

MYCOCANDIDA RIBOFLAVINA

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The following study is based on culture no. 921, resulting from a long series of selections from stock culture no. 321 in the collection of Anheuser-Busch, Inc., St. Louis. The latter was originally isolated from figs by H. J. Phaff and sent under the name *Saccharomyces fragilis* ?.¹

Mycocandida riboflavina Dodge, sp. nov.

Pseudomycelium ex cellulis longe ellipsoideis, 10–11 \times 1.6–2.5 μ , ramis laterali-bus 1–2 (–4) ad quemque nodum, cellulis 1–3. Colonia parva, cremea vel albida, laevis vel subfoveolata, margine tenui. Colonia rugosa cremea, crateriformis, rugis radiantibus, subelevatis, margine crassiori. Gelatina tarde liquefacta. Glucosa, fructosa, mannosa, sucrosaue fermentatae.

Cells in young cultures variable in shape from ellipsoid to long-ellipsoid, or ovoid, often with both ends rather acute, budding polar, but cells not apiculate as in *Kloeckera* (*Pseudosaccharomyces*), mostly single, a few in short chains.

In old liquid cultures (four months) similar to young cultures but chains somewhat longer with 2 (rarely up to 4) short branches at the nodes (up to 3 cells long), cells ellipsoidal to subpyriform, terminal cells short-ellipsoid to nearly spherical; one spherical cell seen with 4 buds at one end and 3 at the other.

On old malt agar cultures, pseudomycelium well developed, cells 10–11 \times 1.6–2.5 μ , branching lateral, only 1 or 2 branches at a node, cells long-ellipsoid or with the end bearing the branch slightly enlarged and more rounded; terminal cells shorter.

No ascospores produced on old cultures (some completely dried out) nor on gypsum blocks nor on Gorodkova agar.

COLONY CHARACTERS AND SECTORING

On malt extract agar (15° Balling), colony small, margin thin, sloping gently to the center, surface smooth with very minute pitting and with some very shallow radial valleys, with a small rugose sector. Transfers from the rugose sector produced colonies with a shallow central crater, with low broad radial folds and a few cross folds, margin circular and somewhat elevated, with a smooth sector occupying about one-sixth the circle. Colonies cream buff with a lighter margin. Transfers from the smooth sector produced colonies with a very low dome in the central crater, sloping gently to the margin, with 4 or 5 radial valleys, surface smooth with some very shallow pits (visible under a 9 \times hand-lens), margin very smooth and slightly elevated. Colonies cartridge buff or darker, margins somewhat lighter. No further sectoring occurred on smooth colonies.

¹Mrak, E. M., H. J. Phaff, R. H. Vaughn, and H. N. Hansen. Yeasts occurring in souring figs. *Jour. Bact.* 44:441–450. 1942.

On Sabouraud glucose agar, colonies with a low central plateau, broadly crenate margins with narrow radial valleys connecting the central plateau to the notches in the margin. Transfers from the smooth type of colony on malt extract agar produced colonies with a nearly smooth center (only a very faint suggestion of a crater), sloping very gently to the margin, surface rather dull from minute pitting (about the limits of visibility with a $9 \times$ hand-lens), no radial valleys nor ridges, margin less elevated, with the faintest suggestion of marginal striation (under $9 \times$ lens). Color pale ochraceous salmon. Transfers from the rugose sector on malt extract produced colonies with a low central dome in a very shallow excentric crater, sloping gently to the margin, a very few slight ridges and radial valleys (arranged as the lamellae of a mushroom). Colony light ochraceous salmon, margin paler. No sectoring was observed on Sabouraud glucose agar.

Yeast decoction agar: colony more elevated, very moist and shining, faint depression in center with about 4 very shallow, radial valleys, pure white. Transfers from either smooth or rough sectors on malt extract agar produced the same type of colony on yeast decoction agar.

BIOCHEMICAL ACTIVITY

In general, liquid cultures produced a slight ring on the sides of the tube, no islets nor pellicle; the liquid remained clear and the sediment finely granular; in old liquid cultures (about 4 months), the ring is a little better developed and the sediment becomes slightly more flocculent. In fermentation tubes of glucose, mannose and lactose, a few islets developed but they were never abundant nor did they coalesce to form a pellicle. Litmus milk was neither acidified nor coagulated; a slight ring and abundant sediment developed in the tubes, showing that growth occurred. Gelatin was very slowly liquefied, complete in 16 weeks, abundant sediment, but no ring nor pellicle.

Fermentation: Gas is produced with glucose, fructose, mannose and sucrose, none with maltose nor lactose. Gas is produced much more slowly than with *Saccharomyces cerevisiae*, not showing until the second day after inoculation. No acid was produced under anaerobic conditions (long arm of fermentation tube); acid with glucose and lactose, none with mannose, maltose nor sucrose under aerobic conditions (short arm of fermentation tube). Sediment abundant in all tubes.

Since our organism was thought possibly related to *Brettanomyces* and since most species of the latter produce an after-fermentation of beer, 95 per cent ethyl alcohol was added to 15° Balling malt extract to make final concentrations of 5–12 per cent ethyl alcohol. At 5 per cent after three weeks, there was good growth with abundant flocculent sediment; at 6 per cent growth was good, but with less and more granular sediment; at 7 per cent growth was poor with very fine granular sediment. No growth at 8–12 per cent, hence the limiting concentration of alcohol lies between 7 and 8 per cent ethyl alcohol.

Two hundred ml. portions of Budweiser beer (4 per cent alcohol) were measured into flasks under aseptic conditions, and one set inoculated with *Myc-*

candida riboflavina, the rest being reserved as a control. The flasks were weighed daily. In 6 weeks, 2 gm. loss of weight was recorded, with a decrease of alcohol to 1.1 per cent (volume) and an increase in non-volatile organic acids measured. Had the organism been a species of *Brettanomyces*, an increase in ethyl alcohol and volatile organic acids would have been obtained.

TEMPERATURE RELATIONS

Week-old cultures in malt extract (15° Balling) were placed in a water bath at the desired temperature for half-hour and hour intervals. Good growth on subsequent plating occurred at 48° C. for a half hour and at all lower temperatures. No growth occurred at 49° C. nor above to 58° C. after half-hour exposures. About 20 colonies per plate were found on plates from cultures exposed to 48° for one hour; hence we conclude that the thermal death point is 49° for a half-hour exposure, and the maximum temperature for growth is 47–48° C. While no extensive experiments were made to determine optimum temperature for growth, such experience as we have had indicates that the optimum is about 30° C.

RELATIONSHIPS

At first examination of young cultures, our organism might be taken for *Kloeckera* (*Pseudosaccharomyces*), as the cells are rather elongate with acute ends, but not truly apiculate. Little or no pseudomycelium has ever been reported in *Kloeckera*, while our organism predominantly produces pseudomycelium in old cultures, placing it in the Eremascaceae Imperfectae. *Brettanomyces*, when grown on potato agar, resembles our organism in morphology, but fails to liquefy gelatin, produces sufficient volatile acid in malt extract to kill the cultures quickly (unless calcium carbonate is added to the medium), and ferments sugars very slowly. Our organism liquefies gelatin slowly, produces no volatile acid under similar conditions, and ferments sugars more rapidly if they be fermented at all.

Both morphologically and physiologically, our organism belongs in *Mycocandida* Langeron & Talice¹, based on the type species *Candida mortifera* Redaelli². Our organism differs from the type species in its fine granular sediment rather than flocculent growth with little sediment, liquefying gelatin more slowly and fermenting fewer sugars but growing better in those it does not ferment. Our organism has longer, slenderer ellipsoidal cells in malt and other media, while *M. mortifera* has nearly spherical ellipsoid cells.

Our organism resembles *Candida Guilliermondi* (Cast.) Langeron & Guerra³ in its fermentative ability, but the latter forms a pellicle on liquid media, fails to liquefy gelatin, renders litmus milk alkaline, fails to assimilate lactose, and has larger cells and pseudomycelium. Although some strains approach the morphology

¹Langeron, M., et Talice, R. V. Nouvelles méthodes d'étude et essai de classification des champignons levuriformes. Ann. Parasitol. Hum. Comp. 10:1–80. 5 pls. 1932.

²Redaelli, P. I miceti come associazione microbica nella tubercolosi polmonare cavitaria. Osservazioni micopatologiche e sperimentali. pp. 21–24. Pavia, 1925.

³Langeron, M., et P. Guerra. Nouvelles recherches de zymologie médicale. Ann. Parasitol. Hum. Comp. 16:429–476. pl. 12–24. 1938.

of *Mycocandida* on some media, in general it has the type of pseudomycelium of *Syringospora* (*Mycotorula*).

Our organism differs from strain 488 (isolated by L. J. Wickersham from sour milk and used by P. R. Burkholder⁴ in patent 2,363,227 under the name *Candida Guilliermondia*) in producing as good growth in lactose as in mannose and sucrose. *Candida Guilliermondi* (Cast.) Lang. & Guerra produced no growth on lactose and maltose while *C. Guilliermondia* Burkholder produced better growth on maltose than on glucose and only slight growth on lactose.

Our organism was thought to be an aberrant strain of *Saccharomyces fragilis* when first isolated and might be considered an asporogenous variant. It has the same general morphology when first transferred to fresh media, but is smaller, the cell walls are not fragile, and it has produced no ascospores by the usual techniques. It liquefies gelatin much more slowly and fails to ferment lactose. I have found no report of *S. fragilis* producing pseudomycelium in old cultures.

In view of the above considerations, our organism is an undescribed species of the Eremasaceae Imperfectae for which I propose the name *Mycocandida riboflavina*.

In conclusion, I wish to express appreciation to Anheuser-Busch for a research grant and permission to study this organism; to Dr. George W. Freiberg for helpful suggestions and photostats of pertinent literature not available in local libraries; to Dr. J. E. McClary for determinations of ethyl alcohol and volatile and non-volatile acids; to Dr. Lilian Nagel for the drawings illustrating morphology; and to Dean George T. Moore, of the Henry Shaw School of Botany of Washington University, for kindly interest in this study.

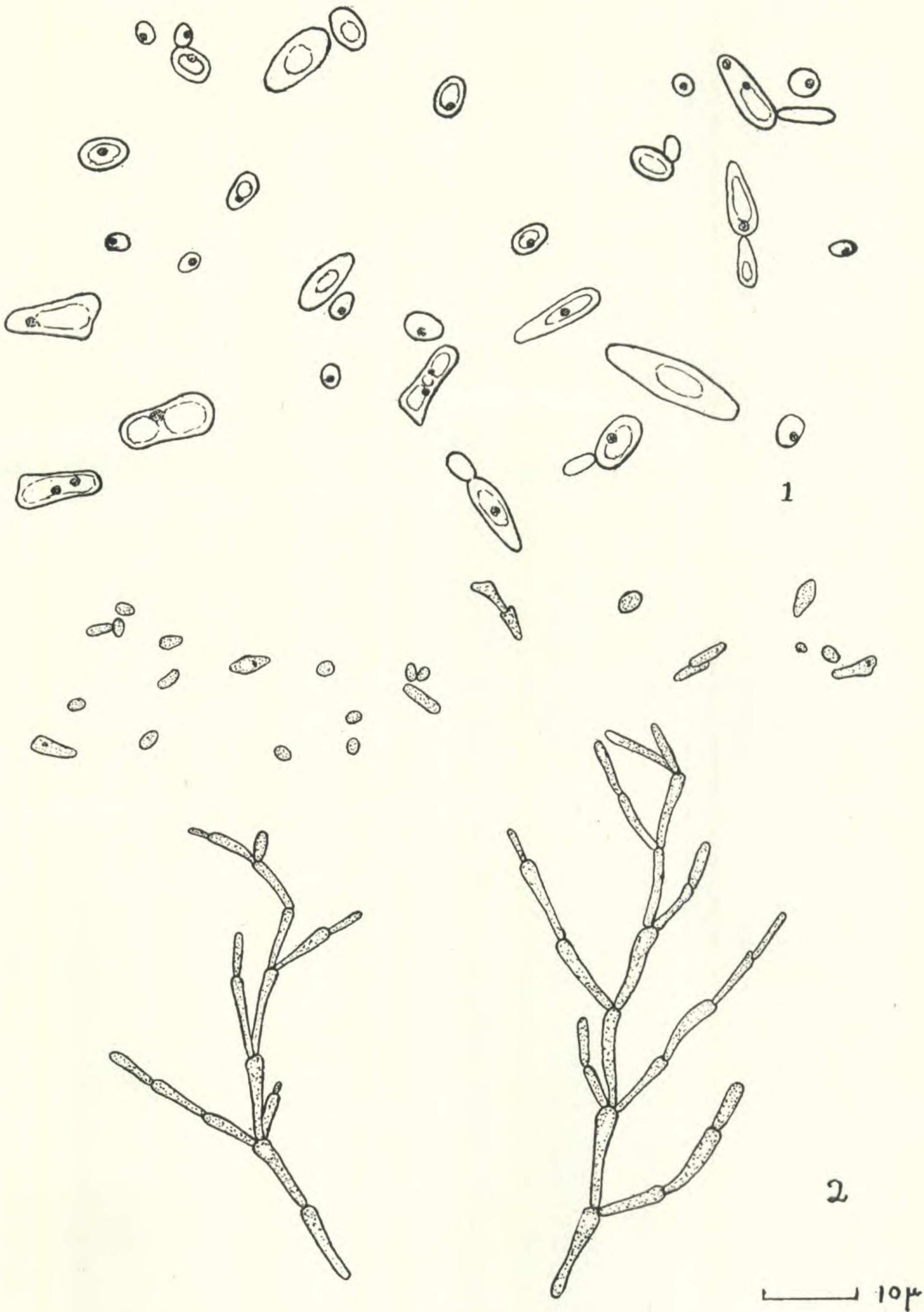
⁴Burkholder, P. R. Fermentation process for the production of riboflavin (vitamin B₂). U. S. Patent Office 2,363,227:1-3. 1944.

EXPLANATION OF PLATE

PLATE 7

Fig. 1. Cells from 3-day agar culture at room temperature.

Fig. 2. Cells from dried-out colony on agar plate, soaked in distilled water and stained with aceto-orcein. 10 × ocular, 90 × objective.



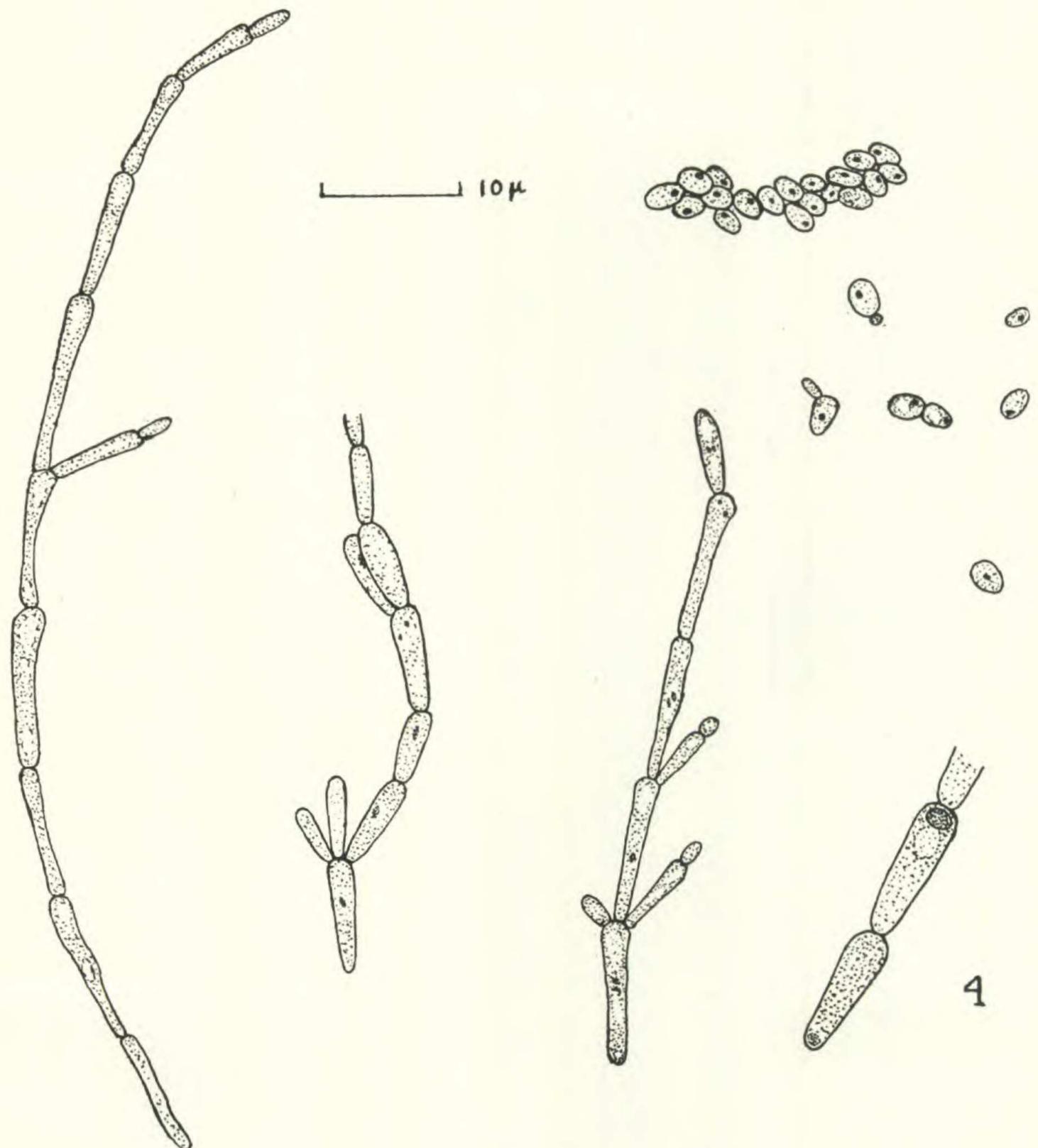
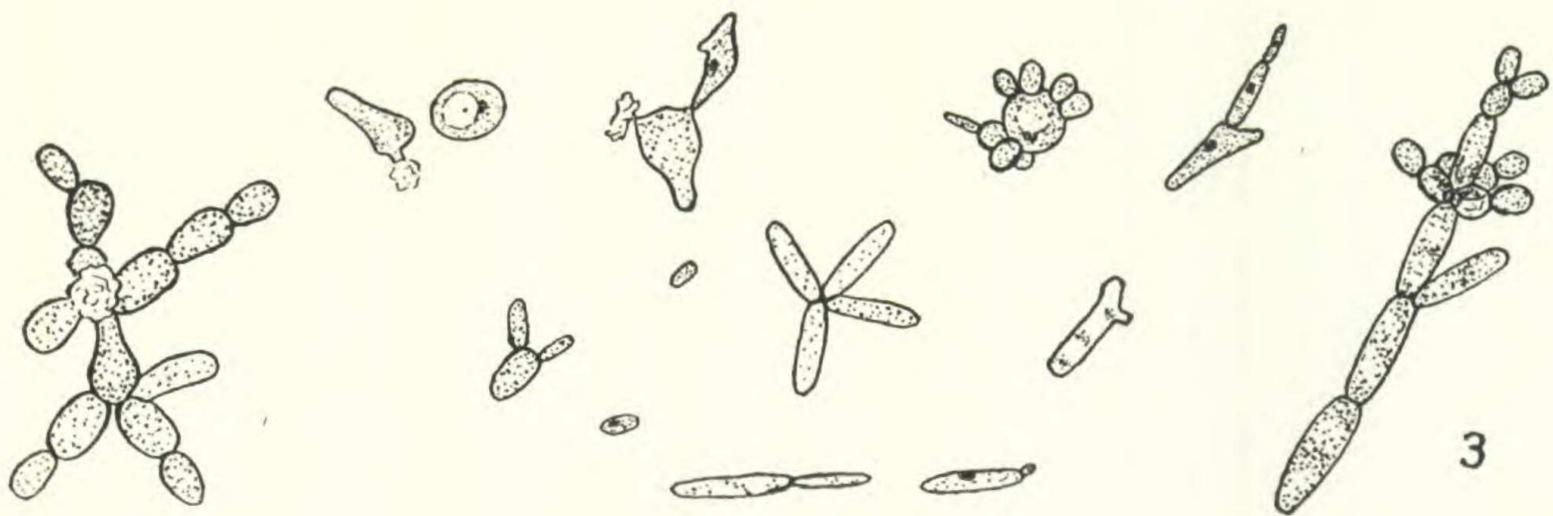
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EXPLANATION OF PLATE

PLATE 8

Fig. 3. Unusual types of cells from old lactose fermentation tube. Free-hand sketches.

Fig. 4. Pseudomycelium and cells from moist colony on agar slant, stained with acetoorcein. 10 × ocular, 90 × objective.



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