | | 8 |
| T. species #3+ | | | 1 (5) | | | 1 |
| T. species #4+ | | 1 (5) | 1 (6) | | | 2 |
| Unknown #1+ | | 1 (5) | | | | 1 |
| Unknown #2 | | 1 (4) | | | | 1 |
| Unknown #4 | 1 (< 2) | | | | | 1 |
| Unknown #5 | | | 3 (4-8) | | | 3 |
| Total: | 4 | 6 | 40 | 51 | 7 | 108 |

* = Dried mesquite sap; ** = Mesquite slime flux with no Drosophila present; *** = Mesquite slime flux with feeding Drosophila; + = indicates species with identical young colony types; ++ = range of concentration (# cells/cc of sample) given as the nearest base 10 exponent.

The yeast flora of Drosophila carbonaria the only species of flies observed to be feeding on the mesquite slime fluxes, was very similar to the flora of the mesquite. Not only were the three yeast most common in the flies identical to the three yeast most common in the mesquite, but also they had very similar isolation frequencies. All other yeast species were present in 6% to 12% of the flies except for C. species #1 which was present in 23% of the flies. Two out of the 17 flies sampled contained no yeast.

As with the citrus and associated flies, the average relative frequencies of certain yeast in the flies were compared to those in the mesquite. The three most common yeast,except for Cr. albidus var. diffluens because it was one of the six yeast species that had identical young colony types, were used in the calculations that are found in Table 6. Although the calculations suggest that D. carbonaria is selecting P. membranaefaciens and avoiding T. species #1, the differences are not statistically significant ($\alpha = .05$).

There was no correlation between the presence or abundance of any particular yeast with the physical characteristics of the mesquite fluxes. The pH values varied from 3.5 to 4, and the temperature of the fluxes varied from 18°C to 20°C. At any one time no more than five flies were observed at any one mesquite slime flux. The single sample of dried mesquite sap, unlike the other mesquite samples, was a yellowish, semitransparent foam which had apparently exuded from a small break in the bark and had become crystallized. The other mesquite samples were thin to thick slimy black to brown exudates which covered the entire

Table 6. A comparison of the average relative frequencies of major yeast between *Prosopis* and associated *Drosophila*.

| | *D. carbonaria* | *Prosopis* |
|---|---|---|
| *P. membranaefaciens* | | |
| $\bar{X}$ | .75 | .51 |
| S | .34 | .32 |
| $S_{\bar{X}}$ | .12 | .08 |
| *T. species* #1 | | |
| $\bar{X}$ | .16 | .37 |
| S | .33 | .30 |
| $S_{\bar{X}}$ | .12 | .08 |
| *C. species* #1 | | |
| $\bar{X}$ | .10 | .13 |
| S | .13 | .28 |
| $S_{\bar{X}}$ | .05 | .07 |

lower side of some branches and appeared to be exuding from very narrow cracks in the branches. Few Drosophila larvae were observed in the bark innundated with black exudate.

## Opuntia

Four species of yeast were isolated from the eight opuntia samples (Table 5), yielding an average of 0.88 species of yeast per sample. Pichia membranaefaciens and Cryptococcus cereanus, the most common yeast, were isolated from 38% and 25% of the samples, respectively. P. species and Torulopsis sonorensis were both represented by single isolates. All species were present in concentrations of $10^6$ to $10^7$ cells per cc. Three of the eight opuntia samples contained no yeast.

There was no correlation between the presence or abundance of any particular yeast with the physical characteristics of the rotting tissue. The pH values varied from 6 to 8.5, but 5 of the samples varied from 7 to 8; temperature varied from 27°C to 37°C.

Drosophila eclosed from only one of the eight rotting opuntia pads. Of the 46 flies that eclosed, 41 were D. hamatofila Patterson and Wheeler 1942, 4 were D. longicornis Patterson and Wheeler 1942, and one was D. melanogaster Meigen 1830. Both T. sonorensis and P. membranaefaciens were present in this opuntia.

## Yeast Distribution and Diversity

A summary of the yeast species restricted to and overlapping among substrates can be found in Table 7. Five of the 38 species (13%) were isolated from more than one substrate, and the number of isolates

Table 7. A summary of the yeast species restricted to and overlapping among substrates studied.

| | *Citrus* and *Drosophila* | *Quercus* | *Platanus* | *Opuntia* | *Prosopis* and *Drosophila* | Total |
|---|---|---|---|---|---|---|
| Species Restricted: | | | | | | |
| Number | 4 | 11 | 0 | 3 | 15 | 33 |
| Percent | 11 | 29 | 0 | 8 | 39 | 87 |
| Species Overlapping: | | | | | | |
| Number | | | | | | 5 |
| Percent | | | | | | 13 |
| *Cr. albidus* var. *albidus* | | +(6)* | +(1) | | +(1) | |
| *Cr. albidus* var. *diffluens* | | +(3) | +(1) | | +(21) | |
| *Kl. apiculata* | +(66) | +(1) | | | | |
| *P. membranaefaciens* | +(1) | | | +(3) | +(22) | |
| *Rh. glutinis* var. *glutinis* | | | +(1) | | +(1) | |

* The number of isolates.

of each of these five species in each substrate is given in the table.

The diversities of yeast species within sources (flies and substrate) are expressed in decits in Table 8. Diversity is used as an index of predictability and, therefore, as a measure of randomness. The higher the diversity the greater the randomness and the less likely one will be able to predict, in this case, the yeast species present in any given sample. The substrate diversities in descending order of magnitude are oak, mesquite, orange and platyopuntia. The yeast diversities and yeast floras of the flies are very similar to those of their respective substrates, suggesting that the flies had been feeding nearly exclusively on their substrates.

Table 9 compares the pH values of the substrates to the yeast concentration and the average number of yeast species in each substrate. This data suggests a negative correlation between substrate pH and average number of yeast species per substrate as well as a negative correlation between substrate pH and concentration of yeast. However, Opuntia, the one exception, exhibited a high pH and a high concentration of yeast.

In addition to yeast, mold and bacterial growth was frequently observed in all samples. Two isolates of a Prototheca sp., one from oak and one from mesquite, were found.

## Physiological and Morphological Characteristics of the Yeast

The yeast species from different substrates exhibited differences in their ability to utilize certain test compounds. The percent of all

Table 8. Diversity of yeast species within sources.

| Source | Diversity |
|---|---|
| *Quercus* | 0.8529 |
| *Prosopis* (all samples) | 0.8393 |
| fluxes | 0.7864 |
| flies present | 0.7321 |
| flies absent | 0.4762 |
| sap | 0.3451 |
| *D. carbonaria* | 0.6822 |
| *Citrus* | 0.4493 |
| *D. pseudoobscura* | 0.5068 |
| *D. arizonensis* | 0.4956 |
| *D. melanogaster* | 0.3130 |
| *Opuntia* | 0.3747 |
| *Platanus* | 0.2594 |

Table 9. A comparison of pH, yeast concentration, and average number of yeast species in each substrate.

| Substrate | pH | Yeast Concentration* | Average Number Yeast Species |
|---|---|---|---|
| Prosopis | 3.5- 4.0 | 4-7 | 4.4 |
| Citrus | 4.0- 5.0 | 6-8 | 2.75 |
| Quercus | 6.0-11.0<br>(8.0- 9.0)** | < 2-3 | 1.78 |
| Opuntia | 6.0- 8.5<br>(7.0- 8.0)** | 6-7 | 0.88 |

* Range of concentration (# cells/cc of sample) in which at least 50% of the yeast isolates are found; given as the nearest base 10 exponent.

** Range of pH for at least 60% of samples.

isolates that utilized each compound tested was calculated for each substrate. Heed et al. (1976) similarly constructed a physiological profile on all described yeast (Lodder 1970) and on yeast from temperate trees utilized by Drosophila (Shehata et al. 1955; Phaff and Knapp 1956; Miller, Phaff and Snyder 1962; Phaff et al. 1972), tropical fruits (da Cunha et al. 1957) and desert cacti utilized by Drosophila. The physiological profile of the yeast reported by Heed and of the yeast reported here are compared in Table 10; those compounds utilized more than 50% of the time by yeast of the four substrates presented herein but not in the table are the following: melezitose-65%-oaks; ribose-55%-opuntia; L-arabinose-50%-oaks, 60%-mesquite; citrate-61%-oranges, 55%-opuntia. The salient morphological and physiological characteristics of each yeast species are given in Appendix A; with the exception of colony morphology, when more than one symbol is used to describe a particular characteristic, e.g., +, L, the symbol appearing first in the sequence represents the most frequently occurring value of that characteristic.

All isolates from the opuntia grew at 37°C while only 30% of the isolates from citrus and mesquite and 15% of the isolates from oaks grew at this temperature. In addition, the growth rate of all the mesquite isolates was much less than that of the other yeast isolates; growth of mesquite yeast on agar medium at five days approximated the growth of all other yeast at three days.

The isolates of Pichia membranaefaciens from the opuntia differed from those of the mesquite in three ways. First, the spores produced by P. membranaefaciens isolates from mesquite were all distinctly hat-shaped

Table 10. A comparison of the physiological profiles (percent utilization) of yeast of *Drosophila* substrates of the three climatic regions with those studied herein.

| | Temperate | | | Tropical | | Desert | | Over-all |
|---|---|---|---|---|---|---|---|---|
| | Trees | *Platanus* and *Quercus* | *Prosopis* | Fruits | *Citrus* | Cacti | *Opuntia* | |
| Glucose (fermentation) | 58-68 | 45 | 13 | 84 | 100 | 19-64 | 11 | 67-71 |
| Galactose | 27-34 | 80 | 43 | 28-40 | 0 | 29 | 11 | 55-61 |
| Sucrose | 40 | 65 | 40 | 38-41 | 3 | 10 | 11 | 64-67 |
| Maltose | 37-39 | 65 | 43 | 38 | 0 | 9 | 11 | 54-58 |
| Trehalose | 63-68 | 50 | 66 | 38-41 | 0 | 19 | 45 | 67-68 |
| Cellobiose | 56-62 | 85 | 30 | 56-66 | 35 | 31-32 | 55 | 53-59 |
| Salicin | 52-61 | 80 | 23 | 56-65 | 35 | 31-32 | 55 | 52-58 |
| Xylose | 56-70 | 65 | 64 | 44 | 29 | 33-78 | 11 | 55-60 |
| Ethanol | 88-99 | 55 | 56 | 41-50 | 61 | 100 | 100 | 80-87 |
| Glycerol | 64-90 | 45 | 47 | 34-50 | 61 | 46-96 | 89 | 75-87 |
| Mannitol | 75-89 | 85 | 83 | 31-41 | 0 | 36 | 55 | 70-78 |
| Glucitol | 71-90 | 85 | 60 | 28-41 | 0 | 36 | 55 | 66-78 |
| Lactate | 36-89 | 35 | 13 | 16-47 | 29 | 20-74 | 45 | 41-56 |
| Succinate | 81-95 | 90 | 15 | 22-47 | 61 | 50-99 | 100 | 54-64 |

with a distinct narrow rim whereas those from opuntia were spherical to ellipsoidal. Secondly, the isolates from opuntia grew at 37°C while those from mesquite did not. Thirdly, of the compounds tested (including glucose) the isolates from mesquite grew rapidly on ethanol only while those from opuntia grew equally well on glucose, ethanol, glycerol, lactate, succinate and citrate.

## DISCUSSION AND SUMMARY

The distribution of the yeast in the substrates studied indicates that there is little overlap of yeast species among substrates (Table 7). Only 13% of all yeast species were isolated from more than one substrate and of these only Pichia membranaefaciens was present in high concentrations in more than one substrate. This could be expected since it is assumed that the substrates differ not only in chemical composition but also to a large degree in the habitat in which they are found. However, mesquite and opuntia can be sympatric, and this could explain the observation that both mesquite and opuntia had high concentrations of P. membranaefaciens.

The oaks exhibited the highest yeast diversity (Table 8) of all yeast sources. However, when the diversity of all mesquite samples is calculated, it is very similar to the oaks. Likewise, in comparing the yeast diversity for the temperate, tropical, and desert Drosophila and substrates, Heed et al. (1976) have found that the temperate trees exhibit a high yeast diversity exceeded only by the temperate Drosophila. Thus the observations that temperate adult Drosophila contain essentially similar yeast flora (Phaff et al. 1956) and that temperate Drosophila discriminate yeast species to a lesser degree than tropical Drosophila (da Cunha et al. 1957) can be somewhat explained by the observation that temperate tree fluxes are unpredictable substrates and,

therefore, to develop high degree of food preference would be dangerous for temperate flies (da Cunha et al. 1957).

Heed et al. (1976) have compiled the number of isolates and species among the common genera of yeast associated with Drosophila. The yeast flora of the oaks (Table 4) is similar to that of the temperate trees in that many genera of yeast are represented. However, Pichia and Hansenula, the predominant genera in temperate trees, were represented by only one isolate of P. pastoris in the oaks. The genus Candida was also under-represented in this study. Since yeast diversity is high in temperate trees, a much larger sample size would be needed to reflect accurately the species present. The species from oaks not isolated from temperate trees or Drosophila in previous studies are C. ingens, Cryptococcus luteolus, Saccharomyces globosus, and Torulopsis wickerhamii.

The yeast flora of mesquite resembles that of the temperate trees since their yeast diversity is high and since Rhodotorula pallida and P. onychis are the only two yeasts of those completely identified that are not found in temperate trees. The mesquite also have a close affinity to the cacti inasmuch as P. membranaefaciens, one of the most common yeasts in mesquite, is also the most common yeast in columnar cacti and desert flies (Starmer et al., in press).

The yeast species and yeast diversity of the platyopuntia are very similar to those of the columnar cacti; the similarity gives additional support to the observation that Drosophila breeding substrates of the Sonoran Desert do not differ significantly in yeast species

composition (Starmer et al., in press). The only major yeast of the columnar cacti not isolated from the Opuntia was C. ingens.

The yeast flora of citrus fruits resembles that of the tropical fruits and Drosophila. Nearly one-half of the yeast are Kloeckera apiculata and its perfect forms Hanseniaspora uvarum and H. valbyensis, and a significant but less frequent yeast is P. fermentans (da Cunha et al. 1957). The yeast flora of fermenting tomato fruits on which D. melanogaster are feeding is similar except that P. fermentans found in Citrus is absent in tomatoes and is replaced by P. kluyveri in the tomatoes as well as in the flies (de Camargo and Phaff 1957).

Only the two substrates, citrus and mesquite, which supported Drosophila populations, each exhibited high frequencies of isolation (>70%) of their three most common yeast species while the other substrates were very inconsistent with respect to yeast species present. This observation may be explained in three ways. First, it is possible that all these substrates had reached a state of microbial composition that was very attractive to certain species of Drosophila. Secondly, it is possible that the Drosophila acted as a vector, and thus the movement of flies from orange to orange or from mesquite to mesquite insured a homogeneous distribution of the predominant yeast. As with the Citrus, all the mesquite samples were taken in an area with a radius of about 300 feet. Thirdly, a combination of both of the above could exist.

The consistency of the yeast flora between the oranges and the Drosophila (Table 1) feeding on the oranges indicates that the flies were feeding, at least recently, only on the oranges. The difference in

relative frequencies of yeast species between substrate and flies suggests either a non-random feeding behavior of the flies or a non-random distribution of yeast species on the substrate. Until yeast preference studies are performed on these flies, the latter is more tenable for the reasons that follow. Since some yeast species are able to form a pellicle, a thin layer of growth on the surface of liquid medium, and are able to form pseudomycelium, branched chains of budding cells (Lodder 1970), these yeasts would tend to concentrate and spread over the surface of the substrate, whereas those lacking a pellicle and pseudomycelium would probably be confined to a smaller surface area. Since adult Drosophila are observed to sponge the surface of their feeding substrates, the yeast growth characteristics are probably important. Kl. apiculata, the yeast that showed a lower concentration in the flies than in the oranges, produces neither a pellicle nor pseudomycelium, whereas P. fermentans and P. kluyveri both produce a pellicle and pseudomycelium. In addition, there was no difference in relative yeast concentration among the three species of Drosophila breeding in the oranges and, therefore, no obvious difference in yeast preferences between these species.

As in the case of the oranges and associated flies, the most common yeast species in the mesquite were also found to be the most common in D. carbonaria (Table 5), indicating that the flies were feeding almost exclusively on the mesquite slime fluxes. Any yeast species not common to both were single isolates (Table 5) or P. onychis, which has not been isolated previously from Drosophila or their breeding substrates,

or Unknown #5. Also, as with the Citrus, the relative frequencies of yeast in the flies differed from those in the mesquite slime fluxes. T. species #1, which produces no pellicle or pseudomycelium, had a lower relative frequency in the flies, and P. membranaefaciens, which produces both a pellicle and pseudomycelium, increased in relative frequency in the flies. C. species #1 produces pseudomycelium but no pellicle. Another explanation for the observed relative frequencies of the yeast may be their differential digestibility in the fly.

It is a well-known fact that the growth of most bacteria is inhibited at low pH values while few, if any, yeasts are inhibited by a pH value of 3.0 and that most yeast readily grow at pH values of 7-8, but optimum growth is normally at pH values of 4.5-6.5 (Phaff, Miller and Mrak 1966). Therefore, at low pH values one would expect to find less competition from bacteria. Data in Table 9 suggest a negative correlation between substrate pH and average number of yeast species per substrate as well as a negative correlation between substrate pH and concentration of yeast. However, Opuntia exhibited a high pH and high concentration of yeast. The values for Opuntia are similar to those obtained by Starmer et al. (in press) who found that the majority of the pH values and concentrations of yeast range from 6-8 and $10^6$-$10^7$, respectively, for the columnar cacti of the Sonoran Desert. Further studies including the determination of bacterial concentrations will be necessary to determine whether bacteria compete with yeast for nutrients. In addition, if only oaks with feeding Drosophila had been sampled, there may have been greater concentrations of yeast present in the oaks. In a one

year quantitative study of the yeast flora in a single slime flux of Ulmus carpinifolia Phaff, Yoneyama and Do Carmo-Sousa (1964) showed that the yeast flora varied from 400 to 790,000 cells per gram of moisture free flux and the pH value ranged between 8.7 and 9.0. This is the only previous study on yeast flora of slime fluxes in which the concentration of yeasts has been determined.

One must remember that when establishing the distribution of yeast, the frequency of yeast species in a substrate can be most accurately determined when each different colony type on the initial isolation plate represents a single yeast species. When many colonies are present, distinct colony types become very important since only one representative of each colony type is isolated. However, when a particular colony type can represent more than one yeast species (the case in the citrus and the mesquite), a more accurate count requires a subsequent series of substrate studies which include many representative isolates of the colony type in question.

## Physiological Factors Affecting Yeast Distribution

The physiological profile of the yeast of a certain substrate suggests the carbon compounds that could be available as an energy and carbon source in that substrate. In addition, a knowledge of the biochemistry of the compound implies whether the yeast are utilizing a complex polymer or whether they are utilizing a metabolic by-product or degredation product. Thus, their physiological profile could explain their role in the succession of microorganisms that utilize the necrotic

tissues of substrates where Drosophila breed. The order of decomposition in leaf litter (Griffin 1972) is sugars, hemicelluloses, cellulose and lignin. This order could explain waves of colonization of fungi utilizing progressively more refractory components and could explain the coexistance between sugar fungi and cellulolytic fungi.

The yeast found in the oranges are considered primary invaders since all were able to ferment glucose rapidly. Hanseniaspora valbyensis (perfect form of Kloeckera apiculata) and Pichia fermentans making up 50% of the isolates of ten yeast species found on the surfaces of intact oranges (Recca and Mrak 1952) gives additional evidence for these yeast being primary invaders. P. kluyveri has previously been isolated from soils under rotting citrus fruits (Spencer and Gorin 1971).

Carbohydrate assimilatory abilities of the yeast in the oranges correlates to some extent with the carbohydrate content of oranges. Sucrose, glucose and fructose (2:1:1) make up approximately 10% of the juice of the orange (Kefford 1959). However, it is surprising that from the oranges no yeast except the single isolate of T. stellata utilizes sucrose. In addition, citrate (1%) and trace amounts of succinate (U. S. Dept. of Agr. 1962) are possible carbon sources.

The yeast found in the oaks appear to be secondary invaders and are able to use a wide variety of compounds. Many of the compounds are degradation products (most likely microbial) of plant structural polysaccharides like cellulose, composed of cellobiose; hemicelluloses, composed of xylose and arabinose; and pectin, containing galactose

(Lehninger 1970). The only carbohydrate in the phloem exudate or sap of many plant species is sucrose; however, small amounts of raffinose, polysaccharides of raffinose plus one or two more galactose molecules, or sugar alcohols such as D-mannitol and glucitol may occur in certain other plants (Crafts and Crisp 1971; Zimmermann 1957, 1958). Ethanol, lactate, and succinate are fermentation products; glycerol is formed from lipid degradation. It becomes apparent that, since sucrose is probably the only or at least the most abundant sugar during fluxing, the yeast in oaks and temperate trees must acquire most of their nutrition from the products of slow degradation of complex molecules. Thus, a varied assimilatory ability would be adaptive for these yeast since fluxing is a transient phenomenon. The probable slow release of available nutrients explains the low concentration of yeast in the oaks, and the probable large number of compounds (nutritional niches) available explains the high diversity of yeast in temperate trees.

The yeast in the mesquite, as in the oaks, are probably secondary invaders. Mesquite sap is composed of 50% L-arabinose and 18% D-galactose (Anderson and Sands 1926), and 60% of all isolates from mesquite assimilate L-arabinose. Since the phloem exudate of most plants is slightly alkaline (Tammes and van Die 1964), the low pH of all the mesquite fluxes suggests a basic difference in the chemistry of the flux or the presence of a microbial community possibly producing organic acids as a result of anaerobic respiration within the flux.

The physiological profile of the yeast in the opuntia and other cacti indicates that these yeast are secondary invaders since they

utilize mainly ethanol and succinate, products of fermentation, and glycerol, a product of lipid degradation.

When one observes the physiological profiles of all yeast across all substrates several trends appear. A high frequency of fermentative ability is associated with substrates with high sugar concentrations, e.g., fruits. A high diversity of yeast that have a high frequency of utilization of a wide variety of compounds is associated with small transient substrates, the tree fluxes. The ability to grow at elevated temperatures (37$^{o}$C) is associated with substrates in desert areas; although the mesquite is a desert plant, the samples were taken from individuals in a temperate habitat, and as a result, the yeast present were not adapted to high temperatures.

APPENDIX A

TAXONOMIC DESIGNATIONS AND CHARACTERISTICS OF THE YEAST SPECIES

The Taxonomic Designation and Total Number of Isolates of Each Species

| No. | Yeast | No. of Isolates |
|---|---|---|
| 1. | *Candida ingens* van der Walt et van Kerken 1961 | 2 |
| 2. | *Candida vini* (Desmazieres *ex* Lodder) van Uden et Buckley *nov. comb.* 1934 | 1 |
| 3. | *Candida sp.* #1 | 10 |
| 4. | *Candida sp.* #2 | 1 |
| 5. | *Cryptococcus albidus* (Saito) Skinner var. *albidus* 1922 | 8 |
| 6. | *Cryptococcus albidus* (Saito) Skinner var. *diffluens* Phaff et Fell 1970 | 25 |
| 7. | *Cryptococcus cereanus* Phaff et al. 1974 | 2 |
| 8. | *Cryptococcus infirmo-miniatus* (Okunuki) Phaff et Fell *nov. comb.* 1931 | 1 |
| 9. | *Cryptococcus laurentii* (Kufferath) Skinner var. *magnus* Loder et Kreger-van Rij 1952 | 3 |
| 10. | *Cryptococcus luteolus* (Saito) Skinner 1922 | 1 |
| 11. | *Cryptococcus sp.* | 1 |
| 12. | *Kloeckera apiculata* (Reess *emend.* Klocker) Janke 1870 | 67 |
| 13. | *Kluyveromyces drosophilarum* (Shehata, Mrak et Phaff) van der Walt 1955 | 4 |

| No. | Yeast | No. of Isolates |
|---|---|---|
| 14. | Pichia fermentans Lodder 1932 | 65 |
| 15. | Pichia kluyveri Bedford 1942 | 43 |
| 16. | Pichia membranaefaciens Hansen 1888 | 26 |
| 17. | Pichia onychis Yarrow 1965 | 2 |
| 18. | Pichia pastoris (Guilliermond) Phaff 1919 | 2 |
| 19. | Pichia sp. | 1 |
| 20. | Rhodotorula glutinis (Fres.) Harrison var. glutinis 1852 | 2 |
| 21. | Rhodotorula minuta (Saito) Harrison var. minuta 1922 | 1 |
| 22. | Rhodotorula pallida Lodder 1934 | 1 |
| 23. | Rhodotorula sp. | 1 |
| 24. | Saccharomyces globosus Osterwalder 1924 | 2 |
| 25. | Torulopsis ernobii Lodder et Kreger-van Rij 1952 | 1 |
| 26. | Torulopsis sonorensis Miller et al. 1976 | 1 |
| 27. | Torulopsis stellata (Kroemer et Krumbholz) Lodder 1931 | 6 |
| 28. | Torulopsis wickerhamii Capriotti 1958 | 3 |
| 29. | Torulopsis sp. #1 | 22 |
| 30. | Torulopsis sp. #2 | 8 |
| 31. | Torulopsis sp. #3 | 1 |
| 32. | Torulopsis sp. #4 | 2 |
| 33. | Torulopsis sp. #5 | 1 |
| 34. | Unknown #1 | 1 |
| 35. | Unknown #2 | 1 |
| 36. | Unknown #3 | 1 |

| No. | Yeast | No. of Isolates |
|---|---|---|
| 37. | Unknown #4 | 1 |
| 38. | Unknown #5 | 3 |
| | | 324 |

## Characteristics of the Yeast Species*

| Yeast Species No. | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| **Morphology:** | | | | | | |
| Colony | W,D,R | W,D,S | W,D,R | W,D,S | Y,G,M | Y,G,M |
| Cell | O,Cy | E,Cy | E | E,V | S | S |
| Pellicle | + | L | - | - | - | - |
| Mycelium | U,Ps | Ps,B | Ps,B | Ps,B | - | - |
| Spores | - | - | - | - | - | - |
| **Physiology:** | | | | | | |
| 37°C | - | - | + | - | - | - |
| Fermentation | - | - | L | - | - | - |
| **Assimilation:** | | | | | | |
| Glucose | + | + | + | w | + | + |
| Galactose | + | w | + | - | +,w | +,w |
| Sucrose | - | - | - | - | + | + |
| Maltose | -,w | - | - | - | + | + |
| Cellobiose | - | - | - | - | + | + |
| Trehalose | -,w | - | L | L | +,L | +,w |
| Lactose | - | - | - | - | + | - |
| Melibiose | - | - | - | - | -,+,w | - |
| Raffinose | - | - | - | - | w,-,+ | -,w |
| Melezitose | - | - | -,w | - | + | + |
| Inulin | - | - | - | - | - | - |
| Soluble starch | - | - | - | - | +,L,- | -,+,w |
| D-Xylose | - | - | L | - | + | + |
| L-Arabinose | - | - | + | - | + | + |
| D-Ribose | - | - | - | - | +,w,- | -,w |
| L-Rhamnose | - | - | - | - | +,w | -,L,w |
| Ethanol | + | + | L | L | -,+,L | L,-,w |
| Glycerol | L | w | + | - | -,w | -,w |
| Erythritol | - | - | - | - | -,+,w | -,+ |
| L-Sorbose | - | - | + | - | -,w,L | -,L |
| D-Arabinose | - | - | - | - | +,-,L | -,+ |
| D-Mannitol | - | + | +,L | + | + | +,w |

* W = white to cream, P = pink, T = tan to white, Bl = black, Y = yellow to cream, G = glossy, D = dull, M = mucoid, S = smooth, R = rough, Sp = spherical, E = ellipsoidal, Cy = cylindrical, A = apiculate, U = true, Ps = pseudo-, B = blastospores, Re = reniform, H = hat, w = weak, L = latent (+ at 10 days).

| Yeast Species No. | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| D-Glucitol | - | + | + | + | +,w,L | L,+,w |
| Salicin | - | - | -,w | - | +,w,- | +,w,L |
| DL-Lactic Acid | - | L | L | - | -,w | - |
| Succinic Acid | + | + | + | - | +,w,- | +,w,L |
| Citric Acid | - | w | L | - | L,-,+ | -,w |
| Inositol | - | - | + | - | + | L,+ |
| Nitrate | - | - | - | - | + | + |

| Yeast Species No. | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|
| Morphology: | | | | | | |
| Colony | W,D,S | P,G,S | W,M,S | W,D,S | W,D,S | W,G,S |
| Cell | E,Cy | S | S | S | S | A,E |
| Pellicle | - | - | - | - | - | - |
| Mycelium | Ps,w | - | - | - | - | - |
| Spores | - | - | - | - | - | - |
| Physiology: | | | | | | |
| 37°C | + | - | - | - | - | - |
| Fermentation | - | - | - | - | - | + |
| Assimilation: | | | | | | |
| Glucose | + | + | + | + | + | + |
| Galactose | - | + | + | + | + | - |
| Sucrose | - | + | + | + | w | - |
| Maltose | - | + | + | + | w | - |
| Cellobiose | w,+ | + | + | + | + | + |
| Trehalose | + | + | + | + | + | - |
| Lactose | - | + | + | - | + | - |
| Melibiose | - | + | -,w | + | - | - |
| Raffinose | - | + | w | + | w | - |
| Melezitose | -,w | + | +,- | + | - | - |
| Inulin | - | - | - | - | - | - |
| Soluble starch | -,w | w | w | w | - | - |
| D-Xylose | + | + | + | + | + | - |
| L-Arabinose | w | + | + | + | + | - |
| D-Ribose | L | L | w,+ | + | L | - |
| L-Rhamnose | w,L | L | + | L | + | - |
| Ethanol | + | - | -,w | - | + | - |
| Glycerol | + | - | -,w | - | w | - |
| Erythritol | + | - | - | w | - | - |
| L-Sorbose | + | - | +,L | w | w | - |
| D-Arabinose | w | L | L,+ | + | + | - |
| D-Mannitol | + | w | + | + | L | - |
| D-Glucitol | + | L | L,+ | L | - | - |

| Yeast Species No. | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|
| Salicin | + | - | + | w | - | + |
| DL-Lactic Acid | -,w | - | +,L | w | - | - |
| Succinic Acid | L | - | +,- | + | w | - |
| Citric Acid | - | - | -,w | w | L | - |
| Inositol | + | L | +,L | L | + | - |
| Nitrate | - | + | - | - | + | - |

| Yeast Species No. | 13 | 14 | 15 | 16 | 17 | 18 |
|---|---|---|---|---|---|---|
| Morphology: | | | | | | |
| Colony | W,D,S | W,D,R | W,D,S | T,D,R | W,D,R | W,G,S |
| Cell | Sp,E | E | E | E | E | S |
| Pellicle | + | + | + | +,- | + | - |
| Mycelium | Ps | Ps,B | Ps | Ps,B | Ps | - |
| Spores | Re | H | H | H,Sp | H,Sp | H |
| Physiology: | | | | | | |
| 37°C | + | + | - | -,+ | + | - |
| Fermentation | + | + | + | -,w | + | + |
| Assimilation: | | | | | | |
| Glucose | + | + | + | w,+ | + | + |
| Galactose | + | - | - | - | - | +,L |
| Sucrose | + | - | - | - | + | w |
| Maltose | + | - | - | - | + | w |
| Cellobiose | + | - | - | - | + | - |
| Trehalose | w,L | - | - | - | + | w |
| Lactose | - | - | - | - | - | - |
| Melibiose | - | - | - | - | - | - |
| Raffinose | + | - | - | - | + | - |
| Melezitose | + | - | - | - | + | - |
| Inulin | - | - | - | - | - | - |
| Soluble starch | - | - | - | - | - | - |
| D-Xylose | w | L | w | -,L | + | w |
| L-Arabinose | - | - | - | - | + | - |
| D-Ribose | - | - | - | - | - | w |
| L-Rhamnose | - | - | - | - | + | + |
| Ethanol | + | + | + | + | + | + |
| Glycerol | +,L | + | L,+ | -,+ | + | + |
| Erythritol | - | - | - | - | - | -,w |
| L-Sorbose | L | - | - | - | - | - |
| D-Arabinose | - | - | - | - | - | - |
| D-Mannitol | +,L | - | - | - | + | + |
| D-Glucitol | + | - | - | - | + | + |
| Salicin | + | - | - | - | + | -,w |
| DL-Lactic Acid | +,- | +,w | w | w,- .+ | + | w |

| Yeast Species No. | 13 | 14 | 15 | 16 | 17 | 18 |
|---|---|---|---|---|---|---|
| Succinic Acid | + | + | + | w,+ | + | + |
| Citric Acid | w | +,w | L,w | -,+ | - | w |
| Inositol | - | - | - | - | - | - |
| Nitrate | - | - | - | - | - | - |

| Yeast Species No. | 19 | 20 | 21 | 22 | 23 | 24 |
|---|---|---|---|---|---|---|
| Morphology: | | | | | | |
| Colony | W,G,S | P,G,S | P,G,S | Y,D,S | Y,D,S | W,D,S |
| Cell | E | Sp | Sp | Sp | Sp | Sp,E |
| Pellicle | - | - | - | - | + | - |
| Mycelium | - | - | - | - | Ps,w | - |
| Spores | H | - | - | - | - | E |
| Physiology: | | | | | | |
| 37°C | + | - | - | - | - | + |
| Fermentation | - | - | - | - | - | + |
| Assimilation: | | | | | | |
| Glucose | + | + | + | + | + | + |
| Galactose | + | L | L | + | w | + |
| Sucrose | + | + | + | w | w | - |
| Maltose | + | + | - | - | w | -,w |
| Cellobiose | + | + | + | - | + | - |
| Trehalose | + | + | + | + | - | + |
| Lactose | - | - | L | - | - | - |
| Melibiose | w | - | - | - | - | - |
| Raffinose | w | w,+ | w | - | - | - |
| Melezitose | L | + | + | - | - | - |
| Inulin | - | - | - | - | - | - |
| Soluble starch | w | -,w | - | - | - | -,w |
| D-Xylose | L | + | + | + | - | -,w |
| L-Arabinose | w | -,w | + | + | L | - |
| D-Ribose | + | L,+ | - | - | - | - |
| L-Rhamnose | + | -,L | - | - | - | - |
| Ethanol | + | -,+ | + | - | L | w |
| Glycerol | + | + | + | w | L | - |
| Erythritol | + | - | - | - | - | - |
| L-Sorbose | L | + | w | - | + | - |
| D-Arabinose | - | + | L | - | - | - |
| D-Mannitol | + | + | - | + | + | - |
| D-Glucitol | + | + | - | L | + | - |
| Salicin | + | + | + | - | + | - |
| DL-Lactic Acid | - | - | - | - | - | - |
| Succinic Acid | + | + | + | - | + | - |
| Citric Acid | + | -,+ | - | - | - | - |
| Inositol | - | - | - | - | - | - |
| Nitrate | + | + | - | - | - | - |

| Yeast Species No. | 25 | 26 | 27 | 28 | 29 | 30 | 31 |
|---|---|---|---|---|---|---|---|
| **Morphology:** | | | | | | | |
| Colony | W,D,S | W,D,S | W,G,S | W,D,S | W,G,S | W,G,S | W,G,S |
| Cell | Sp | Sp,E | E | Sp,E | Sp | Sp,E | E |
| Pellicle | - | - | - | - | - | - | - |
| Mycelium | Ps,- | - | - | Ps,w | - | Ps,w | Ps,w |
| Spores | - | - | - | - | - | - | - |
| **Physiology:** | | | | | | | |
| 37°C | - | + | - | - | - | +,- | - |
| Fermentation | + | + | + | + | L,w | - | L |
| **Assimilation:** | | | | | | | |
| Glucose | + | + | + | + | + | +,w | + |
| Galactose | w | - | - | + | + | w,- | w |
| Sucrose | + | - | + | w | + | -,w | w |
| Maltose | + | - | - | - | + | -,w | L |
| Cellobiose | + | + | - | + | - | -,w | - |
| Trehalose | -,w | - | - | - | w | L | + |
| Lactose | - | - | - | - | - | - | - |
| Melibiose | - | - | - | - | - | - | - |
| Raffinose | - | - | + | w,- | - | - | - |
| Melezitose | + | - | - | - | + | - | w |
| Inulin | - | - | - | - | - | - | - |
| Soluble starch | w | - | - | - | w | - | - |
| D-Xylose | + | w | - | + | + | - | + |
| L-Arabinose | - | + | - | - | + | -,w | w |
| D-Ribose | w | + | - | - | - | - | + |
| L-Rhamnose | - | - | - | L | - | +,L | - |
| Ethanol | + | + | - | + | + | L,w,- | + |
| Glycerol | + | w | - | + | + | w | + |
| Erythritol | - | - | - | - | + | - | + |
| L-Sorbose | - | - | - | - | + | - | + |
| D-Arabinose | w | + | - | - | - | - | w |
| D-Mannitol | + | + | - | + | + | + | + |
| D-Glucitol | + | + | - | + | + | + | + |
| Salicin | + | + | - | + | - | w,- | - |
| DL-Lactic Acid | L | w | - | + | - | - | - |
| Succinic Acid | + | + | - | + | - | w,+ | - |
| Citric Acid | w | - | - | - | - | - | L |
| Inositol | - | - | - | - | - | - | - |
| Nitrate | + | - | - | + | - | - | + |

| Yeast Species No. | 32 | 33 | 34 | 35 | 36 | 37** | 38*** |
|---|---|---|---|---|---|---|---|
| **Morphology:** | | | | | | | |
| Colony | W,D,S | W,G,S | W,D,S | W,G,S | Bl,D,R | P,D,S | P,G,S |
| Cell | Sp,E | Sp | A,E | Sp | Sp,Cy | Sp | E |
| Pellicle | - | + | - | - | - | - | - |
| Mycelium | - | - | Ps,w | - | U,Ps | - | U,Ps,B |
| Spores | - | - | - | - | - | - | - |
| **Physiology:** | | | | | | | |
| 37°C | L,- | - | - | + | - | - | - |
| Fermentation | L | + | - | L | - | - | - |
| **Assimilation:** | | | | | | | |
| Glucose | + | + | + | + | + | + | + |
| Galactose | w | + | w | + | - | + | + |
| Sucrose | + | + | w | + | + | w | + |
| Maltose | + | + | w | + | + | - | + |
| Cellobiose | + | + | w | w | + | - | + |
| Trehalose | + | - | + | + | + | - | + |
| Lactose | - | - | - | - | + | - | + |
| Melibiose | - | - | - | - | + | - | - |
| Raffinose | - | - | - | - | + | w | w |
| Melezitose | + | + | - | + | + | w | + |
| Inulin | - | - | - | - | - | - | w |
| Soluble starch | - | - | - | - | + | - | + |
| D-Xylose | L | + | - | + | + | w | + |
| L-Arabinose | - | - | - | + | + | + | + |
| D-Ribose | L | - | - | - | + | - | w |
| L-Rhamnose | - | - | - | - | + | - | w |
| Ethanol | + | + | - | w | - | - | w |
| Glycerol | + | + | - | + | w | + | w |
| Erythritol | + | - | - | - | L | - | + |
| L-Sorbose | L,- | - | - | + | L | w | - |
| D-Arabinose | - | - | - | - | w | + | L |
| D-Mannitol | + | + | + | + | + | + | + |
| D-Glucitol | + | + | + | + | + | L | + |
| Salicin | + | + | w | - | + | - | L |
| DL-Lactic Acid | w,- | w | - | w | w | - | w |
| Succinic Acid | - | + | - | + | + | - | w |
| Citric Acid | + | L | - | - | L | - | w |
| Inositol | - | - | - | + | w | - | L |
| Nitrate | + | - | - | + | + | - | + |

** Exhibited large oval cells and budding on a broad base.

*** Colony turned pink after two weeks of growth.

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