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THE TAXONOMY OF YEASTS ISOLATED FROM *DROSOPHILA* IN THE YOSEMITE REGION OF CALIFORNIA

by

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The present paper gives a taxonomic account of the yeast flora isolated from the intestinal canal of wild species of *Drosophila* occurring at altitudes from 4500 feet to 10.000 feet, in the Yosemite region of the Sierra Nevada in California. The relationships between these yeasts and the species of *Drosophila* from which they were obtained have been described in a separate publication (9). Besides a general discussion of the yeasts isolated, a number of new species will be described. In addition, the yeast flora found by us will be compared with the results of surveys by other investigators.

METHODS.

Methods of collecting the flies, dissection of the crops and isolation procedures have been described in detail by PHAFF, *et al.* (9). Identification was first attempted by the system of LODDER and KREGER – VAN RIJ (5) or that of WICKERHAM for *Hansenula* (17). If there was doubt about the identity of an isolate with a known species, an authentic culture was usually obtained and a detailed comparison was made. This included a study of their assimilatory ability using a large number of carbon compounds as recommended by WICKERHAM (17). If a culture could not be identified by either system or by comparison with other yeasts described in the literature, it was considered a new species. During the initial study the replica plating method was used to determine assimilation reactions (14). This method is especially useful and time saving when a large number of strains are isolated, allowing a rapid tentative grouping of the cultures. For confirmation the method of WICKERHAM (17),

using a liquid synthetic medium in test tubes, was applied to all new species and in cases of doubt. The carbon compounds used are given in Table 2. The yeasts were also tested for their ability to grow in a vitamin free medium (17) and acid production was determined after growth on chalk agar (see PHAFF and KNAPP, (10).

RESULTS.

The 241 cultures isolated are listed in Table 1. The association of the different yeasts with the various species of *Drosophila* from which they were isolated has been discussed in detail by PHAFF, *et al.* (9).

Genus *Saccharomyces*. This genus is represented by a great number of isolates. The most common yeast was considered to be a new species in our previous paper (9) and was termed provisionally *S. montanus*. Although the fermentation characteristics are the same as those of *S. cerevisiae* and the key to the species of the genus *Saccharomyces* given by LODDER and KREGER – VAN RIJ (5) leads to this species, we considered it to be different for the following reasons. Its cell dimensions are smaller than their group II (small celled group), many of the cells conjugated before sporulation and, perhaps most important of all, it assimilated many more carbon compounds than does *S. cerevisiae*. However, on rechecking some of the older species of *Zygosaccharomyces* described by STELLING-DEKKER (15), we noticed great similarities between our isolates and *Zygosaccharomyces fermentati* described originally by NAGANISHI in 1928, who isolated it from the sediment of a peppermint beverage. LODDER and KREGER – VAN RIJ placed this species in *S. cerevisiae* and considered them synonyms. We obtained an authentic transfer of the former *Z. fermentati* from the C.B.S.[1]) culture collection and found that our isolates were indeed remarkably similar to this yeast, including the assimilation pattern of the 34 carbon compounds used. Since there was little variation in the carbon assimilation reactions among our 36 isolates of this yeast, we believe that *Z. fermentati* is a well defined and valid species. In line with the now well accepted practice to abolish *Zygosaccharomyces* as a separate genus, the species should be termed *S. fermentati* (Naganishi) nov. comb. This, however, is not possible since LODDER and KREGER – VAN RIJ (5) transferred *Torulaspora fermentati* (Saito) to *Saccharomyces* and

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TABLE 1.

Yeasts isolated from *Drosophila* in the Yosemite Region of California.

Species of yeast	No. of isolates	Areas in which isolated [1])
Sporulating yeasts		
1. *Saccharomyces montanus* (Naganishi) nov. comb.	36	M; PF
2. *S. veronae* Lodder et Kreger-van Rij 1952	30	M; AV; T
3. *S. cerevisiae* Hansen var. *tetrasporus* (Beijerinck) nov. comb.	22	M
4. *S. drosophilarum* Shehata, Mrak et Phaff 1955	13	M
5. *S. drosophilarum* Shehata, *et al.* var. *acellobiosa* nov. var.	2	M
6. *S. florentinus* (Castelli) Lodder et Kreger-van Rij 1938	2	M; PF
7. *S. dobzhanskii* Shehata, Mrak et Phaff 1955	1	PF
8. *S. wickerhamii* nov. spec.	2	YC; AV
9. *S. uvarum* Beijerinck 1898	1	PF
10. *S. kluyveri* nov. spec.	1	M
11. *Pichia fermentans* Lodder 1932	2	M
12. *Pichia xylosa* nov. spec.	1	M
13. *P. membranaefaciens* Hansen 1888	1	M
14. *P. pini* (Holst) Phaff 1936	1	M
15. *P. pastori* (Guilliermond) Phaff 1919	2	M
16. *Hansenula angusta* Wickerham 1951	19	M; AV; PF
17. *Hanseniaspora valbyensis* Kloecker 1912	6	M
18. *H. uvarum* (Niehaus) Shehata *et al.* 1932	6	M
19. *H. osmophila* (Niehaus) nov. comb. 1932	5	M
Non-sporulating yeasts		
20. *Kloeckera apiculata* (Reess emend. Kloecker) Janke 1870	15	M; PF; T
21. *K. magna* (De'Rossi) Janke 1920	13	M
22. *Torulopsis stellata* (Kroemer et Krumbholz) Lodder 1931	10	M; PF; T
23. *T. glabrata* (Anderson) Lodder et de Vries 1917	1	AV
24. *T. pinus* Lodder et Kreger-van Rij 1952	1	AV
25. *T. inconspicua* Lodder et Kreger-van Rij 1952	1	AV
26. *T. colliculosa* (Hartmann) Saccardo 1903	1	M
	195	

[1]) Areas studied are indicated as follows: M = Mather area; AV = Aspen Valley; SJ = Smoky Jack; YC = Yosemite Creek; PF = Porcupine Flat; T = Tioga Pass area. For a more detailed description of the areas indicated, see COOPER, D. M. and DOBZHANSKY, TH. 1956. Ecology (in press).

TABLE 1 (continued).

Species of yeast	No. of isolates	Areas in which isolated
	195	
27. *Cryptococcus diffluens* (Zach) Lodder et Kreger-van Rij 1934	5	M; YC; T
28. *Cr. laurentii* (Kufferath) Skinner 1920	2	M; PF
29. *Rhodotorula aurea* (Saito) Lodder 1934	1	PF
30. *Rh. minuta* (Saito) Harrison 1922	1	AV
31. *Rh. glutinis* (Fres.) Harrison 1852	1	M
32. *Rh. mucilaginosa* (Jörg.) Harrison 1909	1	PF
33. *Candida krusei* (Cast.) Berkhout 1910	10	M; AV
34. *C. catenulata* Diddens et Lodder 1942	6	AV
35. *C. parapsilosis* (Ashf.) Lang. et Talice var. *intermedia* Kreger-van Rij et Verona 1949	6	SJ
36. *C. parapsilosis* (Ashf.) Lang. et Talice 1928	2	M; AV
37. *C. mesenterica* (Geiger) Diddens et Lodder 1910	4	M; PF; T
38. *C. mycoderma* (Reess) Lodder et Kreger-van Rij 1870	2	M; PF
39. *C. guilliermondii* (Cast.) Lang. et Guerra 1912	1	M
40. *Trichosporon aculeatum* nov. spec.	2	AV; T
41. *Tr. fermentans* Diddens et Lodder 1942	1	M
42. *Oospora lactis* (Fres.) Saccardo	1	M
Total number of isolates	241	

retained the name *fermentati*, as they did not recognize NAGANISHI's yeast as a valid species. Furthermore SAITO's *Torulaspora fermentati* or *Saccharomyces fermentati* (if one considers the genera synonymous) has priority since it was described in 1923, five years before NAGANISHI described his organism. To avoid further confusion it is proposed to change the name *Zygosaccharomyces fermentati* Naganishi to *Saccharomyces montanus* Naganishi nov. comb. A comparison of the carbon assimilation reactions of this yeast and *S. cerevisiae* is given in Table 2.

Another very common species is *Saccharomyces veronae* of which 30 isolates were obtained. This organism was originally termed *Zygosaccharomyces drosophilae* by SHEHATA and MRAK (11) although these authors did not describe it as a new species at that time. In 1952 LODDER and KREGER – VAN RIJ (5) described a new species, *S. veronae*, which was isolated by VERONA from grapes in Italy.

SHEHATA *et al.* in a later paper (13) recognized the identity of the organisms isolated from *Drosophila* with *S. veronae*.

The next frequent organism (22 isolates) is designated as *S. cerevisiae* var. *tetrasporus*. Our cultures appear to be quite similar to the yeast which BEIJERINCK isolated from the exudate of an oak. He named it *S. tetrasporus*, although he did not describe it. STELLING-DEKKER (15) who studied this organism, named it *S. mangini* var. *tetrasporus*, based on an erroneous observation that maltose was not fermented (5, pg. 128). This error was probably based on a delayed fermentation of maltose, which we also frequently have observed in our strains. Other typical features of our isolates are the very high percentage of sporulating cells on malt agar and the small size of the cells as compared to a standard culture of *S. cerevisiae*. These properties agree well with the description given by STELLING-DEKKER and we feel that the cultures are sufficiently distinct to separate them as *S. cerevisiae* var. *tetrasporus*. The carbon assimilation patterns of our isolates were all alike and they resemble *S. cerevisiae* in that respect except that α-methyl-D-glucoside was used regularly by the variety but not by a group of isolates of bakers yeast studied by us (see Table 2).

S. drosophilarum (13 isolates) has been described recently by SHEHATA, MRAK and PHAFF (13). It is a yeast with kidney-or crescent-shaped ascospores. We isolated two cultures which resembled *S. drosophilarum* a great deal. The principal difference is that these two isolates can not attack cellobiose or salicin and they therefore appear to lack a functional beta-glucosidase. Since they are otherwise very similar we will tentatively designate them as *S. drosophilarum* var. *acellobiosa*, rather than create a new species. Pending further studies regarding the relationship between the species and the variety a description of the variety will not be given at this time.

Two cultures were obtained which corresponded very well to *S. florentinus*, a small-celled, melibiose fermenting yeast, originally isolated by CASTELLI from grape must treated with sulfur dioxide. Our cultures sporulated after 4 days on malt extract. Asci were formed after isogamic conjugation or without conjugation. According to LODDER and KREGER – VAN RIJ (5) the type species (only one strain has been isolated) lost the ability to sporulate. A comparison of the carbon assimilation pattern of our strains with that of the authentic culture proved to be the same. It is given in Table 2.

Saccharomyces dobzhanskii, a yeast with kidney-shaped ascospores,

was recently described by SHEHATA *et al.* (13). These authors isolated a single strain in the Southern California mountains. A single culture was also isolated in the present survey. The two cultures agreed in all respects.

Saccharomyces wickerhamii, another yeast with kidney- to crescent-shaped ascospores, was isolated twice and must be considered a new species since both the fermentation and assimilation characteristics are different from any known species. It will be named after Dr L. J. WICKERHAM in recognition of his extensive contributions to the taxonomy of yeasts.

Standard description of *Saccharomyces wickerhamii* nov. spec.

Growth in malt extract: After 2 days at 25°C. cells are oval to slightly elongate (2.1 – 5.2) × (3.1 – 8.7) μ, occurring singly, in pairs and in branched chains. A slight sediment is present but no ring or pellicle. In older cultures a spotty ring develops, occasionally islets.

Growth on malt agar: After 1 month the growth is yellowish-buff. The surface is slightly glossy, smooth with an indication of sectoring at the edge. Texture butyrous. Cross section of the streak is almost flat. The margin is slightly irregular but not hairy.

Slide cultures: A pseudomycelium was not present after 10 days.

Sporulation: Abundant on malt agar in 3 days. Spores kidney-to crescent-shaped, usually 4 per ascus. Conjugation prior to ascus formation has not been observed. The asci rupture very soon after maturation.

Fermentation: glucose +, galactose + (weak), sucrose + (slow). Maltose, raffinose and lactose are not fermented.

Assimilation: glucose, galactose, L-sorbose (latent), sucrose, cellobiose, lactose, D-xylose, ethanol (with the formation of a thin, smooth pellicle), glycerol, D-sorbitol (latent and weak), salicin, lactic acid and succinic acid (weak). The other compounds are not utilized. Arbutin is split weakly.

Assimilation of KNO_3: negative.

Acid production on chalk agar: weak.

Growth in a vitamin free medium: absent.

The cultures were isolated from *Drosophila montana* and *D. pinicola* in two different areas. Culture F-394A has been designated as the type species.

One culture was isolated which could be identified with *Saccharomyces uvarum*. An authentic culture of this melibiose fermenting yeast was obtained from the C.B.S. culture collection and its assimilation pattern proved to be identical with that of our strain.

Since the complete assimilation reactions have not been reported in the literature they are given in Table 2.

Another melibiose fermenting yeast was isolated which could not be identified with previously described species. It resembles *S. microellipsodes* in its fermentation characteristics, except that galactose is fermented strongly. Furthermore it can assimilate maltose, ethanol, D-sorbitol, D-mannitol and α-methyl-D-glucoside, which cannot be used by *S. microellipsodes*. We propose to name the new yeast in honor of Professor A. J. KLUYVER in recognition of his stimulation of the many taxonomic studies on yeast carried out in his laboratory.

Standard description of *Saccharomyces kluyveri* nov. spec. [1]).

Growth on malt extract: After 3 days at 25°C. cells oval to long-oval (2.3 – 7.0) × (2.6 – 11.5) μ, occurring singly, in pairs and in well developed chains. Only a sediment is present. In older cultures a fairly well developed ring develops gradually, but a pellicle is not formed.

Growth on malt agar: After one month the growth is cream colored. Center of the streak has a few craters, but the surface is otherwise smooth and semi-glossy. Texture is butyrous. The cross-section of the streak is convex. The margin is somewhat irregular with an indication of a few tufts of pseudomycelium.

Slide cultures on potato dextrose agar: A few tufts of primitive pseudomycelium were observed, particularly underneath the cover-slip. There is little differentiation between pseudomycelium cells and blastospores.

Sporulation: spores are formed abundantly after 12 days on vegetable agar. Nearly always 4 spherical spores per ascus and there is no evidence of conjugation prior to spore formation. The spores have a smooth surface and contain a small refractive granule. The asci do not rupture at maturity.

Fermentation: glucose +, galactose +, sucrose +, raffinose + (complete). Maltose and lactose are not fermented.

Assimilation: glucose, galactose, L-sorbose (latent and weak), maltose, sucrose, melibiose, raffinose, melezitose (latent and weak), ethanol (with the production of a slight pellicle), D-mannitol, D-sorbitol and α-methyl-D-glucoside. Lactic and succinic acid appear to be utilized very weakly. Arbutin is not split. The other compounds are not assimilated.

Assimilation of KNO_3: negative.

Acid production on chalk agar: rather weak.

Growth in a vitamin free medium: absent.

The culture was isolated from *Drosophila pinicola* at Mather.

[1]) A second strain of this species was recently isolated in our laboratory from the exudate of a willow in Davis, California.

TABLE 2.

Assimilation data for some yeasts isolated from *Drosophila*. V indicates variable results (some strains assimilate the compound, others do not or only weakly). L indicates latent utilization. W signifies weak growth.

	D-glucose	D-galactose	L-sorbose	Maltose	Sucrose	Cellobiose	Trehalose	Lactose	Melibiose	Raffinose	Melezitose	Inulin	Soluble starch	D-xylose	L-arabinose	D-arabinose	D-ribose
S. montanus	+	+	+	+	+	+	+	—	—	+	+	+	—	—	+	—	—
S. cerevisiae	+	+	—	+	+	—	L+	—	—	+	+	—	—	—	+	—	—
S. cerevisiae var. *tetrasporus*	+	+	—	+	+	—	V	—	—	+	+	—	—	—	L	—	—
S. florentinus	+	+	—	+	+	—	+	—	+	+	+	—	—	—	+	—	—
S. uvarum	+	+	—	+	+	—	+	—	+	+	+	—	—	—	+	—	—

	L-rhamnose	Ethanol	Glycerol	i-Erythritol	Adonitol	Dulcitol	D-mannitol	D-sorbitol	α-methyl-D-Glucoside	Salicin	Gluconic acid	Ca-2-keto-gluconate	K-5-keto-gluconate	D-L lactic acid	Succinic acid	Citric acid	i-Inositol
S. montanus	—	W	—	—	—	—	+	+	+	L	—	+	—	+	+	—	—
S. cerevisiae	—	V	L or	—	—	—	—	—	—	—	—	—	—	—	L	—	—
S. cerevisiae var. *tetrasporus*	—	— or W	—	—	—	—	V	—	+	—	L or —	—	—	+	—	—	—
S. florentinus	—	W	V	—	—	—	+	+	+	—	W	—	W	V	+	—	—
S. uvarum	—	—	V	—	—	—	—	—	+	—	—	—	—	—	—	—	—

Genus *Pichia*. Seven isolates were obtained belonging to *Pichia*. The two cultures of *P. fermentans* and the one of *P. membranaefaciens* fitted the standard descriptions very well. Complete carbon assimilation reactions of these cultures were not determined, since the isolates were lost during the early phases of the investigations. The fourth culture does not form a pellicle on malt extract, although it produces a well developed pseudomycelium. It fits in the amended diagnosis of the genus *Pichia* given in the preceding paper (10a). Since the carbon assimilation reactions are quite different from previously described species it is considered to be a new species. It has been named *Pichia xylosa*, since it utilizes the sugar D-xylose in contrast to most species of this genus.

Standard description of *Pichia xylosa* nov. spec.

Growth in malt extract: After 3 days at 25°C. cells short-oval, $(2.1 - 4.9) \times (3.5 - 7.0)\ \mu$, occurring singly, in pairs and in clusters up to about nine cells. A spotty ring begins to form and a sediment, but no film. After 3 weeks a heavy ring and sediment are present and a few islets, which may have formed from fragments of the ring.

Growth on wort agar: After 1 month whitish to cream colored,

semi-glistening with some craters and sectors on the surface. Texture butyrous. Cross section rather low convex. Margin irregular with a very short fringe of pseudomycelium.

Slide culture on potato-dextrose agar: After one week a well developed pseudomycelium present. Aerobically the Blastodendrion type is typical whereas anaerobically (under the coverslip) a Mycotoruloides type is more common.

Sporulation: Fairly abundant on malt agar after 10 days. Spores hat-shaped, 2-4 per ascus (mostly four). Conjugation prior to sporulation was not observed. The asci rupture readily at maturity. Spores contain a small refractive granule.

Fermentation: Only glucose is fermented (slowly). A full tube of gas is produced in about 10 days.

Assimilation: Glucose, maltose, sucrose, cellobiose, trehalose, D-xylose, L-rhamnose (weak), ethanol, glycerol, D-mannitol, D-sorbitol, salicin, gluconic acid, lactic acid, succinic acid and citric acid. Arbutin is split. The other compounds are not utilized.

Assimilation of KNO_3: negative.

Growth in vitamin free medium: negative.

Acid production on chalk agar: rather weak.

The remaining 3 isolates were designated as *Saccharomyces pastori* (2 cultures) and *S. pini* (1 culture) in our previous paper (9). In line, however, with the arguments presented by PHAFF (10a) who favored a transfer of these two species to the amended genus *Pichia*, these yeasts will be considered as *Pichia pastori* and *Pichia pini* respectively.

Genus *Hansenula*. *H. angusta* was the only species isolated and it is represented by 17 isolates. Their properties corresponded quite well with the description given by WICKERHAM, except that our isolates were unable to utilize D-arabinose and D-xylose, which are reported to be variable or positive for the type species. This property of our strains has been confirmed by Dr WICKERHAM and it is therefore best to consider the utilization of these compounds as variable. Otherwise the cultures are quite similar. *H. angusta* appears to have its natural habitat in association with insects in deciduous trees (WICKERHAM, personal communication) and it seems likely that the flies obtained the yeast from such trees. It is interesting, however, that this yeast was not isolated from the fluxes of oaks (10) which represent the principal deciduous tree in the areas indicated in Table 1.

Genus *Hanseniaspora*. Three species were found, *H. valbyensis* (6 isolates), *H. uvarum* (6 isolates) and *H. osmophila* (5 iso-

lates). The last two species may also be considered as members of the genus *Kloeckeraspora* Niehaus. However, we have followed SHEHATA *et al.* (13) who proposed to transfer the species of this rather doubtful genus to *Hanseniaspora*. *H. uvarum* and *H. osmophila* have retained their ability to sporulate fairly well over a period of 3 to 4 years. NIEHAUS (7) reported that his strains lost this ability soon after isolation. We usually found one spore per ascus, occasionally two. The two species are differentiated by the fact that *H. osmophila* assimilates maltose, whereas *H. uvarum* cannot utilize this sugar.

Genus *Kloeckera*. This genus is generally considered the imperfect form of *Hanseniaspora* although not all species of *Kloeckera* have known perfect forms. Both *Kloeckera apiculata* (15 isolates) and *Kloeckera magna* (13 isolates) were quite common. We agree with LODDER and KREGER – VAN RIJ that *K. apiculata* is the imperfect form of *Hanseniaspora valbyensis*. *Kloeckera magna* is probably the imperfect form of *H. osmophila*. It is interesting that we isolated both the perfect and the imperfect forms of these two yeasts in our survey. We have confirmed the earlier observation of WICKERHAM and BURTON (16) that cellobiose is always among the very few carbon compounds which the apiculate yeasts can utilize. Some of our strains were able to cause a weak fermentation of cellobiose. It seems likely that these yeasts have a competitive advantage in the forest, by being able to use this breakdown product of cellulose produced by extracellular enzymes of other organisms.

Genus *Torulopsis*. Of the 5 species of *Torulopsis* only *T. stellata* was found in significant numbers (10 isolates from 3 areas). According to LODDER and KREGER – VAN RIJ (5) the C.B.S. collection has only one strain, isolated in 1931 from "Trockenbeerenauslese" by KROEMER and KRUMBHOLZ (2,3). This material consists of grapes with a very high sugar content shriveled on the vine. It is of interest that isolates of this species die rather rapidly on malt-agar slants. After 4 to 6 months the streak cultures are quite brown and very few cells are viable. *Torulopsis glabrata* has been obtained usually from human sources and appears to be associated with certain infections. The first culture from other sources was reported by PHAFF *et al.* (8) who isolated it from shrimp caught in the Gulf of Mexico. The single isolate from *Drosophila* is another example of a non-pathological origin. All isolates belonging

to *Torulopsis* corresponded well with the descriptions given by LODDER and KREGER-VAN RIJ.

Genus *Cryptococcus*. Five isolates were found to be similar to *Cr. diffluens* and two to *Cr. laurentii*. All produced starch-like compounds and contained very low concentrations of carotenoid pigments, which fits the definition of the genus. It might be mentioned that the species of this genus, as defined by LODDER and KREGER – VAN RIJ (5), constitute mixtures of different metabolic types. The reason is that these authors used only five sugars in differentiating these non-fermentative yeasts. If more carbon compounds are used large differences between strains of the present species can be detected. Already the addition of raffinose and melibiose to the customary five sugars shows up distinct differences. At this time, however, we do not wish to create a number of additional species on this basis in *Cryptococcus* (or in other imperfect genera as well) until such genera can be studied in detail. In addition the taxonomic position of the genus *Cryptococcus* has been somewhat weakened by the studies of NAKAYAMA *et al.* (6) who showed that strains of certain species of *Cryptococcus* were able to produce appreciable quantities of β-carotene, giving the cultures a yellow appearance.

Genus *Rhodotorula*. In line with the above statements we have decided to retain the species *Rhodotorula aurea* (Saito) Lodder in the genus *Rhodotorula* rather than consider it as a synonym of *Cr. laurentii* as was done by LODDER and KREGER – VAN RIJ (5). One isolate was obtained which corresponded exactly to the description given by LODDER (4). Although it produces a starch-like compound, the isolate was distinctly orange-yellow on malt extract slants and significant quantities of carotenoid pigments could easily be demonstrated in this yeast by the procedure of NAKAYAMA *et al.* (6). In this respect one might say that this yeast occupies an intermediary position between *Cryptococcus* and *Rhodotorula* as was also the case with *Rhodotorula peneaus* described by PHAFF *et al.* (8). The other species of *Rhodotorula* (*R. minuta, R. glutinis* and *R. mucilaginosa*) checked well with the descriptions given in the literature (4,5). The authors have evidence that these species are also made up of a number of physiological types.

Genus *Candida*. *Candida krusei* was the most common species encountered. It was found that the 10 strains isolated could be placed in three distinctly different groups with regard to their

carbon assimilation behavior. According to the criteria used by LODDER and KREGER-VAN RIJ (fermentation and assimilation of glucose only) they would be considered identical. In view of the discoveries by WICKERHAM and BURTON (18, 19) that many imperfect yeasts may be haploid heterothallic mating types of perfect species, we have refrained from subdividing such well known yeasts as *Candida krusei* into several additional new imperfect species, until a systematic comparative study can be made of a considerable number of strains that are now classified as a single species. WICKERHAM has shown quite clearly (19) that only strains with approximately the same assimilation scheme may be expected to belong to complementary mating types. Such detailed carbon assimilation patterns are not yet known for most imperfect yeasts. We have not attempted, therefore, to induce *C. Guilliermondii* (one isolate) to sporulate by mixing it with known mating types, even though WICKERHAM has shown that certain strains of this species constitute the imperfect form of *Endomycopsis guilliermondii* (19). Thus, all species of *Candida* were identified solely by the system of LODDER and KREGER-VAN RIJ and it was found that the isolates checked reasonably well with the descriptions given by these authors.

Genus *Trichosporon*. The single isolate of *Trichosporon fermentans* agreed well with the standard description of this species. Two cultures belonging to the genus *Trichosporon* could not be identified with any of the species described by LODDER and KREGER-VAN RIJ or by other workers, because of biochemical characteristics which differ from the described species. In addition they are morphologically very interesting in that numerous very sharply pointed cells, resembling needles, develop in bunches on some of the oval budding cells (see fig. 1). Because of the very characteristic needle shaped cells, the name *Trichosporon aculeatum* is proposed. One culture was isolated from *D. pinicola* near Mather (altitude 5000 ft.) and the other from *D. occidentalis* near Tioga Pass (altitude 10.000 ft.). Culture No. F-145A has been chosen as the type.

Standard description of *Trichosporon aculeatum* nov. spec.

Growth on malt extract: After 3 days at 25°C. an extensive mycelium is formed. Diameter 3.5-10.0 μ. Hyphal tips are frequently swollen. Arthrospores and small clusters of blastospores are present. The size of the blastospores is $(3.5 - 4.0) \times (8.0 - 10.5)$. In older cultures some oval cells have outgrowths of needle-like cells, which are rounded off at the base and pointed at the other end. The needle-

like cells break off easily and many are found to be free floating in liquid preparations. The length of the needle-shaped cells is 14.0 to 23.0 μ. A heavy moist pellicle develops and eventually a mold-like growth forms throughout the flask.

Streak culture on malt agar: Color grey, surface semidull, felt-like texture, tough, strongly spreading, rather flat, with mycelial border.

Slide cultures on potato dextrose agar: Strong development of true mycelium with arthrospores in zig-zag formation. Blastospores at the septa.

Fermentation: absent (occasionally a few gas bubbles).

Carbon compounds assimilated: glucose, sucrose, maltose, cellobiose, trehalose, melezitose (latent), ethanol (weak), adonitol (latent), mannitol, sorbitol, α-methyl-D-glucoside, salicin (weak), Ca-2-ketogluconate (weak), succinic acid and citric acid.

Assimilation of nitrate: absent.

Splitting of arbutin: positive.

Production of starch-like compounds: absent.

Growth in a vitamin free medium: negative.

Acid production on chalk-agar: none.

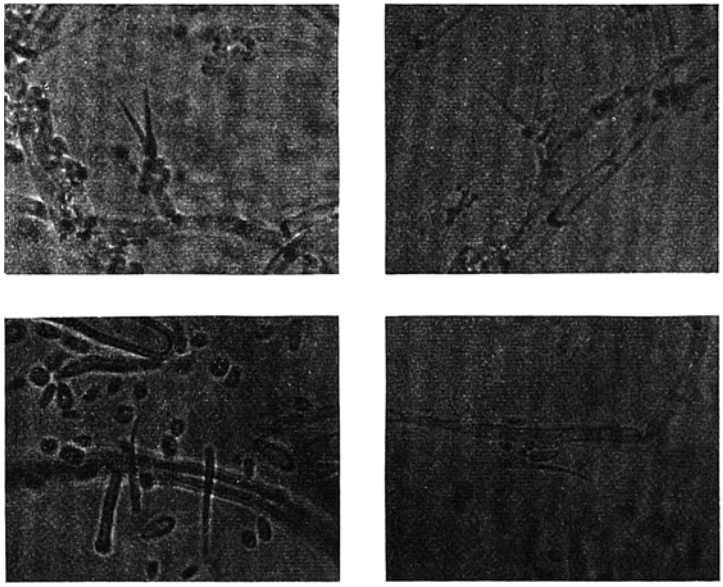

Fig. 1. *Trichosporon aculeatum* showing the typical needle shaped cells as well as true mycelium, budding cells (blastospores) and a few arthrospores.

Discussion.

The yeasts which have been isolated from *Drosophila* form a

rather characteristic group in that the large majority show considerable fermentative ability. This yeast flora is quite different from the one obtained from slime fluxes and mushrooms in the same area (10) which constituted primarily an oxidative group of yeasts. It seems valid to conclude that the flies do not obtain the yeasts from these sources. Since SHEHATA and MRAK (12) showed that *Drosophila* flies digest yeast cells rapidly, the yeasts found in the crops of these insects are not permanently associated with them as parasites or commensals, but rather are ingested with their food obtained from as yet unknown sources in the area (9). Only a few systematic studies have been published on the yeasts found in *Drosophila*. Our yeasts corresponded in general with the yeast flora found by SHEHATA *et al.* (13) in flies collected primarily in Southern California, although we obtained a larger number of species. Another extensive survey was made by DUDGEON (1) who unfortunately identified her yeasts only to the genus. The isolates obtained from 21 species of *Drosophila* collected in 12 different states of the United States, (not including California) were placed in the genera *Saccharomyces*, *Hansenula*, *Pichia*, *Debaryomyces* and *Candida* (genera commonly found) and *Kloeckera* and *Torulopsis* (occurring more rarely). One significant difference compared to our survey, is the fact that *Debaryomyces* was not found by us or by SHEHATA *et al.* (13). Since species were not described by DUDGEON (1) further study will be needed to clear up this discrepancy. It would be of interest to know, for example, whether *Debaryomyces fluxorum* described by PHAFF and KNAPP (10) was present among these isolates.

LODDER and KREGER – VAN RIJ (5, pg.3) have expressed their concern that the use of a large number of carbon compounds in the assimilation tests might lead to an undesirable and impracticable splitting of existing species into dwarf species. Whereas it is probably not essential to retain all of the presently used carbon compounds for species differentiation in a particular genus, their initial use serves to indicate which compounds may be most useful for differentiating purposes after all species in a genus have been studied (16). In this connection it is quite encouraging that our results show that as many as 36 strains of a species could be isolated from nature and that only minor variations in assimilatory behavior were noted. In addition, several of the species showed essentially identical assimilation patterns when they were compared with type species obtained from the C.B.S. culture collection. Thus, increasing

the number of carbon compounds for taxonomic purposes, does not appear to lead to an uncontrolled increase in number of species.

LATIN DESCRIPTIONS OF NEW SPECIES.

Saccharomyces wickerhamii nov. spec.

In musto maltato cellulea ovoideae, (2.1 — 5.2) × (3.1 — 8.7) μ, singulae, binae aut catenatae. Sedimentum et anulus crassus formantur. In agaro maltato (post unum mensem) cultura flavifusca, prope nitida, glabra, mollis, plana, margine parum undulato. Pseudomycelium nullum. Asci formatur ex transformatione cellularum vegetativarum diploidearum. Ad 4 reniformae ascosporae in quoque asco. Glucosum, galactosum (exiguum) et saccharosum (exiguum) fermentatur at non maltosum, raffinosum et lactosum. In medio minerali glucosum, galactosum, sorbosum (exiguum), saccharosum, cellobiosum, lactosum, xylosum, alcohole aethylicum (pellicula tenuis, glabra), glycerolum, sorbitolum (exiguum), salicinum, acidum lacticum, acidum succinicum (exiguum) assimilantur at non nitras kalicus. Arbutinum finditur exigua. Isolata ex *Drosophila montana* et *Drosophila pinicola*.

Saccharomyces kluyveri nov. spec.

In musto maltato cellulae ovoideae aut long ovoideae (2.3 — 7.0) × (2.6 — — 11.5) μ, singulae, binea aut catenatae. Sedimentum et anulus formatur. In agaro maltato (post unum mensem) cultura flavalbida, interdum crateriforma aut glabra, prope nitida,mollis, margine parum undulato, interdum parum piloso. Pseudomycelium primitivum formatur. Asci formatur ex transformatione cellularum vegetativarum diploidearum. Ad 4 ascosporae rotundae in quoque asco. Glucosum, galactosum, saccharosum, raffinosum et melibiosum fermentatur at non maltosum et lactosum. In medio minerali glucosum, galactosum, sorbosum (exiguum), maltosum, saccharosum, melibiosum, raffinosum, melezitosum (exiguum), alcohole aethylicum (pellicula tenuis), mannitolum, sorbitolum, alpha-methyl-D-glucosum assimilantur at non nitras kalicus. Arbutinum non finditur. Isolata ex *Drosophila pinicola* in Mather, California.

Pichia xylosa nov. spec.

In musto maltato cellulae ovoideae (2.1 — 4.9) × (3.5 — 7.0) μ, singulae, binae aut catenatae. Sedimentum, anulus et interdum insulae formantur. In agaro maltato cultura flavalbida, parum nitida, interdum crateriforma, mollis, prope plana, margine parum undulato et piloso. Pseudomycelium cum blastosporis abundat. Peleiformae ascosporae ad 4 aut 2 in quoque asco. Asci formantur ex transformatione cellularum vegetativarum diploidearum. Fermentatio exigua glucosi solius. In medio minerali glucosum, maltosum, saccharosum, cellobiosum, trehalosum, xylosum, rhamnosum (exiguum), alcohole aethylicum, glycerolum, mannitolum, sorbitolum, salicinum, acidum gluconicum, acidum lacticum, acidum succinicum, acidum citricum assimilantur at non nitras kalicus. Arbutinum finditur. Isolata ex *Drosophila miranda* in Mather, California.

Trichosporon aculeatum nov. spec.

In musto maltato cellulae ovoideae aut cylindricae (3.5 — 4.0) × (8.0 — — 10.5) μ, mycelium verum cum arthrosporis et blastosporis. Blastosporae ovoideae aut aculeatae. Anulus et sedimentum, bene crescit post dies 21. In agaro maltato cultura griseola, non nitida, tenax, prope plana, margine piloso. Fermentatio nullum. In medio minerali glucosum, saccharosum, maltosum, cellobiosum, trehalosum, melezitosum (exiguum), alcohole aethylicum (exiguum), adonitolum (exiguum), mannitolum, sorbitolum, alpha-methyl-D-glucosum, salicinum (exiguum), calcium-2-ketogluconicum (exiguum), acidum succinicum, acidum citricum assimilantur at non nitras kalicus. Arbutinum finditur. Isolata ex *Drosophila pinicola* et *Drosophila occidentalis* in Sierra Nevada, California.

Summary.

A survey was made of the yeasts occurring in the intestinal tract of wild species of *Drosophila* occurring in the Yosemite Region of California. Two hundred and forty one yeasts, representing 42 species and varieties, were identified. Each isolate was obtained from a different fly. Almost half of the isolates belong to *Saccharomyces*. The most common species in this genus were *S. montanus* (36 isolates), *S. veronae* (30 isolates), *S. cerevisiae* var. *tetrasporus* (22 isolates) and *S. drosophilarum* (13 isolates). Further species are listed in Table 1. *Zygosaccharomyces fermentati* Naganishi was shown to be a distinct species and not a synonym of *S. cerevisiae*. In order to avoid confusion with another yeast of the same name, it has been proposed to change the name *Z. fermentati* to *S. montanus* Naganishi. Two new species of *Saccharomyces* were described, *S. wickerhamii* and *S. kluyveri*. *S. mangini* var. *tetrasporus* has been renamed *S. cerevisiae* var. *tetrasporus*. A non-cellobiose attacking strain of *S. drosophilarum* has been designated tentatively *S. drosophilarum* var. *acellobiosa*. A new species of the genus *Pichia* was described as *P. xylosa*. *Saccharomyces pastori* and *Saccharomyces pini* were transferred to the genus *Pichia* on the basis of arguments given in the preceding paper. A new species of *Trichosporon* was described as *Tr. aculeatum* on the basis of the presence of characteristic needle-like cells. Common species besides those mentioned in *Saccharomyces* were *Hansenula angusta* (19), *Kloeckera apiculata* (15), *Kl. magna* (13), and *Torulopsis stellata* (10). Other genera represented were *Hanseniaspora, Cryptococcus, Rhodotorula, Candida* and *Oospora*. Evidence was obtained that many species of imperfect genera consist of distinctly different physiological types.

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