Cryptococcus vishniacii sp. nov., an Antarctic Yeast

HELEN S. VISHNIAC† AND WALTER P. HEMPFLING

Department of Biology, River Station, University of Rochester, Rochester, New York 14627

Antarctic soil samples collected by W. V. Vishniac contained (among other microbes) 98 isolates of undescribed, imperfect yeasts. These isolates clustered as 16 biotypes of greater than 90% similarity to each other but less than 90% similarity to all previously described yeast species. We propose to include all such isolates in the new species *Cryptococcus vishniacii*, named in honor of W. V. Vishniac. *C. vishniacii* grows at 4°C and below but not at 26°C and above, is cream-colored in mass, lacks pseudomycelia, is nonfermentative, produces "amylose," assimilates (at least) glucose, maltose, melezitose, trehalose, and xylose, and uses nitrate-N. The type strain, MTSW 304Y268 (= ATCC 36649), does not grow at 21 to 22°C or above and assimilates (additionally) L-arabinose, D-glucuronic acid, raffinose, L-rhamnose (weakly), sucrose, succinate, and citrate.

The Dry Valleys of South Victoria Land (Antarctica) are the most extreme cold deserts known. It may be anticipated that this highly stressed environment, low in microbial abundance and diversity (6), contains unique populations. Microbiological examination of soil samples collected in the Dry Valleys by W. V. Vishniac and Z. Bowen in 1973 yielded 98 isolates of previously undescribed yeasts. A conservative interpretation of our phenetic studies places all of these isolates in a single new species for which we propose the name *Cryptococcus vishniacii* (in honor of Wolf Vladimir Vishniac, 1922-1973, who died while investigating the microbial biota of Antarctica).

MATERIALS AND METHODS

A list of some strains used in this study is given in Table 1. Although all 98 isolates were characterized, we have, in the interest of brevity, listed only a single isolate of each biotype from each soil sample. Isolation procedures are described elsewhere (H. S. Vishniac and W. P. Hempfling, J. Gen. Microbiol., in press).

Stock cultures were both incubated and held, at 4°C, on slants of malt agar (Difco Laboratories, Detroit, Mich.) and GPY medium (glucose, 0.5%; peptone, 0.5%; yeast extract, 0.3%) with or without phosphate buffer (KP; pH 6.8, 10 mM). Unless otherwise specified, all concentrations were weight/volume percentages.

Standard methods currently in use in yeast taxonomy (12) were modified as required by available equipment, the sensitivity of some isolates to ammonium ions, and the characteristic low-temperature optima of these yeasts. Unless otherwise indicated, tests were performed at 10°C, using Difco nitrogen-, carbon-, and vitamin-free yeast bases solidified with 1.8% agar

(Difco) or made semisolid (in lieu of agitation of liquid cultures) with 0.1% agar or agarose (Sigma Chemical Co., Saint Louis, Mo. 63178). Optical densities or semisolid cultures were read at 660 nm in 1-cm-light-path cuvettes, using a Hitachi-Coleman Perkin-Elmer model 124 spectrophotometer.

Temperature tolerance and morphology (Dalmat plates) were studied by using malt agar, GPY agar, corn meal agar (Difco), and Y-1 (see below) agar.

Vitamin independence was examined by using culture vessels which had been heated to at least 400°C in a muffle furnace for a minimum of 2 h. Agarose (0.1%) did not interfere with detection of vitamin requirements of authentic strains. Results of tests with unshaken liquid media were consonant with those using (more optimal) semisolid media.

Sugars other than monosaccharides and D-glucuronic acid were sterilized by filtration through membrane filters (0.22-µm average pore size; Millipore Corp., Bedford, Mass.). Monosaccharides, sugar alcohols, and other organic acids were autoclaved separately as 10% solutions and added aseptically to prepared base. Soluble starch and inulin were autoclaved in the basal medium. Ethanol and decane were supplied by diffusion in closed containers to known users as well as to the test yeasts.

The D-glucose content of certain substrates was assayed by the glucose oxidase method (Glucostat Special, Worthington Biochemicals Corp., Freehold, N.J.); trehalose and raffinose did not contain detectable glucose (less than 0.005%, wt/wt); sucrose contained 0.1% (wt/wt), and melezitose contained slightly over 1% (wt/wt). The agar used contained unidentified substances (staining brown with Gram iodine) which were cleared by some isolates. Appropriate controls indicated that neither substrate contamination with D-glucose nor agar contaminants allowed significant growth.

Substrate assimilation tests were performed by inoculating duplicate (with and without either the recommended amino acids of Wickerham or 10 to 20 mg of Difco Casamino Acids per 100 ml) sets of dry agar plates with 14 isolates each, using a multipoint inoc-

† Present address: Department of Cell, Molecular and Developmental Biology, Oklahoma State University, Stillwater, OK 74074.

TABLE 1. Localities and sources for representative strains of *Cryptococcus vishniacii*

Serial no.	Laboratory no./biotype	Name	Source	Habitat
1.	MYSW 202Y252/12	<i>Cryptococcus vishniacii</i>	Site 202: North flank of Mt. Baldr (6,220 feet [ca. 1,896 m])	Soil (coarse, weathered dolerite gravel)
2.	202Y347/4			
3.	202Y349/11			
4.	202Y375/13			
5.	302Y253/12		Site 302: Center Cliff, near middle of east slope, Mt. Baldr (4,870 feet [ca. 1,485 m])	Soil (dolerite terrace)
6.	302Y259/14			
7.	302Y265/10			
8.	302Y309/4			
9.	302Y310/16			
10.	302Y312/5			
11.	303Y200/3		Site 303: Foot of glacier (4,700 feet [ca. 1,433 m])	Soil (dolerite moraine)
12.	303Y202/6			
13.	303Y206/9			
14.	303Y216/1			
15.	303Y336/15			
16.	303Y338/8			
17.	303Y354/4			
18.	303Y364/7			
19.	303Y365/2			
20.	303Y368/13			
21.	304Y267/12		Site 304: Slope of Baldr's Bathtub, 250 yards (ca. 229 m) from its center on course of 17° (4,660 feet [ca. 1,421 m])	Soil (sandstone, sand)
22.	304Y268/7	(type)		
23.	306Y212/6		Site 306: Escarpment, northwest part of valley, 300 yards (ca. 183 m) from cliff (4,750 feet [ca. 1,448 m])	Soil (sandstone, sand)
24.	306Y250/12			
25.	309Y215/4		Site 309: Labyrinth, south of Center Cliff, 250 feet (ca. 76 m) from frozen pond, and 30 feet (ca. 9 m) above (2,840 feet [ca. 866 m])	Soil (mixed sand, basalt columns)
26.	ATCC 12307	<i>C. albidus</i> , var. <i>diffuens</i> (type)		
27.	ATCC 22461	<i>C. bhutanensis</i> (type)		
28.	ATCC 22462	<i>C. himalayensis</i> (type)		
29.	IAM 4963	<i>C. himalayensis</i> (type)		

* MYSW, W. P. Hempfling, Department of Biology, University of Rochester, Rochester, N.Y.; ATCC, American Type Culture Collection, Rockville, Md.; IAM, Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan.

ulator (flat-tipped prongs 2 mm in diameter) charged from a well template containing just slightly milky suspensions of the yeast isolates in sterile distilled water or basal medium. The inoculator was drawn forward slightly during inoculation to give a streak approximately 4 by 9 mm. The absence of interactions between isolates was established by comparing the growth of selected isolates inoculated singly on slants with their growth on plates seeded by multiple inoculation.

Assimilation test plates were scored at about 7 days, 10 days, and 2, 3, and 4 weeks, after which they were flooded with Gram iodine to test for "amylose" production. Growth was judged "±" if visibly greater than the inoculum (a thin film viewed by oblique light), "+" if a complete but translucent layer, "++" if completely opaque to transmitted light from laboratory overhead lighting sources, or "+++" if opaque and markedly overflowing the bounds of the inoculum streak. Only strong (++ or greater), rapid (within 2

weeks), and unequivocal (continuous growth) responses were scored as positive; all other responses were scored as negative. Although minor variations in rapidity of growth occurred with variations in inoculum size and plate dryness, results scored as positive were replicable. Slow or weak responses, as well as some indeterminate responses which appeared to be dependent upon the presence of added amino acids, were not replicable.

The Wickerham media have failed to support the growth of some imperfect yeasts (3). These media, containing 38 mg-ions of ammonium ions per liter proved unsuitable for assimilation tests involving ammonium-sensitive yeasts during this study. The substrate responses of such isolates (and all other dubious results of testing by standard methods) were investigated by using medium Y-1. Medium Y-1 base contained NH_4NO_3 (1.25 mM), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (2.0 mM), potassium phosphate at pH 6.0 (7 mM), and 10 ml of a chelated trace metals solution per liter (14). Y-1, with substrates and vitamin supplements added aseptically as required, was used as a semisolid medium and was distributed in 10-ml amounts per 25-ml Erlenmeyer flasks. The responses obtained with this medium were reasonably replicable. We accordingly scored low growth (optical density at 660 nm of less than 1 after 10 days at 10°C from an inoculum of ca. 10^5 cells) as "positive (weak)." Y-1 media, with $(\text{NH}_4)_2\text{SO}_4$ (20 mM) substituted as the N-source, were inhibitory to *C. bhutanensis* and to those strains of *C. vishniacii* listed (see Table 2) as ammonium sensitive.

Salt (NaCl) tolerance was determined on Y-1 agar with 11 mM (0.2%) glucose. Since duration of incubation influences apparent tolerance, results were judged relative to the reported salt tolerances of the authentic cultures used. Comparative tests on GPY medium indicated distinctly lower salt tolerances, attributed to the possible salt content of the medium.

The characters used for single-linkage cluster analysis (modeled from reference 10) were: red- or orange-colored colonies; presence of pseudomycelia; fermentation of D-glucose and sucrose; assimilation of glucose, D-galactose, L-sorbose, sucrose, maltose, cellobiose, trehalose, lactose, melibiose, raffinose, melezitose, inulin, soluble starch, D-xylose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, ethanol, glycerol, erythritol, ribitol (adonitol), galactitol (dulcitol), D-mannitol, D-glucitol (sorbitol), methyl α -D-glucoside, salicin, lactate, succinate, citrate, inositol, and arbutin; growth with nitrate-N, ammonium-N, no vitamins, and 3, 6, and 9% NaCl; growth at 10, 20, 21 to 22 (GPY agar), 26, and 31°C; liquefaction of gelatin; urease production; and "amylose" production. Characters of known species were taken from the report of Barnett and Pankhurst (1), in which definite values were assigned. When a character was given as "variable" or "undetermined," we used our own results for authentic strains. Our results for authentic strains were identical to those of Barnett and Pankhurst (using the data of the Centraalbureau voor Schimmelfcultures) with the exception of some for *C. himalayensis*. Type cultures of *C. himalayensis* from both sources assimilated adonitol (ribitol) (Sigma), as do nearly all yeasts that utilize D-ribose (1). Neither culture assimilated lactose, nor was citrate or succinate assimilated. The assimila-

tion of the latter acids is sometimes temperature dependent (Vishniac and Hempling, in press); tests were conducted at 10, 20, and 23°C. Our characterizations of *C. vishniacii* isolates will not necessarily be repeatable by others if the Wickerham ammonium-containing media are used indiscriminately or if tricarboxylic acid cycle substrates are tested at temperatures near the upper limit for growth. Evison and Rose (2) have shown the effects of stressing temperatures on tricarboxylic acid cycle functions in *Candida* A3E-2.

RESULTS

Latin diagnosis of *Cryptococcus vishniacii* sp. nov. Coloniae in agar colore cremeo, aut politae et mucosae aut asperae et durae; in mediis liquidis difficiliter crescit, pelliculam haud, annulum exigue, et sedimentum lente formans. Crescit calore minus 4°C, optime 10°C aut major, sed non 26°C. Cellulae ovoideae ($4.5 \times 3 \mu\text{m}$ ad 8.5×5 aut $9 \times 4.5 \mu\text{m}$) aut subsphaeroideae (5.5 ad $7 \mu\text{m}$ d.) in diversas partes gemmant. Capsula exigua ad nulla. Nec ascosporas nec pseudomycelium vidi.

Nec glucosum nec sucrosus fermentat. Assimilat acidum citricum, acidum D-glucuronidum, acidum succinum, L-arabiosum, glucosum, maltosum, melezitiosum, raffiniosum, L-rhamnosum (lente), sucrosus, trehalosum xylosumque. Dum L-arabiosum, glucosum, sucrosus xylosumque assimilatur, amylosus formatum est. Non assimilat acidum lacticum, amylosus, D-arabiosum, cellobiosus, decanus, erythritolum, ethanolum, galactitolum (dulcitolum), D-galactosus, D-glucitolum (sorbitolum), glycerolum, inositolum, inulinum, lactosus, D-mannitolum, melibiosus, methyl- α -D-glucosidum, ribitolum (adonitolum), D-ribosus, salicinum nec L-sorbosus. Crescit cum nitrato; crescit sine vitaminis. Gelatinum et urea non finduntur.

Typus 304Y268 e solo, Mons Baldr 4660 p.

Differt in varietas quae sucrosus raffiniosumque non assimilant aut L-arabiosus aut L-rhamnosus non assimilant aut cellobiosus salicinumque assimilant. Effectus in methyl- α -D-glucosidum, acidum citricum succinumque, ammonum, vitaminis, natrium chloridum, calor-emque et mutantem.

Standard description of *Cryptococcus vishniacii* sp. nov. This species is typified by isolate 304Y268 isolated from soil (sandstone, sand) from Mt. Baldr (at 4,660 feet [ca. 1,421 m]) South Victoria Land, Antarctica, and deposited in the American Type Culture Collection (Rockville, Md.) as ATCC 36649. The species is considered to include all isolates showing greater than 90% similarity to the type isolate.

Isolate 304Y268 possesses all of the common characters of the species: colonies on agar are

TABLE 2. *Characteristics of Cryptococcus vishniacii* biotypes^a

Biotype	Isolate	Assimilation of:					Growth with:					Growth at various temp
		Sucrose (raffinose)	Cellobiose (salicin)	L-Arabinose	L-Rhamnose	Methyl α -D-glucoside	Citrate	Succinate	Ammonium	No vitamins	NaCl	
1.	303Y216	0 (0)	0 (0)	0	+	0	0	+	v	+	2	2
2.	303Y365	0 (0)	0 (0)	0	+	0	0	+	+	+	2	(≥ 2)
3.	303Y200	+	0	0	+	+	0	+	+	+	2	2
		(+)	(0)		(weak)	(weak)						
4.	309Y215											
	303Y354	+a	0	0	+	0	0	+	+	+	2	2
	302Y309	(+)	(0)		(weak)							
	202Y347											
5.	302Y312	+	0	0	+	0	0	+	I	+	2	2
		(+)	(0)		(weak)							
6.	306Y212	+a	0	0	+	0	0	0	+	+	2	2
	303Y202	(+)	(0)		(weak)							
7.	304Y268 ^b	+a	0	+a	+	0	+	+	+	+	2	1
	303Y364	(+)	(0)		(weak)							
8.	303Y338	+a	0	+a	+	0	+	+	+	+	2	(≥ 2)
		(+)	(0)		(weak)							
9.	303Y206	+	+	0	0	+	+	+	+	+	1	3
		(+)	(+)									
10.	302Y265	+a	+	+a	0	+a	+	+	+	+	2	4
		(+)	(+)									
11.	202Y349	+	+	+a	0	+a	+	+	+	s	2	(≥ 3)
		(+)	(+)									
12.	202Y252											
	302Y253	+	+	+a	0	+	+	0	+	+	2	3
	303Y267	(+)	(+)									
	306Y250											
13.	202Y357	+	+	+a	0	+	+	0	I	+	2	(≥ 3)
	303Y368	(+)	(+)									
14.	302Y259	+a	+	+a	0	0	+	0	+	+	2	4
		(+)	(+)				(weak)					
15.	303Y336	+a	0	+a	0	+	0	0	+	+	2	(≥ 2)
		(+)	(+)									
16.	302Y310	+a	+	+a	+	+	+	+	+	+	2	4
		(+)	(+)									

^a +, Positive; +a, positive and amylose produced; 0, negative; v, variably inhibited; I, inhibited; s, stimulated by vitamin supplement. Results for sucrose (raffinose) and cellobiose (salicin) are so coupled since they were (with one exception) identical. This similarity may depend upon the activity of a single enzyme in each case. The apparent exception (biotype 15) may resemble *Rhodoturula minuta* in possessing a β -glucosidase more actively hydrolyzing salicin than cellobiose (1). Growth in the presence of NaCl was scored as 1 where growth at 3% was greater than that of *C. albidus* var. *diffluens*, but growth at 6% was equally absent and was scored as 2 where growth at 6% approximately equalled that of *C. bhutanensis* (weak and delayed: Goto and Sugiyama [5] recorded growth at 6% but not growth at 9%) but not that of *C. himalayensis* (scanty growth at 9%: Goto and Sugiyama [5]). Temperature responses were complicated by variation in the array of temperatures available at different times during our study. They were scored as 1 for optimal growth at 10°C (rather than 4 or 20°C), 2 for optimal growth at 20°C (rather than 10°C) but no growth at temperatures of 21 to 22°C, ≥ 2 for optimal growth at 20°C but no growth at 23 to 24°C when temperatures of 21 to 22°C were not available for testing, 3 for growth on corn meal agar (an impoverished medium for these isolates) but not on GPY agar at 21 to 22°C, 4 for growth on GPY agar at 21 to 22°C, and ≥ 3 for growth on Y-1 agar at 23 to 24°C.

^b Type species.

cream colored, either glistening and mucoid (GPY agar) or tough and granular (malt extract agar) in appearance and texture. Growth in liquid media is limited; no pellicle is formed, a scanty annulus and slowly produced sediment giving the only evidence of growth. Cells are ovoid (4.5 by 3 μ m to 8.5 by 5 or 9 by 4.5 μ m) or subspherical (5.5 to 7 μ m in diameter) with a scanty (1 μ m) to no visible capsule. Budding is usually but not necessarily polar. Pseudomycelia are not formed and ascospores were not observed. Fermentation does not occur. Glucose, maltose, melezitose, trehalose, and xylose are assimilated, and D-arabinose, decane, erythritol, ethanol, galactitol (dulcitol), galactose, D-glucitol (sorbitol), glycerol, inositol, inulin, lactic acid, lactose, D-mannitol, melibiose, ribitol (adonitol), D-ribose, soluble starch, and sorbose are not assimilated. Nitrate-N is utilized, but neither gelatin nor urea is split. "Amylose" is produced during growth on glucose or xylose. There is no growth at 26°C.

In addition to these characters common to all isolates, 304Y268 assimilates L-arabinose and sucrose with amylose production and assimilates citric acid, D-glucuronic acid, raffinose, L-rhamnose (weakly), and succinic acid but not cellobiose or salicin. All biotypes of *C. vishniacii* assimilate D-glucuronic acid and fail to assimilate D-glucosamine. 304Y268 grows well on the Wickerham media, does not require vitamins, grows about as well as *C. bhutanensis* in the presence of 6% NaCl, and has an optimal temperature for growth nearest to 10°C (of the available temperatures), growing well at 4°C, poorly at 20°C, and not at all at 21 to 22°C.

Character differences of typical isolates belonging to biotypes with greater than 90% similarity to biotype 7 (304Y268) are given in Table 2. A similarity dendrogram including these isolates and the nearest previously described species is given as Fig. 1.

DISCUSSION

Generic assignment. The history of the genus *Cryptococcus* has been discussed by Lodder (8), Phaff and Fell (9), and Kreger-van Rij (7). *Cryptococcus* is an anasco sporogenous genus, lacking pseudomycelia, defined by a complex of additional characters which has included the absence of red or orange colonies, absence of fermentation, production of starchlike compounds (amylose) at low pH, and inositol assimilation. Golubev et al. (4) have suggested that D-glucuronic acid assimilation is diagnostically equivalent to the utilization of *myo*-inositol, the character considered decisive by some workers

FIG. 1. Percent similarity of biotypes of *Cryptococcus vishniacii* and the most similar yeast species. D, *C. albidus* var. *diffluens*; B, *C. bhutanensis*; P, *C. (T.) pignaliae*; M, *C. (T.) mannifaciens*; T, *C. terreus*; H, *C. himalayensis*. *C. vishniacii* biotypes clustered at 93.5%.

(9). If the diagnosis of *Cryptococcus* is amended by this suggestion, *C. vishniacii* meets all of the historical criteria for inclusion in this genus.

Many systematic microbiologists would prefer to follow van Niel (13) in refusing to give any single physiological character generic weight. Our generic assignment is based upon numerical analysis of the similarity between our individual biotypes and all described species of yeasts (of whatever genus) as tabulated by Barnett and Pankhurst (1). The known yeasts most similar to *C. vishniacii* include species of *Cryptococcus* (*C. albidus* var. *diffluens*, *C. bhutanensis*, *C. himalayensis*, and *C. terreus*) and of *Candida* (*Torulopsis*) (*Candida mannifaciens* and *Candida pignaliae*), but the closest linkage is to other species of *Cryptococcus* (Fig. 1.). *C. bhutanensis* Goto and Sugiyama (5), like *C. vishniacii*, fails to assimilate inositol but does assimilate D-glucuronic acid (4).

Specific assignment. *C. vishniacii* differs from *C. albidus* var. *diffluens* ATCC 12307 in colonial morphology, lacking the slimy translucent appearance and cohesion of the latter; in lower cardinal temperatures; in greater salt tolerance; in lack of urease; in ability to grow in vitamin-free media; and in failure to assimilate inositol. The type isolate of *C. vishniacii* also differs in inability to assimilate cellobiose.

C. vishniacii resembles *C. bhutanensis* in colonial morphology and degree of encapsulation as well as in failure to assimilate inositol, but differs from the latter in slightly lower temperature limits (*C. bhutanensis* grew under our conditions at 26 to 27°C but not at 31°C) and in

failure to assimilate mannitol, soluble starch, sorbose, sorbitol (glucitol), and D-arabinose or to require vitamin supplements *C. bhutanensis* fails to produce amylose consistently on Wick-erham media but does so on media containing lower ammonium levels and suitable substrates.

The phenetic characterization of imperfect yeasts provides an inadequate measure of genetic dissimilarity. A single difference in substrate utilization may be associated with a relatively wide difference in guanine-plus-cytosine composition (11). A comparison of guanine-plus-cytosine composition in the biotypes of *C. vishniacii* might well indicate that they represent independently derived genotypes converging in an uniquely stressed environment. In the absence of such data, the erection of a single species appears appropriately conservative.

ACKNOWLEDGMENTS

This study was supported by a grant (NGR-33-019-002) from the Office of Space Sciences, National Aeronautics and Space Administration, Washington, D.C. H.S.V. is grateful for support as Florence B. Seibert Fellow of the American Association of University Women during part of this study.

We wish to thank K. Yamasato and the Culture Collection of the Institute of Applied Microbiology of the University of Tokyo for *C. himalayensis* IAM 4963, R. R. Colwell for reagents, H. J. Phaff for advice, and the following members of the MYSW laboratory who participated in isolation and analysis: T. Kurtz, P.L.R. Farrell, C. Rice, and I. Uydess. We are indebted to L. Rodrigues de Miranda of the Centraalbureau voor Schimmelcultures for stimulating discussions.

REPRINT REQUESTS

Address reprint requests to: Dr. H. S. Vishniac, Department of Cell, Molecular and Developmental Biology, Oklahoma State University, Stillwater, OK 74074.

ADDENDUM

All biotypes of *C. vishniacii* assimilate D-glucuronic acid and fail to assimilate D-glucosamine.

LITERATURE CITED

1. Barnett, J. A., and R. J. Pankhurst. 1974. A new key to the yeasts. North Holland Publishing Co., Amsterdam.
2. Evison, L. M., and A. H. Rose. 1965. A comparative study on the biochemical basis of the maximum temperatures for growth in three psychrophilic microorganisms. *J. Gen. Microbiol.* **40**:349-364.
3. Fell, J. W. 1974. Distribution of yeasts in the water masses of the southern oceans, p. 510-523. *In* R. R. Colwell and R. Y. Morita (ed.), *Effect of the ocean environment on microbial activities*. University Park Press, Baltimore.
4. Golubev, V. I., O. N. Okunev, and N. V. Vdovina. 1974. Assimilation of i-inositol by yeasts as a diagnostic character. *Mikrobiologiya* (English transl.) **43**:890-893.
5. Goto, S., and J. Sugiyama. 1970. Studies on Himalayan yeasts and moulds. IV. Several asporogenous yeasts including two new taxa of *Cryptococcus*. *Can. J. Bot.* **48**:2097-2101.
6. Horowitz, N. H., R. E. Cameron, and J. S. Hubbard. 1972. Microbiology of the Dry Valleys of Antarctica. *Science* **176**:244-245.
7. Kreger-van Rij, N. J. W. 1969. Taxonomy and systematics of yeasts, p. 5-78. *In* A. H. Rose and J. S. Harrison (ed.) *The yeasts*, vol. 1, Academic Press Inc., New York.
8. Lodder, J. 1970. General classification of the yeasts, p. 1-33. *In* J. Lodder (ed.), *The yeasts—a taxonomic study*. North Holland Publishing Co., Amsterdam.
9. Phaff, H. J., and J. W. Fell. 1970. *Cryptococcus* Kutzing emend. Phaff et Spencer, p. 1088-1091. *In* J. Lodder (ed.), *The yeasts—a taxonomic study*. North Holland Publishing Co., Amsterdam.
10. Sneath, P. H. 1972. Computer taxonomy, p. 29-98. *In* J. R. Norris et al., *Methods in microbiology*, vol. 7A. Academic Press Inc., New York.
11. Starmer, W. T., H. J. Phaff, M. Miranda, and M. W. Miller. 1978. *Pichia cactophila*, a new species of yeast found in decaying tissue of cacti. *Int. J. Syst. Bacteriol.* **28**:318-325.
12. van der Walt, J. P. 1970. Criteria and methods used in classification, p. 34-113. *In* J. Lodder (ed.), *The yeasts—a taxonomic study*. North Holland Publishing Co., Amsterdam.
13. van Niel, C. B. 1971. The yeasts—a taxonomic study (review). *Science* **172**:552.
14. Vishniac, W., and M. Santer. 1957. The thiobacilli. *Bacteriol. Rev.* **21**:195-213.